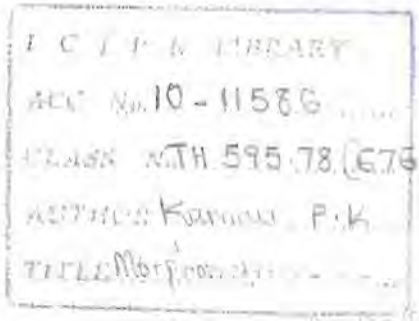
MORPHOMETRICS AND DNA BARCODING FOR THE IDENTIFICATION OF WILD SILK MOTHS FROM SELECTED SITES IN KENYA

Peter Kuria Kamau

A thesis submitted in partial fulfillment for the Degree of Master of Science in Biochemistry in the Jomo Kenyatta University of Agriculture and Technology

2010



DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature..... Date.....20/05/2010.....

Peter Kuria Kamau

This thesis has been submitted for examination with our approval as University Supervisors

1. Signature..... Date.....20/05/2010.....

Dr. Daniel Masiga

ICIPE, Kenya

2. Signature..... Date....21st May 2010

Dr. Joseph Ng'ang'a

JKUAT, Kenya

3. Signature..... Date.....20/05/2010.....

Dr. Esther Kioko

MUSEUM, Kenya

4. Signature..... Date.....21.05.2010

Prof. Mabel Imbuga

JKUAT, Kenya

DEDICATION

I dedicate this thesis to my father George Kamau, my mother Jane Kamau, my two sisters and to my grand parents for their unwavering support during the entire period of my research.

ACKNOWLEDGEMENTS

I would like to give thanks to the Almighty GOD for giving me the strength and ability to do my work to completion.

I would also like to appreciate my supervisors Dr. Joseph Nganga, Dr. Esther Kioko, Dr. Daniel Masiga and Prof. Mabel Imbuga for their continued support and guidance during the entire period of my work. Special thanks to Prof. Suresh Raina the head of Commercial Insects Programme at *icipe* for giving me the opportunity to carry out my research on the Kenyan wild silk moths, within this programme.

I am also grateful to the World Federation of Scientists, for awarding me a scholarship during the period of my research.

I would also like to thank my fellow students and staff in the Commercial Insects Programme and the Molecular Biology and Biotechnology departments for their support, encouragement and constant consultations.

May the Almighty GOD bless you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

BOLD	Barcode of Life Data Systems
Bp	Base pair
B	Beta
°C	Degree Celsius
COI	Cytochrome Oxidase I
CTAB	Cetlyltrimethylammoniumbromide
ddH_20	Double distilled water
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotides triphosphates
EDTA	Ethylene DiamineTetraacetic Acid
g	Gravitational force
Fig	Figure
ICIPE	International Centre of Insect physiology and Ecology
K2P	Kimuar-2-Parameter
Kb	Kilobase
M	Molar
mM	Millimolar
MEGA	Molecular Evolutionary Genetics Analysis
Min	Minute
mt DNA	Mitochondrial DNA
p	Significance level
PCR	Polymerase Chain Reaction

Pmol/µl	Picomoles per microlitres
PCA	Principal Component Analysis
PTC	Peltier Thermal Cycler
rDNA	Ribosomal DNA
SAS	Statistical Analysis System
T.A.E.	Tris-acetate-EDTA
T.E.	Tris EDTA
µl	Microlitres
U.V. light	Ultra Violet light
Volts	Voltage
%	percentage

ABSTRACT

Wild silk moth farming is a unique eco-friendly agro-practice with the potential of raising people's standards of living. However, the species identity is poorly understood in East Africa. This undermines efforts in conservation practices. Accurate identification is important in understanding the biology and ecology of the different species. Cocoons of wild silk moths were collected from Eastern and Rift valley provinces in Kenya. The samples included *Gonometa* species, *Epiphora bauhiniae*, and *Argema besanti*. Morphometric studies of the Kenyan wild silk moth species were performed based on three morphometric characters, which included forewing length, forewing width and body length measurements. Voucher specimens of each moth species were pinned and stored appropriately. Mophometric measurements of the moth voucher specimens were then taken. DNA was extracted from the middle left leg of the moths using the CTAB DNA extraction protocol. The COI region of the mitochondrial DNA was amplified using universal primers and direct sequencing was done on the cleaned PCR product. Analysis of the COI region was initially done using the Chromas software program to edit the sequences. Clustal X software program was then used to do multiple sequence alignments to check for any polymorphism within the sequences. This was followed by drawing of a Neighbour-joining tree using Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 to observe the graphical depiction of the patterning of divergences between the species. Multiple sequence alignment of the cytochrome oxidase I gene helped discover two congeneric *Gonometa* species found in Mwingi District that had not been described before. This alignment indicated variable sites at positions 217, 412 and 542 in these sequences, which separated the two species. This

approach also provided an understanding of the genetic variation that exists among these different wild silk moth species. This has now provided an avenue for investigating issues of species distribution and abundance, which will contribute not only to the understanding of their biology and ecology, but also to their conservation and utilization for income generation in these marginal areas of Kenya.

Keywords: Morphometry, Cytochrome Oxidase 1, Congeneric species

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Moths

Moths are insects closely related to the butterflies, both being of the order Lepidoptera (Table 1). This order is then broken down to different families of moths and butterflies. There are thought to be 150,000 to 250,000 different species of moths with thousands of species yet to be described. Most species of moths are nocturnal (active at night), but there are diurnal (active during day time) and crepuscular (active primarily during twilight, i.e., at dusk and dawn) species.

Table 1. Classification of moths

Kingdom	Animalia (Animals)
Phylum	Arthropoda (Arthropods)
Class	Insecta (Insects)
Order	Lepidoptera (Butterflies and Moths)

1.2 Family Saturniidae

This is a family of moderate size in number (about 1000 species) as previously demonstrated by Pinhey (1975). Some of the genera in this family include *Epiphora*, *Argema*, *Decarchorda* and *Labobunaea* among others. Antennae of the males in this family are strongly bipectinate, however in females the antennae are less bipectinate. The females have stouter bodies and the wings are more rounded

at the margins or apex of the fore wings. The fore wings are always broad in this family. The proboscis are vestigial or undeveloped so they do not visit flowers.

Many have characteristic hyaline patches, often ringed with coloured eyespots on the middle of the hind wings (Holloway *et al.*, 1987).

1.3 Family Lasiocampidae

This is a family of about 1500 species world wide as previously exemplified by Pinhey (1975). Some of the genera in this family include *Gonometa, Phyllodesma Tolype* and *Malacosoma* among others. Species in this family are small to moderately sized hairy, thick-bodied moths. Like Sartunidae they have no proboscis or frenulum but wings are generally broad. The antennae are bipectinate in both sexes. Wing colouration is most often grey, brown or reddish brown. A strong feature of this family is strong sexual dimorphism. While resting the drawn back, broad hind wings of these moths protrude forward. Sometimes the fore wings are held obliquely up, the hind wings flat, like crumpled leaves (Holloway *et al.*, 1987).

1.4 Life Cycle of Moths

Moths go through four life stages (Fig. 1) in their development, egg, larva pupa and adult (Holloway *et al.*, 1987). There is great diversity in each of these stages in different species and families of moths (Zagulajev, 1988). Eggs may be fairly smooth or finely pitted, armed with minute prominences, fluted, rounded or shaped in some other mould.

The female may lay eggs separately, frequently on the underside of a leaf, or in clusters often rather haphazardly (Ngoka *et al.*, 2008).

The larvae develop through several moults and may change in size of each stage (instar), and also in shape, colour and other features.

The larva produces liquid silk through openings in the head called spinnerets. This liquid is then coated with sericin, a water soluble gum, and solidifies on contact with air. Within 2-3 days of spinning, the larva is completely encased in a cocoon. Silk fibers from cocoons of these moths have been used in textile manufacture in different parts of the world for many years (Merzheevskaya, 1988).

On emerging from its cocoon a moth may exude a liquid, sometimes an alkali, to soften the walls of the cocoon, after which it expands its wings by pulsations of fluid through the veins. A watery liquid, consisting of waste products sometimes tinted with excess pigments, may also be ejected. Wings must then dry and stiffen before the moths are ready to fly (Sholtz & Holme, 1996).

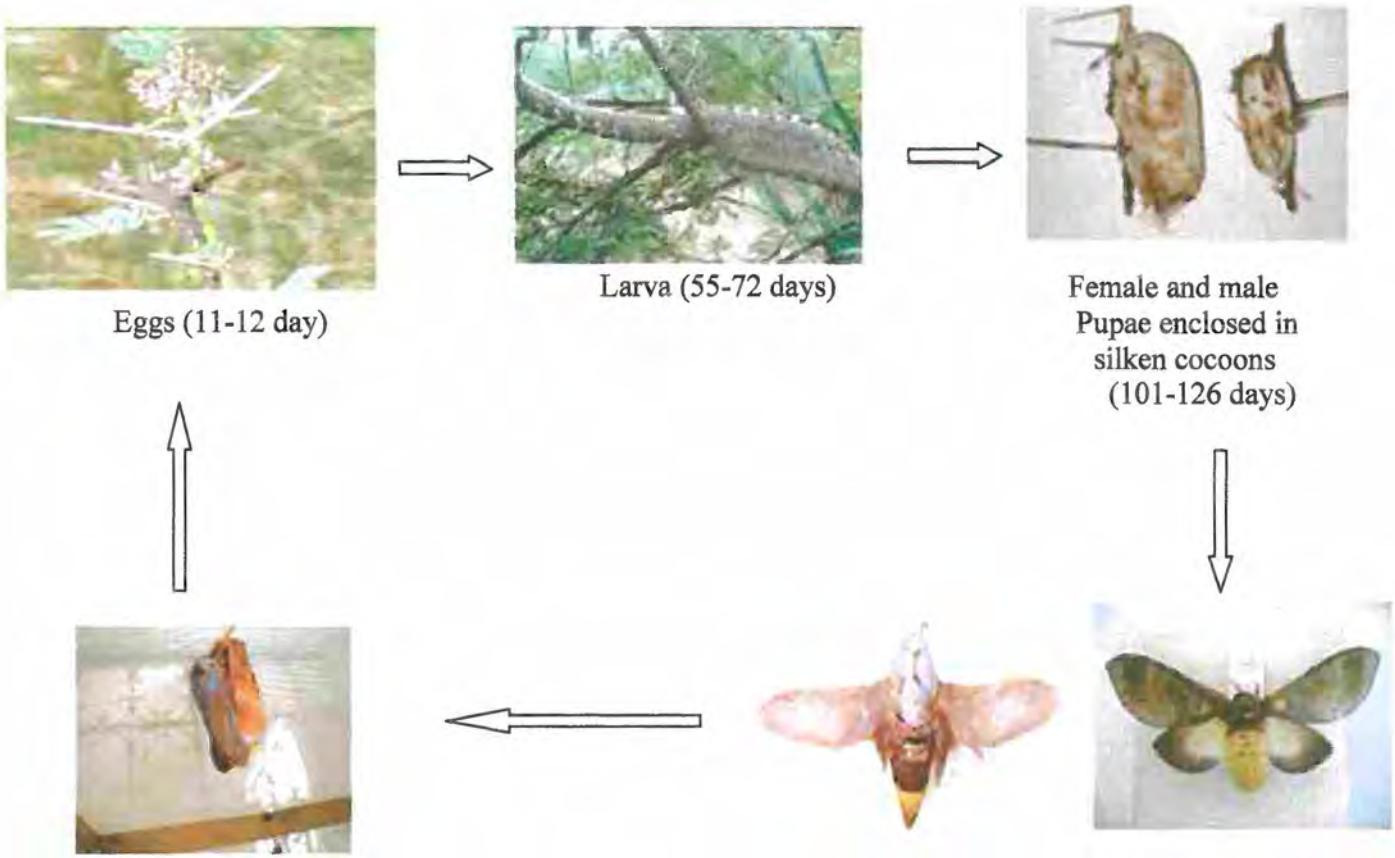


Figure 1. Developmental cycle of *Gonometa* species. Wild silk moths have four stages in their development from the egg to the adult moth (Fening, 2008).

1.5 Wild silk moths

Silk moths occurring in the wild are known as wild silk moths. The majority of the wild silk moth species found in Africa belongs to Saturniidae, Lasiocampidae and Thaumetopoeidae family (Ngoka, 2003). The African silk moth with highest diversity is found in the family Lasiocampidae, and a recent survey in East Africa had a record of 33 species in 17 genera (Kioko *et al.*, 2000). The Genus *Gonometa* in the family Lasiocampidae has been reported to produce silk for commercial purposes (Hartland-Rowe, 1992; Akai *et al.*, 1997; Raina *et al.*, 1999). Silk moths go through four stages of development, as do most insects: egg, larva, pupa and adult. The adult (imago) stage is the reproductive stage. The larva is the feeding stage. The larva pupates in a cocoon before emerging as a moth. Since the silkworm grows so much, it must shed its skin while it is growing. These stages-within-a-stages are called instars.

1.6 Commercial wild silk moth

Insects of the order Lepidoptera that produce silk are divided into two main categories, i.e. mulberry and non-mulberry silk moths (Mahendran *et al.*, 2006a). Mulberry silk is produced mainly by the commercial silk moth *Bombyx mori* that is in the family *Bombycidae* (Mahendran *et al.*, 2006b). The wild silk moth *Gonometa postica* produces shashe silk, which is of high quality and thus rivals the mulberry silk from *Bombyx mori* (Veldtman *et al.*, 2002). Silk from the *Gonometa* species is more preferable compared to silk from other wild silk moth species since it is more fine, has a natural gold colour and dyes well (Hartland-Rowe, 1992).

The African wild silk moths *Gonometa* spp, *Argema* spp and the *Epiphora* spp are of good commercial quality, certainly good enough for the discerning fashion and home décor industries (Erasmus, 2007). As a result of this potential, rearing of these wild silk moths can provide the marginalized communities with good income activity.

Sericulture is an eco-friendly, though highly labour-intensive activity and is well suited to the economy of developing countries like Kenya, which faces the enormous problem of creating gainful employment to the growing labour force in rural and peri-urban areas. In the silk producing sector, reeling, spinning, dyeing, warping, weaving, finishing and processing of silk fabric produces employment to a large number of people (Adolkar *et al.*, 2006).

1.7 Morphological Characteristics of Kenyan Wild Silk Moths

1.7.1 *G. postica*

Males of *G. postica* are small compared to the females. Their appearance is also different in comparison to the females (Ngoka *et al.*, 2008). They are light brown in colour. They have a triangular margin area in the hind wing. The abdomen is red brown in colour with terminal yellow portion. The antennae are bipectinate. Proboscis in this species is absent. Females of *G. postica* are much larger than males. The forewings are light brown with suffused whitish grey bands. The hind wings are rounded, cream with broad reddish brown borders. The abdomen of the females is light yellow. They also lack a proboscis and have bipectinate antennae (Pinhey, 1975).

1.7.2 *G. negrottoi*

The males of *G. negrottoi* are small compared to the females. Their appearance is also different in comparison to the females. These males are greyish brown in colour. They lack the triangular margin area of the hind wing present in *G. postica* males. The abdomen is red brown in colour with end being yellow. The antennae are bipectinate. Proboscis in this species is absent. Females of *G. negrottoi* are darker in colour compared to the *G. postica.* The forewings are greyish brown with suffused whitish grey bands. The hind wings are rounded, cream with broad greyish brown borders. The abdomen of the females is light yellow. They also lack a proboscis and have bipectinate antennae (Pinhey, 1975).

1.7.3 *A. mimosae*

The wings are green with divided yellow and reddish brown spots. Their hind wings have long strap like tails. They have a wingspan of about 10-12cm. They also have eyelike margins on their wings. The fore wings have distinct grey coloured furry leading edges. The antennae in this species are bipectinate and the proboscis is vestigial (Holloway *et al.*, 1987).

1.7.4 *A. besanti*

The wings are green with divided reddish brown spots, which are suffused with brown bands. Their hind wings have long strap like tails. They have a wingspan of about 8-9cm. The fore wings have distinct reddish brown leading edges. They also have eyelike

markings on their wings. The antennae in this species are bipectinate and the proboscis is vestigial (Holloway *et al.*, 1987).

1.7.5 *E. bauhiniae*

They have maroonish brown wings with white areas. They have a wingspan of 7-8cm. The margins of the wings are yellow in colour. Large round hyaline spots occur in the middle of each wing. The antennae in this species are bipectinate and the proboscis is vestigial (Holloway *et al.*, 1987).

1.8 Limitation of Morphology and Morphometrics in Species Identification

Morphometrics, which is a taxonomic technique in species identification, has got its own shortcomings when the species in question are very similar. Classification of species on the basis of morphological attributes may be riddled with problems because morphological features may be variable with the environment (Shouche & Patole 2000). DNA barcoding therefore becomes essential in providing alternative practical solutions in identification of species.

1.9 DNA Barcoding

DNA barcoding has been proposed as a potent new technique for rapidly identifying known species, discovering unknown species and indicating cryptic species (Blaxter, 2003; Hebert *et al.*, 2003; Marshall, 2005). This identification system is based on the principle that sequence diversity within a short standardized portion of the genome can offer discrimination at the species level (Hebert *et al.*, 2003; Marshall, 2005). The region

being applied in the identification of animals is a 648-bp fragment of the 5' end of mitochondrial (mt) cytochrome oxidase I (COI) that can be readily recovered from diverse species with a limited set of primers (Kevin *et al.*, 2007). Several studies have now established that sequence diversity in the 648-bp region near the 5' end of the mitochondrial (COI) gene provides strong species-level resolution for varied animal groups including birds (Hebert *et al.*, 2004), fish (Ward *et al.*, 2005), springtails (Hogg & Hebert, 2005) and moths (Hebert *et al.*, 2003; Janzen *et al.*, 2005). Genomic approaches to taxon diagnosis exploit diversity among DNA sequences to identify organisms (Kurtzman 1994; Wilson, 1995). These sequences can be viewed as genetic 'barcodes' that are embedded in every cell. Mitochondrial genome has been shown to be a better target for analysis than the nuclear genome because it lacks introns, has limited exposure to recombination and due to its haploid mode of inheritance (Saccone *et al.*, 1999). These mitochondrial genes also have the advantage of being present in high copy number in each cell, therefore facilitating their recovery and amplification (Stoeckle & Ausubel, 2003). Evolution of the COI gene is relatively rapid to not only allow the discrimination of closely allied species, but also phylogeographic groups within a species (Cox & Hebert 2001; Wares & Cunningham, 2001).

Barcode sequence data provides a shared genomic keystone for the variable repertoire of genes that can be used to build phylogenetic trees; which can be used as a link between the deeper branches of the trees to its shallow, species level branches (Hajibabaei *et al.*, 2007). The COI gene has been proposed as a standard marker, and there has been an attempt to build up a global and complete COI database of eukaryotes except plants (Chase *et al.*, 2005).

An advantage of DNA barcoding is the cost effectiveness for species identification, especially in bioinventory and biomonitoring programs (Smith *et al.*, 2005). Another major benefit of, and rationale for DNA barcoding lies in the rapid acquisition of molecular data. In contrast, morphological data gathering can be time consuming, in some cases totally confusing and in others almost impossible (Litaker *et al.*, 2007; Evans *et al.*, 2007; Huang *et al.*, 2007).

1.10 Mitochondrial DNA in Barcoding

Species identification has been focused on the mitochondrial genome of animals (Fig 2) because it lacks introns, has limited exposure to recombination and exhibits haploid mode of inheritance (Saccone *et al.*, 1999). Robust primers also assist the routine recovery of specific segments of the mitochondrial genome (Folmer *et al.*, 1994; Simmons & Weller, 2001). In the past phylogenetic work has often focused on the mitochondrial genes encoding ribosomal (12S, 16S) DNA, but their broad use in taxonomic analyses is hampered by the prevalence of insertions and deletions that greatly complicate sequence alignments (Doyle & Gaut, 2000). The 13 protein-coding genes in the animal mitochondrial genome are better targets since deletions are rare. There is no particular compelling reason to focus analysis on a specific gene, however the cytochrome oxidase I gene (COI) has two important advantages. To start with, the universal primers for this gene are very robust, enabling recovery of its 5' end from representatives of almost all animal phyla (Folmer *et al.*, 1994; Zhang & Hewitt, 1997). Secondly, COI appears to have a greater range of phylogenetic signals than any other mitochondrial gene. In line with other protein coding genes, its third-position

nucleotides show a higher incidence of base substitutions, leading to a rate of molecular evolution that is about three times greater than that of 12S or 16S rDNA (Knowlton & Weigt, 1998). The evolution of the COI gene is quite rapid to not only allow the discrimination of closely allied species, but also phylogeographic groups within a species (Cox and Hebert 2001; Wares & Cunningham 2001).

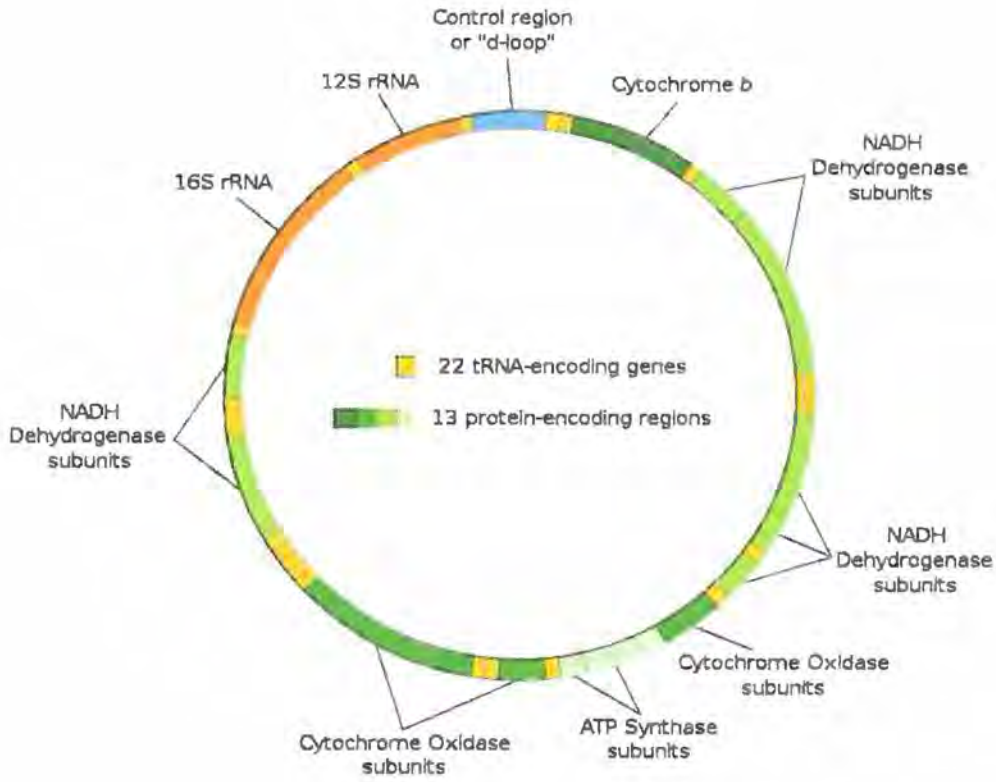


Figure 2. Structure of an animal Mitochondrial DNA (http://commons.wikimedia.org/mitochondrial_DNA) [Date accessed 1 October 2008]. Mitochondrial DNA in wild silk moths is structured in a similar manner to the animal mitochondrial DNA

1.11 The Barcode of Life Data System Work bench

The barcode of Life Data System (BOLD) (www.barcodinglife.org) was originally developed as an informatics workbench for a single, high volume DNA barcode facility (Hajibabaei *et al.*, 2005). It has now progressed into a resource for the DNA barcoding community (Ratnasingham & Hebert, 2007). BOLD now provides an integrated bioinformatics platform that supports all phases of the analytical pathway from specimen collection to tightly validated barcode library. The barcode sequence of an unknown specimen is usually compared with a library of reference barcodes sequences derived from individuals of known identity (Fig 3) (Hajibabaei *et al.*, 2007). A specimen is identified if its sequence closely matches one in the barcode library. Otherwise the new record can lead to a novel barcode sequence for a given species (i.e. a new haplotype or geographical variant), or it can suggest the existence of a newly encountered species (Hajibabaei *et al.*, 2007).

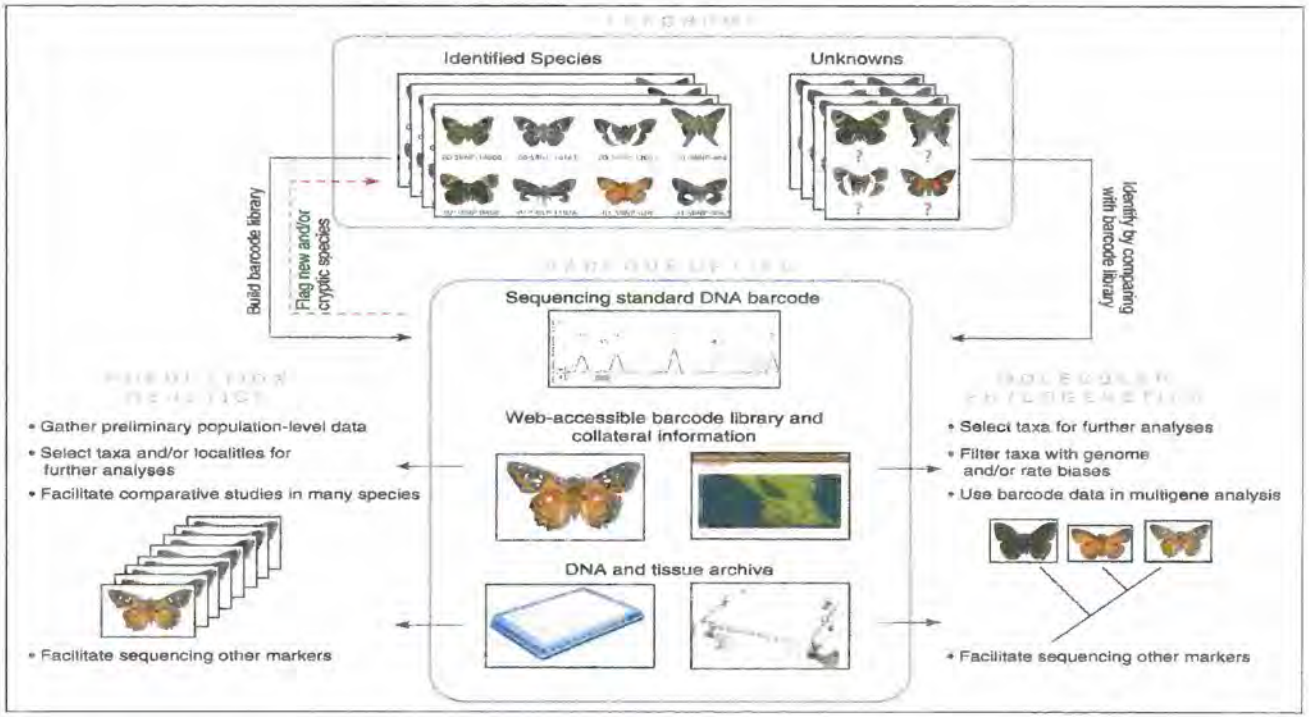


Figure 3. DNA barcoding workflow. DNA barcoding libraries can support the conventional taxonomic workflow by high-throughput identification of unknown specimens and by helping to draw attention to new and cryptic species (Hajibabaei *et al.*, 2007).

1.12 Limitations of DNA Barcoding

DNA-based species identification depends on distinguishing intraspecific from interspecific genetic variation (Stoeckle, 2003). The ranges of these types of variation are unknown and may differ between groups. It may be difficult to resolve diverged species or new species that may have arisen through hybridization. There is no universal DNA barcode gene, nor a single gene that is conserved in all domains of life and exhibits enough sequence divergence for species discrimination. The validity of DNA barcoding therefore depends on establishing references from taxonomically confirmed specimens (Stoeckle, 2003).

1.13 Statement of Problem

The wild silk moth of the Genus *Gonometa, Argema* and *Epiphora* in Kenya, which have the potential for commercial wild silk production, have not been studied and the taxonomy of these species is still poorly known. DNA barcoding studies and morphometric studies of *Gonometa, Argema* and *Epiphora* species of wild silk moths will provide tools for solving taxonomic problems of species identification. DNA barcoding will offer a better understanding of the species; their biology and ecology hence assist in planning for their conservation through planting of acacia trees and commercial utilization in silk production. This will provide income-generating ventures to farmers in the marginal areas of Kenya.

1.14 Justification of the Study

Due to the increasing demand for silk in the world market there is need for people living adjacent to forests to take advantage of this opportunity and utilize the silk moth species thus providing an alternative source of income. In Kenya, as elsewhere in the world, there is increasing concern for biodiversity and its sustainable utilization and conservation. Since some solutions lie in introducing economic incentives that integrate conservation with economic development of the local people (Fening *et al.*, 2008), characterization of *Gonometa, Argema* and *Epiphora* species wild silk moths are needed, for their conservation and economic utilization.

This study of the Kenyan *Gonometa, Argema* and *Epiphora* species will provide tools for clear identifications and hence better understanding of their biology and ecology.

1.15 Hypothesis

Morphological characterization and sequence diversity of the mitochondrial DNA, 648 base pair fragment of cytochrome oxidase subunit I can discriminate among *Gonometa, Argema* and *Epiphora* species thus resolving taxonomic impediments.

1.16 Main Objective

Morphometric and genetic characterization to identify and classify five species of wild silk moths in Kenya.

1.16.1 Specific Objectives

i. To assess the variability of the Kenyan species of wild silk moths based on morphometric characteristics.

ii. To determine the feasibility of using molecular tools to discriminate between intraspecific and interspecific genetic variations of the COI genes in the Kenyan species of wild silk moths.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Experimental design

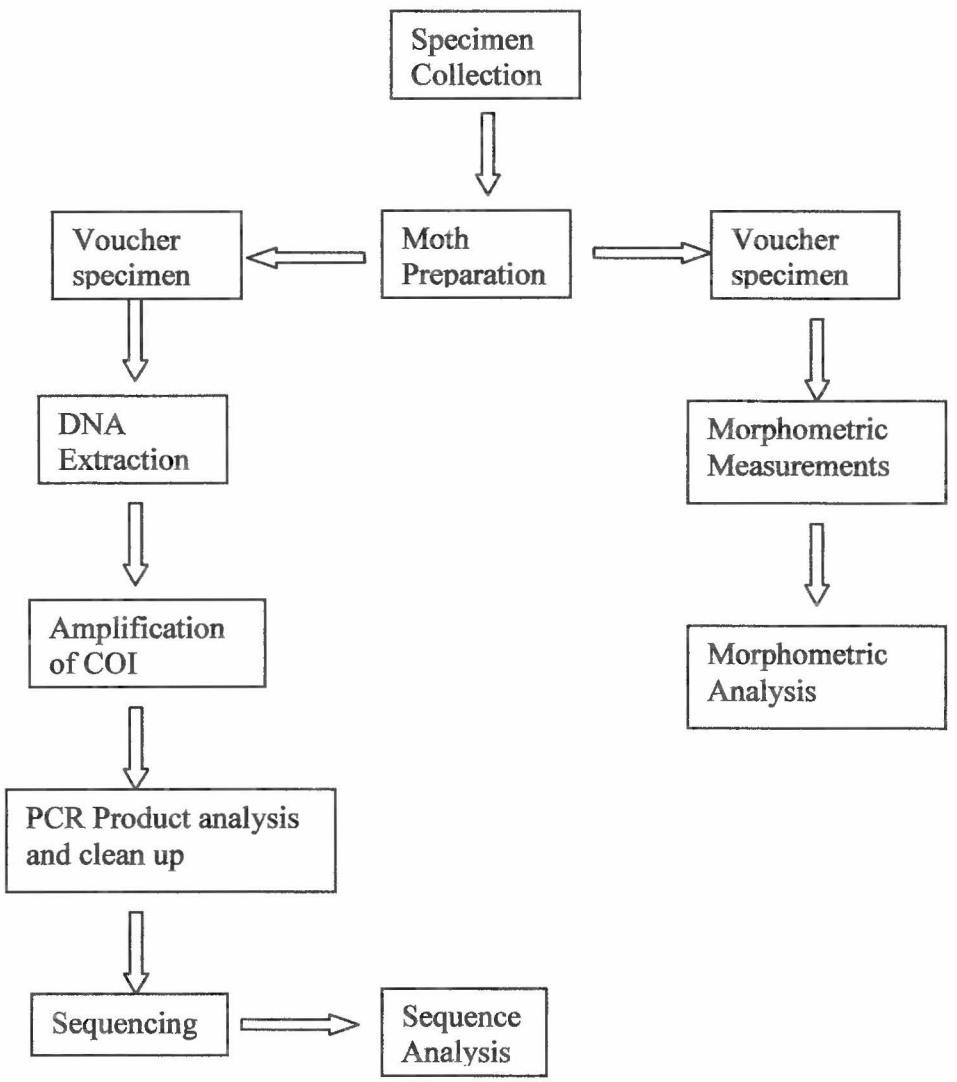


Figure 4. Experimental design flow chart. This chart shows a summary of the work involved in this study.

2.2 Specimen Collections

The cocoons of the wild silk moths were collected from 8 localities (Table 2) in Kenya, at the beginning of the year 2007 and from mid 2008 to early November 2008 (Appendix 1). The locations included Mwingi, Arabuko Sokoke, West Pokot, Ngomeni, Central Pokot and Mararal. The cocoons (Plate 1-6) were put in separate cages (Plate 7) depending on the location of collection. This was to ensure that the moths did not mix after emergence, which would have made it difficult to know the locality of the individual moths.

Table 2. Wild silk moths sampling locations

Locality	**Latitude**	**Longitude**	**Altitude**
Nuu	S 01° 03' 451'	E 038° 21' 438'	2447ft
Ngomeni	S 00° 37' 016'	E 038° 22' 070'	3157ft
Nguni	S 00° 48' 354'	E 038° 18' 891'	2088ft
Mituki	S 00° 44' 965'	E 038° 06' 370'	1632ft
Arabuko Sokoke	S 03° 22'6'	E 039° 47'097'	465ft
Central Pokot	N 01° 24'732'	E 035° 32' 056'	3937ft
West Pokot	N 01° 24' 752'	E 035° 31' 836'	3772ft
Maralal	N 01° 37' 01'	E 037° 34' 59'	2983ft

Plate 1. *E. bauhiniae* (Cocoon)

Plate 2. *A. besanti* (Cocoon)

Plate 3. *G. negrottoi* (Female) (Cocoon)

Plate 4. *G. negrottoi* (Male) (Cocoon)

Plate 5. *A. mimosae* (Cocoon)

Plate 6. SM27 (Cocoon)

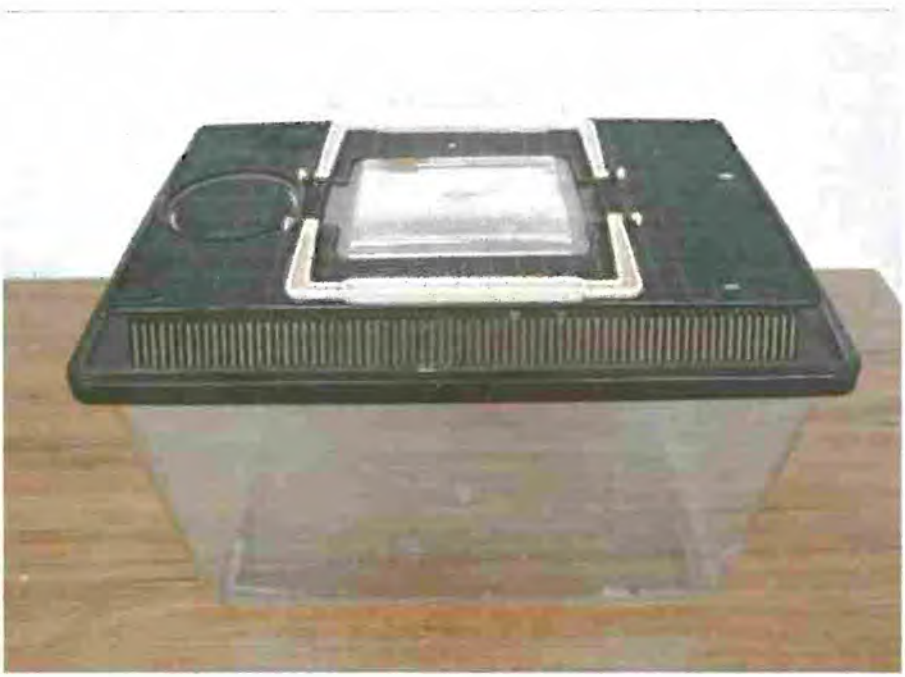


Plate 7. Cage used to separately keep the cocoons. Cocoons collected from different localities were separated in different cages.

2.3 Preparation of Moths

Moths emerging from the cocoons were killed by freezing. This was necessary to facilitate pinning. The moths were pierced through the thorax using insect pin size 4 and set on a pinning board as outlined previously by Holloway et al. (1987). The wings were spread on the setting board using pins to avoid removing of scales on the wings. The spread wings were covered with a tracing paper and pinned on top of the wings to maintain the spread form of the wings. The moths on the pinning board were dried in an oven at 35^{0}C for 48 hours. Each dried moth was given an identification number (Appendix 2) and a photograph was taken. The moth specimens have now been deposited as permanent vouchers at ambient temperature in *icipe*'s insect collection room.

2.4 Morphometric Measurements

Three morphometric characters of the 52 specimens of the Kenyan wild silk moths; were carefully measured using a pair of vernier caliper for morphometric analysis. These included body lengths, forewings width and the forewings breadth of these wild moths. Measurements of the cocoons length and width were also done using a pair of vernier caliper.

2.5 DNA Extraction

Genomic DNA was extracted from a leg following the CTAB procedure (Wagner *et al.*, 1987). About 1 ml of isolation buffer (2X CTAB) (Appendix 3) containing 8µl of β-Mercaptoethanol was preheated in a 65°C water bath. The left middle leg of the moths was macerated using 100 µl of the isolation buffer. An equal volume of chloroform/isoamylalcohol (24:1) was then added to the slurry and mixed gently. The mixture was then mixed on an ENVIRON-SHAKER 3597-1 orbital shaker (150g) for 1 hour. The mixture was spun at 13000g at room temperature for 20 minutes in a BIOFUGE fresco centrifuge. The aqueous (top) phase containing the DNA was pippeted out and transferred to clean 1.5 ml tubes. Two-thirds volume of -20° C isopropanol was added, mixed gently and incubated at -20° C overnight to precipitate DNA. The DNA was pelleted by centrifugation at 13000g for 15 minutes in a BIOFUGE fresco centrifuge. The supernatant was gently discarded and the pellet washed with 300 µl of 70% ethanol for 5 minutes followed by centrifugation at 13000g for 10 minutes in a BIOFUGE fresco centrifuge. The tube was air dried in a fume hood at room temperature for 30 minutes. The DNA was resuspended in 30 µl of ddH_20 and stored at -20° C till ready for use.

2.6 Amplification of COI (Barcode region)

Total DNA isolated from the wild silk moths was used in the amplification of the cytochrome oxidase region of the mt DNA. Primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994) were used to amplify the 5' end of the mitochondrial gene cytochrome oxidase subunit 1 (COI) gene in a PTC-100 thermocycler. PCR amplifications were carried out in 20µl reaction volumes containing 1µl DNA extract, 1µl of each primer (at 10pmol/µl), 0.5µl of Taq DNA polymerase, 2.5µl 10X PCR Buffer (Genescript), 2.5µl 2.5mM dNTPs and double distilled water. The amplification regime consisted of an initial denaturation of 94° C 2 min, 30 cycles of 1 min denaturing at 94° C, 1 min annealing at 45° C and 2 min extension at 72° C, followed by a final extension of 10 min at 72° C.

2.7 PCR Product analysis and clean-up

Gel electrophoresis was carried out in a 1% agarose gel. The gels were run in T.A.E. buffer at a constant voltage of 70 volts for 1 hour. The gels were stained with ethidium bromide in which bands of approximate 650 bps in size were observed under the U.V. light.

Clean up of the PCR product was done through the alcohol-salt precipitation method. 185µl of TE buffer was added to 15µl of the PCR product. 20µl of 3M sodium acetate (pH 5.2) was then added which was followed by the addition of 440µl of cold absolute ethanol. The samples were then incubated at -20° C for 1 hour. This was followed by

centrifugation at 12000g for 20 min to precipitate the DNA in a BIOFUGE fresco centrifuge. The DNA pellets were washed with 300µl of 70% ethanol and centrifuged for 5 min at 12000g and the ethanol discarded. The DNA pellets were air dried for 1 hour. The DNA was finally resuspended in 30µl of ddH_20 and sequenced bidirectionally, using a commercial service (Macrogen, South Korea).

2.8 Data Analysis

2.8.1 Morphometric Analysis

Analyses of the male *Gonometa* species morphometric characters were performed using the Statistical Analysis System software version 9.1.2 (SAS institute Inc., 2004). Principal Component Analysis (PCA) was performed based on Variance-Covariance matrix for the three morphometric characters to detect presence of possible clusters of the male *Gonometa* species from the different locations among the scatter scores from a plotted plane graph of the first two principal components. Further multivariate analysis of variance was performed to test the equality of the mean vector across the males of *Gonometa* species from Nuu, Nguni and Mituki in Mwingi district. Principal Component Analysis was performed only on the males of the *Gonometa* species; since two sympatric groups were identified with a single difference in the wing morphology of these males. However, the females were identical making it difficult to match the males with the females for a reliable identification

2.8.2 Sequence Analysis

Sequences were edited to remove ambiguous base calls and primer sequences using the ChromasPro version 1.33 software program (Copy © 2003-2005 Technelysium Pty Ltd). Sequences were then aligned using ClustalX software (Thompson *et al.*, 1997) to establish consensus sequences and manually edited. Kimura two-parameter (K2P) model of base substitution (Kimura, 1980) was used to calculate the pairwise genetic ratios in MEGA software version 4 (Tamura *et al.*, 2007). A Neighbour-joining tree was also produced using the MEGA V 4.0, to provide a graphical depiction of the patterning of divergences between the species in this study (Saitou & Nei, 1987). The sequences were also translated to protein using translate software, an online translation tool from the Expasy website (http://www.expasy.ch/tools/dna.html). The barcode of Life Data System (BOLD) allowed us to compare the barcode sequence of specimens in this study to the firmly authenticated reference barcodes sequences obtained from individuals of known identity (Hajibabaei *et al.*, 2007). Identification of our specimen was determined by how closely their sequences matched the ones in the barcode library.

CHAPTER THREE

3.0 RESULTS

3.1 Morphometrics

The measurements of the voucher specimens' (Plate 8-15), forewings length, forewings width and the body lengths of the five different species (Table 3) of the wild silk moths in Kenya showed the variability in size of these silk moths ranging from 2-6cm in length. The female *A. mimosae* had the largest sized wings of all the wild silk moths under this study. The cocoon size also varied among the different wild silk moth species in this study ranging from 3-5cm in length (Fig 5). The female *Gonometa* species had the largest cocoons whereas the *Epiphora* species had the smallest cocoons.

Projection of the male *Gonometa* species on the two principal axes showed no clear separation of these species from the different localities (Fig 6). The first two components accounted for about 95% of the variation (PC 1=88 % and PC 2=6.8 %). The multivariate analysis of variance applied on the morphometric variables of the male *Gonometa* species from the three localities, suggested no evidence for difference of the species, $F_{6,34} = 1.42$, $p>0.05$

Plate 8. *G. negrottoi* (Male)

Plate 9. *G. negrottoi* (Female)

Plate 10. *G. postica* (Male)

Plate 11. *G. postica* (Female)

Plate 12. *A. mimosae*

Plate 13. *A. besanti*

Plate 14. *E. bauhiniae* (Male)

Plate 15. *E. bauhiniae* (Female)

Table 3. Mean and standard deviation values of morphometric characters

Moth species	Location	Sex	Number	Mean (Body length)	Mean (Forewing length)	Mean (Forewing breadth)
G. negrottoi	Nuu	Male	12	2.653±0.315 cm	2.361±0.292 cm	0.746±0.116 cm
G. negrottoi	Nguni	Male	4	2.887±0.085 cm	2.435±0.197 cm	0.765±0.037 cm
G. postica	Mituki	Male	6	2.617±0.214 cm	2.217±0.232 cm	0.677±0.118 cm
G.negrottoi	Nuu	Female	6	3.746±0.224 cm	4.392±0.163 cm	1.742±0.107 cm
G.negrottoi	Nguni	Female	4	3.917±0.128 cm	4.413±0.131 cm	1.813±0.063 cm
G.negrottoi	Ngomeni	Female	3	4.048±0.477 cm	4.569±0.252 cm	1.847±0.146 cm
E.bauhiniae	Pokot	Female	9	2.172±0.172 cm	4.911±0.326 cm	2.966±0.270 cm
E.bauhiniae	Pokot	Male	11	1.798±0.116 cm	4.586±0.319 cm	2.746±0.249 cm
A.mimosae	Mwingi & Arabuko sokoke	Female	5	3.434±0.287 cm	6.109±0.074 cm	3.927±0.307 cm
A.mimosae	Mwingi & Arabuko sokoke	Male	5	2.716±0.202 cm	5.685±0.283 cm	3.449±0.132 cm
A.besanti	Ngomeni	Female	2	2.675±0.106 cm	4.348±0.354 cm	2.448±0.071 cm
A.besanti	Ngomeni	Male	3	2.316±0.115 cm	3.933±0.115 cm	2.045±0.229 cm

Three morphometric characters which include Body lengths, forewing lengths and breadths were measured for the five species of Kenyan wild silk moths in this study.

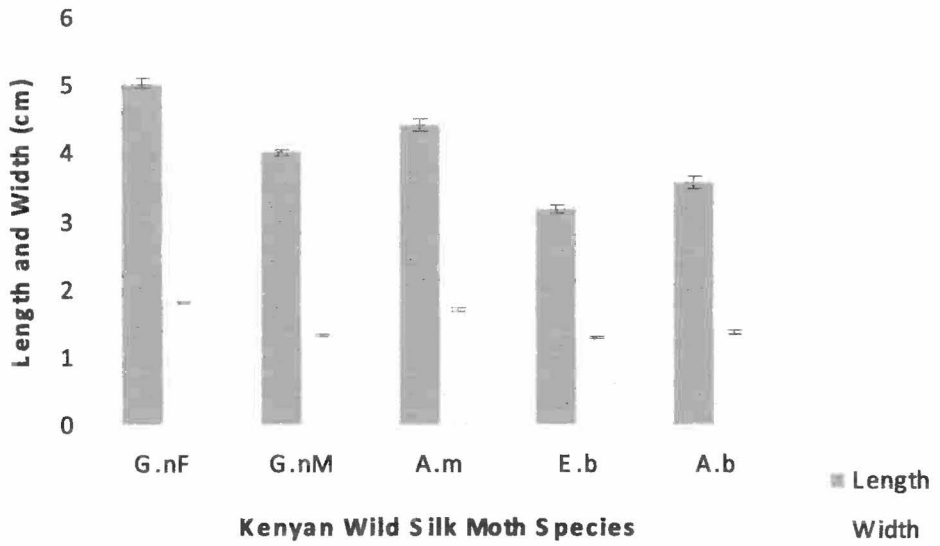


Figure 5. Mean length and width (cm) of Kenyan wild silk moth cocoons. This bar chart shows the variation in size of the cocoons of the different species of Kenyan wild silk moths used in this study. G. nF- *G. negrottoi* Female, G. nM- *G. negrottoi* Male, A. m- *A. mimosae*, E. b- *E. bauhiniae*, A. b- *Argema besanti*

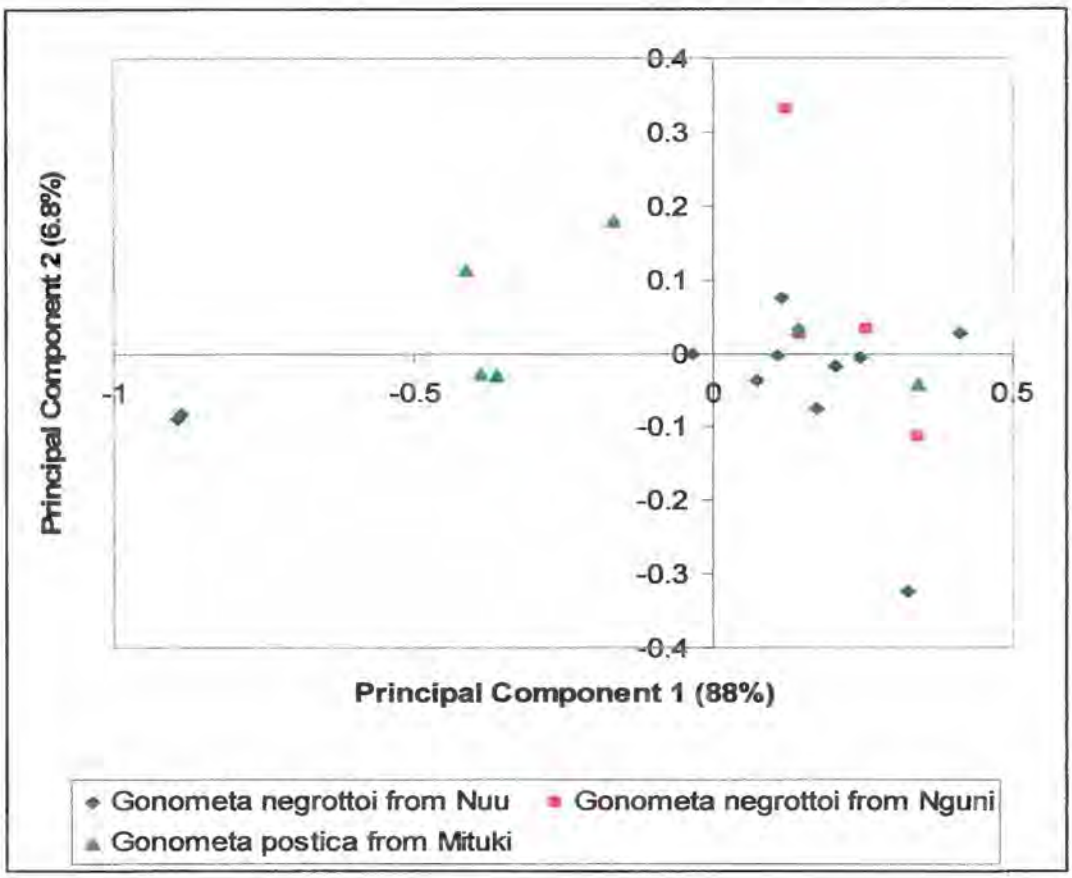


Figure 6. Projection of mean vectors of the *Gonometa* male species. The figure explains a multivariate analysis of variance applied to test for equality of the mean vector across the male *Gonometa* species from three localities.

3.2 DNA Barcoding

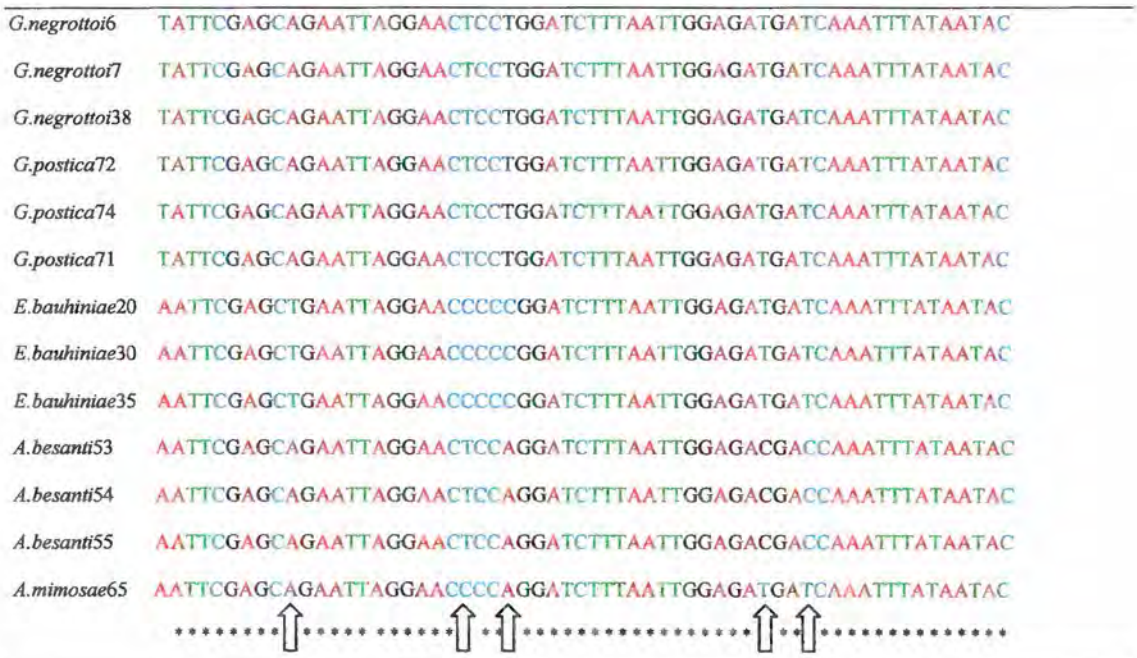
The Folmer primers (Folmer *et al.*, 1994) amplified the target region of COI of all the samples (Plate 16). As expected no variation in size was detectable on agarose gels among the COI amplicons from all 52 specimens of the Kenyan wild silk moth examined in this study. Alignment of all 52 sequences revealed nucleotide variations among the different species (Table 4). Each of the 52 specimens included in the Neighbour-joining profile possessed a distinct COI sequence (Fig 7). COI sequences in the 52 specimens represented by the individual clusters were either identical or most similar to other sequences of the same species. There were no identical COI sequences between species, thus all species were separable by genetic distances.

Pairwise comparison among the 52 specimens of the different silk moth species revealed sequence variation ranging from 0-15.49% (Table 5). The average genetic divergence within the same species ranged from 0-0.08% (Table. 6). However, the average genetic divergence between wild silk moths' species belonging to different species ranged from 0-14.67% (Table 7).

All 52 DNA sequences were conceptually translated into COI amino acid sequences, to exclude the possibility of any of the sequences representing pseudogenes. Lengths of all predicted amino acids sequences were 214 residues. Comparison among amino acid sequences revealed 10 substitutions randomly distributed across the sequences (Table 8). Five hydrophilic amino acids substitutions (Serine and Threonine), four hydrophobic amino acids substitutions (Isoleucine and Leucine) and one positively charged amino acid (Arginine) substitution were present. The amino acid sequence variation range was 0% to 3.81% (Table 9).

Plate 16. Agarose gel electrophoresis of *E. bauhiniae, A. besanti, A. mimosae* and *G. negrottoi* COI fragments. Bands of the correct size were clearly visible under the U.V. light. The marker (M) used was 1kb smart DNA ladder, where A-200 bp, B-400 bp, C-600 bp, D-800 bp and E-1000 bp. The *E. bauhiniae* COI fragments were labeled 20, 30, and 35. *A. besanti* COI fragments were laballed 62, 51 and 52. *A. mimosae* fragment was labeled 53 whereas *G. negrottoi* fragment was labeled 65. The COI fragment in the four species had a size of over 600 base pairs.

Table 4. Multiple Sequence Alignment



Multiple sequence alignment of a fraction of the COI gene in five different species of the Kenyan wild silk moths used in this study. Homology of bases in the four species is shown by asterisks (*) at the bottom of the alignment. Arrowheads indicate mutation sites within the aligned sequences.

Table 5. Pairwise genetic ratios of COI sequences

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
[1]*G.negrottoi*	0.0000													
[2]*G.negrottoi*	0.0000													
[3]*G.negrottoi*	0.0000	0.0000												
[4]*G.postica*	0.0046	0.0046	0.0046											
[5]*G.postica*	0.0046	0.0046	0.0046	0.0000										
[6]*G.postica*	0.0046	0.0046	0.0046	0.0000	0.0000									
[7]*E.bauhiniae*	0.1455	0.1455	0.1455	0.1418	0.1418	0.1418								
[8]*E.bauhiniae*	0.1455	0.1455	0.1455	0.1418	0.1418	0.1418	0.0000							
[9]*E.bauhiniae*	0.1473	0.1473	0.1473	0.1436	0.1436	0.1436	0.0015	0.0015						
[10]*E.bauhiniae*	0.1473	0.1473	0.1473	0.1436	0.1436	0.1436	0.0015	0.0015	0.0000					
[11]*A.besanti*	0.1201	0.1201	0.1201	0.1183	0.1183	0.1183	0.1309	0.1309	0.1327	0.1327				
[12]*A.besanti*	0.1201	0.1201	0.1201	0.1183	0.1183	0.1183	0.1309	0.1309	0.1327	0.1327	0.0000			
[13]*A.besanti*	0.1201	0.1201	0.1201	0.1183	0.1183	0.1183	0.1309	0.1309	0.1327	0.1327	0.0000	0.0000		
[14]*A.mimosae*	0.1549	0.1549	0.1549	0.1511	0.1511	0.1511	0.1382	0.1382	0.1401	0.1401	0.0724	0.0724	0.0724	0.0000

The pairwise similarity calculation using the K2P model of representative samples of the species *G. negrottoi*, *G. postica*, *E. bauhiniae*, *A. besanti* and *A. mimosae* show the genetic ratios among the individual wild silk moth species. The Kenyan wild silk moths in this study had a pairwise similarity genetic ratio ranging from 0 to 0.1549

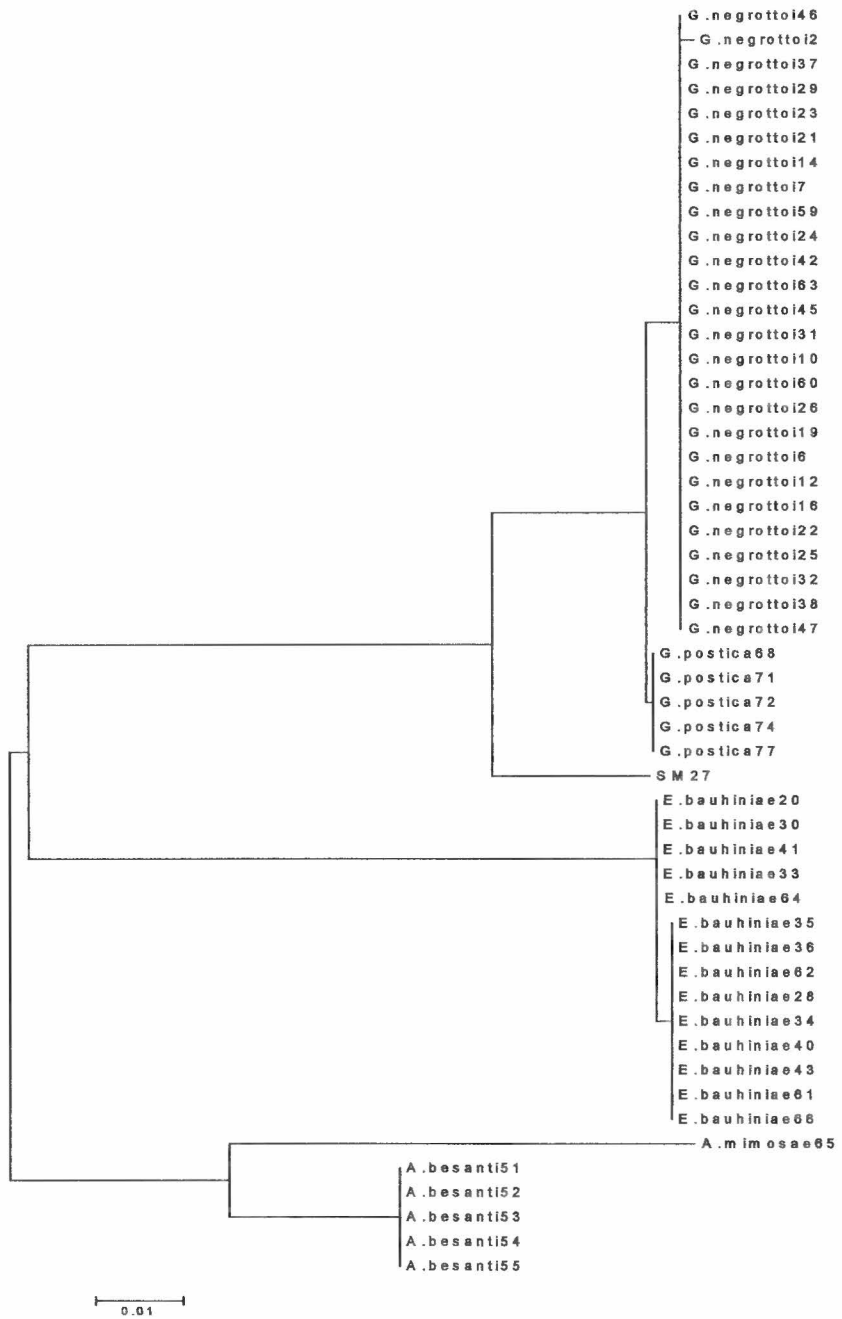


Figure 7. Neighbour-joining tree of COI sequences of Kenyan wild silk moths. A graphical depiction of the patterning of the COI divergences between the species of the Kenyan wild silk moths in this study

Table 6. Intraspecific genetic variation

Species name	Family	Intraspecific genetic variation
G. postica	Lasiocampidae	0.0000
G.negrottoi	Lasiocampidae	0.0001
A. besanti	Sartunidae	0.0000
E. bauhiniae	Sartunidae	0.0008

The above table shows the genetic variation within four different Kenyan wild silk moth species.

Table 7. Interspecific genetic variation

Species	Interspecific genetic variation			
	1	2	3	4
1.*G.postica*	0.0000	0.0000	0.0000	0.0000
2.*G.negrottoi*	0.0046	0.0000	0.0000	0.0000
3.*A.besanti*	0.1183	0.1201	0.0000	0.0000
4.*E.bauhiniae*	0.1430	0.1467	0.1321	0.0000

The above table shows the genetic variation between four different Kenyan wild silk moth species.

Table 8. Amino acid variability of a sample of COI protein sequences

*E.bauhiniae*28	TLYFIFGIAGIVGTSLRLLIRAELGTPGSLIGDDQIYNTIVTAHAFIIIFFMVIPIIIGGFGNLVPLILGAPDIAFPRIN
*E.bauhiniae*40	.....
*E.bauhiniae*35	.....
*E.bauhiniae*62	.....
*E.bauhiniae*20	.....I.....
*E.bauhiniae*30	.....I.....
*A.besanti*53	.....S.....I.....I.....
*A.besanti*54	.....S.....I.....I.....
*A.besanti*55	.....S.....I.....I.....
*A.mimosae*65	.....S.....I.....
*G.negrottoi*6	.....S.....I.....
*G.negrottoi*7	.....S.....I.....
*G.negrottoi*12	.....S.....I.....
*G.postica*68	.....S.....I.....
*G.postica*77	.....S.....I.....
*G.negrottoi*2	.....S.....S.....I.....

Above is a table of the first 80 amino acid residues of the total 214 amino residues coded by 648-bp subunit of the mitochondrial cytochrome oxidase 1 (5'COI) gene, which shows some of the variations occurring in these amino acids.

(A dot indicates an identical amino acid)

Table 9. Pairwise differences of COI protein sequences

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
[1]*A.besanti*	0.0000													
[2]*A.besanti*	0.0000													
[3]*A.besanti*	0.0000	0.0000												
[4]*A.besanti*	0.0000	0.0000	0.0000											
[5]*A.mimosae*	0.0047	0.0047	0.0047	0.0047										
[6]*E.bauhiniae*	0.0236	0.0236	0.0236	0.0236	0.0189									
[7]*E.bauhiniae*	0.0236	0.0236	0.0236	0.0236	0.0189	0.0000								
[8]*E.bauhiniae*	0.0236	0.0236	0.0236	0.0236	0.0189	0.0000	0.0000							
[9]*E.bauhiniae*	0.0189	0.0189	0.0189	0.0189	0.0141	0.0047	0.0047	0.0047						
[10]*G.negrottoi*	0.0284	0.0284	0.0284	0.0284	0.0236	0.0381	0.0381	0.0381	0.0333					
[11]*G.negrottoi*	0.0284	0.0284	0.0284	0.0284	0.0236	0.0381	0.0381	0.0381	0.0333	0.0000				
[12]*G.negrottoi*	0.0284	0.0284	0.0284	0.0284	0.0236	0.0381	0.0381	0.0381	0.0333	0.0000	0.0000			
[13]*G.postica*	0.0284	0.0284	0.0284	0.0284	0.0236	0.0381	0.0381	0.0381	0.0333	0.0000	0.0000	0.0000		
[14]*G.postica*	0.0284	0.0284	0.0284	0.0284	0.0236	0.0381	0.0381	0.0381	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000

The pair wise similarity calculation using the K2P model of representative samples of the species *Gonometa postica, Gonometa negrottoi, Epiphora bauhiniae, Argema besanti* and *Argema mimosae* show the amino acid sequence variation ranging from 0 to 0.0381

CHAPTER FOUR

4.0 DISCUSSION

The aim of this study was to use morphometric analysis and also utilize the COI sequence in providing species level resolution in five species of wild silk moths in Kenya. The use of the COI sequence variation in differentiating species has been clearly demonstrated by earlier studies in birds (Hebert *et al.*, 2004); fish (Ward *et al.*, 2005); spring tail (Hogg & Hebert, 2005) and moths (Hebert *et al.*, 2003; Hajibabaei *et al.*, 2005). This study also used the COI region of the mitochondrial DNA to determine the genetic variations among some of the Kenya's wild silk moths.

The morphometric analyses of the *Gonometa* species males were not able to discriminate between the two congeneric species. The projection of the scatter plot suggested the two congeneric species i.e. *G. postica* and *G. negrottoi* from Mwingi district were not different. This perhaps could have been because the only clear morphological difference between the two congeneric *Gonometa* species is the presence of the triangular hyaline portion of the hind wings in *G. postica* and its absence in *G. negrottoi*. PCA of female *Gonometa* species was not done since females of the two congeneric species are morphologically similar thus indistinguishable from each other.

The COI region was easily amplified and recovered from all species as shown in (plate 16). Alignment of the COI sequences were straight forward, as indels were absent, which supports results from earlier work showing the scarcity of indels in this gene (Mardulyn & Whitefield, 1999). The simplest test of species identification by DNA barcoding is whether two different species share a common COI sequence (Hebert *et al.*,

2004); none was found in this study. However sequence variation was found in the COI region occurring in some wild silk moths within the same species. The intraspecific genetic variation within the species was far less compared to the interspecific genetic variation between the species. This is in agreement with expectations that intraspecific variation is much smaller than between species (Hebert *et al.*, 2004). These Kenyan wild silk moths showed consistent genetic variation that facilitated identification (Kress & Erickson, 2008). These different species also clustered differently in the Neighbour-joining tree.

A BLAST search of the COI sequences of the entire specimens used in this study, were done against the National Centre for Biotechnology Information (NCBI) database. In spite of the variability of amino acids of the translated COI gene in the different species, the BLAST search results of all the specimens were Cytochrome Oxidase I protein. This would therefore seem to indicate the mutations of this genes occurred at the third position of the codon where degeneracy of a gene is observed. This also proved that the COI gene of all the species used in this study were not pseudogenes. It also demonstrated the conserved nature of the cytochrome oxidase I gene.

BOLD has become very instrumental in identifying specimens whose identities are unknown or have raised questions by querying the BOLD identification engine (Ratnasingham & Hebert, 2007). The query sequence must have a minimum of 300 base pairs from the barcode region of the COI (Ratnasingham & Hebert, 2007). The sequences that were used to query the BOLD identification engine had lengths of 658 base pairs (Appendix 4). Entry of the sequence of the *Gonometa* species without the

triangular hyaline portion of the hind wings into the BOLD identification engine gave results of 100% specimen similarity to *G. negrottoi*. When this was repeated using sequences of the *Gonometa* species with the triangular hyaline portion of the hind wings, the BOLD identification engine gave back results of 100% specimen similarity to *G. postica*. The two congeneric *Gonometa* species found in Mwingi district (Kenya) exhibited significant species level genetic variability and deviation to separate them. BOLD was able to also identify *E. bauhiniae* and *A. mimosae* from their fragment of COI.

COI barcodes of all the species used in this study were identified using the identification engine and compared to the reference barcodes of the BOLD systems. No reference sequences were available in BOLD library sequence to identify *A. besanti* and SM27 in this study. This was because BOLD did not have COI barcodes in its reference library for these particular species. The barcodes of the entire wild silk moth species in this study were then deposited into BOLD (Appendix 5). This study has therefore contributed to the pool of scientific knowledge by adding the COI sequences of the species *A. besanti*, which BOLD did not have in its COI reference library.

However, it is clear that DNA barcoding is not a substitute for conventional taxonomic approaches; but rather it seeks to flag cases of deep genetic divergences among individuals grouped as a single species that may indicate overlooked species (Filipe *et al.*, 2007). This study provides evidence that a COI based identification system is effective for identifying wild silk moth species. This conclusion reflects the fact that

there is a much lower level of sequence variation among members of a species than closely allied species.

4.1 Conclusion

In conclusion this study reaffirms the utility of COI sequence in providing species level resolution. Through this study it was possible to identify two congeneric *Gonometa* species, which had initially been described as *Gonometa postica*. DNA barcoding proved to be more objective and precise than morphometrics in distinguishing between *G. negrottoi* and *G. postica*. DNA barcoding gave an insight of the genetic variation that exists among the different Kenyan wild silk moth species in this study. Neighbour-Joining tree based on K2P distances was able to provide a graphical depiction of the patterning of divergences among the different wild silk moth species. This study however failed to identify a single species (SM27) from Mararal that had not been described before. BOLD systems did not have a reference COI sequence in its library similar to that of (SM27), thus it was impossible to identify it from BOLD. In spite of that, DNA barcoding is quite crucial when the morphological differences are not clear to distinguish closely allied species. In such cases identification can be done only on character based on genetic differences (Desalle *et al.*, 2005).

4.2 Recommendations

In spite of the fact that the cytochrome oxidase I (5' COI) gene in the mitochondrial DNA is sufficient for identification of species; more studies should be done to identify a gene with similar properties as COI in the nuclear DNA. Such a gene in the nuclear DNA would authenticate results obtained from DNA barcoding of the COI gene in species identification. This will increase confidence in using DNA markers in species separation especially where there is little discriminatory morphological variation. I would also recommend further characterization to be done on sample SM27 that was not identified in the course of this study. I would also propose extensive sampling of the wild silk moth cocoons to be done as this will yield a more definitive picture of the taxonomic status, diversity, population structure and phylogeography of the wild silk moths. The data obtained in this study will become very useful in contributing to conservation of the Kenyan wild silk moths and also in the utilization of these wild silk moths for income generation activities by farmers in the marginalized areas of Kenya.

REFERENCES

Adolkar V., Raina S., Nguku E., Kioko E. and Ngoka B. (2006). *Conservation and utilization of commercial insects.* In: Raina S; Muli E; Nguku E. and Kioko E. (Eds), Prospects for the development of organic wild and mulberry silk products: Proceedings of the trainers' course and IV international workshop on the conservation and utilization of commercial insects, 14th November to 8th December 2006 *icipe* headquarters, Duduville, Nairobi, Kenya. Development of Sericulture and Apiculture products for the poor in fragile ecosystems using the value chain approach.Pp. 138-144.

Akai H., Nakatomi R., Kioko E. and Raina S. K. (1997). Fine structure of cocoon and cocoon filament from African Gonometa Silkmoth (Lasiocampidae). *International Journal of Wild Silk moth and silk* **3**: 15-22.

Blaxter M. (2003). Molecular systematics: counting angels with DNA. *Nature* **421**: 122-124.

Chase M. W., Salamin N., Wilkinson M., Dunwell J. M., Kesanakurthi R. P., Haidar N. and Savolainen V. (2005). Land plants and DNA barcodes: short-term and longterm goals. *Philosophical Transanction of the Royal Soc*iety. B **360**: 1889–1895.

Cox A. J. and Hebert P. D. N. (2001). Colonization, extinction and phylogeographic patterning in a freshwater crustacean. *Molecular Ecology* **10**: 371–386.

DeSalle R., Egan M. G. and Siddall M. (2005). The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transanction of the Royal Soc*iety B **360**: 1905–1916.

Doyle J. J. and Gaut B. S. (2000). Evolution of genes and taxa: a primer. *Plant Molecular Biology* **42**:1–6.

Erasmus, J. (2007). 'Going wild over indigenous silk' [online]. Available from http://www.mediaclubsouthafrica.com. [cited 30 October 2008].

Evans K. M., Wortley A. H. and Mann D. G. (2007). An assessment of potential diatom "barcode" genes (cox1, rbcL, 18S and ITS rDNA) and their effectiveness in determining relationships in Sellaphora (Bacillariophyta). *Protist* **158**: 349–364.

Fening O., Kioko E., Suresh K. R. and Mueke J. M. (2008). Monitoring wild silk moth, *Gonometa postica* Walker, abundance, host plant diversity and distribution in Imba and Mumoni woodlands in Mwingi, Kenya. *International Journal of Biodiversity Science and Management* **4**: 104-111.

Filipe C., Jeremy R., James B., Sujeevan R., Robert D., Hajibabaei M. and Hebert P. D. (2007). Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* **64**: 272-295.

Folmer O., Black M., Hoeh W., Lutz R. and Vrijenhoek R. (1994). DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biological Biotechnology* **3**: 294–299.

Hajibabaei M., deWaard R., Ivanova V., Ratnasingham S., Dooh T., Kirk L., Mackie M. and Hebert P. D. N. (2005). Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society* B **360**: 1959–1967.

Hajibabaei M., Singer G. A., Hebert P. D. N. and Hickey D. A. C. (2007). DNA barcoding: how it complements taxonomy, molecular Phylogenetics and population genetics. *Molecular Phylogenetics and Evolution* **520**: 1-6.

Hartland-Rowe R. (1992). The biology of the wild silkmoth *Gonometa rufobrunnea* Aurivillus (Lasiocampidae) in northeastern Botswana, with some comments on its potential as a source of wild silk. *Botswana Notes and Records* **24**: 123–133.

Hebert P. D., Cywinska A., Ball S. L. and deWaard J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society,* B **270**: 313–321.

Hebert P. D., Penton E. H., Burns J. M., Janzen D. H. and Hallwachs W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. *Proceedings of the National Academy of Sciences* USA **101**: 14 812–14 817.

Hogg I. D. and Hebert P. D. N. (2005). Biological identifications of springtails (Hexapoda: Collembola) from the Canadian arctic, using mitochondrial barcodes. *Canadian Journal of Zoo*logy **82**: 749–754.

Holloway J. D., Bradely J. D. and Carter D. J. (1987). *CIE Guides To Insects of Importance Lepidoptera*. C.A.B International publishers. Wallingford Oxon U.K.

Huang J., QinXu Q., Sun Z. J., Tang G. L. and Su Z. Y. (2007). Identifying earthworms through DNA barcodes. *Pedobiologia* **51**: 301–309.

Janzen D. H., Hajibabaei M., Burns J. M., Hallwachs W., Remigio E. and Hebert P.D.N. (2005). Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. *Philosophical Transanction of the Royal Soc*iety B **360**: 1835-1845.

Kevin C. R. K., Stoeckle M., Carla J. D., Lee A. W., Charles M. F. and Paul D. N. Hebert P. D. N. (2007). Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes* **10**: 1-9.

Kimura M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*. **16**: 111–120.

Kioko E. N., Raina S. K. and Mueke J. M. (2000). Survey on diversity of wild silkmoth species in East Africa. *East Africa Journal of Science* **2**: 1–6.

Knowlton N. and Weigt L. A. (1998). New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society Londo*n B **265**: 2257–2263.

Kress W. John and Erickson L. David. (2008). DNA barcodes: Genes, genomics, and bioinformatics. *Proceeding of the National Academy of Sciences* USA **105**: 2761-2762.

Kurtzman C. P. (1994). Molecular taxonomy of the yeasts. *Yeast* **10**: 1727–1740.

Litaker R. W., Vandersea, M. W., Kibler S. R., Reece K. S., Stokes N. A., Lutzoni F. M., Yonish B. A., West M. A., Black M. N. D. and Tester P. A. (2007). Recognizing dinoflagellate species using ITS rDNA sequences. *Journal of Phycology* **43**: 344–355.

Mahendran B., Ghosh S. K. and Kundu S. C. (2006a). Molecular phylogeny of silk-producing insects based on 16S Ribosomal RNA and Cytochrome Oxidase subunit I genes. *Journal of Genetics* **85**: 32-38.

Mahendran B., Sudip K. and Kundu S. C. (2006b). Molecular phylogeny of silk producing insects based on Internal Transcribed Spacer I DNA. *Journal of Biochemistry and Molecular Biology* **39**: 522-529.

Mardulyn P. and Whitfield J. B. (1999). Phylogenetic signal in the COI, 16S, and 28S genes for inferring relationships among genera of Microgastrinae (Hymenoptera: Braconidae): evidence of a high diversification rate in this group of parasitoids. *Molecular Phylogenetics and Evolution.* **12**: 282–294.

Marshall E. (2005). Taxonomy. Will DNA barcodes breath life into classification? *Science* **307**: 1037.

Merzheevskaya O. I. (1988). Larvae of Owlet moths. Amerind publishers limited. Smithsonian Institute. Washington, D. C.

Ngoka B. (2003). *A study on Population Trends of African Wild Silk Moth, Gonometa sp. At Kamaguti, Uasin Gishu District, Kenya.* M.sc. Thesis. Kenyatta University, Nairobi, Kenya.

Ngoka B., Kioko E., Raina S., Mueke M. and Kimbui D. (2008). Semi-captive rearing of the African wild silkmoth *Gonometa postica* (Lepidoptera:Lasiocampidae) on an indigenous and a non-indigenous host plant. *International Journal of Tropical Insect Science*. **27**: 183-190.

Pinhey E. C. G. (1975). Moths of southern Africa. Tofelberg Publishers Limited. National Museum Bulawayo. South Africa.

Raina S. K., Kioko E. N. and Mwanycky S. W. (Eds) 1999. *Proceedings of the First International Workshop on the Conservation and Utilization of Commercial Insects.* Nairobi, 18th – 21st1997. ISBN 92 9064 12 3. 252 pp.

Ratnasingham S. and Hebert P. D. N. (2007). BOLD: The Barcode of Life Data System. *Molecular Ecology Notes* **10**: 1-10.

Saccone C., DeCarla G., Gissi C., Pesole G. and Reynes A. (1999). Evolutionary genomics in the Metazoa: the mitochondrial DNA as a model system. *Gene* **238**: 195–210.

Saitou N. and Nei M. (1987). The Neighbour-Joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406-425.

SAS Institute Inc. (2004) SAS/STAT® User's Guide, Version 9.1.2 Cary, NC, USA.

Sholtz C. H. and Holme E. (1996). Insects of Southern Africa. Butterworths publishers limited. University of Pretoria. South Africa.

Sholtz C. H. and Holm E. (1996). Insects of Southern Africa. Butterworths publishers limited. University of Pretoria. South Africa.

Shouche Y. S. and Patole M. (2000). Sequence analysis of Mitochondrial 16S ribosomal RNA gene fragment from several mosquito species. *Journal of Bioscience* **24**: 361-366.

Simmons R. B. and Weller S. J. (2001). Utility and evolution of cytochrome *b* in insects. *Molecular Phylogenetic Evolution* **20**: 196–210.

Smith M. A., Fisher B. L. and Hebert P. D. N. (2005). DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: The ants of Madagascar. *Philosophical Transansaction of the Royal Society* B **360**: 1825-1834.

Stoeckle M. (2003). Taxonomy, DNA, and the Barcode of Life. *Bioscience* **53**: 9.

Stoeckle M. and Ausubel J. H. (2003). Barcode of life: In Scientific Rationale and Strategy: proceedings of the Taonomy and DNA seminar, March 2003, Cold spring Harbor Laboratory.

Tamura K., Dudley J., Nei M. and Kumar S. (2007). MEGA 4. Molecular Evolution Genetics Analysis (MEGA) Software Version 4.0. *Molecular Biology and Evolution* **24**: 1596-1599.

Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F. and Higgins D. G. (1997). The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**: 4876-4882.

Veldtman R., McGeoch M. A. and Scholz C. H. (2002). Variability in pupal size in Southern Africa wild silk moths: implications for sustainable harvesting. *African Entomology* **10**: 127-136.

Wagner D. B., Furnier G. R., Saghai-Maroof M. A., Williams S. M., Dancik B. P. and Allard R. W. (1987). Chloroplast DNA polymorphisms in Lodgepole and Jack pines and their hybrids. *Proceedings of the National Academy of Science USA*. 84:2097-2100.

Ward R. D., Zemlak T. S., Innes B. H., Last P. R. and Hebert P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transanctions of the Royal Society* B **360**: 1847-1857.

Wares J. P. and Cunningham C. W. (2001). Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution* **12**: 2455–2469.

Wilson K. H. (1995). Molecular biology as a tool for taxonomy. *Clinical Infectious Diseases* **20**: 192–208.

Zagulajev A. K. (1988). Clothes moths. Oxonion publishers limited. Smithsonian Institute. Washington, D. C.

Zhang D. X. and Hewitt G. M. (1997). Assessment of the universality and utility of a set of conserved mitochondrial primers in insects. *Insect Molecular Biology* **6**: 143–150.

APPENDICES

Appendix 1: Dates of collections of cocoons and emergence of moths from the cocoons

Number	Location	Date of Arrival at ICIPE campus	Emergence date from cocoons
1	Nuu	05/08/2008	27/10/2008
2	Nuu	05/08/2008	15/09/2008
3	Nuu	05/08/2008	20/10/2008
4	Nuu	05/08/2008	07/10/2008
4	Nuu	05/08/2008	02/10/2008
6	Nuu	05/08/2008	02/10/2008
7	Nuu	05/08/2008	25/09/2008
8	Nuu	05/08/2008	17/09/2008
9	Nuu	05/08/2008	31/10/2008
10	Nguni	10/07/2008	02/10/2008
11	Nguni	10/07/2008	26/10/2008
12	Nguni	10/07/2008	05/11/2008
13	Ngomeni	08/07/2008	27/10/2008
14	Ngomeni	08/07/2008	09/10/2008
15	Ngomeni	08/07/2008	21/09/2008
16	Nguni	10/07/2008	31/10/2008
17	Nguni	10/07/2008	04/10/2008
18	Nguni	10/07/2008	29/09/2008
19	Nuu	05/08/2008	15/09/2008
20	Nuu	05/08/2008	09/09/2008
21	Nuu	05/08/2008	26/09/2008
22	Nuu	05/08/2008	02/10/2008
23	Nuu	05/08/2008	04/10/2008
24	Nuu	05/08/2008	11/10/2008
25	Nuu	05/08/2008	24/10/2008
26	Nuu	05/08/2008	27/10/2008
27	Mituki	07/02/2007	05/04/2007
28	Mituki	07/02/2007	08/03/2007
29	Mituki	07/02/2007	02/04/2007
30	Mituki	07/02/2007	20/03/2007
31	Mituki	07/02/2007	02/04/2007
32	Central Pokot	16/08/2008	01/10/2008
33	Central pokot	16/08/2008	07/10/2008
34	Central Pokot	16/08/2008	26/10/2008
35	West Pokot	7/10/2008	17/10/2008

36	West Pokot	7/10/2008	07/11/2008
37	Central Pokot	16/08/2008	12/10/2008
38	Central Pokot	16/08/2008	18/10/2008
39	Central Pokot	16/08/2008	04/11/2008
40	West Pokot	7/10/2008	07/10/2008
41	West Pokot	7/10/2008	12/10/2008
42	West Pokot	7/10/2008	26/10/2008
43	West Pokot	7/10/2008	27/10/2008
44	West pokot	7/10/2008	04/11/2008
45	West Pokot	7/10/2008	08/11/2008
46	Ngomeni	26/10/2008	28/10/2008
47	Ngomeni	26/10/2008	28/10/2008
48	Ngomeni	26/10/2008	28/10/2008
49	Ngomeni	26/10/2008	28/10/2008
50	Ngomeni	26/10/2008	28/10/2008
51	Arabuko Sokoke	11/06/2008	07/11/2008
52	Maralal	24/09/2008	06/10/2008

Appendix 2: Specimen Information

Sample ID	Field ID	Institution Storing	Sample Donor
SM46	SM46	*icipe*	Florence kiilu
SM2	SM2	*icipe*	Florence kiilu
SM37	SM37	*icipe*	Florence kiilu
SM29	SM29	*icipe*	Florence kiilu
SM23	SM23	*icipe*	Florence kiilu
SM21	SM21	*icipe*	Florence kiilu
SM14	SM14	*icipe*	Florence kiilu
SM7	SM7	*icipe*	Florence kiilu
SM59	SM59	*icipe*	Florence kiilu
SM24	SM24	*icipe*	Esther Kioko
SM42	SM42	*icipe*	Esther Kioko
SM63	SM63	*icipe*	Esther Kioko
SM45	SM45	*icipe*	Florence kiilu
SM31	SM31	*icipe*	Florence kiilu
SM10	SM10	*icipe*	Florence kiilu
SM60	SM60	*icipe*	Esther Kioko
SM26	SM26	*icipe*	Esther Kioko
SM19	SM19	*icipe*	Esther Kioko
SM6	SM6	*icipe*	Florence kiilu
SM12	SM12	*icipe*	Florence kiilu
SM16	SM16	*icipe*	Florence kiilu
SM22	SM22	*icipe*	Florence kiilu
SM25	SM25	*icipe*	Florence kiilu
SM32	SM32	*icipe*	Florence kiilu
SM38	SM38	*icipe*	Florence kiilu
SM47	SM47	*icipe*	Florence kiilu
SM68	SM68	*icipe*	Esther Kioko
SM71	SM71	*icipe*	Esther Kioko
SM72	SM72	*icipe*	Esther Kioko
SM74	SM74	*icipe*	Esther Kioko
SM77	SM77	*icipe*	Esther Kioko
SM20	SM20	*icipe*	Clement Ngoriareng
SM30	SM30	*icipe*	Clement Ngoriareng
SM41	SM41	*icipe*	Clement Ngoriareng
SM33	SM33	*icipe*	Clement Ngoriareng
SM64	SM64	*icipe*	Clement Ngoriareng
SM35	SM35	*icipe*	Clement Ngoriareng
SM36	SM36	*icipe*	Clement Ngoriareng
SM62	SM62	*icipe*	Clement Ngoriareng
SM28	SM28	*icipe*	Clement Ngoriareng
SM34	SM34	*icipe*	Clement Ngoriareng
SM40	SM40	*icipe*	Clement Ngoriareng
SM43	SM43	*icipe*	Clement Ngoriareng
SM61	SM61	*icipe*	Clement Ngoriareng
SM66	SM66	*icipe*	Clement Ngoriareng

SM65	SM65	*icipe*	Boniface Ngoka
SM51	SM51	*icipe*	Florence kiilu
SM52	SM52	*icipe*	Florence kiilu
SM53	SM53	*icipe*	Florence kiilu
SM54	SM54	*icipe*	Florence kiilu
SM55	SM55	*icipe*	Florence kiilu

Appendix 3: Molecular Reagents

a. Components of DNA extraction buffers

2XCTAB buffer

100mM Tris-HCL pH 8·0

1.4M NaCl

2% CTAB (hexadecyltrimethylammonium bromide)

TE Buffer

10mM Tris-HCL pH 8·0

0.25mM EDTA

b. Buffer solution

1X TAE Buffer

Prepare 50X: 242g Tris base

57·1ml glacial acetic acid

100ml 0.5M EDTA (pH 8·0)

Dilute to 1X TAE working solution with dH_20

c. Molecular Marker

1kb Smart DNA ladder

Analysis of 0.3µl of the DNA ladder on agarose gel by ethidium bromide staining generates 14 discrete band patterns (in base pairs) 1000, 800, 600, 400, 200,

Appendix 4: Representative COI sequences

>*Gonometa negrottoi*
AACATTATATTTTATTTTTGGAATTTGAGCTGGAATAGTAGGAACTTCTTTAA
GCTTACTTATTCGAGCAGAATTAGGAACTTCTGGATCTTTAATTGGAGATGA
TCAAATTTATAATACAATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTA
TAGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAAT
ATTAGGGGCTCCAGATATAGCATTTCCACGAATAAATAATATAAGATTTTGA
TTATTACCTCCATCTCTTACTCTTTTAATTTCAAGAAGAATTGTAGAAAATGG
AGCAGGTACAGGATGAACTGTATATCCACCATTATCATCAAATATTGCTCAT
AGAGGAAGTTCAGTAGATTTAGCAATTTTTTCTCTTCACTTAGCAGGAATTTC
TTCAATTTTAGGAGCAATTAACTTTATTACTACTATTATTAATATACGATTAA
ATAATATATCATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGAATTACA
GCATTTTTACTTTTACTATCTCTCCCAGTTCTTGCAGGAGCAATTACAATATT
ATTAACAGATCGAAATCTTAATACATCTTTTTTCGATCCAGCTGGAGGAGGA
GATCCTATTTTATATCAACATTTATTT

>*Gonometa postica*
AACATTATATTTTATTTTTGGAATTTGAGCTGGAATAGTAGGAACTTCTTTAA
GCTTACTTATTCGAGCAGAATTAGGAACTCCTGGATCTTTAATTGGAGATGA
TCAAATTTATAATACAATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTA
TAGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAAT
ATTAGGAGCTCCAGATATAGCATTTCCACGAATAAATAATATAAGATTTTGA
TTATTACCTCCATCTCTTACTCTTTTAATTTCAAGAAGAATTGTAGAAAATGG
AGCAGGTACAGGATGAACTGTATATCCACCATTATCATCAAATATTGCTCAT
AGAGGAAGTTCAGTAGATTTAGCAATTTTTTCTCTTCACTTAGCGGGAATTTC
TTCAATTTTAGGAGCAATTAACTTTATTACTACTATTATTAATATACGATTAA
ATAATATATCATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGAATTACA
GCATTTTTACTTTTATTATCTCTCCCAGTTCTTGCAGGAGCAATTACAATATT
ATTAACAGATCGAAATCTTAATACATCTTTTTTCGATCCAGCTGGAGGAGGA
GATCCTATTTTATATCAACATTTATTT

>*Epiphora bauhiniae*
AACTTTATATTTTATTTTTGGAATTTGAGCTGGTATAGTAGGAACCTCCTTAA
GATTACTAATTCGAGCTGAATTAGGAACCCCCGGATCTTTAATTGGAGATGA
TCAAATTTATAATACTATTGTAACGGCCCATGCATTTATTATAATTTTTTTTA
TAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAATA
TTGGGAGCCCCTGATATAGCTTTCCCACGAATAAATAATATAAGTTTTTGAC
TTTTACCCCCCTCTTTAACTTTATTAATTTCTAGAAGAATTGTAGAAAATGGA
GCTGGTACAGGTTGAACAGTCTATCCCCCCCTTTCTTCTAACATTGCTCATGG
AGGAAGATCTGTAGATTTAGCCATTTTTTCATTACATCTTGCTGGAATTTCTT
CAATTTTAGGTGCTATTAATTTCATTACAACAATTATTAATATACGAATAAAT
AATTTAGCTTTTGATCAAATACCTTTATTTGTTTGAGCTGTTGGTATTACAGC
TTTCCTATTATTATTATCTCTTCCAGTTTTAGCAGGTGCTATTACTATACTTTT

AACAGATCGAAATCTTAATACTTCATTTTTTGACCCTGCTGGAGGAGGAGAT
CCAATTTTATATCAACATCTTTTT

>*Argema besanti*
AACTTTATATTTTATTTTTGGTATTTGAGCTGGAATAGTTGGAACTTCTTTAA
GTTTATTAATTCGAGCAGAATTAGGAACTCCAGGATCTTTAATTGGAGACGA
CCAAATTTATAATACAATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTA
TAGTTATACCTATCATAATTGGAGGATTTGGAAATTGATTAATTCCCTTAATA
TTAGGAGCTCCTGATATAGCTTTCCCACGAATAAATAATATAAGTTTTTGAC
TTCTTCCCCCATCTCTTACATTATTAATTTCTAGAAGAATTGTTGAAAATGGA
GCAGGAACAGGATGAACAGTTTATCCCCCACTTTCATCTAATATTGCTCATG
GAGGAACTTCAGTTGATTTAGCTATTTTTTCTCTTCATTTAGCAGGAATTTCT
TCTATTTTAGGAGCTATTAATTTTATTACTACAATTATTAATATACGAATAAA
TAATTTATCATTTGATCAAATACCTTTATTTGTTTGAGCAGTTGGAATTACAG
CTTTTTTACTTCTTTTATCCCTTCCTGTTCTAGCTGGAGCTATTACTATACTAT
TAACTGATCGTAATTTAAATACCTCATTTTTTGATCCTGCTGGAGGTGGAGAT
CCTATTCTTTATCAACATTTATTT

>*Argema mimosae*
AACTCTATATTTTATCTTTGGTATTTGAGCAGGAATAGTTGGAACTTCCTTAA
GCTTATTAATTCGAGCAGAATTAGGAACCCCAGGATCTTTAATTGGAGATGA
TCAAATTTATAATACAATTGTAACAGCTCATGCTTTTATTATAATTTTTTTCA
TAGTTATACCTATTATAATTGGAGGATTTGGAAATTGACTAGTTCCTTTAATA
TTAGGAGCCCCTGACATAGCTTTCCCACGAATAAATAATATAAGTTTCTGAC
TACTCCCCCCATCTCTTACATTACTAATTTCAAGAAGAATTGTTGAAAATGG
AGCAGGAACAGGATGAACAGTTTATCCCCCTCTTTCATCTAATATTGCTCAT
GGTGGAACTTCAGTTGATTTAGCTATTTTTTCCCTTCATTTAGCTGGTATTTCT
TCTATCTTAGGGGCTATTAATTTTATTACTACAATTATTAATATACGAATAAA
TAATTTATCATTTGATCAAATACCTTTATTTGTCTGAGCAGTTGGTATTACAG
CTTTCTTATTACTTTTATCTCTCCCTGTTTTAGCTGGGGCTATTACTATATTAC
TAACTGACCGTAATCTAAATACCTCATTTTTTGACCCTGCAGGTGGGGGAGA
TCCTATTCTTTACCAACATTTATTT

>SM27
AACATTATATTTTATTTTTGGAATCTGAGCTGGAATAATTGGAACTTCTTTAA
GTTTACTTATTCGAGCAGAATTAGGAACTCCTGGATCTTTAATTGGAGATGA
TCAAATTTACAATACAATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTA
TAGTTATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAAT
ATTAGGAGCCCCAGATATAGCATTTCCACGAATAAATAATATAAGATTTTGA
TTATTACCCCCTTCTCTTACCCTTTTAATTTCAAGAAGAATTGTTGAAAGTGG
AGCAGGTACAGGATGAACTGTATACCCACCATTATCATCAAATATTGCTCAT
AGAGGAAGTTCAGTAGATTTAGCTATTTTTTCCCTTCATTTAGCTGGAATTTC
TTCAATTTTAGGAGCAATTAATTTTATTACTACTATTATTAATATACGATTAA
ATAATATATCATTTGATCAAATACCTTTATTCGTTTGAGCAGTAGGAATTAC
AGCATTTTTACTTTTATTATCTCTTCCAGTTCTTGCAGGAGCAATTACAATAT

TATTAACAGATCGAAATCTTAATACATCCTTTTTTGATCCAGCTGGAGGAGG
AGATCCTATTTTATATCAACATTTATTT

Appendix 5: Taxonomy & Identification details

Sample ID	Phylum	Class	Order	Family	Genus	Species	Identifier	Identifier Email	Identifier Institution
SM46	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM2	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM37	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM29	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM23	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM21	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM14	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM7	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM59	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM24	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM42	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM63	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM45	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM31	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM10	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM60	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM26	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM19	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM6	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM12	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM16	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM22	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM25	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*

SM32	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM38	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM47	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*negrottoi*	Peter Kuria	pkamau@icipe.org	*icipe*
SM68	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*postica*	Peter Kuria	pkamau@icipe.org	*icipe*
SM71	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*postica*	Peter Kuria	pkamau@icipe.org	*icipe*
SM72	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*postica*	Peter Kuria	pkamau@icipe.org	*icipe*
SM74	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*postica*	Peter Kuria	pkamau@icipe.org	*icipe*
SM77	Arthropoda	Insecta	Lepidoptera	Lasiocampidae	*Gonometa*	*postica*	Peter Kuria	pkamau@icipe.org	*icipe*
SM20	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM30	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM41	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM33	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM35	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM36	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM62	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM28	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM34	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM40	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM43	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM61	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM66	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Epiphora*	*bauhiniae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM65	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Argema*	*mimosae*	Peter Kuria	pkamau@icipe.org	*icipe*
SM51	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Argema*	*besanti*	Peter Kuria	pkamau@icipe.org	*icipe*
SM52	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Argema*	*besanti*	Peter Kuria	pkamau@icipe.org	*icipe*
SM53	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Argema*	*besanti*	Peter Kuria	pkamau@icipe.org	*icipe*
SM54	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Argema*	*besanti*	Peter Kuria	pkamau@icipe.org	*icipe*
SM55	Arthropoda	Insecta	Lepidoptera	Sartunidae	*Argema*	*besanti*	Peter Kuria	pkamau@icipe.org	*icipe*