UNIVERSITY OF CAPE COAST

AN INVESTIGATION INTO THE POPULATION STRUCTURE OF *Bactrocera dorsalis* FROM FOUR AGRO ECOLOGICAL ZONES IN GHANA

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BY

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Thesis submitted to the Department of Conservation Biology and Entomology of the School of Biological Science, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Entomology.

DECEMBER 2023

DECLARATION

Candidate's Declaration

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

Candidate's Signature: .... Date: ....

Name: Ekene Kwabena Nwaefuna

Supervisors' Declaration

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

Principal Supervisor's Signature: .... Date.........................

Name: Professor Alexander Egyir-Yawson

Co-Supervisor's Signature: .... Date: ....

Name: Professor Rofela Combey

ABSTRACT

Fruit flies pose significant challenges as pests in the global cultivation of fruits and vegetables. This study reports how landmark based geometric morphometry and cuticular hydrocarbon analysis were used to discriminate populations of *Bactrocera dorsalis* from four Agro-ecological zones. The images of the right forewing of 706 samples were captured and imported into a TPS utility program to generate a TPS file. The TPS file was then imported into TPS Dig, version 1.40 to facilitate digitization of landmarks. Raw ‘x’ and ‘y’ coordinates were imported into Morpho J 1.07a for the analysis. For the cuticular hydrocarbon analysis, infested fruits were collected from the four Agro-ecological zones and incubated. The developing larvae were raised until they reached adulthood. Adult flies, aged 9 days, were used for Hexane extraction and Gas Chromatography–Mass Spectrometry (GCMS). Geometric morphometry utilized Procrustes ANOVA and Partial Least Square analyses to assess the significance of variation among populations. To gain better insights into population differences, Principal Component Analysis (PCA) was utilized to identify the wing feature contributing to the most variation, while Discriminant Function Analysis (DFA) was applied to determine cluster classifications of the populations. For the cuticular hydrocarbon analysis, a heat map was used to visualize the data and subjected to Principal Component Analysis (PCA). Significant variations were observed in the shape of the wing among the populations of the pest from four Agro ecological zones. Samples from the transition and coastal zones were most distant from each other while samples from Northern and Eastern Volta zones were most similar. This may have implications for population structure of the pest in Ghana.

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DEDICATION

“To my family in memory of my Late father”.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BNARI	Biotechnology and Nuclear Agriculture Research Institute
CHC	Cuticular Hydrocarbon
DFA	Discriminant function Analysis
GAEC	Ghana Atomic Energy Commission
IPM	Integrated Pest Management
MoFA	Ministry of Food and Agriculture
PCA	Principal Component Analysis
PPRSD	Plant Protection and Regulatory Services Directorate
REPMC	Radiation Entomology and Pest Management Centre
SIT	Sterile Insect Technique
UCC	University of Cape Coast

CHAPTER ONE

INTRODUCTION

Background of the Study

Fruit flies, classified under the family Tephritidae, are widely acknowledged as significant pests of fruits and vegetables and constitute the predominant group of agricultural pests within the Diptera order (Foote et al., 1993). The group contains over 4500 species that are distributed across both temperate and tropical areas across continents (Christenson & Foote 1960; Hendrichs et al., 2000; Benelli et al., 2014). This pest has expanded over a longitudinal distance of approximately 5000 km from north to south encompassing a continuous expanse of 8.3 million km^2. (Goergen et al., 2011). Its recognition as pests is because of the reproductive behaviour of adult females. All female fruit flies oviposit eggs into tissues of fruits. The developing larvae then feed off the pulp of the fruit until maturity where they exit to pupate (Christenson et al., 1960; Qin et al., 2015). Among 4500 globally known species, there are approximately 250 species that cause significant destruction to various fruits and vegetables.

In Africa, these tephritid flies are responsible for about 20 – 80% losses to fruit and vegetable farmers (Muchiri, 2012). These losses result from damage inflicted by larval foraging and a decrease in international trade opportunities, constrained by quarantine limitations enforced by importing countries to prevent the introduction and spread of undesirable pests. Control efforts have ranges from usage of insecticide, good sanitation practices, deployment of traps, to other genetic alteration techniques. Effective management of fruit flies is essential to alleviate these adverse effects. This could also help address

Sustainable Development Goals 1 and 7 which have to do with the elimination of severe poverty and hunger as well as ensuring the sustainability of the environment. To successfully design control and management strategies and subsequently implement them, there is the need for knowledge on the population structure of these species (Roderick, 1996).

Bactrocera dorsalis ranks among the foremost notorious varieties of fruit flies, inflicting significant destruction to fruits and vegetables in areas where they have been reported. This species is significant due to its high dispersal and invasive capabilities, resulting in its widespread distribution across various regions worldwide (Mwatawala et al., 2006). *B. dorsalis* has acclimated to diverse ecological and climatic conditions extending from rainforests in lowland areas to arid savannahs. The pest is primarily found in Asia (Bhutan, India, Sri Lanka) and extensively distributed across Africa (Angola, Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Comoros, Congo, Democratic Republic of the Congo, Cote d'Ivoire, Equatorial Guinea, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya, Liberia, Mali, Mauritania, Mozambique, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo, Uganda, Zambia) (Ekesi et al., 2009; Geurts et al., 2012).Given these unique dispersal, adaptation, and invasive characteristics of *B. dorsalis*, this study was conducted to investigate their population structure across four different ecological zones in Ghana and to understand the ecological influence of these zones on the species.

Bactrocera dorsalis originated from Sri Lanka and has dispersed to every corner of the globe.

In Africa, the species was initially identified in Kenya (Lux et al., 2003) and later confirmed in Ghana in 2005 (Billah et al., 2006). Following its detection in Ghana, the pest has since dispersed all over the nation (PPRSD, 2006; Utomi, 2006).

The species was initially referred to as *Bactrocera invadens*. but phylogenetic analysis was unsuccessful in resolving the Pest different from *Bactrocera dorsalis,* resulting in its synonymization with *B. dorsalis* (Hendel) (Schutz et al., 2015; San Jose et al., 2013).

Bactrocera dorsalis, known for its polyphagous nature, exhibits a wide-ranging appetite and can infest an extensive variety of hosts. This includes not only cultivated fruits but also wild fruits, showcasing its adaptability and ability to impact both agricultural and natural ecosystems. The diverse array of hosts makes *Bactrocera dorsalis* a significant concern for the agricultural sector and biodiversity, as its presence poses a threat to both cultivated crops and native plant species. *Bactrocera dorsalis,* a highly adaptable pest, has demonstrated its infestation capabilities across a diverse array of more than 90 plant species, spanning over 40 families. Notably, cultivated fruits such as Mango (*Mangifera indica* L. - Anacardiaceae), banana (*Musa* sp. - Musaceae), and citrus fruits, including Lemon (*Citrus limon*), Tangerine (*Citrus reticulata*), and Sweet Orange (*Citrus sinensis*) - all belonging to the Rutaceae family, are part of its preferred hosts.

This polyphagous pest extends its impact beyond commonly cultivated fruits to include Marula (*Sclerocarya birrea* - Anacardiaceae) and *Terminalia catappa* L. (Combretaceae), highlighting its capacity to attack a variety of plant species belonging to different botanical families. The wide spectrum of hosts underlines the significance of *Bactrocera dorsalis* as a potent*ial* threat to both agricultural crops and native plant ecosystems. Also wild plants that ensure there are ample reproductive foundations for the pest during the non-fruiting period of cultivated plants.

In a controlled laboratory study focusing on host preference, mango (*Mangifera indica*) and banana (*Musa sp*.) emerged as the predominant choices among the nine planted varieties that were systematically investigated (Moquet et al., 2020). This observation underscores the specific attraction and preference demonstrated by *Bactrocera dorsalis* toward these fruits.

The findings of this study shed light on the pest's selective behaviour when presented with a range of cultivated species. The higher affinity for mango and banana as host plants highlights the significance of understanding the preferences and behavior of *Bactrocera dorsalis*, providing valuable insights for effective pest management strategies in agricultural settings where these favored crops are cultivated. Such research outcomes play a crucial role in devising targeted approaches to reduce damage caused by this polyphagous pest on economically important crops.

The synergy achieved by combining different control strategies in a coordinated manner provides a more sustainable and environmentally friendly approach to pest management. This integrated approach not only enhances the efficacy of pest control but also minimizes the potential negative consequences associated with the overuse of any single control method. The Sterile Insect Technique (SIT), for instance, is frequently employed to reduce, eradicate, or confine them (Klassen and Curtis, 2005). SIT requires mass rearing of these insects. For long-term control, mating compatibility is needed between mass-reared colonies and wild populations. The harmony in compatibility can be influenced by ecological diversification and can influence selection of mates and insemination, especially in polyandrous pests. In such cases, females can select sperm from multiple males after mating (Tregenza & Wedell, 2000). Polyandry is observed in numerous invasive fruit fly pests, including *B. dorsalis*, and a lack of mating compatibility between natural and modified populations has the potential to compromise the effectiveness of a control program. Population structure of a species reports systematic variations in populations of the species. These variations may be influenced by the combined effect of differences in environmental factors like temperature, humidity, and rainfall, as documented by Danjumah et al., (2020). when the population structure of *Bactrocera dorsalis* in the southern Guinea Savannah zone of Nigeria was examined on an annual basis. The collective influence of climatic variables, including temperature, humidity, and rainfall, differs across different habitats and exerts distinct effects on the populations of species dwelling within these environments. These population variations can result in population structuring as was reported by Getahum et al. (2014), when a population study was carried out on ecological populations of *Glossina pallipides* from Ethiopia using cuticular hydrocarbon analysis and wing morphometry. Cuticular hydrocarbon analysis and wing morphometry are commonly used to study population variations (Getahum et al., 2014). Geometric morphometry of wings underscores variation in the shape of wings of populations (Lawing & Polly, 2009) of the species under study while cuticular hydrocarbon profiling in population studies investigates the hydrocarbon preferences and concentrations in different populations of the species being examined. (Kota et al., 2021, Badu et al., 2022). Differences in environmental conditions exist across four Agro-ecological zones in Ghana, encompassing the Coastal Savannah zone, the eastern-Volta zone, the Transition zone, and the Guinea Savannah zone where commercial mango cultivation takes place may be resulting in population structuring of *Bactrocera dorsalis* populations from Ghana.

Problem Statement

Bactrocera dorsalis is currently responsible for significant economic losses to fruit and vegetable farmers in Ghana. The species has outcompeted and displaced the indigenous *Ceratitis cosyra* to become responsible for the most severe damage to fruits, especially mango.

Several control measures are being deployed to minimize the harm inflicted by this pest. These methods include the application of insecticides, good sanitation practices, and deployment of traps baited with attractants. These methods may become laborious and sometimes harmful to the environment, while others may not be effective as a stand-alone measure. An effective approach to control or eliminate this pest may involve the application of sustainable pest control practices, ideally integrating the insect sterility program or a genetic modification of the pest.

For effective implementation and success of this approach, the population structure of the pest must be known. To successfully implement a large regional sustainable pest control programme, understanding the population structure of the pest within the ecological landscape of the region is crucial. There is not adequate information on how the ecological landscape of Ghana is shaping the population of this invasive species since its detection in Ghana, more than 10 years ago. There is therefore the need for a study to investigate the population structure of *Bactrocera dorsalis* within a geographical and ecological context of Ghana.

To evaluate variability among species or populations of a species, various methods have been employed (Khamis et al., 2012; Yi et al., 2016; Badu et al., 2022). including microsatellite genetic analysis (Yi et al., 2016) that measures actual gene flow between populations, Geometric morphometry of wings (Khamis et al., 2012, Badu et al., 2022) that investigates alterations in the wing morphology between populations, and Cuticular hydrocarbon analysis (Badu et al., 2022) that measures qualitative and quantitative variation in hydrocarbons extracted from the epicuticle of an insect. All these methods have been very useful tools for variability assessments, especially in insects. In this study, attempts were made to include all afore-mentioned tools to describe the population structure of ecological populations of *Bactrocera dorsalis* from Ghana. However, only two of the tools were successful, Geometric morphometry of wings, and cuticular hydrocarbon profiling and are reported in this thesis.

Significance of the Study

Bactrocera dorsalis is one of the most economically important tephritid pests affecting fruit production in Ghana. Its rapid spread and adaptability across diverse ecological zones pose significant challenges for effective management. Studying the population structure of this pet using morphometric and ecological methods is crucial for understanding how environmental variation influences its phenotypic plasticity and potential for adaptation.

Morphometric analysis, particularly of wings, provides a cost-effective and reliable approach to detect subtle morphological differences among populations. These differences may reflect ecological adaptations to local environments such as temperature, humidity, altitude, and host plant availability. By comparing populations across Ghana's distinct ecological zones—such as the coastal savannah, forest, and transitional zones—this study can reveal whether geographically isolated populations exhibit morphological divergence due to ecological pressures.

In addition, correlating morphometric variation with ecological data helps identify environmental factors driving population differentiation. This eco-morphological approach provides insights into how *B. dorsalis* responds to habitat heterogeneity, which is critical for predicting its spread and developing region-specific pest control strategies. Ultimately, the results will support the design of targeted surveillance and management programs that consider ecological variability, helping to reduce fruit losses, improve crop quality, and enhance Ghana's competitiveness in international fruit markets.

A prior investigation conducted by Wilson et al. (2002) revealed an escalation in the population of savannah blackflies (*Simulium damnosum s. str.* and *S. sirbanum*) within the forest region of southern Ghana, potentially carrying with them the more virulent form of *Onchocerca volvulus*, because of deforestation (changing ecology). In a study published in 2007, Yawson et al. used microsatellite loci to show that ecological barriers proved to be significantly more crucial obstacles to the flow of genes, influencing intra- and inter-form variation in the two forms of the *Anopheles gambiae* mosquito, a vector for malaria, even though inter-form hybridization occurred at a significant level. Other studies on the eco-epidemiology of leishmaniasis have implicated certain species of sand flies as disease transmitters by incorporating biotic relationships with transmission potential. These studies explored connections between ecological elements and shifts in extent of vector presence and dissemination potential, arising from a blend of human activities and natural occurrences like global warming. In the fruit fly, *B. dorsalis*, it is not known whether new environments or ecologies have favoured the emergence of novel phenotypes and/or genotypes or the preferential selection of existing ones.

Population segregation or subdivisions may pose obstacles to the effectiveness of any genetic control strategy. The successful introduction of desired traits into wild populations, particularly those with distinct subdivisions, may require the introduction of multiple strains (Lanzaro et al., 1998; Coluzzi et al., 2002). Therefore, comprehending the population structure is a pivotal factor in effectively planning and implementing existing pest control methods. Additionally, insufficient knowledge about the population structure could negatively affect scheduled eradication programs, specifically those targeted at diminishing mating effectiveness between bioengineered and/or induced sterility controlled male fruit flies, and natural populations. The existence of population sub-structuring or ecological barriers within pest and vector populations may present challenges that could impede the spread of desired genes, (Lanzaro et al., 1998; Lanzaro and Tripet, 2003). The ultimate question is whether ecological diversity in the distribution range of *B. dorsalis* will affect its population structure. In natural populations, genetic diversity plays a crucial role in adaptation and persistence across varied environmental conditions, shaped by the interplay of population dynamics and the surrounding environment. Collectively, these factors can affect transfer of genetic material among populations, consequently shaping the population configuration of an organism across its entire habitat (Ronce et al., 2001).

The creation of landscape barriers through ecological diversification has been suggested as a factor that can hinder gene flow, including swift chromosomal evolution, leading to reproductive divergence and speciation (Schneider, 2000).

Hence, gaining insight into the interplay between a fruit fly species and its evolving environment can offer valuable information about population configuration.

This knowledge is essential not just for comprehending the evolutionary process but also, in the context of pests and infection carriers, for formulating effective mitigation measures.

Objectives

The main objective of the study was to investigate the population structure of *Bactrocera dorsalis* in Ghana.

The specific objectives were as follows.

1. To determine the wing features accounting for the most variation in wing shape among *Bactrocera dorsalis* populations from different Agro – ecological zones in Ghana using geometric morphometry.
2. To use Geometric Morphometry to investigate possible variations among populations of *B. dorsalis* from four ecological zones in Ghana.
3. Use discriminant function analysis and cross validation to investigate cluster classifications of populations of *B. dorsalis* from four Agro – ecological zones in Ghana.

4. To investigate differences in cuticular Hydrocarbon profiles of samples of *B dorsalis* species from four different Agro-ecological zones in Ghana.
5. To determine if sexual dimorphism is evident from the cuticular hydrocarbon profiles of *Bactrocera dorsalis* samples gathered from four differing Agro – ecological zones in Ghana.

Research Questions

1. What wing feature is the most important feature accounting for the most variation in wing shape among populations of *Bactrocera dorsalis* species collected from four different Agro – ecological zones in Ghana?
2. Are there significant variations among populations of *B. dorsalis* from four ecological zones in Ghana from Geometric morphometry of wings?
3. How different are cuticular Hydrocarbons isolated from Hexane washes of *B. dorsalis* samples from four Agro-ecological zones in Ghana?
4. Is there sexual dimorphism from the cuticular profiles of *B. dorsalis* from different Agro – ecological zones in Ghana?

Delimitations

The damage caused by this invasive pest cannot be overemphasized; from on-farm losses to post-harvest losses. This pest continues to wreak havoc in fruits and vegetable farms. This research work collected *B. dorsalis* from four Agro ecological regions in Ghana. The ecological zones were Coastal (Southern) savannah, (Forest), Transition, and Guinea savannah (Northern) zone. Similarly, fallen infested fruits were collected from these zones also for the cuticular hydrocarbon analysis. The fruit fly rearing facility at the Ghana Atomic Energy commission was used to rear flies.

In this study, the population structure of *Bactrocera dorsalis* in Ghana was described by utilizing morphological variations observed through wing shape changes identified by geometric morphometry, as well as physiological variations highlighted by variations in cuticular hydrocarbon profiles. Different samples of *B. dorsalis* were collected from four distinct Agroecological zones for this analysis. It also used data from the above-mentioned methodologies to infer clustering of populations within and between ecologies.

This research work only utilized *B. dorsalis* from major mango orchards in the country. Even though the pest is polyphagous, the sampling for this study was limited to *B. dorsalis* obtained from mango farms. The study focused on describing the structure of populations of *B. dorsalis* within Ghana by comparing populations collected from different ecologies.

Limitations

The genetic structure of *B. dorsalis* was not described in this study. Nevertheless, geometric morphometry and cuticular hydrocarbon profiles have demonstrated their efficacy as potent tools that can serve as proxies for genetic analyses, effectively representing patterns of gene (Martínez-Abadías et al., 2016, Nannan et al., 2022). These morphological and physiological methods are adequate in describing the structure of *B. dorsalis* populations in Ghana. This study does not include any genetic analysis, and all inferences, conclusions and recommendations made were from morphological variations of the populations. Genuine effort was made to include microsatellite genetic analysis to determine actual gene flow among populations in this study but there were challenges with the acquisition of reagents for a real time PCR and when DNA was extracted and shipped to the US for analysis, the quality of the DNA didn’t allow for successful reactions thus the genetic analysis was excluded from this study.

Organization of the Study

This thesis comprises six chapters. The initial chapter offers a brief overview of the study, including background information, as well as study objectives and an outline of the extent of the work. The second chapter critiques available literature. It discusses the biology of the pest as well as the ecology as it pertains to Ghana. It also provides adequate taxonomic history of the pest and lastly identifies knowledge gaps. Chapter three outlines the methodologies used in the study. It gives a full account of how the research was carried out. It also described the sampling sites and how data was collected and analyzed. Chapter four outlines all the results generated from the study represented in maps and charts. Chapter five discusses the results in relation to similar studies carried out elsewhere as well as implications for control of the pest. Chapter six contains the study's findings and recommendations.

CHAPTER TWO

LITERATURE REVIEW

Introduction

The objective of this research is to characterize the populations *of Bactrocera dorsalis* within the context of changing ecologies. This research provides the prevailing population structure of the pest in Ghana as it pertains to ecological diversification and anthropogenic land use. This chapter contains secondary data or information obtained from published journal articles, conference proceedings, books, and reports on the subject matter. It also includes information from student thesis and dissertations, and other available sources culminating in a comprehensive literature review. It brings to bear information available from a historical perspective on the general biology of the insect, economic importance of the pest, ecological landscape of Ghana and the varied land use patterns. It also describes the principles of geometric morphometry and cuticular hydrocarbon analysis as applied in this research to delineate the population configuration of *Bactrocera dorsalis*. Finally, research gaps were identified, some of which this study seeks to fill.

Theoretical Framework

The concept of population structuring revolves around the organization of variations within and between populations, influenced by the cumulative impact of evolutionary mechanisms. These mechanisms include recombination, mutation, genetic drift, demographic history, natural selection, and ecology. Defining a population through the utilization of its population structure and understanding the obvious interactions among populations from different ecological backgrounds forms the theoretical framework of this study.

Understanding how a population of a pest from a particular ecology varies from the same pest from a different ecology helps bring to bear how the pest is evolving within an ecological space and can inform policy makers, farmers, and researchers on how to manage the pest. The *Bactrocera dorsalis* complex (*B. dorsalis*, *B. papaya*, and *B. philipinensis*) is reported to be undergoing rapid evolution (Khamis et al., 2012). The presence of genetically distinct but morphologically similar species within this economically significant pest poses significant challenges in formulating effective control methods for these pests, and these challenges need to be addressed. To effectively control these pests, a comprehensive understanding of their biology and taxonomy is necessary. Recent considerations for managing this group involve various control methods, including the application of the induced insect sterility, pheromone use, the detection and mapping of fruit fly – free zones as well as the implementation of quarantine measures and risk analysis (Schutze et al., 2012; Krosch et al., 2013; Norrbom et al., 2013; Perre et al., 2014).

A significant contributor to the emergence of new diseases is anthropogenic land-use change, which may lead to unique interactions among vectors, hosts, and diseases. Considering these changes (which influence ecosystems), it seems logical to believe that they may have an impact on comparable relationships between agricultural pests and their hosts as well as diseases.

Habitat fragmentation is a common outcome of anthropogenic land utilization and can result in substantial shift in the form, function, and composition of habitats, as reported in some tropical rainforest communities (Laurance et al., 2011). The fact that landscape changes may cause genetic differences in the population of a species is also worth mentioning as an example of an event that might cause population differentiation (bottleneck effect). As a result, both genetic and environmental processes are involved in population divergence (Kim & McPheron, 1993).

History of *Bactrocera dorsalis* (Hendel)

Bactrocera dorsalis (Hendel) was initially identified in Formosa now called Taiwan in the second decade of the 20th century (Hardy 1973; Drew & Hancock, 1994; Aketarawong et al., 2014), and it has since been discovered in several other countries throughout the Asia-Pacific region (Xie, 1937; White & Elson Harris, 1992; Aketarawong et al., 2007.). *B. dorsalis* was initially discovered in 1996 near the airport on the Mauritius Island (White, 2006), however it was pronounced eliminated in 1998 (White, 2006). Following that, *Bactrocera dorsalis* was initially captured in East Africa in 2003 (Ekesi et al., 2006), and subsequently, it swiftly spread across East and West Africa, into Ghana in 2005. The fly was initially thought to be a different species and named as *Bactrocera invadens* but later synonymized with *Bactrocera dorsalis*. In 2005, *Bactrocera dorsalis* extended its distribution to the central and southern regions of the continent (Mwatawala et al., 2006; Magagula et al., 2015; Manrakhan et al., 2015*)*. In addition, *B. dorsalis* has been found in areas of Oceania, South America, and North America, as well as other locations. According to Aketarawong et al (2014), *Bactrocera dorsalis* has been identified in Hawaii (1945), Guam (1947), Florida (1960), and California (1960) within the United States, as well as in Suriname (1975) in South America (Philips, 1946; White & Elson Harris, 1992*)*. The pest has invaded and spread to most provinces in southern China, and it is now spreading into northern China (Wu et al., 2000 Chen et al., 2011; Li et al., 2012;). Although numerous reports have documented recent incursions of *Bactrocera dorsalis* in various regions worldwide, our comprehension of the global pattern of its invasion and spread remains limited.

Biology of *Bactrocera dorsalis*

Bactrocera dorsalis are multi-voltine species (i.e., it undergoes several breeding cycles annually) and are found within climates characterized by tropical and subtropical conditions and do not go through a developmental break throughout their life span. They undergo a comprehensive metamorphic process, progressing through stages such as eggs, larvae, pupae, and the eventual emergence of adult flies. *Bactrocera dorsalis* completes its lifecycle quicker in tropical conditions; the warmer the environment, the faster it develops. The larvae inflict the most damage as their feeding activity on the fruit's flesh results in its destruction.

Nevertheless, the larva remains concealed within the fruit as the female fly lays its eggs inside it. It may be more judicious to focus on controlling the adult stage of the pest.

Life Cycle of *Bactrocera dorsalis*

Under favourable conditions, the transition from egg to adult generally takes approximately 16 to 35 days. The fully developed larvae exit the fruit, drops to the ground, and undergoes pupation within a puparium, exhibiting colours ranging from tan to deep brown. Pupation takes place in the soil. After emergence, it takes about nine days for the adult to attain sexual maturity. Cool temperature has the potential to significantly lengthen the developmental phases. Under ideal circumstances, in its lifetime, a female has the potential to lay over 3,000 eggs: however, under natural conditions, the typical yield ranges from 1,200 to 1,500 eggs per female (Ekesi & Billah, 2007).

It is preferable for oviposition to occur on ripe fruit, although immature fruit may still be targeted.

The Adult

The length of the adult's body is approximately 8.0 mm, making it bigger than a housefly (*Musca domestica*). The wings, measuring about 7.3 mm in length, are primarily hyaline. The fly exhibits a variety of colours, but its thorax is particularly distinctive with distinct yellow and deep brown to black patterns, creating a noticeable contrast with the remainder of its body.

A typical abdominal pattern comprises two latitudinal black lines and a longitudinal middle line that extends from the base of the third segment to the tip of the abdomen. The markings have the potential to create a T-shaped pattern, although the precise arrangement can vary significantly. A strongly pointed ovipositor is seen on the end of the abdomen of the female (Ekesi & Billah, 2007).

The egg

Upon emerging, adult flies attain sexual maturity within 9 to 10 days and promptly initiate mating at this stage. The adult female fly has a long and extendable ovipositor located at the end of its abdomen, allowing it to deposit eggs into the skin of suitable hosts, especially during the ripening or ripe stage of fruits and vegetables, the ovipositor can pierce the host as deep as 2-5 mm (Ekesi & Billah, 2007). In a 24-hour period, a single *Bactrocera sp* has the capacity to lay anywhere from 400 to 1000 eggs. (FAO/AusAID/UNDP/SPC, 1997). The eggs, resembling bananas in shape, are laid in clusters ranging from

3 to 8, although certain species may lay individual eggs. Typically, the egg exhibits a glistening white appearance, with slight darkening as it approaches the time of hatching. The egg is whitish, elongated, and elliptical, measuring approximately 1.17 x 0.21 mm, with no sculpturing present on the chorion. The rotting of the fruit tissues around the eggs is attributed to the introduction of saprophagous bacteria from the fly's intestinal flora during oviposition. The bacteria trigger fruit rot, acting as a food source for the larvae. The eggs hatch into larvae within a period of 3-12 days, a process influenced by both the environmental conditions and host physiology (Ekesi & Billah, 2007).

The eggs hatch to larvae, undergoing two moults (with three larval instars in total), and subsequently, they consume the fruit pulp for a period ranging from 6 to 35 days. In the third larval stage, resembling a typical maggot, the length measures approximately 10 mm, and it exhibits a creamy white colour. The initial segment showcases a single band of small spines encircling the entire body. The outer part of the larva's anterior respiratory organs, known as spiracles, is located on each side of the pointed or head end. It exhibits a pronounced and deflected lobe on each side, adorned with numerous small tubercles. The posterior section has a smooth transition. When looking at the segment from the back of the larva, the spiracles at the rear are situated on the third dorsal segment. The mature larva exits the fruit, descends to the ground, and forms a puparium, which can vary in colour from tan to deep brown and measures 4.9 mm in length, (Ekesi & Billah, 2007).

The pupae

The puparia are located underground, typically buried about 2-5 cm in the soil. The adult emerges from the pupal case within a period of 10 to 20 days, influenced by the prevailing environmental conditions. Upon the completion of pupation, a winged fly emerges from the soil or debris and initiates the process of digging its way out. (Ekesi & Billah, 2007).

Taxonomic History

Bactrocera dorsalis belongs is a species complex. The species complex *B.* dorsalis is a part of the subgenus *Bactrocera.*; as a result, the name *Bactrocera (Bactrocera) dorsalis* may be used to refer to this species complex. Initially considered a single species widespread across Asia, *B. dorsalis* underwent subsequent classification, leading to the recognition of distinct species including *Bactrocera carambolae, Bactrocera dorsalis, Bactrocera papayae, and Bactrocera philippinensis* were identified as distinct species by Drew and Hancock in 1994. The original habitat of true *B. dorsalis* was primarily located within continental Asian countries situated north of the Malay Peninsula. *B. dorsalis,* was officially identified by Drew et al. in 2005 following the observation of established populations in East Africa (Lux et al., 2003) and West Africa (Vayssières, 2004). The scientific community has considered *B. philippinensis* as a synonym for *B. papayae*, as indicated by Drew and Romig in 2013. Comprehensive research undertaken by multidisciplinary teams involved defining species boundaries through the integration of morphological, molecular, cytogenetic, behavioural, and chemo ecological data. This collaborative effort was part of an FAO/IAEA Coordinated Research Project (CRP) focused on addressing challenges related to the Sterile Insect

Technique (SIT)application and international trade by resolving cryptic species complexes among tephritid pests. Consequently, *B. invadens* and *B. papayae* were merged as synonyms with *B. dorsalis*, while *B. carambolae* retained its status as a distinct species.(Schutze et al., 2015a). *Records of B. pedestris (Bezzi)* outside the Philippines are largely attributed to misidentifications *of B. dorsalis*.

Economic Importance

Bactrocera dorsalis is a severe threat to horticulture output in East, Central, and West Africa, causing significant yield losses (White & Elson-Harris et al., 1992; Muhammad et al., 2004; ICIPE, 2007). In Africa, in the south of the Sahara a total of 915 fruit fly species are distributed among 148 genera. Out of these, 299 species develop in either wild or cultivated hosts, or both (Ekesi et al., 2010). In Ghana, *Bactrocera dorsalis* is notably the most widespread fruit fly species (Ekesi et al., 2006). Due to their extensive distribution, rapid reproduction, large populations, polyphagous behaviour, and resistance to insecticides, these insects result in significant harvest decline in agricultural produce (Muhammad et al., 2004; Baral et al., 2006; Mumford et al., 2006). The presence of *Bactrocera* species reduces both the quantity and quality of fruits and vegetables, posing a threat to their production and market desirability. This impedes the growth of local and international trade in these crops, resulting in substantial economic losses for growers. These flies can easily cross international borders without detection. Consequently, they have been classified as a worldwide quarantine insect pest. European Union (EU) countries that bring in agricultural harvest enforce stringent restriction measures Identification of even one immature stage of the pest during importation triggers the seizure and incineration of the whole product. This situation can potentially lead to a complete ban on exports from the originating country. These losses are projected to exceed USD 42 million annually in Africa and reach a global estimate of USD 1 billion USD, (FAO, 2010). Most African nations south of the Sahara are prohibited from exporting mangoes to both the European Union and the U. S. A, (Lux et al., 2003b; Ndiaye et al., 2008; Vayssieres, 2009a; FAO, 2010). Furthermore, the financial repercussions of fruit fly damages in Africa have intensified following South Africa's recent restrictions on the importation of mangoes and avocados from Kenya, as well as mangoes, bananas, and citrus fruits from Mozambique.

Control of *Bactrocera dorsalis*

Chemical Control

Pest control has previously been limited to broad-spectrum chemical insecticides used by mango producers (Waiganjo et al., 2009). According to research on pesticide usage in emerging countries, the number of pesticides used is increasing, (Wilson & Tisdell, 2001). Despite this, fruit fly pesticide resistance has not yet reached the point where treatment is fully useless, since they still function to some degree. Pesticides have been demonstrated to be ineffective in managing key pests of fruits and vegetables; while also incurring high costs (Manene, 2010) To overcome this challenge, farmers often triple the number of spraying schedules expecting to improve effectiveness. For example, Kenyan farmers raised the frequency of cover sprays to 6 to 8 times during blooming and fruit development, surpassing the recommended frequency of 3 to 4 times (Krain et al., 2008). Farmers are attempting to "improve" the efficiency of pesticides by blending different types of pesticides and utilizing higher doses, (Sithanantham et al., 2004; Adetonah, 2007; Gitonga, 2009). Farmers' desperation may be observed in these non-profitable yet nonetheless adopted alternative strategies. Harmful cycle of pesticide resistance may compromise the ability of natural enemies to manage pests and raise concerns about the long-term sustainability of Agrochemical-dependent agriculture (Wilson & Tisdell, 2001). Chemical pesticides may still exhibit some effectiveness if the pesticide resistance of target pests has not fully developed. This implies that the flow of benefits maintains a positive discounted net present value, suggesting that farmers will continue to use them despite increasing costs until they become economically impractical or until a more viable alternative emerges.

Conversely, the excessive application of chemicals for insect management has demonstrated its unsustainability and presents environmental hazards. If the insect population grows and stays permanently above pre-pesticide levels, it is considered no longer economically feasible to employ pesticides. Therefore, pesticides are no longer recommended (Wilson & Tisdell, 2001; Adetonah et al., 2007).

Baiting application technique (BAT)

This procedure entails the use of bait sprays or bait traps, and the bait is composed of a protein that releases ammonia along with a lethal substance, such as a Spinosad-based insecticide with lower toxicity. The food bait entices both sexes of the adult fruit fly to a 1 square meter area on the host tree canopy, where the attractant is applied. The fly ingests the attractant and the pesticide and subsequently die rapidly—usually in less than 60 minutes—prior to laying eggs in the fruits. Each season, sixteen bait spray sessions are necessary on average.

Work by Ekesi & Billah (2007) and Ekesi (2010) are two examples of this method. Baiting strategies are not highly effective on their own, especially during periods of high pest populations. However, they prove to be a valuable tool in fruit fly management by diminishing the number of pre-reproductive females (Lux et al., 2003b).

The male annihilation technique (MAT)

This procedure involves capturing large numbers of male flies using a male bait to control or eliminate their populations and prevent them from mating (eradicationCotton wicks, infused with a sex pheromone such as Methyl Eugenol and coated with a less hazardous pesticide, are positioned within tree traps. When Methyl Eugenol traps are employed in conjunction with bait sprays, fungicides, parasitoids, and orchard sanitation, numerous fruit fly monitoring studies have highlighted their outstanding effectiveness in trapping and eradicating male fruit flies.

This leads to a notable decrease in the percentage of fruit infestation during the growing season. MAT exhibits the qualities of an environmentally friendly pheromone that selectively targets male flies without posing a threat to other beneficial insects. Its suitability for integration into a control program arises from its non-toxicity to other beneficial insects (Muhammad et al., 2004; Ekesi & Billah, 2007).

Biological control

The engagement of biological control agents such as *Oecophylla longinoda* (red ants), *Fopius arisanus* (parasitoid wasps), and *Metarhizium* sp (fungal pathogens) in fruit fly management reduces infestation by preying on adult fruit flies, consuming third-instar larvae, eliminating pupae in the ground, and driving away fruit flies through the repulsive effects of "pheromones" left by these agents (Adandonon et al., 2009). The utilization of parasitoids presents various benefits. Notably, the introduced natural enemy's persistence and activity do not necessitate farmer intervention. Consequently, the introduced natural enemy continues to thrive in the environment without incurring additional costs for the farmer, representing a significant advantage.

Moreover, it is harmless to both the farmer and the consumer, as well as environmentally friendly. Additionally, the use of parasitoids does not lead to the development of resistance by fruit flies.

Mango producers are encouraged to use safer (less harmful) pesticides in their orchards if they must apply pesticide cover sprays to prevent harming these biological agents (Mohamed et al., 2009; Vayssières et al., 2009b). The introduction of fungal pathogens into the soil creates an inhospitable environment for adult fruit flies, as well as for the larval and pupal stages of the fly's life cycle. Nonetheless, due to its non-toxic nature to beneficial parasitoids and its potential to remain on the farm for more than 365 days, it requires only one application per season. It is essential to note that these conventional biological control measures are most effective when applied in conjunction with the other methods highlighted above (Ekesi & Billah, 2007).

Complementary methods

Behavioural approaches aim to minimize fruit fly damage without directly suppressing pest numbers, and their effectiveness has been demonstrated. Farm sanitation, mechanical fruit protection, and post-harvest fruit treatment represent a few of the employed methods. Farm sanitation is necessary as poorly maintained or abandoned farms contribute to an escalation in the fruit fly populations on the farm. Consistent gathering and disposal of all dropped fruits on the ground throughout the season are essential, giving special attention to those containing fruit fly larvae. White & Elson-Harris (1992) and Rwomushana et al. (2008) carried out population dynamics studies on fruit flies, revealing a robust relationship between the volume of fruit flies in fruits littered in farms and those in fruits on the plant. This underscores the crucial significance of farm hygiene. Fruits that have fallen to the ground are collected and either buried in a deep hole or burnt. In the practice of fruit bagging, fruits are safeguarded by placing them in a brown or transparent paper bag during the prematurity stage, ideally around one month before the completion of harvesting. Picking certain fruits while they are still green, like papaya and banana, can be a preventive measure against fruit fly damage. However, for mangoes, this approach is not effective due to the infestation risk posed by fruit fly species such as *B. dorsalis*, leading to the premature dropping of immature fruit. Following harvest, hot water treatment has proven to be advantageous, particularly in regions like South Africa, where it effectively eliminates all maggots in mangoes before harvesting (Ekesi & Billah, 2007).

IPM methods for fruit fly control

Integrated pest management utilizes monitoring and decision-making processes to identify the most appropriate, cost-effective, and environmentally friendly methods of pest control. It reduces pest damage while causing the least amount of disruption to the natural balance of the Agro-ecosystem and posing the least amount of danger to public health. It achieves this by reducing the overall use of chemical pesticides in agriculture. Consequently, the reliance on chemical pest management is diminished over time (Varela et al., 2006).

Farmers should embrace integrated pest management (IPM) strategies that are both compatible and economically viable. When effectively implemented and carefully overseen, these strategies can collectively enhance production objectives, leading to both immediate economic benefits and long-term sustainability (Sullivan et al., 2000; Vayssières et al., 2009b).

IPM, as an agricultural pest control strategy, occupies a conceptual middle ground between conventional and organic farming. The adoption of integrated pest management (IPM) offers a practical and cost-effective alternative to conventional agriculture by substantially reducing the expenses associated with chemical pesticide use. Moreover, it provides an alternative to organic agriculture, as it has been demonstrated in numerous cases not to significantly hinder crop productivity, (Govindasamy & Italia, 1999). In developing countries, the adoption of IPM strategies is frequently an exception rather than the norm, primarily due to the increased demand for labour in these regions. As a result, they are only implemented on a small scale. Overall, Integrated pest management strategies prioritize the restoration of the natural balance between pests and their hosts within ecological systems. As many integrated pest management approaches are tailored to specific pests and are shaped by host-plant relationships and the crop ecosystem, the potential exists to establish a lucrative fruit industry in regions where these approaches are applied. An excellent example can be found in South Africa, where the management costs and residual losses attributed to fruit flies constitute less than one percent of the total product value (Baral et al., 2006; FAO, 2009).

The limited knowledge among farmers about the biology of fruit flies, coupled with a lack of understanding of safer control measures, serves as a substantial obstacle to improving productivity, (Sithanantham et al., 2004). The anticipation is that infestations will persist in reducing earnings and market competitiveness for mango producers, serving as an ongoing barrier to accessing profitable export markets. As a result, it is essential to introduce and educate farmers on cost-effective and ecologically sound integrated pest management (IPM) alternatives. Furthermore, the risks associated with the improper use of chemical pesticides have led scientists, policymakers, donors, and development organizations to endorse the implementation of integrated pest management (IPM) alternatives for crop protection in the developing world, with a specific emphasis on Africa (Adetonah et al., 2007).

In the context of fruit flies, two components of IPM can be employed: (a) the suppression strategy, aiming to reduce the pest population and minimize production losses, and (b) the eradication approach, focusing on the removal of fruit flies to establish "fruit fly-free" zones. However, the latter is a costly procedure that needs to be implemented across an entire region and is only justified when a highly profitable sector is at risk. The suppression of fruit flies can be achieved through various methods, including the bait application technique (BAT), the male annihilation technique (MAT), orchard sanitation, and the utilization of biocontrol agents such as parasitoids, predators, and pathogens.

The proposed IPM-based strategy comprises a set of approaches, each making a substantial contribution to the overall approach when integrated with the other methods. The likelihood that any of the components outlined above can be used as a stand-alone fruit fly control method is thus minimal (Ekesi & Billah, 2007).

Sterile insect technique (SIT)

When appropriately employed, the sterile insect technique (SIT) has the potential to reduce the population of agricultural pests in the field, maintaining its ecological friendliness, biological and non-insecticidal nature, (Whyard *et al*., 2015). The subsequent generation can be diminished by introducing diseased or infertile males into the fields to mate with wild females (Thailayil *et al*., 2011). SIT is a widely embraced strategy, especially in the management of tephritid fruit flies, as well as for the control of tsetse flies, screwworms, and mosquitoes, (Dyck et al., 2005). Currently, male flies that have been sterilized are often mass reared using various methods, including chemosterilants, symbionts, and radiation treatments. While radiation is highly effective, its use is constrained by the need for suitable radiation sources. Additionally, the somatic damage caused to the insect by radiation inevitably hampers the ability of male flies to compete with wild females for mating resources, (Collins et al., 2008; Norikuni, et al., 2008). The inability of males to reproduce leads to a decrease in the overall effectiveness of the pest population, (Ant et al., 2012). When conventional SIT methods proved ineffective in reducing the population of the olive fruit fly due to altered mating behavior in SIT-flies, genetically modified SIT demonstrated significant potential in pest population control, (Ant et al., 2012). Furthermore, symbionts like Wolbachia are employed to induce sterility in male insects before their release into the wild for reproduction. For this method of pest management to be successful, the sterile flies must demonstrate competitiveness and compatibility with wild females. Therefore, conducting local population structure research is essential to gather information for the effective design and implementation of area wide IPM with a SIT component to ensure its success.

Ecological Landscape of Ghana

Globally, ecological zones vary in terms of composition and organizational structure. A steady modification of each ecological zone's land resource occurs because of human usage and management, together with natural environmental processes. Climate change and increased land use intensity in the twentieth century have accelerated changes in landscape composition and layout across all the world's ecozones. For instance, the global conversion of forests to alternative land use patterns has led to a loss of interconnected ecosystem services, amounting to 13 million hectares. (FAO, 2010). Over 26% of these changes have occurred in Africa. The rapid loss of forest cover in Africa can be attributed to a heavy reliance on land-based resources, as approximately 70% of the continent's population depends on the forest as their primary source of income or food (World Bank, 2006). In 2013, Ghana's deforestation rate was 65,000 km^2 per year, and it is projected that at the current rate, all the country's forests will be depleted within 25 years. (Boafo, 2013). Ghana is divided into seven distinct ecological zones, primarily characterized by variations in climate, including rainfall patterns, temperature, and other environmental factors, as well as land use, (Dinar et al., 2008; FAO, 2005). Ecological zones and their typical climate and soil types, influenced by the distribution of natural resources, have an impact on the environment and human well-being, catering to various socioeconomic demands. These transformations can also influence the evolution of species, including the development of insect pests.The invasive pest *Bactrocera dorsalis* likely encountered diverse ecologies in Ghana to ensure its success. The ecozones bear significant spatial and temporal implications for the sustainable management of landscape structure and composition. Human-induced observable changes in Ghana encompass variations in land use, deforestation, and urbanization, among other factors. Additionally, there has been an increase in the construction of dams and dugout reservoirs in the interior savannah, which is a positive development (Obour et al 2016). All these factors contribute to the fragmentation of the landscape. A diverse landscape structure characterizes Ghana's terrain, which includes a distinctive blend of natural environment and human activity (Hanson, 2001). The fragmentation of the landscape into smaller segments, resulting from both human and natural factors (Wu, 2013), serves as the primary transformative force in this Agro-ecological zone, as it does in others(Berger & Botequilha, 2002). Ghana's climate exhibits a broad range of variations, from the tropical unimodal monsoon type in the north to the bimodal equatorial type in the south (Boafo, 2013). Generally, rainfall increases from south to north, serving as the main source of moisture for agricultural purposes throughout the country (Boafo, 2013). Each of the seven ecozones has a distinct mean annual rainfall, with the wet evergreen forest experiencing the highest mean annual rainfall exceeding 2200 mm, followed by rainforest (2200 mm), deciduous forest (1500 mm), transitional zone (1300 mm), Guinea savannah (1100 mm), Sudan savannah (1000 mm), and coastal savannah (800 mm) (Boafo J, 2013). Drought, flooding, and landslides are the predominant natural disasters in Ghana. The dry, dusty harmattan winds, prevalent from January to March, particularly impact the nation during this period (Attua & Laing, 2001; Yiran, et al., 2012;). Sandy loam and loam represent the two most prevalent soil texture classes in

Ghana; nevertheless, coarse sandy and clay loams can be encountered in lower latitudes, along with sandstone. The most significant soil types include Alfisols, plinth luvisols, and their combinations (Armah et al., 2011).

Oxisols, ochrosols, acid gleysols, and lateritic soils constitute the soil types present in the southern regions of Ghana, known as the forest zone. Southern hemisphere soils are generally porous, well-drained, and typically loamy when compared to the soils in the northern savannah zones. The northern soils are nutrient-deficient and primarily depend on humus and fertilizer for growth (MOFA, 1998). Approximately 47 percent of the northern savannah zones are unsuitable for crop cultivation, while another 25 percent are considered marginal, leaving only 28 percent that are deemed suitable. The three savannah regions in the northern part of the country are characterized by savannah grassland interspersed with bands of drought-resistant trees, including baobab (*Adansonia digitata*), dawadawa (*Parkia biglobosa*), shea (*Vitellaria paradoxa*), neem (*Azadirachta indica*), and acacia (*Acacia nilotica*) at varying densities. In the southern part of the country, the landscape is predominantly occupied by evergreen and semi-deciduous forests, featuring tropical hardwoods like mahogany, odum (ebony), silk cotton, and kolas as the predominant components of the forest cover. The varied ecologies present in Ghana could have a notable impact on the population structure of *Bactrocera dorsalis.*

Influence of ecology on the structure of insects

Ecology plays a crucial role in shaping the structure and diversity of insect populations. Various ecological factors, including temperature resource availability, and relative humidity, significantly impact insect morphology. In a study by Rashwin (2021), it was found that insects in diverse ecosystems, such as tropical rainforests, exhibit greater morphological diversity compared to those in more homogeneous environments like agricultural fields. This diversity is attributed to the wide range of niches and resources available in complex habitats, which drive adaptive morphological changes Climate and environmental conditions, such as temperature and humidity, are critical ecological factors affecting insect physiology and structure. A study by Boesing, Nichols, and Metzger (2017) highlighted that insects in temperate regions often exhibit seasonal morphological changes to cope with varying climatic conditions. These adaptations include changes in body size, coloration, and reproductive strategies to enhance survival and reproduction in fluctuating environments (Boesing et al., 2017)

These findings underscore the importance of ecological factors in determining the structural characteristics of insect populations, which in turn influence their roles in ecosystems and their interactions with other species.

Geometric Morphometry

Traditional morphological approaches employ multivariate statistics to quantify variables such as the length, height, and breadth of structures, distances between landmarks, and, in some instances, angles and ratios, among other factors (Rohlf & Marcus, 1993; Adams et al., 2004; Zelditch et al., 2012). However, these approaches may not always effectively capture all aspects of bodily structures, and extracting information about shape and size from the collected Collecting information about shape and size from the data can be challenging, (Rohlf, & Marcus 1993; Zelditch et al., 2012; Tatsuta, 2012). Geometric morphometrics, a method developed towards the end of the twentieth century, has the potential to overcome these limitations, (Adams et al. 2004; Rohlf, & Marcus 1993; Zelditch et al., 2012).

Geometric morphometrics is a methodology that utilizes 2D or 3D coordinates of naturally identifiable locations, referred to as landmarks, to document the geometry of the object under investigation, (Adams et al. 2004; Zelditch et al., 2012). By employing a consistent coordinate system, these landmarks can be superimposed to unveil information about shape and size, while also minimizing variability in translation and rotation of the targeted structures. (Rohlf & Marcus 1993; Adams et al. 2004; Zelditch et al., 2012). In comparison to traditional methods, geometric morphometrics offers greater flexibility in data acquisition, preserving the geometry of morphological structures throughout the analysis (Adams et al. 2004), and demonstrating enhanced discriminating power (Parsons et al., 2003; Bernal, 2007; Maderbacher, 2008; Viscosi et al., 2009). Geometric morphometry involves comparing the relative positions of landmarks among individuals or groups, utilizing the "Procrustes paradigm" to standardize shape variation. This approach proves to be an effective method for delineating morphological differences in populations of *B. dorsalis* through shape variations.

Cuticular Hydrocarbons

Cuticular hydrocarbons (CHCs) are hydrophobic compounds situated on the outermost layer, known as the epicuticle, present in all insects and other arthropods, serving as the primary defense mechanism against desiccation in insects. As a secondary function, they play a crucial role in communication both within and between species across various scenarios, encompassing tasks such as species and gender identification, signaling fertility, chemical mimicry, and various other functions that have been identified specifically in social insects, such as mating and reproduction. Therefore, CHCs have proven to be an incredibly effective identification tool in various fields of insect biology, (Blomquist & Bagnères, 2010; Kather & Martin, 2012). Qualitative and/or quantitative specificity associated with sex and mating status has been observed in a wide range of insect species, including numerous dipterans (Howard and Blomquist, 2005).

Population Structure of *Bactrocera dorsalis*

Most of the recent research has concentrated on the invasion, spread, and establishment of this pest in Sub-Saharan Africa. Initially identified in Kenya in 2003 (Lux, et al., 2003), the fly subsequently appeared in Ghana in 2005. Within a period of 14 years, it has extended its presence across all sub-Saharan Africa, with only limited areas in South Africa remaining unaffected by the pest (Manrakhan, et al., 2015). Research and management of the pest have faced challenges due to its unclear taxonomic history. Over the past few decades, the fly has been referred to by various names, including *B. invadens*, *B. dorsalis* (Drew, Tsuruta & White), *B. papayae* (Drew and Hancock), and *B. philippinensis* (Drew & Hancock). Through a substantial international collaborative effort a few years ago (De Meyer et al., 2015), these three taxa were officially recognized as junior synonyms of *B. dorsalis* (Drew & Romig, 2013; Schutze et al., 2015a; Schutze et al., 2015b). While this synonymization aids in clarifying taxonomic identification and helps in certain aspects of pre- and post-harvest control and market access (Hendrichs et al., 2015), it also introduces recent challenges within the scientific community. An organism with a geographic range spanning from Africa to Asia and the Pacific Ocean might be anticipated to display macrogeographic population structuring, (Virgilio et al., 2010; Ascunce et al., 2011; Gloria-Soria et al., 2016;). However, there is no evidence supporting this hypothesis. As per the International Plant Protection Convention (FAO, 2011), which defines a "pest" as "any species, strain, or biotype of plant, animal, or pathogenic agent injurious to plants or plant products," the synonymization of taxa does not eliminate the concern that different geographic populations may still exhibit high levels of population structuring, posing potential quarantine and trade issues at the "strain" level. Despite comprehensive sampling across the entire geographic range of *B. dorsalis* (Qin et al., 2018), no macrogeographic population structuring was observed. This outcome aligns fully with earlier studies that covered individual components of the range, (Khamis et al., 2009; Schutze et al., 2012; Shi et al., 2012; Choudhary et al., 2016).

However, these studies primarily examined macrogeographic population structure. The impact of microhabitats or microecology on the local population structure of the pest in Ghana remains unexplored. This information is crucial for effectively planning and implementing control (eradication or suppression) strategies for this pest.

Research Gaps

Bactrocera dorsalis continues to destroy the horticulture industry in Ghana, there is a lack of comprehensive knowledge regarding the population structure of the pest in Ghana, and this information is crucial for the strategic planning of effective areawide Integrated Pest Management (IPM) strategies with a Sterile Insect Technique (SIT) component.

The effect of different Agro-ecological zones as found in Ghana, on the spread of *Bactrocera dorsalis* (population structure) since its invasion into the country in 2005 has been poorly studied.

Use of insecticides and some control methods are crude and not environmentally friendly, SIT and other genetic modification approaches may prove effective. These methods cannot be successful without knowledge of population structure.

Effect of changes in Agro – ecological zones on the evolution of *B. dorsalis* in Ghana is lacking, this can inform on species segregation, isolation, sub-divisions and possible speciation.

CHAPTER THREE

MATERIALS AND METHODS

Introduction

This chapter explains into detail all the materials and methodology used in this research. The methodologies are explained into detail to ensure the reproducibility of the procedures. All assumptions and modifications made to standard procedures are explained. The chapter explains how the study was designed to generate results to achieve the objectives. The sampling method is described, and georeferenced sampling sites are clearly shown with a map to indicate where the samples were collected. Finally, this chapter describes how the principles of geometric morphometry and cuticular hydrocarbon profiling were applied to investigate the population structure of *B. dorsalis* within a biogeographic context.

Study Design

The study was structured in two main parts: geometric morphometry and cuticular hydrocarbon profiling. Whole flies were collected from farms (representing populations) from four Agroecological zones for the geometric morphometric analysis while Mango fruits infested with larvae were collected from all four Agro-ecological zones, and the ensuing larvae were nurtured until reaching adulthood, and used for the cuticular hydrocarbon profile.

Agro Ecological Zones

The four main Agroecological zones (ecozones) in which commercial mango is cultivated in Ghana are, Southern (Coastal), Forest (Eastern-Volta), Transition, and Northern (Guinea Savannah) Zones. Environmental conditions (Rainfall, Temperature, Relative humidity and dew point) characterizing these ecological zones were recorded daily for one year throughout 2018 the year of data collection and represented in monthly averages and reported in graphs.

Sample sites and sampling for morphometric analysis

- The sampling was conducted along Ghana's commercial mango growing enclave, representing four (4) Agro ecological zones, namely Coastal Savanah, Eastern Volta, Transition zone and Guinea Savannah (Northern) Zone. Whole flies were collected from five (5) farms within each Agroecological zone for the morphometric analysis. Collections were made from twenty farms - five (5) farms per Agro ecological zone (Table 1) (Figure 1). 5 Methyl eugenol baited traps were set per acre farmland and harvested after 24 hours (Ekesi et al., 2009). Thirty (30±) whole flies (Table 1) were selected per farm to represent a population. Flies were Identified as *Bactrocera dorsalis* by entomologists at the PPRSD using identification keys: Identification of *Bactrocera* species (Drew et al 2013) Flies with damaged wings were removed. Seven hundred and six (706) whole undamaged right forewings of male *Bactrocera dorsalis* were used for the geometric morphometry aspect of this study

Table 1: Georeferenced Sample Sites and Sample Size

Farm	**Locality**	**Region**	**GPS**	**ID**	**No. of samples**
	Coastal Savannah Ecozone			GH_1	**192**
Mikleb farm	Loye, Awutu Senya	Central	N05^{0}33.738’, W002^{0}30.239’	GH_1V_1	39
Eve-Lyn farm	Oyarifa	Greater Accra	N05^{0}44.599’, W001^{0}10.753’	GH_1V_2	40
Prudent/Blueskies farm	Ayikuma,	Greater Accra	N05^{0}56.764’, W001^{0}01.655’	GH_1V_3	36
Peter & Tetteh farm (Peterbeck),	Dodowa,	Greater Accra	N05^{0}54.071’, W001^{0}04.579’	GH_1V_4	40
Awur farm,	Vume, Sogakope	Volta	N06^{0}00.748’, E000^{0}31.856’	GH_1V_5	37
	Eastern Volta Ecozone (semi deciduous forest)			GH_2	**155**
Alphonse farms,	Vakpo	Volta	N06^{0}52.925’, E000^{0}18.599’	GH_2V_1	34
Bomart farm	TafiAbuife	Volta	N06^{0}52.386’, E000^{0}21.481’	GH_2V_2	30
Abraham farm	Kpong	Volta	N06^{0}07.692’, E000^{0}03.951’	GH_2V_3	29
Obeng farm	Somanya	Eastern	N06^{0}02.475’, E000^{0}00.211’	GH_2V_4	31
Sabastian farm	Somanya	Eastern	N06^{0}02.599’, E000^{0}00.336’	GH_2V_5	31
	Transitional Zone			GH_3	**169**
Gyiele Nurah farm	Ejura	Eastern	N07^{0}25.161’, W001^{0}27.791’	GH_3V_1	30
Kobbiman farm	Nkoranza	Eastern	N07^{0}37.088’, W001^{0}43.363’	GH_3V_2	38
Nana Yaw Gyan farm	Wenchi	Bono	N07^{0}48.350’, W002^{0}11.432’	GH_3V_3	23
Djeabour farm	Techiman	Bono	N07^{0}41.876’, W002^{0}02.986’	GH_3V_4	39

Table 2: Cont’d

Effah Nimo farm	Kintampo	Bono	N08^{0}02.222’, W001^{0}40.407’	GH$_3$V$_5$	39
		Northern Zone		GH$_4$	**190**
Nakohinaa farm	Libga, Tamale	Northern	N09^{0}35.330’, W000^{0}50.865’	GH$_4$V$_1$	35
ITFC head office farm	Gushie, Savelugu, Tamale	Northern	N09^{0}48.965’, W000^{0}51.661’	GH$_4$V$_2$	37
Dawuni Salifu farm	Gushie, Savelugu, Tamale	Northern	N09^{0}48.253’, W000^{0}52.136’	GH$_4$V$_3$	40
Abdul Razark farm	Tuunayille, Savelugu, Tamale	Northern	N09^{0}48.240’, W000^{0}53.698’	GH$_4$V$_4$	39
ITFC main farm	Dipale, Savelugu, Tamale	Northern	N09^{0}47.829’, W000^{0}55.536’	GH$_4$V$_5$	39
Total					**706**

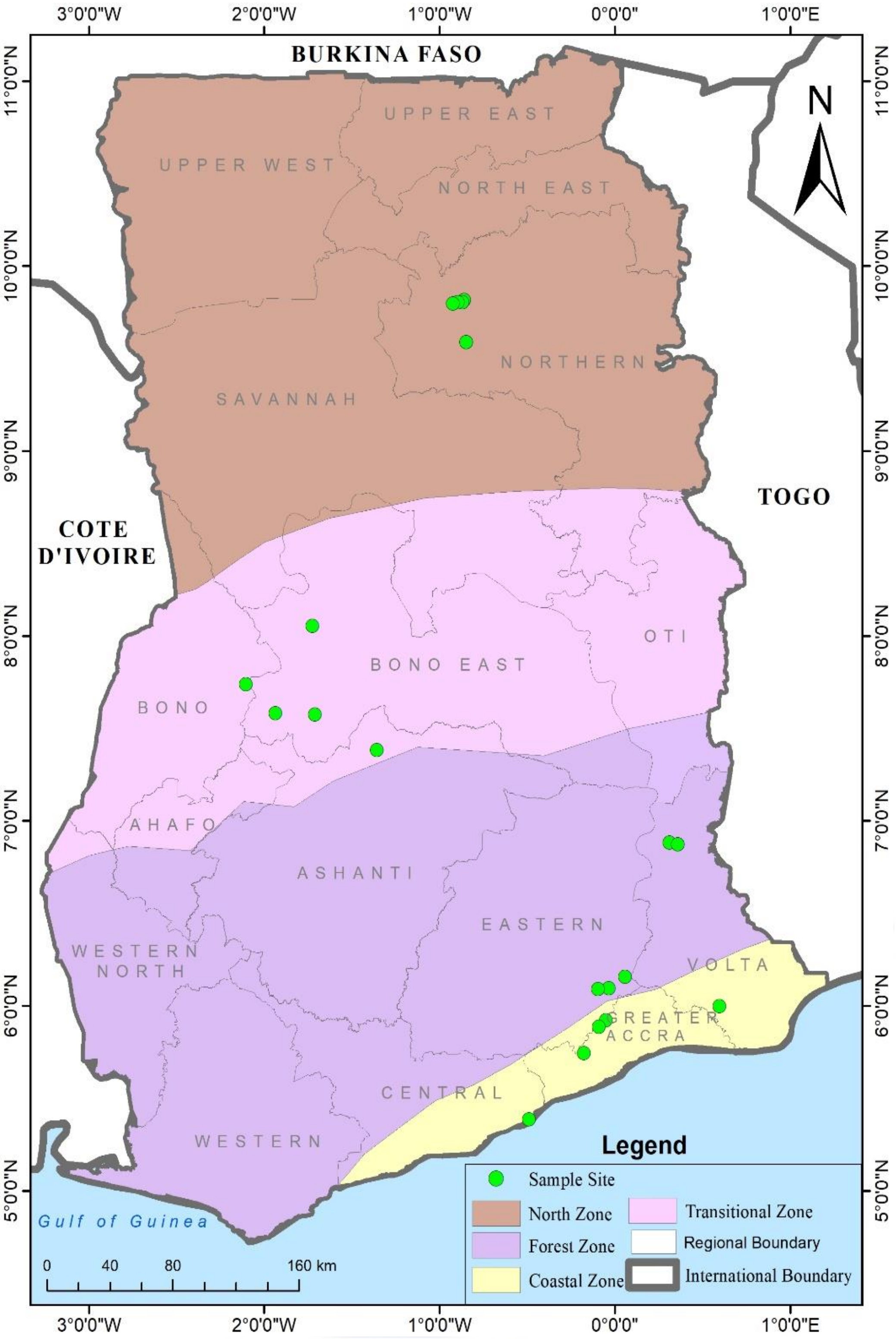

Figure 1: Map showing Agro-ecological zones and sample sites.

Geometric Morphometrics

The right forewing was delicately extracted from each fly using forceps and placed in a petri dish. Following an ethanol rinse, the wings were carefully mounted on a microscope slide, ensuring even placement without distortions, and facing the same direction to facilitate measurements. (Combey et al., 2013) The mounted wings were covered with cover slips and secured with adhesive. Each mounted wing was labeled with a sample identification number. Using a camera connected to a stereomicroscope with Leica Application Suite version 3.4.1, images of each mounted wing were captured. All the captured images were then imported into the TPS Util computer program to generate a TPS file. This TPS file was subsequently imported into TPS Dig version 1.40 (Rohlf, 1998) for the digitization of anatomical landmarks (Table 2) (Figure 2). The raw x and y coordinates of the landmarks for each wing were loaded into Morpho J 1.7a for further analysis.

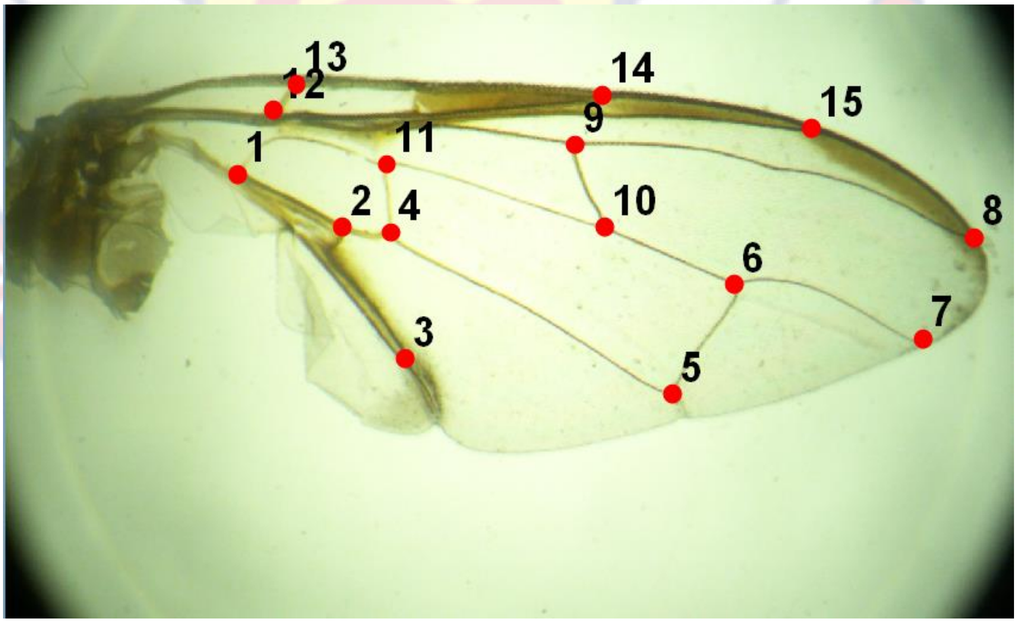

Figure 2: Image of the right forewing showing the position of 15 anatomical landmarks.

Table 3: Position of landmarks on the wing of *B. dorsalis*

Landmark	Position
Landmark 1	Basal junction of cell bm
Landmark 2	Junction of vein CuA_1 and CuA_2
Landmark 3	Junction of vein A_1 and CuA_2
Landmark 4	Junction of vein CuA_1 and dm-bm cross vein
Landmark 5	Junction of vein CuA_1 and dm-cu
Landmark 6	Junction of vein m and dm-cu
Landmark 7	Termination of vein m
Landmark 8	Termination of vein R_{4+5}
Landmark 9	Junction of vein R_{4+5} and r-m cross vein
Landmark 10	Junction of vein m and r-m cross vein
Landmark 11	Junction of vein m and dm-bm cross vein
Landmark 12	Anterior most point of the suture located toward the base of vein SC
Landmark 13	Inner antero-distal corner of cell bc
Landmark 14	Junction of vein R1 and costal vein
Landmark 15	Termination of vein R_{2+3}

Analyses of morphometric data in Morpho J

Outliers in samples were removed prior to analyses. Outliers were defined as samples with Mahalanobis squared distance of more than 100. Classifiers (zones and farms) were set in Morpho J, and populations within the same Agro ecological zones were grouped together, also populations were grouped based on the farms they were collected from. Procrustes superimposition was performed on the data in Morpho J. This process aligns shapes and minimizes differences between them, ensuring that only genuine shape variations are measured within the population. (Rohlf, & Slice, 1990).

Procrustes ANOVA and Partial Least Squares were employed to assess the significance of variation among populations, categorized by Agroecological zones and the farms from which the populations were collected. Additionally, multivariate statistical analyses such as Procrustes Fit, Principal Component Analysis, discriminant function analysis, and Cross-validation were conducted to further evaluate the variation among populations.

Principal Component Analysis (PCA) was utilized in this study to identify the principal wing character(s) contributing to most of the variation within populations among the four ecological zones based on wing shape differences. PCA simplifies data and enhances interpretability by creating new coordinates that are linear representations of the original data and are uncorrelated. PC plots were employed to illustrate groups of individuals within the four Agroecological zones. Eigen vectors were normalized, and the distribution of the groups within the space was assessed (Özden, 2008). The range of variance on each PC axis was calculated based on the Eigen values obtained from the Principal Component. Discriminant Function Analysis (DFA) was employed to evaluate effective classification and predict cluster membership. DFA scores were utilized to identify populations that differed from others, and the percentage variance was calculated. Cross-validation information was employed to assess the predictive accuracy of the dataset.

Sample Collection for Cuticular Hydrocarbon Profiling

Mango fruits infested with *Bactrocera dorsalis* gathered from the four Agro-ecological zones, namely Coastal Savannah, Forest Savannah, Transition, and Guinea Savannah zones. Naturally infested fruits from three farms representing three populations from each Agroecological zone were used for this study. The fruits were collected from mango orchards and were transported to the fruit fly rearing facility of the Biotechnology and Nuclear Agriculture Research Institute (BNARI). Placed on sawdust in containers, the fruits allowed the larvae to mature and pupate. The larvae and pupae were maintained at 25±2 °C, 80–90% RH, and a 12:12 (L: D) photoperiod. Emerged adult flies were provided with a standard diet, with water supplied ad libitum. Six mature flies (3 males and 3 females) aged 9 days from each ecological zone were used for the solvent extraction of CHCs.

Solvent extraction of cuticular hydrocarbons

Each of the flies was placed in a sterilized transparent 125 mL reagent bottle containing 100 µl analytical grade hexane. The fully submerged specimen was agitated for 10 minutes and removed with sterile forceps. The extract was labelled accordingly and evaporated to dryness, and used in the chemical analysis (Blomquist et al., 2010).

Chemical analysis

The chemical analysis involved Gas Chromatography-Mass Spectrometry (GC–MS) of the cuticular extracts, conducted at the instrumentation laboratory of the Chemistry Department, University of Cape Coast, Ghana. The extract was diluted with 1.0 mL of hexane (GC grade), and a 1.0 µl aliquot of the resulting solution was injected (in split less injection mode) into a Shimadzu GC–MS (QP 2020) equipped with an Rx-5MS column (29.5 m x 0.25 mm diameter x 0.25 µm thickness), using helium as the carrier gas with a column flow rate of 1.49 ml/min. The injection temperature was set at 250°C. The temperature program included an initial stage at 50°C for 1 min, followed by an increase to 220°C and then held at 310°C. All mass spectra were obtained by electron impact, and the ion source temperature was maintained at 210 °C. Identification of n-alkanes was achieved by comparing retention times and mass spectra of unknowns with three referenced libraries of mass spectra (NIST-14s, NIST-14, and WILEY-8), (Bloomqist and Bagneres, 2010).

Statistical analysis of cuticular hydrocarbon data

The concentrations of all 54 hydrocarbons identified after GCMS analysis of hexane washes of both male and female flies were entered into Microsoft excel and the names of the hydrocarbons coded with a simple code (A001 – A054). The excel file was imported into R statistic version 4.3.0 and used for the analysis. A heat map was employed to present compounds organized as matrices, and a dendrogram was generated using correlation-based distances. Additionally, a Principal Component Analysis (PCA) was conducted with hierarchical clustering based on Pearson’s correlation to illustrate the clustering of entities.

CHAPTER FOUR

RESULTS

Introduction

This chapter presents the results obtained from the methods outlined in the preceding chapter. The results are reported in graphs, tables, and a map. First, the environmental data collected during the year of data was collected that characterized each Agro-ecological zone was reported. Then results of population variations, underscored by geometric morphometry of the wings of *B. dorsalis* are reported. Lastly, results from the cuticular hydrocarbon profile of populations of *B. dorsalis* and how they cluster between ecological zones and sexes are reported.

Environmental Conditions Characterizing the Ecological Zones

Environmental parameters were recorded to characterize the conditions that describe each of the ecological zones that were demarcated for the study. Specifically, temperature, relative humidity, dew point and rainfall levels were recorded daily for a year to describe the environmental conditions characterizing each ecological zone. Temperature ranged from 25.7°C to 34.6°C across ecological zones. The Guinea Savannah zone recorded the highest temperature, while the Transition zone registered the lowest temperature. Temperature trends generally showed two peaks across the ecological zones. The First peak was recorded from February and March after which a decline was observed throughout the rest of the months till August when the temperature started to rise once more, reaching its peak in October and November (Figure 3). There were no significant differences in temperature observed between the ecological zones.

(F = 2.123, *p* = 0.110).

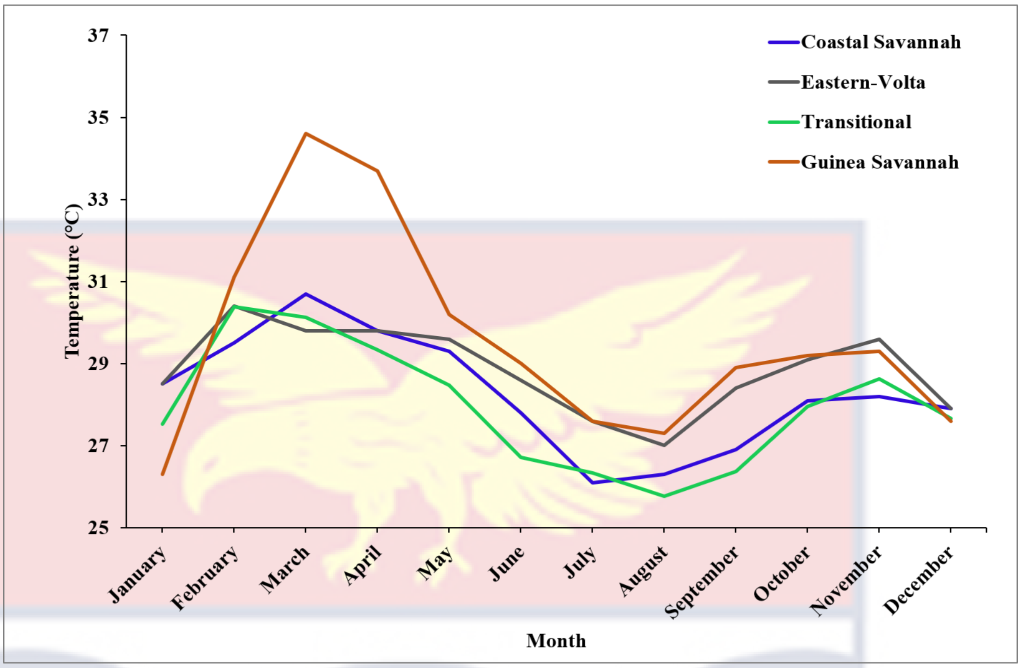

Figure *3*: Trends in temperature recorded in each of the four ecological zones from January to December in 2018.

Humidity fluctuated between 29.7% and 86.6% across ecological zones, with the highest humidity observed in the Coastal Savannah (southern) ecological zone and the lowest in the Guinea Savannah (Northern) ecological zone. However, these differences were not statistically significant (F = 1.102, p = 0.359). In the Transition and Guinea Savannah ecological zones, humidity increased from January, reaching a peak between May and August, followed by a decline until the end of the year. In the Eastern Volta ecological zone, a sharp increase in humidity occurred in May, maintaining a relatively uniform value until October, when a sharp decline was recorded (Figure 4). In the Coastal

Savannah, humidity appeared to peak in July, with lower peaks in February and November.

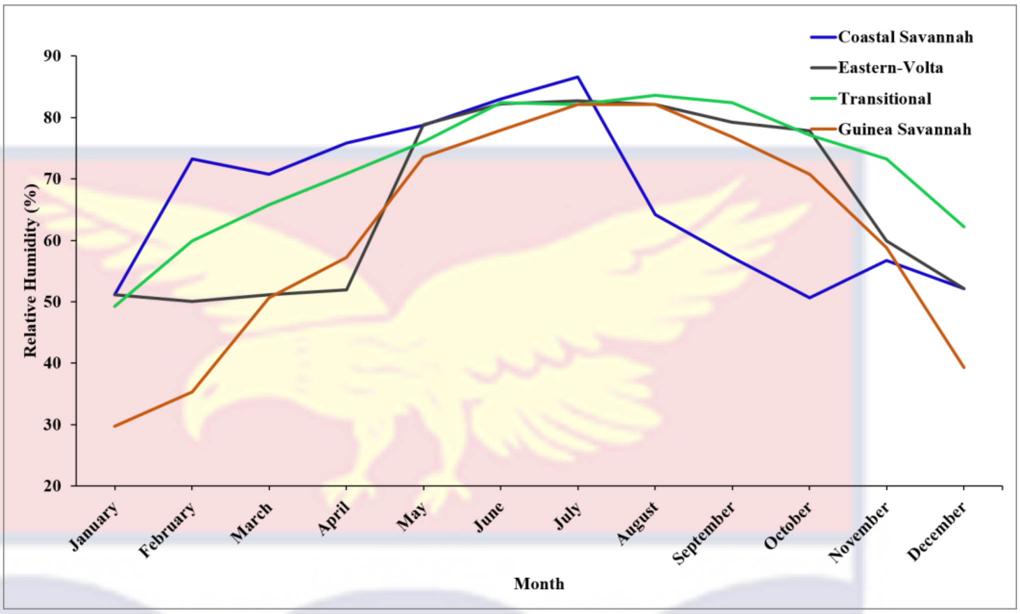

Figure 4: Humidity levels recorded from January to December across the four ecological zones.

Dewpoint ranged from 1°C recorded in January in the Guinea Savannah ecological zone to 25.4°C recorded in June in the Eastern-Volta ecological zone. Differences in dewpoint were not significant between the ecological zones ($F = 0.458$, $p = 0.713$). Dewpoint values increased from very low values in January for Coastal Savannah, Transition and Guinea Savannah ecological zones and peaked after March after which values were consistent till after September where a decline was recorded for the Guinea Savannah ecological zone and after October where a decline was recorded for the Coastal Savannah and Transition ecological zones (Figure 5). In the Eastern Volta ecological zone, dewpoints increased steadily from January to April and a sharp increase was recorded in May which was maintained with occasional declines and increase across the months till October where values declined to the end of the year (Figure 5).

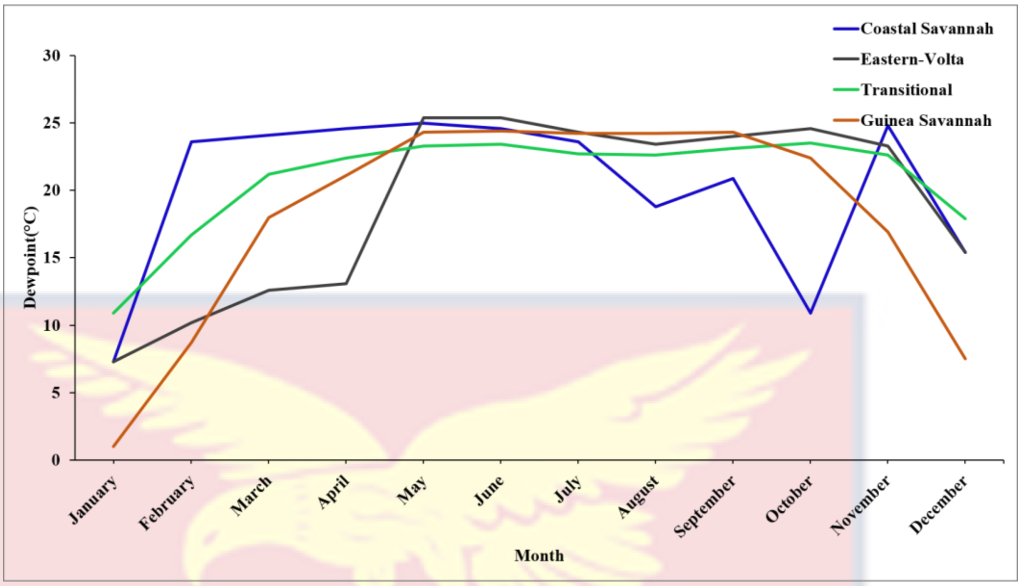

Figure 5: Dewpoint levels recorded from January to December across the four ecological zones.

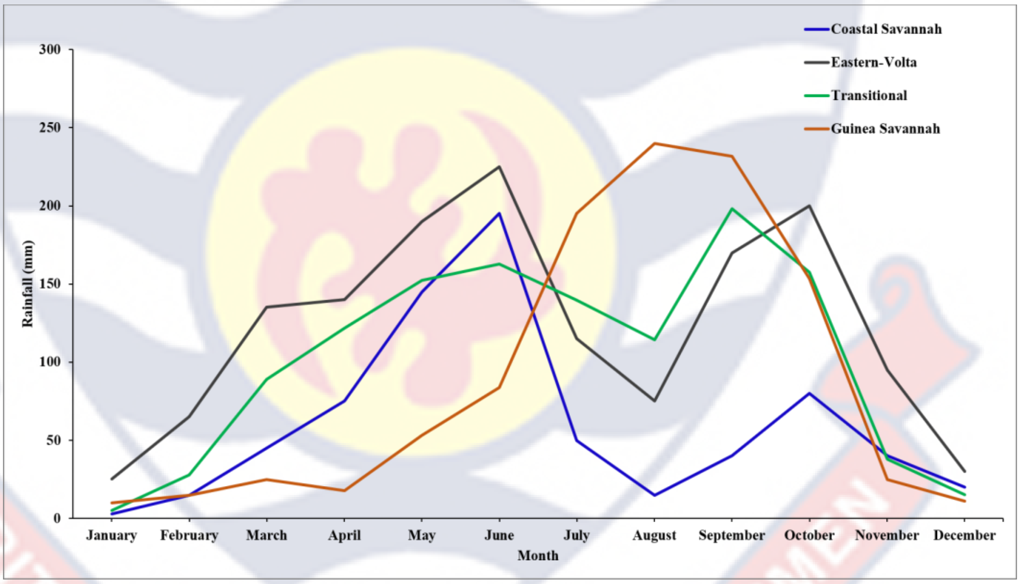

Figure 6: Rainfall levels recorded from January to December across the four ecological zones.

Rainfall exhibited a bimodal pattern in all ecological zones, with peaks in June and between September and October, except for the Guinea Savannah ecological zone, which displayed a single peak in rainfall between July and September (Figure 6). However, rainfall values were not significantly different in the different ecological zones throughout the months (F = 1.598, p = 0.204). The minimum recorded rainfall level was 3 mm in the Coastal Savannah in January, while the maximum was 239.7 mm recorded in August in the Guinea Savannah ecological zone. Population variations using geometric morphometry of wings of *Bactrocera dorsalis* (syn. *B. invadens*) from four Agro-ecological zones in Ghana.

Geometric morphometric techniques were employed to evaluate the variability of *B. dorsalis* populations across different Agroecological zones. Before analyzing differentiation, all samples were aligned to a principal axis to eliminate size and orientation variations and retain shape variations in the samples. A Cumulative frequency plot of Mahalanobis distance (squared) (Figure 7) shows that both curves rise steeply and level off quickly, meaning most of the data points were clustered within a small Mahalanobis distance. This is a fitness test of the data set indicating that any variation observed is inherent in the wing and not introduced.

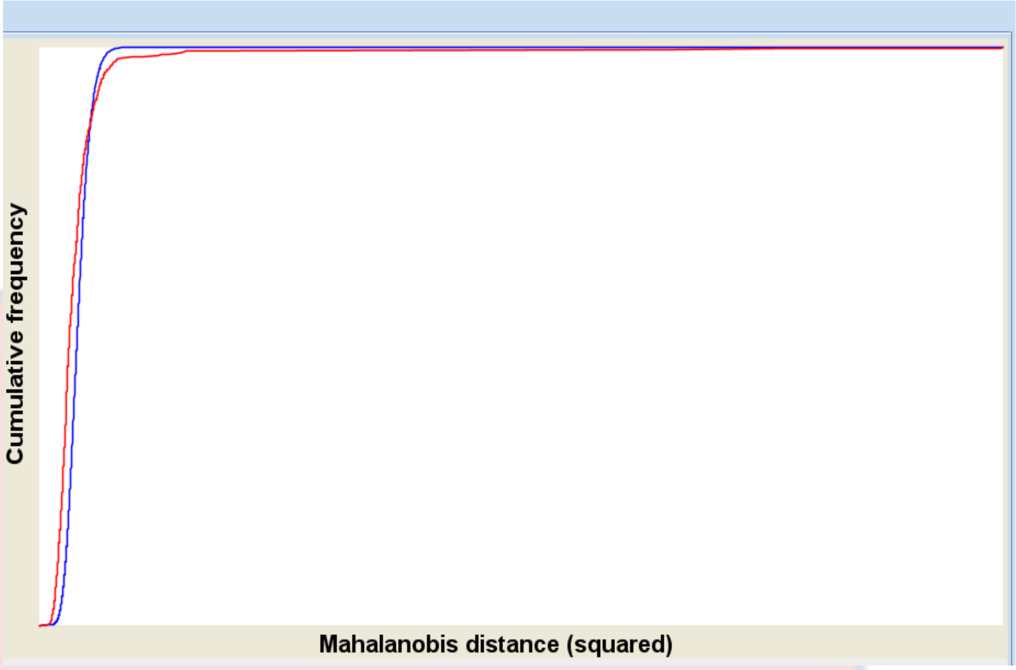

Figure 7: Cumulative frequency plot of Mahalanobis distance (squared)

Variation between ecological zones

Differences in wing shape of *B. dorsalis* were significant between the four ecological zones ($F = 5.16$, $p < 0.0001$) and between individual farms they were collected from across all ecological zones ($F = 3.06$, $p < 0.0001$). Principal component analysis (PCA) was performed to pinpoint the wing features that primarily contributed to the observed variability between the ecological zones. The PCA results indicated that landmark 14, followed by landmarks 7, 8, 10, 6, and 15, made the most significant contributions to the variation in shape between the ecological zones, listed in decreasing order of their contributions (Figure 7a).

Landmark 14, which corresponds to the Junction of vein R1 and costal vein (Please see Table 2 in materials and methods) contributed 23.24% to the total variation while landmarks 7, 8, 10, 6 and 15 contributed high percentages as compared to the rest of the landmarks but lower than that of landmark 14 in order of decreasing contribution (Figure 8b). A Scatter plot of samples showed overlaps between samples collected from the four ecological zones and no group was clearly distinct from another (Figure 8c).

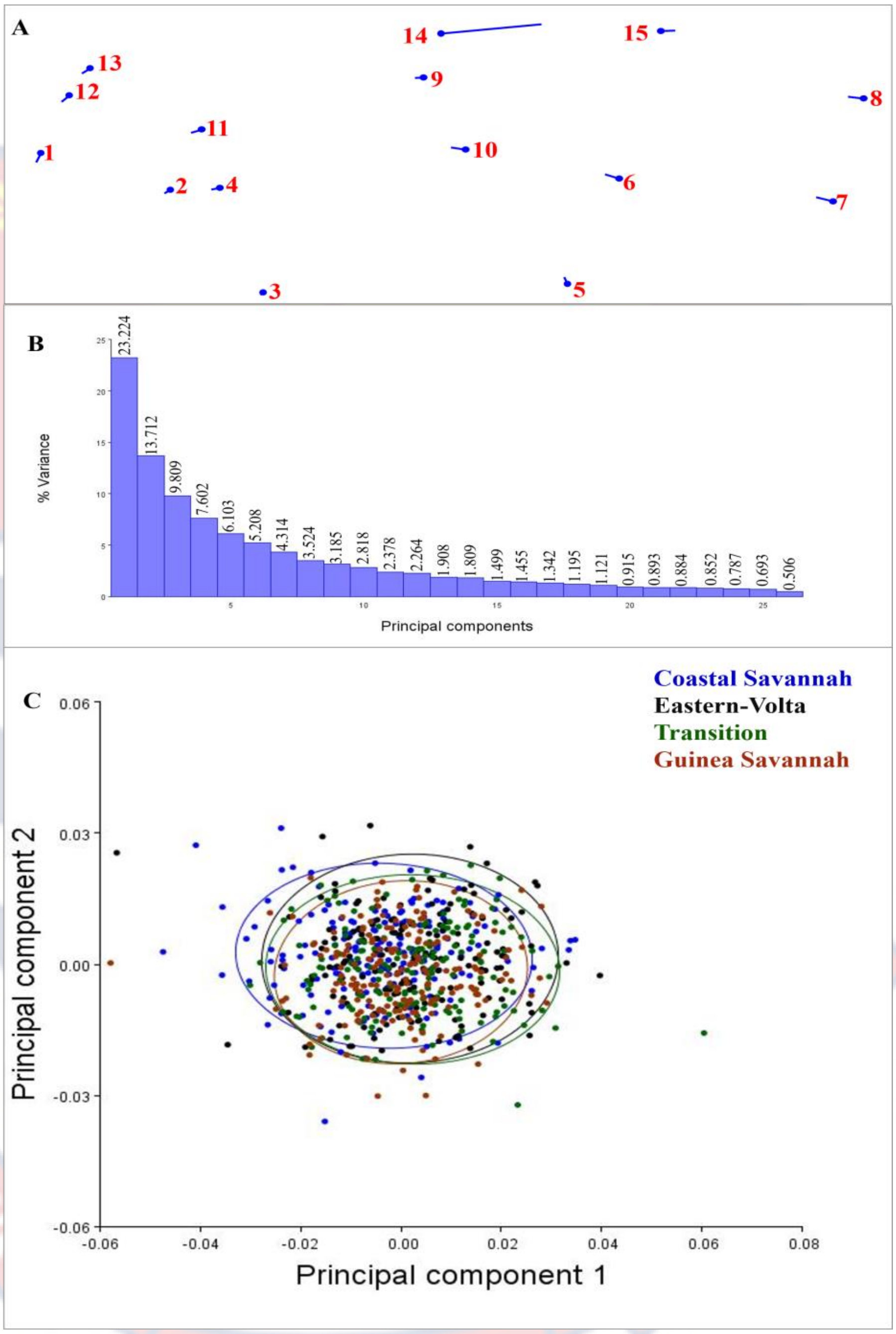

Figure 8*:* Results of principal component analysis showing (A) contribution of each landmark to the changes in shape, (B) eigenvalues and percentage contribution of each landmark, (C) scatter plot of samples, showing variation between wing shapes of *B. dorsalis* collected from each ecological zone.

Discriminant function and cross-validation analyses were subsequently carried out to determine the percentage classification of samples into the four ecological zones. In general, a notable difference was observed in the mean wing shape of *B. dorsalis* when comparing each pair of ecological zones. (Table 5).

Table 4: Mahalanobis distances (p-values) between mean wing shape of *B. dorsalis* collected from the four ecological zones.

	Eastern Volta	**G. Savannah**	**Transition**
Coastal Savannah	1.385 (< 0.0001)	1.316 (< 0.0001)	1.15 (< 0.0001)
Eastern Volta		1.295 (< 0.0001)	1.148 (< 0.0001)
Guinea Savannah			0.889 (0.0001)

B. dorsalis populations collected from the various ecological zones recorded high classifications (> 60%) to each of the ecological zones when compared with each other. Samples from the Coastal savannah ecological zone showed 79.06% classification when compared with samples from the Eastern Volta ecological zone which recorded 73.5% classification, with 75.4% and 66.9% cross validation for both ecological zones respectively (Figure 9A, D). A similar observation was also made when populations from coastal savannah zone were compared with samples from the Guinea savannah ecological zone (Figure 8B, E) and from the Transition zone (Figure 9C, F)

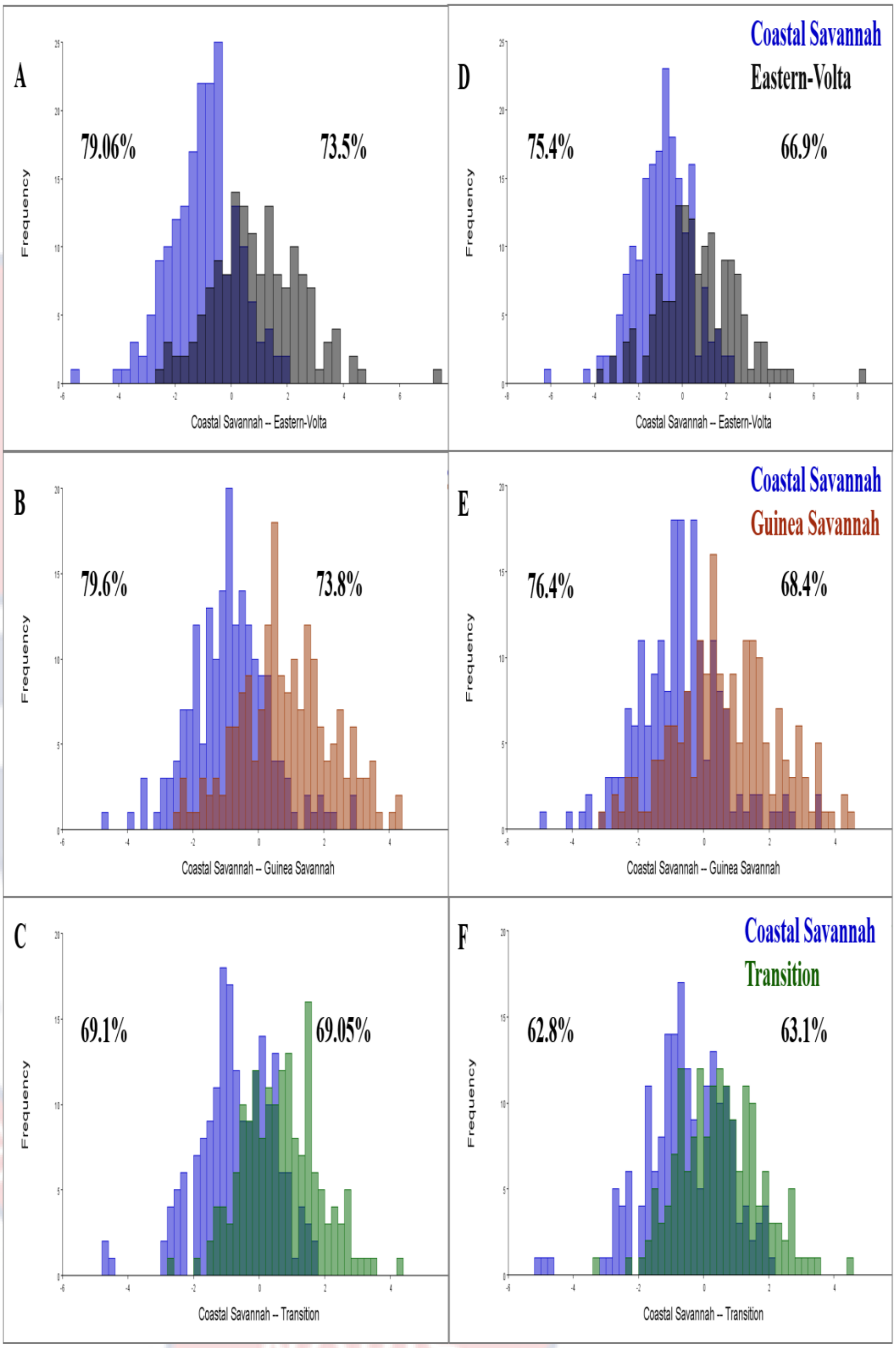

Figure 9: Discriminant function (A – C) and cross validation (D – F) analysis showing classifications of *B. dorsalis* samples collected from the Coastal savannah ecological zone in comparison with those collected from the Guinea savannah Eastern Volta and transition zones.

Similarly, higher significant percentages of classification were recorded between samples collected from the Eastern-volta ecological zone and Guinea Savannah ecological zones (Figure 10A, D), between Eastern-volta ecological zone and Transition zone, and between Guinea savannah and Transition zone (Figure 10C, F).

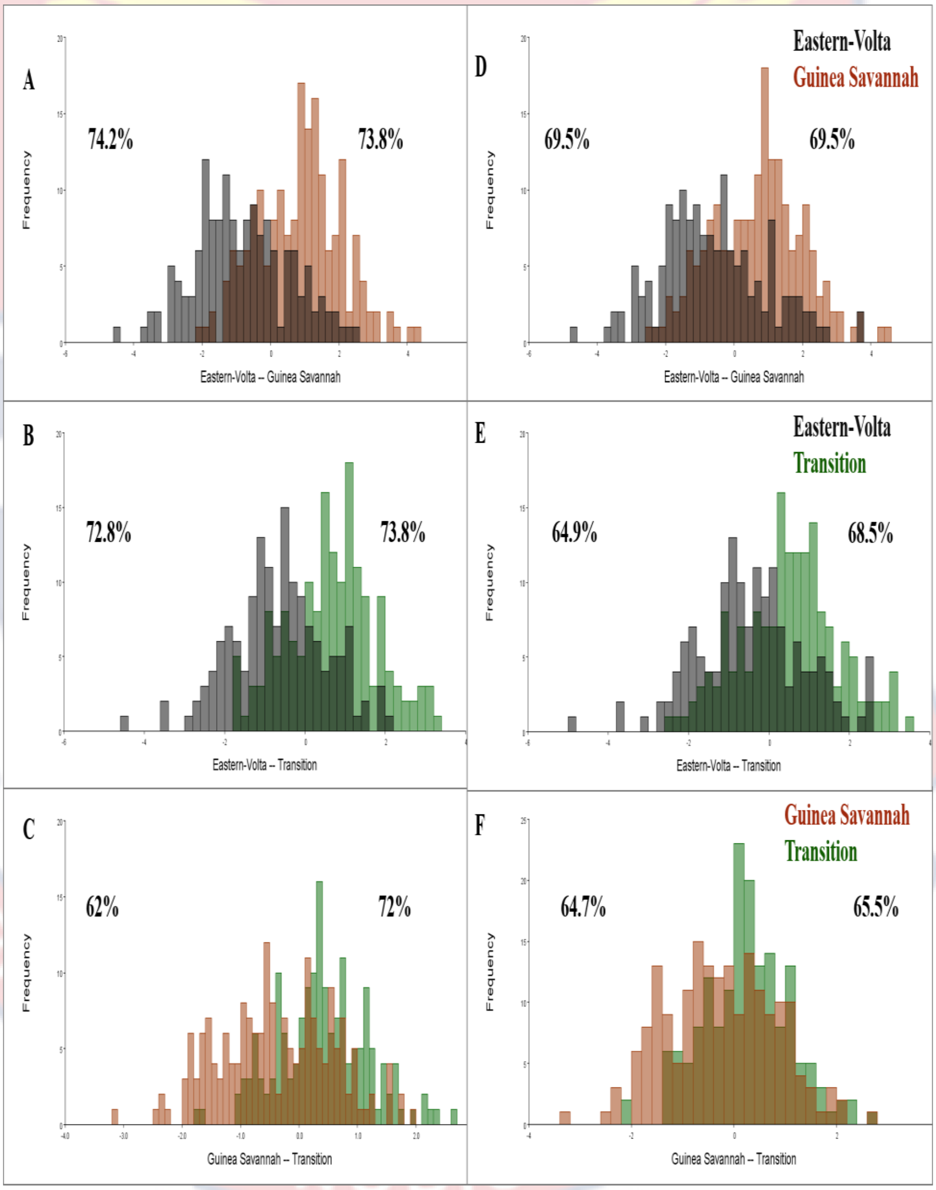

Figure 10: Discriminant function (A – C) and cross validation (D – F) analysis illustrating the classifications of *B. dorsalis* samples collected from the Eastern Volta ecological zone in comparison with those from the Guinea Savannah and Transition zones, as well as classifications between the Guinea Savannah and Transition zones.

Variation among farms in which *B. dorsalis* samples were collected regardless of ecological zones.

Samples of *B. dorsalis* collected from individual farms when compared with other farms regardless of the ecological zone showed significant differences in their wing shapes (*F* = 3.06, $p < 0.0001$). Like the variation between ecological zones, landmark 14 contributed most to the variation of *B. dorsalis* between farms (Figure 11A). The first 4 principal components (landmarks 14, 8, 7 and 15) contributed more than 50% to the overall variability of wing shapes of *B. dorsalis* between the farms (Figure 11B). The graph of shape changes also showed that almost all landmarks contributed some amount of variation to the overall variation observed between individuals recorded from different farms (Figure 11A).

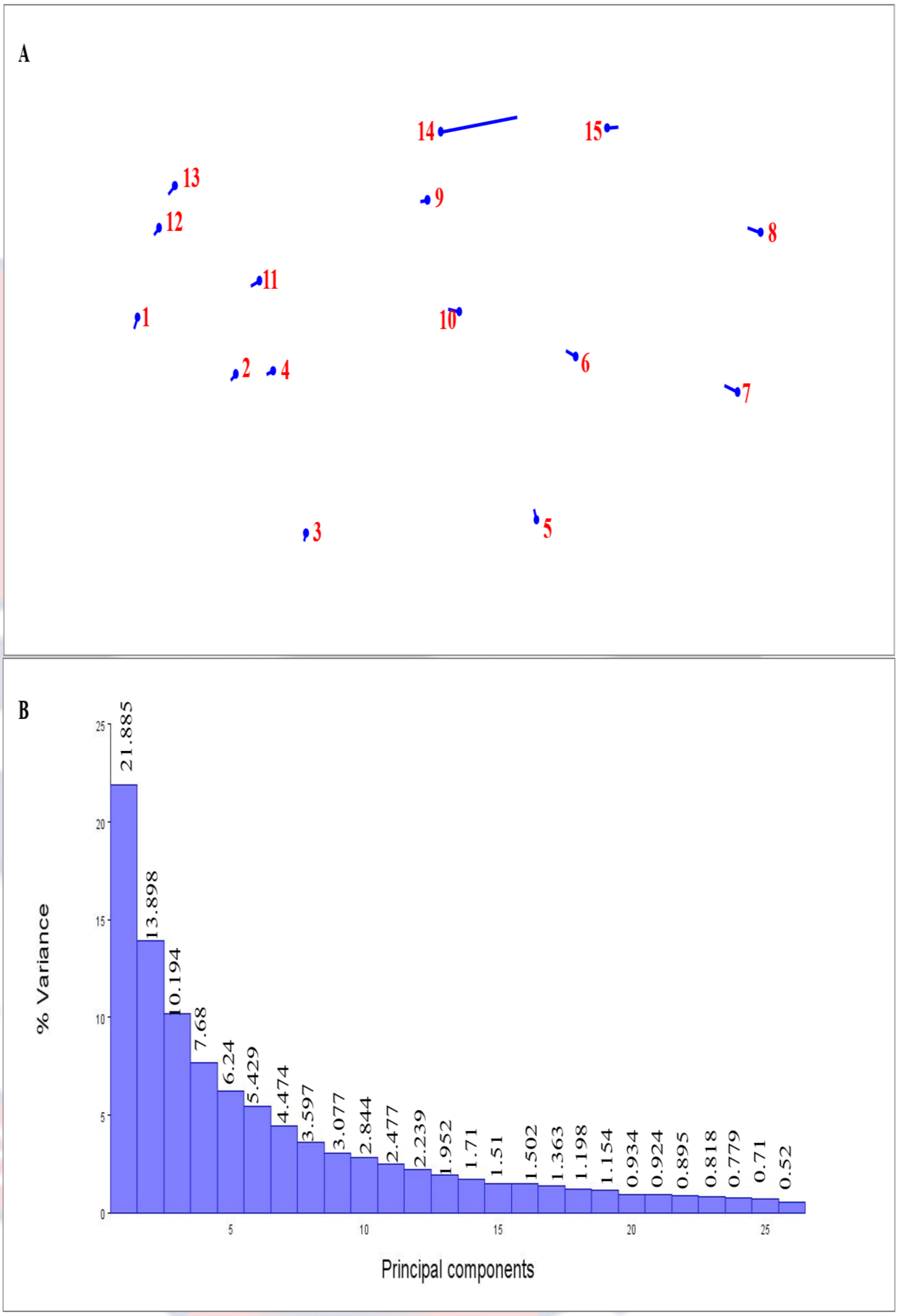

Figure 11**:** Shape changes showing contribution of each landmark (A) and the percentage variance contributed by each principal component (B) to the variability between individuals of *B. dorsalis* collected from different farms regardless of ecological zone.

Variation was then compared between farms within each ecological zone. Results of discriminant function analysis showed significant differences between farm 1 (GH1V1) in the coastal savannah ecozone and farms 3 (GH1V3), 4 (GH1V4), 5 (GH1V5) however, its difference when compared with farms 2 (GH1V2) was not significant. Significant differences in wing shapes were recorded between farm 2 and farms 3, 4 and 5 also between farm 3 and 4 but difference between farm 3 and 5, and 4 and 5 was not significant (Table 6A).

In the Eastern Volta ecological zone, significant differences were also observed between farms. However, differences were between farm 1 (GH2V1) and farms 3 (GH2V3), 4 (GH2V4) and 5 (GH2V5) and between farm 2 and farms 3, 4 and 5. Other comparisons did not show significant differences (Table 6B).

In the Transition zone, significant differences were recorded between samples from farm 1 (GH4V1) and farms 4 (GH4V4) and 5 (GH4V5) however, differences with farms 2 (GH4V2) and 3 (GH4V3) was not significant. Samples from farm 2 were significantly different from samples from farms 3, 4 and 5. Samples from farm 3 were also significantly different from samples from farm 4 but not different from farm 5 (Table 6C).

In the Guinea savannah ecological zone, it appeared samples from all farms were very similar as non-significant differences were recorded between samples. Significant differences were only recorded between farm 1 (GH4V1) and 5 (GH4V5) and between farm 4 (GH4V4) and 5 (Table 6D).

Table 5: Mahalanobis distances (p-values) of mean wing shape of *B. dorsalis* between study farms in each ecological zone.

A		**Coastal Savannah**		
Farm	**1**	**2**	**3**	**4**
2	1.44 (0.434)			
3	2.42 (0.001)	2.21 (0.005)		
4	2.93 (<0.0001)	2.54 (<0.0001)	1.90 (0.048)	
5	3.08 (<0.0001)	2.60 (0.0001)	1.83 (0.106)	1.384 (0.553)
B		**Eastern-Volta**		
Farm	**1**	**2**	**3**	**4**
2	1.64 (0.538)			
3	1.49 (<0.0001)	3.44 (0.0009)		
4	4.23 (<0.0001)	3.34 (0.0006)	1.899 (0.455)	
5	3.32 (<0.0001)	2.86 (0.0030)	2.22 (0.116)	2.313 (0.053)
C		**Transition**		
Farm	**1**	**2**	**3**	**4**
2	3.40 (<0.0001)			
3	3.06 (0.015)	3.12 (0.0012)		
4	1.39 (0.734)	2.74 (<0.0001)	2.54 (0.021)	
5	1.19 (0.934)	2.84 (<0.0001)	2.29 (0.082)	1.76 (0.096)
D		**Guinea Savannah**		
Farm	**1**	**2**	**3**	**4**
2	1.78 (0.189)			
3	1.74 (0.188)	1.35 (0.629)		
4	1.88 (0.093)	1.597 (0.259)	1.28 (0.693)	
5	2.26 (0.008)	2.083 (0.013)	1.47 (0.382)	2.38 (0.0007)

Cuticular Hydrocarbon Profile of Ecological Populations of *Bactrocera dorsalis* from Ghana

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of hexane washes from male and female *Bactrocera dorsalis* samples collected from four Agroecological zones identified a total of 54 hydrocarbons. This composition included a complex mixture of straight-chain and methyl-branched saturated and unsaturated hydrocarbons, exhibiting a diverse range of carbon backbones.

Cuticular extracts from samples collected across all four ecological zones revealed the presence of eleven hydrocarbons, including Pentadecane-

2,6,10,13-tetramethyl-pristane, Hexacosane, Benzene-1,1'(3,3-dimethyl-1-butenylidene), 1h-indene-2,3-dihydro-1,1,3-trimethyl-3-phenyl, 1-propen, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl, Octacosane-2-methyl, Nonacosane-2-methyl, Triacontane, 2-methylhentriacontane, Dotriacontane, and 2-tetradecyl-1-octadecene. The hydrocarbon with the highest concentration was A051, representing phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl], which was isolated from male flies from the transition zone. Seven hydrocarbons (Tetrapentacontane, Tetracosane, Hexatriacontane, Eicosane-2,6,10,14,19-pentamethyl-, Benzene, 1,1'-(1-methylethylidene) bis [4-methyl-, Tetracosane-2,6,10,15,19,23-hexamethyl-, and Dotetracontane) were exclusively present in samples collected from the Coastal Savannah ecological zone. The remaining hydrocarbons were found in more than one ecological zone, with variations observed only in concentrations.

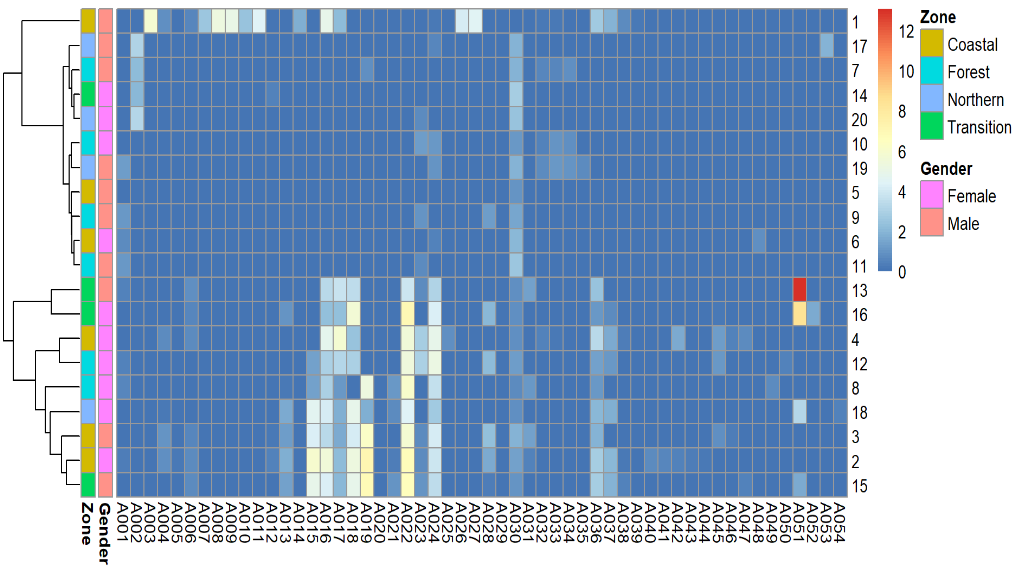

Figure 12: Heat map of percentage concentrations of all 54 cuticular hydrocarbons grouped by ecozones and sex.

Based on the heat map (figure12), the hydrocarbon with the highest concentration was A051, representing phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- with a percentage concentration of 13.07% (refer to Appendix 1). This hydrocarbon was isolated from male flies in the Transition zone. In contrast, the lowest hydrocarbon concentration was A005, representing 2-propen-1-one, 1,2-diphenyl, with a percentage concentration of 0.17%, and it was isolated from female flies in the Coastal Savannah zone.

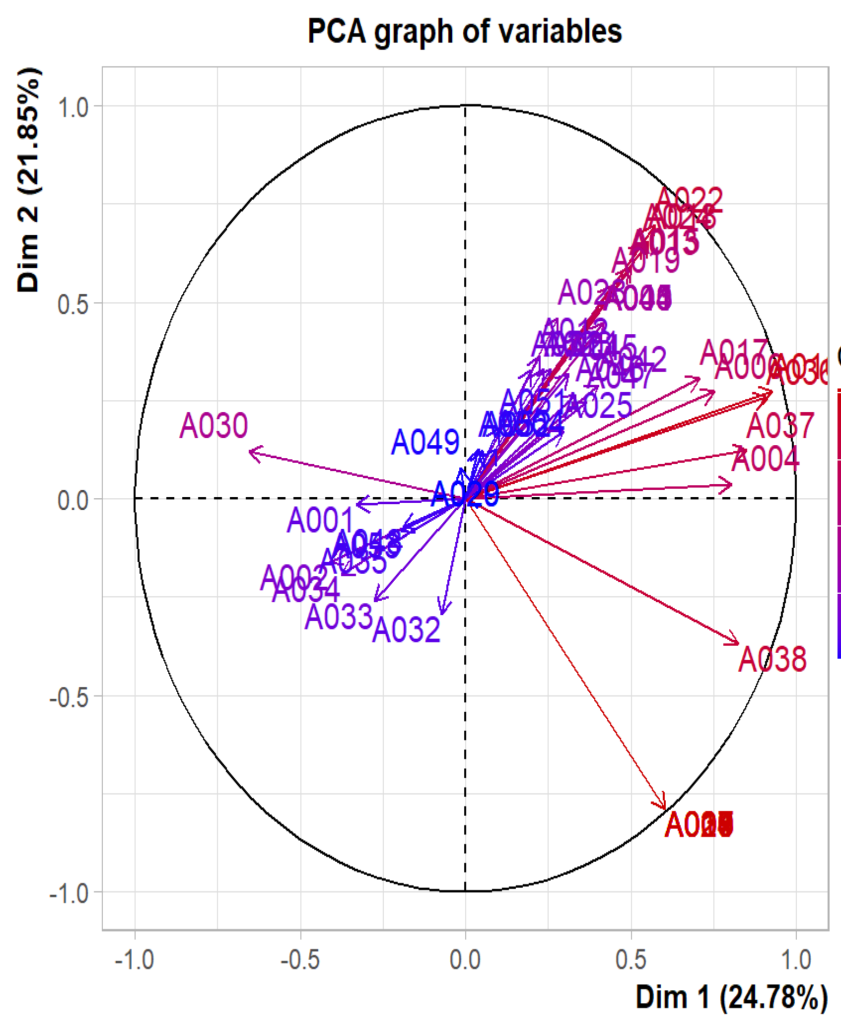

Figure 13: A PCA of GCMS data of 54 compounds identified in hexane washes populations of *Bactrocera dorsalis* from four Agroecological zones in Ghana.

The PCA (Figure 13) presents four different components showing the relationship among 54 hydrocarbons (A001 – A054) (Appendix1). Each arrow indicates the contribution of each hydrocarbon to a given principal component.

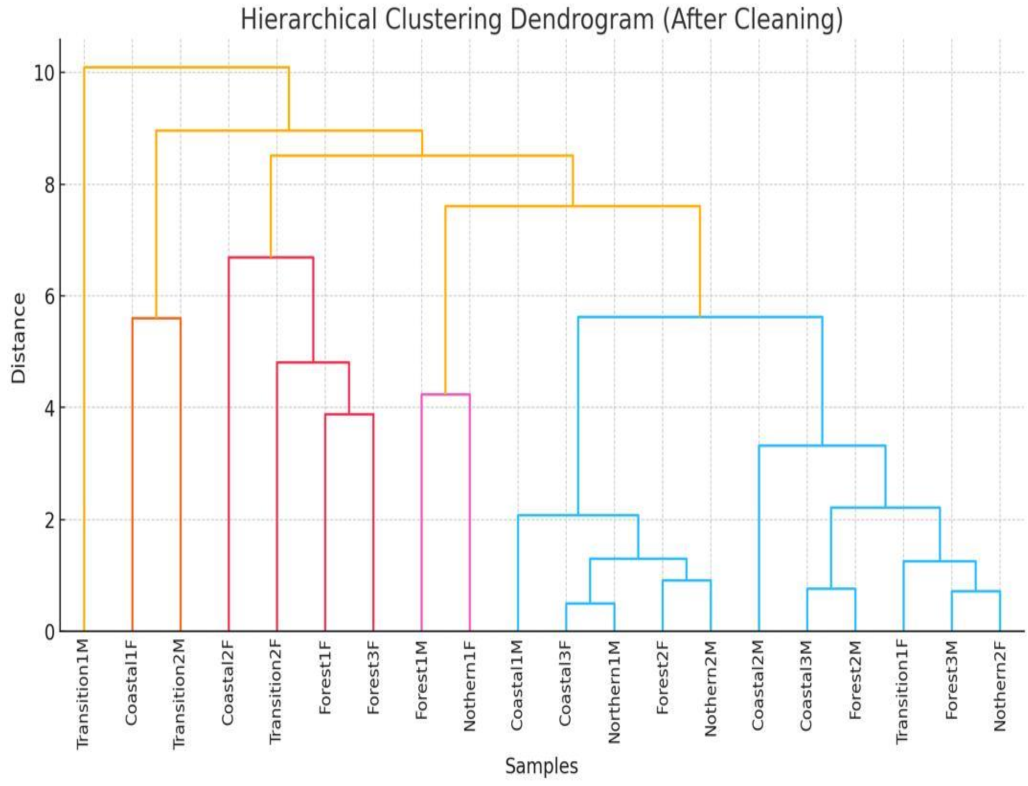

Figure 14: A Dendrogram showing the relationship among samples of *Bactrocera dorsalis* from all four Agro-ecological zones

The dendogram (figure14) shows how *B. dorsalis* from the four Agro-ecological zones are related to each other.

CHAPTER FIVE

DISCUSSION

Introduction

This chapter delves into a comprehensive discussion of the results presented in the previous chapter. It includes a comparative analysis with findings from analogous studies conducted in other regions. Furthermore, the implications of the results for the control of this pest in Ghana are thoroughly examined.

5.1 Variability Assessments among populations.

Factors influencing variability in ecosystems may impact the entire ecosystem, its habitats, and the populations residing within these ecological spaces. However, the responses to these factors manifest not instantly at the species level but rather at the individual level. Variability in responses to climatic variables and ecosystem changes is evident among individuals within species (Clark et al., 2011). The success of species and populations is significantly influenced by the extent of genetic and phenotypic variability (Forsman & Wennersten, 2016). In the case of insect pests, such variations play a role in the species' ability to thrive in diverse ecosystems and inflict considerable damage to crops. Evaluating variability is crucial for comprehending population structures and devising effective strategies to mitigate pest impact. Consequently, variability assessments play a pivotal role in comprehending the structural organization of individuals within species across diverse ecosystems and habitats. To examine variability among individuals of *B. dorsalis*, this study employed Geometric Morphometry techniques and Cuticular hydrocarbon profiling.

Geometric morphometry has proven to be a valuable tool for distinguishing populations by capturing variations in wing shape. The significance of wing shape lies in its potential to impact wing kinetics, influencing strength and beat patterns during flight (Wootton, 1981). This, in turn, may have implications for distribution potential and taxonomic studies. In the context of the *B. dorsalis* complex, wing shape could play a functional role in mating systems, like observations in the Neotropical tephritid genus Blepharoneura, where wing shape was found to influence audible signals produced during courtship (Marsteller et al., 2009). In the case of *Bactrocera dorsalis* (complex), geometric morphometrics has been employed to differentiate species within the *Bactrocera dorsalis* complex (Schutze et al., 2012). Krosch et al. (2013) conducted work combining molecular techniques with metric analysis to describe the population structure of *Bactrocera dorsalis*. They concluded that integrating these methods provided a finer definition of the population compared to using only one method. Environmental variables have shown promising results in geometric morphometric analysis, as seen in the study of hind wing shape changes in *Diabrotica virgifera verifier* (Coleoptera: Chrysomelidae). The shape was found to vary according to the major types of soil in Croatia (Benítez et al., 2013). Cuticular hydrocarbons have been used for variability assessments between species, individuals of a species and even gender. Cuticular hydrocarbons play very specific roles in mediating various intra and interspecific interactions within and between species (Blomquist, Nelson, De Renobales, 1987; Blomquist & Bagnères, 2010). Hence, the set of hydrocarbons that characterize an individual of a species differs from that of another species.

Dipterans, especially, exhibit a relatively stable cuticular hydrocarbon profile that undergoes variations throughout their entire life span and during adulthood (Jackson & Bartelt, 1986; Ferveur, 2005; Vaníčková et al., 2012). Cuticular hydrocarbons have proven to be excellent chemotaxonomic markers for insect species identification and for assessing variability within and between species (Blomquist et al., 2010; Kather & Martin 2012). In tephritid fruit flies, such as the *Anastrepha fraterculus* species complex and the African Ceratitis FAR species complex (*C. fasciventris, C. anonae,* and *C. rosa*), cuticular hydrocarbon profiles have been successfully utilized to delineate putative species (Vaníčková et al., 2014, 2015). However, there has been no comprehensive analysis to date on the impact of ecological zone differences on the cuticular hydrocarbon profiles of *B. dorsalis* populations in Ghana. 5.2 Variation in environmental conditions in the four Agro-ecological zones and its effect on segregation of population of *Bactrocera dorsalis*.

The study results revealed that the southern region (coastal savannah zone) exhibits a bimodal rainfall pattern with an annual rainfall of 723 mm. This zone is characterized by consistently warm temperatures throughout the year, featuring a major season with 100-110 days and a minor season with 50 major days for planting. The crops cultivated in this region are predominantly seasonal, with a focus on root crops and maize as the primary food crops. Moving to the eastern-Volta zone there is also a bimodal rainfall pattern with an annual precipitation of 1465 mm. The major season provides 150–160 planting days, and the dominant land use system is centered around forests and plantations. Similarly, the transition zone exhibits a bimodal rainfall pattern, with a total annual rainfall of 1221mm. This zone serves as a transitional area between the forest and Guinea savannah zones. There are 180 – 200 growing days in this zone. The Guinea savannah (northern) zone has a unimodal rainfall pattern, with an annual rainfall of 1165 mm. There are 180 – 200 growing days in the zone. It is the biggest Agro ecological zone in Ghana.

The varied environmental conditions and varied land use systems in the various Agro ecological zones can influence species segregation. The eastern-volta or forest zone has the highest amount of annual rainfall and planting days. This can influence availability of food like wild fruits and vegetables and in turn influence species dispersal and segregation. Abiotic environmental factors like relative humidity and temperature can affect species distribution. These factors can also vary among different habitats within the same Agro ecological zone. Warm temperatures can influence fast development of insect species and favour proliferation of species. Some abiotic factors like Rainfall can influence some biotic factors like availability of food that in turn influences species distribution. Individuals of *B. dorsalis* are faced with different climatic conditions within each ecological zone. Though the environmental parameters recorded did not differ significantly among the ecological zones, the interaction of these parameters may affect individuals differently in each ecological zone. Studies have shown that though areas may be similar in individual climatic variables, the overall net effect of the sum of all climatic variables differs significantly for each area under study (Kurukulasuriya, & Mendelsohn, 2018). In Ghana, Agro-ecological zones are differentiated mainly based on the dominant vegetation type that characterize the area. Variation across these Agro-ecological zones is evident not only in the vegetation but also in the types of food crops supported (Asante et al., 2019), climatic variable patterns (Abbam et al., 2018; Asare-

Nuamah & Botchway 2019), and soil conditions (Buri et al., 2010). Numerous studies have highlighted significant differences in species distributions, abundance, and diversity among these Agroecological zones (Gaston, 2000; Nboyine et al., 2012; Combey et al., 2013; Koffi et al., 2020), underscoring the impact of these variations on overall biodiversity.

Population variations using geometric morphometry of wings of *Bactrocera dorsalis* from four Agro-ecological zones in Ghana.

Geometric morphometry facilitates the evaluation of population variation in *B. dorsalis* across various Agro-ecological zones in Ghana, focusing on the wing shapes of the individuals. To ensure that the observed variability among populations was genuinely inherent in the wing shape rather than introduced variability due to differences in size and orientation, wing images were subjected robust analysis in Morpho J.

Several studies have demonstrated that size does not significantly differ. For instance, in studies on *Bactrocera dorsalis* flies emerging from various fruits in South Africa, the centroid wing size did not change significantly, but the wing shape varied considerably when geometric morphometry was used (Pieterse et al., 2017b). Similarly, Kwapong et al. (2017) showed distinctions in Honeybees (*Apis mellifera adansonii*) from different Agro-ecological zones. Therefore, the study results exclusively reflect shape differences among populations of *B. dorsalis*. In general, there were significant differences in the wing shapes of *B. dorsalis* among populations from the four Agro-ecological zones. Wing shapes reflect inherent changes in the development of insects, leading to the hypothesis that variations in the wing shapes of *B. dorsalis* are influenced by the climatic conditions characterizing their habitats. The study results support this hypothesis, aligning with findings from other studies that have investigated variations in wing shapes among species in areas with diverse climatic conditions. For example, in a study by Alves et al. (2016), it was concluded that the wing shapes of the *fly Polietina orbitalis* were influenced by individual responses to local environmental conditions, including elevation, precipitation, and temperature.

The selected landmarks, representing key points on the wings of *B. dorsalis*, contributed varying degrees of variation to the overall variability observed among populations from different Agro-ecological zones. Principal Component Analysis (PCA) indicated that the variation across the four ecological zones was notably influenced by the positioning of landmark 14, corresponding to the junction of vein R1 and the costal vein. Features of interest in the wings, likely impacted by variation in *B. dorsalis* response to climatic conditions, include vein R1 and the costal vein. Additionally, landmarks 7, 8, 10, 6, and 15 also contributed significantly to the overall variability. Landmarks 6, 7, and 10 were positioned around vein m, either at its termination or at a junction with another vein. Vein m emerges as another relevant feature to consider in understanding how environmental factors influence the species.

Variations in the costal vein and vein R1 have been observed in *B. dorsalis* when studying the impact of different host plants on wing shape (Pieterse et al., 2017b). This suggests that local conditions not only alter wing shapes but also influence the venation of wings, contributing to the overall wing shape changes.

Despite the significant differences in wing shapes among the zones, a scatter plot representing the wing shapes did not indicate distinct clustering of populations with similar habitats. It is noteworthy that individuals of a species often share many traits, making it unlikely for marked clustering to occur even when there are significant differences in wing shapes. Additionally, the individuals were exclusively sampled from mango plantations, suggesting a consistent diet of mango throughout the larval stage of their lifecycle. Considering that various host plants can impact wing shapes (Pieterse et al., 2017a), it is unsurprising that individuals from different ecological zones exhibited close clustering. Discriminant function and cross-validation analyses demonstrated that individuals were reliably classified into ecological zones with an accuracy exceeding 60%, and significant differences were observed when comparing ecological zones. Variations in environmental conditions across the Agro-ecological zones likely contributed to the observed differences in wing shapes. Temperature and relative humidity fluctuations can impact the flight behavior of insect species. The higher temperatures in the Northern (Guinea Savannah) Zone may explain the variations observed between this zone and the Coastal Savannah Zone. The presence of dual rainy seasons from the Transition Zone to the Coastal Savannah Zone might enhance food availability, potentially influencing population movements among zones. The substantial rainfall in the Eastern Volta Zone could also contribute to the observed variability within that zone.

Interestingly, variations were noted when comparing populations of *B. dorsalis* within each ecological zone. While the expectation was that individuals from the same ecological zone would be more similar, significant differences were observed in some instances. These differences could be attributed to potential variations in local climatic conditions among farms, as well as differences in the nutritional composition of mango fruits in the various farms. A similar study by Badu et al. (2022) found significant differences in the wing shapes of blowflies collected from a single location.

The observed variations have implications for potential pest control strategies tailored to each ecological zone. However, further studies are needed to confirm this and assess the species' response to various control measures in different ecological zones, which was beyond the scope of this study.

Cuticular Hydrocarbon Profile of Ecological Populations of *Bactrocera dorsalis* from Ghana

In general, there were no distinctive qualitative differences in cuticular hydrocarbons isolated from *Bactrocera dorsalis* samples collected from the four Agroecological zones. The observed variations were mainly quantitative, aligning with findings such as those reported by Goh et al. (1993), where indistinct qualitative differences in cuticular hydrocarbons were observed when investigating two strains of the *Bactrocera dorsalis* complex from Malvasia using GCMS. A similar result was also observed in the study of cuticular chemistry of the Queensland fruit fly *Bactrocera tyroni* from Australia (Park, 2020). These findings underscore the notion that species within the *Bactrocera* genus typically do not exhibit qualitative differences in their cuticular hydrocarbon profiles.

From the heatmap (Figure 12), the hydrocarbon with the highest concentration was phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl], isolated from male flies in the transition zone. This compound, known for its antioxidant properties (Eda Gunes et al., 2018), may serve to protect the oxidative balance in all tissues of the fly's digestive tract. The elevated concentration of this compound in male flies from the transition zone could be attributed to increased oxidative stress, possibly arising from the indiscriminate use of pesticides by farmers in that zone. Additionally, genetic factors of the fly may also contribute to such variations, independent of sex. Low to moderate concentrations of Benzene, 1,1'-3,3-dimethyl-1-butenylidene bis, 1-propen, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-, 1h-indene, 2,3-dihydro-1,1,3-trimethyl-3, phenyl-, 2-methylheptacosane, and Octacosane, 2-methyl- were identified in male *B. dorsalis* specimens from the transition and coastal ecological zones, as well as in females from all four ecological zones. These compounds may constitute fundamental components of the epicuticle, contributing to its form and function. Additionally, a moderate concentration of Triacontane was detected in male flies from the transition and coastal savannah ecological zones, as well as in female flies from the coastal, northern, and forest ecological zones.

The role of this hydrocarbon, like other long-chain alkanes, may be to regulate trans-cuticular water movements. The consistently warm temperatures across all ecological zones may have contributed to the presence of this compound in both male and female flies from all the ecological zones. Similarly, moderate concentrations of Tetracosane, Hexatriacontane, and Tetrapentacontane were exclusively identified in male flies from the coastal savannah Agroecological zone. These hydrocarbons, being straight-chain alkanes, likely function to regulate trans-cuticular water movements (Eda Gunes et al., 2018). Consequently, the efficacy of water-based insecticides may be compromised for the control of this pest in the coastal savannah zone. The cuticular hydrocarbon profile *of B. dorsalis* species did not exhibit sexual dimorphism, except in the transition zone where sexual dimorphism was observed based on the concentration of isolated hydrocarbons rather than the type of hydrocarbons. This observation contrasts the findings of VanõÂčkova et al. (2017), who reported sexual dimorphism in the epicuticular chemistry profile of other sibling species within the *B dorsalis* species complex.

Rom the dendrogram attached to the heat map (fig. 12), two major clusters are evident—one at the top, consisting of hydrocarbons at low concentrations, and the other below, comprising hydrocarbons at relatively higher concentrations. These hydrocarbons span all four Agro-ecological zones and both sexes From the PCA analysis of the 54 hydrocarbons identified through GCMS, pentadecane, 2,6,10,13-tetramethyl-pristane, pentadecane, 2,6,10,14-tetramethyl-benzene, 1-methylethenyl-benzene, 1,2-diethyl-, and Benzene, 1,4-diethyl cluster together. This clustering may indicate that these compounds share a similar function. Given that these compounds are methyl-branched hydrocarbons, they are more likely to be involved in communication, (Martin & Drijfhout, 2009). Insect communication is a fundamental aspect of insect behavior, and these hydrocarbons are not expected to vary based on ecological zone differences. Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl, with the highest concentration, forms a cluster with other hydrocarbons like nonanoic acid, ethyl ester, and 1h-purin-6-amine, [2-

fluorophenyl) methyl. Despite their low concentration, the clustering suggests a potential shared function, possibly linked to the antioxidant property of the cuticle (Eda Gunes et al., 2018). Another cluster includes methyl abieta-8,11,13-trien-18-oate, trans-calamenene, 1,1,2-trimethyl-2-phenylpropyl benzene, and 1h-indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl. These compounds may share a function, as indicated by their clustering. This cluster is close to the Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl cluster, suggesting potential similarities. Lastly, 2-tetradecyl-1-octadecene stands alone but is closer to the Pentadecane, 2,6,10,13-tetramethyl-Pristane cluster than all other clusters, indicating a potential shared function.

The dendogram (Figure 14) shows that the blue clusters (right side) indicate similar samples while orange/pink cluster (left side) indicates samples that are quite distant from each other. Most Northern and some forest samples show high similarity whereas most Transition and coastal samples seem to be quite dissimilar. This provides some evidence of population segregation or structure among the ecological zones. Samples from the Northern Forest and Coastal Zones exhibited some similarity. This may be attributed to the movement of fruits between those zones. Males from the transition zone seem to be most different from females from the coastal zone, a clear indication of some population structure.

Implication for control of the pest

The results reveal notable variability among populations from the four Agro-ecological zones, suggesting potential population subdivisions corresponding to ecological zones. These subdivisions could impact the effectiveness of control strategies reliant on mating compatibility across zones for the dissemination of desired genes or traits. Consequently, the success of a nationwide Sterile Insect Technique (SIT) control strategy might be compromised.

Moreover, the efficacy of water-based pesticides for the control of this pest in the coastal savannah ecological zone might be compromised, requiring the application of higher doses of pesticide by farmers thereby causing more harm to the consumers and the environment.

From the results a nationwide blanket approach for the control of this pest may not be recommended, specific control strategies tailored to each Agro-ecological zone may be most effective.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Introduction

This chapter summarizes the conclusions drawn from the study, providing evidence of the population structure of *Bactrocera dorsalis* across four ecological zones in Ghana. Furthermore, it offers recommendations aligned with the study's findings.

Conclusions

- The primary wing feature contributing to 23.24% of the observed variation among populations of *Bactrocera dorsalis* across four Agro-ecological zones in Ghana is the junction of vein R1 and costal vein.
- Vein R1, the Costal vein, and vein m are the wing features of particular interest in understanding the variations in the wing shape of *Bactrocera dorsalis* in response to differences in Agro-ecological zones.
- Geometric morphometry highlights significant variations in the wing shape among *B. dorsalis* populations from the four Agro-ecological zones in Ghana.
- Discriminant Function Analysis (DFA) and cross-validation reveal a cluster classification accuracy of over 60% among populations of *B. dorsalis* across the four Agro-ecological zones in Ghana.
- The Cuticular Hydrocarbon (CHC) profiles of *B. dorsalis* samples from four Agro-ecological zones displayed quantitative rather than qualitative variations.

- Sexual dimorphism in the Cuticular Hydrocarbon (CHC) profile of *B. dorsalis* samples was observed only in the transition zone, manifested through variations in the concentration of individual CHCs rather than the types of CHCs.
- The results show some evidence of population segregation and structure. Samples from the Transition zone seem to be quite different from the coastal savannah zone while samples from the Northern and Eastern Volta zones were quite similar from their CHC profile, evidence of some structure along ecological lines

Recommendations

- Additional investigations, such as microsatellite studies, are suggested to explore the presence of active gene flow among populations from all four Agro-ecological zones.
- The significant variation observed among samples from the four Agro-ecological zones could suggest potential population segregation along ecological gradients. Consequently, it is essential to take these study findings into account when formulating and executing any area-wide control strategy.

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APPENDIX 1

APPENDIX 1: **CHCS CONCENTRATION AND ASSIGNED CODE**

Code		Coastal zone		Eastern - Volta		Transition zone		Guinea savannah zo	
	CHCs	Male	Female	Male	female	Male	Female	male	femal
A001	Pentadecane, 2,6,10,13-tetramethyl-Pristane		0.36	1.12	0,64	0.41		1.24	
A002	pentadecane, 2,6,10,14-tetramethyl-			2.14				3.03	3.19
A003	5-hexen-3-one, 4,4-dimethyl-1,6,6-triphenyl-	5.82	.						
A004	methyl abieta-8,11,13-trien-18-oate	0.86	0.83						0.26
A005	2-propen-1-one, 1,2-diphenyl-		0.17						
A006	Trans-calamenene	0.53	0.69			3.32	0.54		
A007	Tetrapentacontane	2.59							
A008	Tetracosane	5.35							
A009	Hexatriacontane	5.07							
A010	eicosane, 2,6,10,14,18-pentamethyl-	2.39							
A011	Eicosane, 2,6,10,14,19-pentamethyl-	4.43							
A012	Benzene, 1,1'-(1-methylethylidene) bis [4-methyl-	0.2	0.46				0.49		
A013	Hexacosane	1.27	1.79		0.24	1.35	0.93		1.61
A014	Hexacontane	1.7							
A015	Benzene, 1,1'-(3,3-dimethyl-1-butenylidene) bis-	4.2	5.91		1.33				4.69

A016	1-propen, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-	4.93	5.55	2.93	2.96				4.18
A017	1h-indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	2.2	2.2	3.17	1.17	3.67	2.29		1.77
A018	2-methylheptacosane		5.35	2.99		3.43	5.67		4.91
A019	Octacosane, 2-methyl-		7.13	0.9	5.3	6.92			1.77
A020	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-								
A021	Nonacosane, 2-methyl-		0.36			1.04			
A022	Triacontane		6.78	5.4	5.4	3.9	6.93		4.37
A023	2-methylhentriacontane					0.72			0.71
A024	Dotriacontane	0.26	3.91	5.19	5.19	3.03	4.27	0.6	2.68
A025	Nonanoic acid, ethyl ester		0.8						
A026	Tetracosane, 2,6,10,15,19,23-hexamethyl-	4.13							
A027	Dotetracontane	4.5							
A028	2-methyltriacontane		1.6	1.26		0.39	2.07		
A029	Naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethyl-								
A030	2-tetradecyl-1-octadecene	1.0	1.32	1.94		0.83	2.87	1.83	0.84
A031	Benzene, 1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl) bis-		0.45			1.35		1.85	
A032	Benzene, (1-methylethenyl)-	0.25	0.47	0.6				0.67	
A033	Benzene, 1,2-diethyl-	0.17	0.15	0.65				1.14	
A034	Benzene, 1,4-diethyl-			0.8				0.84	
A035	1,2-benzenedicarboxylic acid, tridecyl ester							0.68	

A036	benzene, 1,1'-(1,4-dimethyl-1-butene-1,4-diyl) bis-	2.74	2.84		1.36	2.4	1.08		2.06
A037	(1,1,2-trimethyl-2-phenylpropyl) benzene	1.93	1.98		1.08	1.92	0.75		1.75
A038	1-phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1r-(1. alpha.,4a.	0.7	0.22			0.4			
A039	isopropyl tetradecanoate	0.22							
A040	n'-(2-benzoylbenzoyl)-4-methylbenzohydrazide #		0.66						
A041	(2-chloro-2,3-dimethylcyclopropyl) benzene		0.62						
A042	methyl tert-butyl(pivaloyl)acetate		0.45						
A043	7,9-di-tert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione		0.59						
A044	s-indacene-1,7-dione, 2,3,5,6-tetrahydro-3,3,5,5-tetramethyl-		0.23						
A045	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.81	1.27		1.06		0.25		
A046	1,2-benzenedicarboxylic acid		0.51						
A047	4,6,7-triethyl-1-methyl-5-vinylindane		0.67			0.42			
A048	1h-purin-6-amine, [(2-fluorophenyl) methyl]-								
A049	2-[4-(1-methyl-2-propenyl) phenyl] propanal			0.7					
A050	2,6-piperidinedione, 4-ethyl-4-methyl-				0.41				
A051	phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-					13.07	8.46		3.23
A052	10-ethyl-2,3,6,7,10,13,17,20,21-nonamethyldocosane						1.64		

A053	3-ethyl-5a,5b,8,8,11a,13b-hexamethylicosahydro-1h-cyclopenta[a]chrysene #							1.9	
A054	naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethyl-							0.42	0.42