Zoological and Entomological Letters

E-ISSN: 2788-8428
P-ISSN: 2788-8436
ZEL 2022; 2(2): 31-35
Received: 02-05-2022
Accepted: 20-06-2022

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DNA barcoding for deciphering the evolutionary lineages of five insect pests of horticultural crops in Ikorodu local government area of Lagos state, Nigeria

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Abstract
Arthropod pest management is dependent on the proper identification of species as quickly as possible. Before now, morphology has been the key methodology, resting basically on the use of established taxonomic keys that have suddenly become plastic because of the introduction of new species and the evolution within old one. The development of the DNA barcoding protocol has proven to be of tremendous help as it relies on sequencing a segment of the cytochrome oxidase 1 gene. Where reference gene sequences exists, this technology is exact and can unambiguously identify pests without errors. The method has become very important with Lepidopteran pests which typically wreak havoc at the larva stages when morphological identification is almost impossible. In this research, we have added first genetic information for *Alpenus maculosus*, *Chilo* species, *Mussidia* species and *Sesamia calamistis* and have further enriched the available reference database upon which further researches can be built. The study also adds quality information to the molecular ecology of Lepidopteran pests around Ikorodu in Lagos State, Nigeria.

Keywords: Agricultural pests, DNA barcoding, Lepidoptera, genetic similarity, barcoding gaps

Introduction
Most of tropical Africa has land area that is suitable for agriculture. The populace have also for ages been dependent on subsistent farming for survival and livelihood. This includes keeping small units of livestock and small holder farms where vegetables are grown. A very major challenge to the agricultural production enterprise are pests of various types, mainly insects that are capable of wrecking serious havoc in very short periods of time, especially prior to harvest. The list of valuable plants subject to damage by lepidopterans is a long one, including many grains, sugar beets and sugarcane, cotton, tobacco, some root crops and leaf crops, many fruits, and timber and shade trees. The damage may involve the leaves, stems, roots, or fruit. Woolens, furs, silk, and even feathers are eaten by fungus moths (tineid moths) of several genera (clothes moths). The greater wax moth (*Galleria mellonella*) causes considerable damage in beehives (Culin 2018) [7]. With poor research and documentation, several generations of subsistent farmers have unwritten histories of pest infestations across time. They are however limited by a proper knowledge of what the names of the pest species. This is not unexpected because of the plethora of types in the insect kingdom and the variation that exists even with a genus. The interactions between pest and pathogenic organisms at the trophic levels pose a serious threat to the whole of the agricultural production enterprise. The movement of animals and pests have had such an unintended consequences like species establishing themselves outside the traditional ranges and out-populating the naturally occurring species. When there are insect plagues, the agricultural economy has been damaged and recovery usually takes several years. Several authors have noted the need for an accurate taxonomical identification as this is key to an integrated pest management approach Tahir *et al*., (2018) [1]. Morphological approaches usually have the following limitations of accuracy, the high difficulty in identifying immature stages because of genic similarities, phenotypic variation (Murugan *et al*., (2015) [8], the time needed for these pest to grow out for the identification of adult stages and the lack of a working reference database for most tropical pest. Molecular methods like DNA barcoding have proven in recent times to be very useful both for identification and the planning of research that is needed to allow for the implementation of control measures. In the last decades, mitochondrial DNA has extensively been used and have proven to be an important tool for species identification.

Several researchers have suggested DNA barcoding in taxonomy as a method to achieve rapid species description in the context of the current biodiversity crisis and also for the cataloguing of insect pests (Herbert *et al*. 2003a, b; Ball and Armstrong 2006) [2, 3, 4]. It major feature being the prompt identification of species especially young indistinct instars and well as fragmentary cuticular body parts. Our ability to identify, monitor and control deleterious insect species depends on our ability to quickly and accurately identify them and put in place proper control measures. In this research, DNA barcoding was used for the identification of seven insect pests attacking agricultural crops in Ikorodu local government area of Lagos State, in Nigeria. Our main goal was to use the mitochondrial sequence information from the CO1 genes to shed more light on these new cum strange insect pests affecting the economically important vegetable crops.

Materials and Methods

Taxonomic collection and preservation

In Ikorodu Local Government area of Lagos State, Nigeria, more than twenty insect pests have been implicated in the huge damage caused to agricultural crops and vegetavles like Kolanuts (*Cola nitida*), Maize (*Zea mays*), Okra (Abelmoschus esculentus) and the African Egg plant (*Solanum macrocarpon*). All pest samples were collected on the farm of the Teaching, Research and Demonstration Farm of the School of Agriculture, Lagos State Polytechnic, Ikorodu, (6.6464° N, 3.5175° E) in Lagos State, Nigeria by picking with forceps and were singly stored in clean glass vials. Fifty insects were collected in all and preserved in 95% Ethanol and kept in the laboratory for further molecular investigations. Insects were separated into groups based on morphological similarities as they were all instar.

Molecular analysis, PCR and sequencing

Small tissues were cut from the twelve groups and stored in cryo-tubes for shipment to the laboratory of Professor Axel Hausmann, Head of the Lepidoptera section of the Bavarian State Collection of Zoology in Germany. The collections were then digitized and the sections sent to the Canadian Centre for DNA barcoding where further extraction of DNA, PCR and sequencing was done.

Sequences were then sent back as chromograms after which they were trimmed and edited using Bio Edit v7.25 (Hall 1999) [5]. Further exploratory sequences analysis was done.

General properties of sequences and genetic divergence

A similarity search for each sequence was performed using the BLASTn. Fifteen distinct sequences were gotten which bore high similarities with sequences in the GenBank and also helped in species assignment in some. DNA comparisons and alignments were performed using the MEGA 7.0 and exploratory analysis was done until the obtained sequences could actually be pinned down as being of a species where possible.

Results and Discussion

Eight of the sequences do not have any index of similarity with sequences on the GenBank indicating that the organism or any similar to it has ever been sequenced or deposited in the GenBank. Seven of the DNA sequences from a portion of the CO1 region are fully shown below.

1. Sample ID - BC ZSM Lep 109678

GGGCAGGAATAGTAGGAACTTCTCTTAGATTATTA
ATTCGAGCTGAATTAGGAAACCCCGGATCTCTGAT
CGGTGATGATCAAATTTATAATACTATTGTTACAGC
CCATGCATTTATCATAATTTTTTTTATAGTTATGCC
CATTATAATTGGAGGATTTGGAAATTGATTAGTAC
CTTTAATATTAGGAGCCCCTGATATAGCTTTCCCCC
GTATAAATAATATAAGTTTTTGACTTCTTCCTCCAT
CATTAACCTTACTAATTTCTAGAAGAATTGTTGAAA
ATGGAGCTGGTACGGGTTGAACTGTTTACCCCCCT
CTCTCTTCTAATATTGCTCATGGTGGCAGCTCAGTT
GATCTAGCAATTTTTTCTTTACATTTAGCTGGGATT
TCCTCAATCTTAGGGGCTATTAATTTTATCACTACT
ATTATTAATATACGAATTAATAATTTATCTTTTGAT
CAGATACCTTTATTTGTTTGAGCTGTAGGGATTACT
GCCTTATTATTACTTCTTTCTTTACCTGTTCTAGCTG
GAGCTATTACTATACTCCTAACAGATCGAAATTTA
AATACATCATTTTTTGACCCTGCAGGAGG

2. Sample ID BC ZSM Lep 109680

GAGCTGGAATAGTAGGAACATCTTTAAGATTATTA
ATTCGAGCTGAATTAGGAAATCCTGGTTCTTTAATT
GGAGATGATCAAATTTATAATACTATTGTAACAGC
TCATGCTTTTATTATAATTTTTTTTATAGTTATACCA
ATTATAATTGGAGGATTTGGTAATTGATTAGTACCT
TTAATATTAGGAGCTCCAGATATAGCCTTCCCCCG
AATAAATAATATAAGTTTTTGACTTCTACCCCCATC
ATTAACTTTATTAATTTCAAGAAGAATTGTAGAAA
ATGGAGCAGGAACAGGATGAACAGTTTATCCCCCT
CTTTCCTCTAATATTGCTCATGGGGGAAGATCAGTT
GATTTAGCTATTTTTTCCCTTCATTTAGCGGGAATT
TCTTCAATTTTAGGAGCTATTAACTTTATCACTACA
ATCATTAATATACGATTAAATAATTTATCATTTGAT
CAAATACCATTATTTGTTTGAGCTGTAGGAATTACA
GCCTTTTTACTCCTTCTTTCACTTCCTGTTTTAGCAG
GAGCTATTACTATACTTTTAACAGATCGAAATTTAA
ATACATC

3. Sample ID BC ZSM Lep 109682

TATTTTTGGAATTTGAGCAGGAATAATTGGAACAT
CACTTAGACTTTTAATTCGAGCTGAATTAGGAACTC
CAGGATCTTTAATTGGTGATGATCAAATTTACAAT
ACTATTGTTACAGCTCACGCATTTATTATAATTTTT
TTTATAGTCATACCAATTATAATTGGAGGATTTGGA
AATTGATTAGTACCTCTAATATTAGGAGCTCCTGAT
ATAGCTTTTCCACGAATAAATAATATAAGATTTTG
ATTACTCCCCCCATCATTAACTTTACTAATTTCTAG
AAGAATTGTAGAAACTGGAGCCGGAACAGGATGA
ACAGTTTACCCCCCACTATCATCTAATATCGCACAT
GCTGGAAGTTCGGTAGATTTAGCAATTTTCTCCCTC
CATTTAGCTGGGATTTCTTCTATTTTAGGAGCTATT
AACTTTATTACAACAATTATTAATATACGAATTAAT
GGATTATCATTTGATCAAATACCATTATTTGTTTGA
TCAGTTGGTATTACAGCTTTATTATTATTACTTTCTT
TACCTGTTTTAGCAGGTGCTATTACTATATTATTAA
CAGATCGAAATTTAAATACATCATTTTTTGATCCAG
CTGGAGGAGGTGATCCAATTCTTTANCAACACTTA
TTT

4. Sample ID BC ZSM Lep 109683

AGGAACATCATTAAGTTTATTAATTCGAGCTGAAT
TAGGAACCCCAGGATCATTAATTGGGGATGATCAA

ATTTATAATACTATTGTTACAGGGCATGCCTTTATT
ATAATTTTTTTTATAGTTATACCTATTATAATTGGA
GGATTTGGTAATTGATTAGTACCTTTAATATTAGGG
GCCCCTGATATAGCCTTCCCACGAATAAATAATAT
AAGATTTTGACTTTTACCTCCTTCCTTAACTCTACTT
ATTTCCAGTAGAATTGTTGAAAACGGAGCAGGAAC
AGGGTGAACTGTGTACCCCCCACTTTCATCTAATAT
TGCTCATGGAGGAAGATCTGTAGATCTAGCTATTTT
TTCCCTTCATTTAGCCGGTATTTCATCTATCCTAGG
AGCTATTAATTTTATTACAACTATTATTAATATAAA
ACTTAATGGCTTATCTTTTGATCAAATACCTTTATT
TGTTTGAGCAGTAGGAATTACAGCTCTTTTATTACT
TTTATCTTTACCAGTATTAGCTGGAGCTATTACTAT
ACTTCTAACTGATCGAAATTTAAAT

5. Sample ID BC ZSM Lep 109684

TAGGAACTTCATTAAGTTTATTAATTCGAGCTGAAT
TAGGAACTCCTGGCTCTTTAATTGGAGATGATCAA
ATTTATAATACTATTGTCACAGCTCATGCTTTTATT
ATAATTTTTTTTATAGTTATACCTATTATAATTGGG
GGATTTGGAAACTGACTTGTACCTTTAATATTAGG
GGCACCAGATATAGCATTCCCACGAATAAATAATA
TAAGATTTTGATTATTACCCCCATCCTTAACCCTTT
TAATTTCAAGTAGAATTGTAGAAAACGGAGCAGGA
ACAGGATGAACAGTATACCCCCCACTTTCATCTAA
TATTGCTCATGGAGGAAGATCAGTAGATTTAGCTA
TTTTTTCTCTTCACTTAGCTGGGATTTCATCTATTTT
AGGAGCAATTAACTTTATTACAACAATTATTAATA
TACGATTAAATAGCCTATCATTTGATCAAATACCCC
TATTTATTTGAGCTGTGGGAATTACTGCCTTTTTAT
TACTATTATCTTTACCTGTTTTAGCGGGAGCCATTA
CTATATTACTTACAGATCGAAATTTAAATACTTC

6. Sample ID BC ZSM Lep 109685

GGAATTTGAGCAGGAATAGTAGGAACTTCATTAAG
TTTATTAATTCGAGCTGAATTAGGAACTCCTGGCTC
TTTAATTGGAGATGATCAAATTTATAATACTATTGT
CACAGCTCATGCTTTTATTATAATTTTTTTTATAGTT
ATACCTATTATAATTGGGGGATTTGGAAACTGACT
TGTACCTTTAATATTAGGGGCACCAGATATAGCAT
TCCCACGAATAAATAATATAAGATTTTGATTATTAC
CCCCATCCTTAACCCTTTTAATTTCAAGTAGAATTG
TAGAAAACGGAGCAGGAACAGGATGAACAGTATA
CCCCCCACTTTCATCTAATATTGCTCATGGAGGAAG
ATCAGTAGATTTAGCTATTTTTTCTCTTCACTTAGC
TGGGATTTCATCTATTTTAGGAGCAATTAATTTTAT
TACAACAATTATTAATATACGATTAAATAGCCTAT
CATTTGATCAAATACCCCTATTCATTTGGGCTGTTG
GAATTACTGCCTTTTTATTACTATTATCTTTACCTGT
TTTAGCGGGAGCCATTACTATATTACTTACAGATCG
AAATTTAAATCTTCATTTTTTGATCCTGCAGGAGGA
GGAGATCCAATTTTATATCAACACTTATT

7. Sample ID BC ZSM Lep 109686

TATTTTATTTTTGGAATTTGAGCTGGAATAGTAGGA
ACATCTTTAAGATTATTAATTCGAGCTGAATTAGG
AAATCCTGGTTCTTTAATTGGAGATGATCAAATTTA
TAATACTATTGTAACAGCTCATGCTTTTATTATAAT
TTTTTTTATAGTTATACCAATTATAATTGGAGGATT
TGGTAATTGATTAGTACCTTTAATATTAGGAGCTCC
AGATATAGCCTTCCCCCGAATAAATAATATAAGTT
TTTGACTTCTACCCCCATCATTAACTTTATTAATTTC
AAGAAGAATTGTAGAAAATGGAGCAGGAACAGGA
TGAACAGTTTATCCCCCTCTTTCCTCTAATATTGCT
CATGGGGGAAGATCAGTTGATTTAGCTATTTTTTCC
CTTCATTTAGCGGGAATTTCTTCAATTTTAGGAGCT
ATTAACTTTATCACTACAATCATTAATATACGATTA
AATAATTTATCATTTGATCAAATACCATTATTTGTT
TGAGCTGTAGGAATTACAGCCTTTTTACTCCTTCTT
TCACTTCCTGTTTTAGCAGGAGCTATTACTATACTT
TTAACAGATCGAAATTTAAATACATCATTTTTGG

The full classifications is shown in Table 1 below.

Table 1: Showing the species of insect pests and their evolutionary lineages

Sample ID	Phylum	Class	Order	Family	Subfamily	Tribe	Genus	Species	Subspecies
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera						
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera						
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera						
BC ZSM Lep 1	Arthropoda	Insecta							
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera						
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera						
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera						
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera						
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera	Erebidae	Arctiinae	Arctiini	Alpenus	Alpenus maculosus	
BC ZSM Lep 1	Arthropoda	Insecta							
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera	Crambidae	Crambinae		Chilo	Chilo sp_AM16	
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera	Pyralidae	Phycitinae		Mussidia	Mussidia sp.	
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera	Noctuidae	Amphipyrinae		Sesamia	Sesamia calamistis	
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera	Noctuidae	Amphipyrinae		Sesamia	Sesamia calamistis	
BC ZSM Lep 1	Arthropoda	Insecta	Lepidoptera	Erebidae	Arctiinae	Arctiini	Alpenus	Alpenus maculosus	

The sequences were all identified as belonging to the phylum Arthropoda, class insect and order lepidoptera which reinforces the damage that insect groups with their very diverse nature do to agricultural crops. It is important to emphasize here that Lepidoptera are the taxonomic order of insects with more species considered of economic importance due to their pest effect in agriculture. Although the order contains more than 150,000 described species, and it is estimated that they can reach 255,000, there are thirteen super families with species that constitute pests. The damage that the pests of the order Lepidoptera cause to the crops happens fundamentally during its larval phase as their adults don't feed on plants typically (Futucrop, 2018) [6]. The fact that nine sequences out of our consensus fifteen don't have any reference in the GenBank and Bar Code of Life database is however an indication that there is a

substantial barcode gap for the Nigerian Lepidopterans and a need to do more as we try to document members of the group. In five of the instances, it was possible to identify them to species level. Sample IDs Sample ID BC ZSM Lep 109680 and Sample ID BC ZSM Lep 109688 were of the family Erebidae, sub-family Arctiinae, tribe arctini, the genus alpenus and the species *Alpenus maculosus*. Sample ID BC ZSM Lep 109682 is identified as belonging to the family crambidae, sub-family cramninae, the genus Chilo and have 100% similarity with Chilo sp_AM16 on the bar code of Life database. Sample ID BC ZSM Lep 109683 is identified as being of the family pyralidae, sub-family phycitinae, genus mussidia and the species Mussidia species, indicating that there has been no exact previous submission for the species. Sample ID's BC ZSM Lep 109684 and BC ZSM Lep 109685 were also identified as belonging to the family noctuidae, sub-family amphipyrinae, the genus sesamia and the species *Sesamia calamistis.*

Fig 1: shows the adult Moth *Alpenus maculosus*

Figure 2 shows the adult *Chipo species*

Fig 3: Showing a typical *Mussidia species*

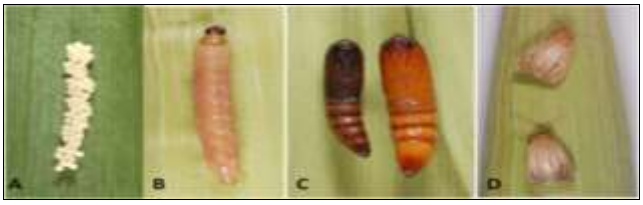

Fig 4: Showing the life stages of *Sessamia calamistis*

Further, we examined the evolutionary relationship between the pests by assessing the Neighbour joining phylogenetic relationship.

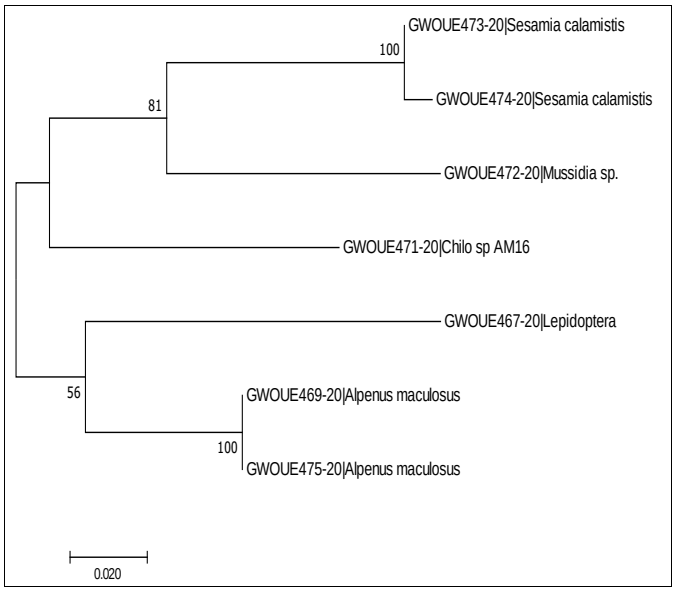

Fig 5: Showing the phylogenetic relationship between the species identified.

Three major clades were formed. The *Sesamia calamistis* despite being from different locations grouped together with a 100% bootstrap value with relationship with the *Mussidia species* despite belonging to different families (Pyralidae and Noctuidae). The *Alpenu maculosus* also mapped together with a 100% bootstrap value despite being taken from different locations. The significance of the lepidopteran group as a potential danger to *Kola nitida, Zea may* cannot be overemphasized. It is also the second report of the species *Alpenus maculosus* in Nigeria at the molecular level.

One empirical take home from this research is the feasibility of using the DNA barcodes as a rapid tool for the identification of species that may be unknown either because they have never been documented or due to their being in the larva stages where a lot of phenotypic similarities within and between species hinders proper identification. It is also an important tool where the key morphological tools are lacking. It however has the major limitation of being dependent on the availability of previous sequences to either the GenBank or the Barcode of Life database. Our inability of identify nine species in an indication of this limitation. There are a relatively small number of barcodes for the Nigerian lepidopterans in both databases. One take home from this research is that the species *Alpenus maculosus, Chilo, Mussidia and Sesamia calamistis* can be readily identified using barcodes and a

need for a more comprehensive sampling and barcoding exists. Further, they are all species that merit concern when considering damage to agricultural crops. More extensive monitoring of the species is also recommended. The inherent benefits of identifying larva stages of these pests cannot also be over-emphasized, as the major damage that they wreck is at the larva stages. The benefits and the overall applicability of DNA barcoding technology for species assignment of lepidopterans in Nigeria justifies this initial efforts and necessitates the expansion of DNA barcoding as a complimentary methodology for identifying agricultural pests of concern as an initial step towards efforts at initiating measures to control them and reduce or minimize the havoc that they can wreck. As the barcoding database expands, so will the accuracy, utility and usefulness of the method become more important in identifying both known and unknown species without ambiguities and as a complimentary method to morphological identification. While DNA barcoding may not be able to provide species level identification for all species at all times, its use as a complimentary identification technique is recommended. This being the first recorded effort at identification of lepidopteran pests in Nigeria at the molecular level will be a prelude to many more efforts at addressing a very serious issue confronting the agricultural sector in Nigeria.

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