which would improve their health condition in the long run when they space the births of their children. The state government should continue to put more effort in empowering women to assist them improve their income generating capacity, by teaching them different sorts of arts and craft which will help them to increase their income and reduce the poverty rate of the women, this will in the long run help to improve their access to family planning services.

References

Adamu, Y. M, Salihu, H. M,& Sathiakumar, N (2003) Maternal Mortality in Northern Nigeria: A Population-Based Study. Euro Journal of Obstetric Gynecology.109 (2): 153-159

Carlifornia Deppartment of Public Health Information (1999)

Easterlin, R. and Crimmins, E. (1985). The fertility Revolution: A supply Demand Analysis Chicago; University of Chicago Press.

Fortney, J. A. (1997) Low income Women's perception of Family Planning. International Family Planning Perspectives 22 pages 150-157

Gombe State Ministry of Health Report 2014

Mahmoud and Ringheim (1997) Family planning programs. Family planning Perspectives 23; pages 122-129.

Mitchel, D. R. (2007): "family Planning; Contraception, Sterilization and Pregnancy Termination" In Katz, V.L., Lenz G.M. , Lobo R. A., Gershenson D.M. Eds. Comprehensive Gynaecology. 5th Ed. Philadelpia P.A.; Mocby EL Sevier, 2007, Chapter 14.

National Population Commission and ICF Macro (2013). Nigerian Demographic Health Survey, 2013. Abuja: Nigeria. NPC and ICF Macro.

National Population Commission (2006). National HIV/AIDS and Reproductive Health Survey (NRHS plus 2007).Federal ministry of Health.

Family Planning and Reproductive Health Report 2004 (Reproline). Office of population and reproductive health, Bureau for Global Health. US Agency for International Health.

Zango, I.M (2001).Male Attitude Towards and Use of Family Planning Techniques in Kano State. Unpublished PhD thesis submitted to the Department of Sociology and Anthropology BU

06.30.2024

JOURNAL OF

Health, Metabolism & Nutrition Studies (JHMNS) Vol. 4 No. 3

MOLECULAR CHARACTERIZATION OF CULEX MOSQUITOES FROM 10 LOCATIONS IN BAUCHI METROPOLIS

***ABDULHAKEEM AKANO SHITTU; **RICHARD J KUTSHIK; **ISHAYA Y LONGDET; & *ABDULAZEEZ LAWAL**

*Department of Biochemistry, Abubakar Tafawa Balewa University, Bauchi, Bauchi State, Nigeria. ** Department of Biochemistry, University of Jos, Jos, Plateau State, Nigeria.

sabdulhakeem@atbu.edu.ng

Abstract

Mosquitoes transmit numerous pathogens which are responsible for numerous diseases and a driver of numerous emerging infectious diseases around the world. Correct identification of mosquito vectors is critical to defining pathogen transmission pathways and is the first step in preventing pathogen transmission. The present study characterizes culex species using mitochondrial DNA (mtDNA) cytochrome oxidase subunit I (COI) and ribosomal RNA internal transcribed

Introduction

Mosquitoes are the principal and the most important vectors of infectious disease-causing agents that greatly affect global health (1,2)(Famakinde, 2018; Motayo et al, 2016). More than half the global population are at risk of exposure to mosquito-transmitted disease such as Malaria which led to 212 million clinical cases and about 429,000 deaths worldwide in 2016,

E-ISSN 3026-8664 P-ISSN3027-2238

spacer 2 (ITS2) gene sequences. A total of 604 mosquitoes were collected from 10 locations in Bauchi Metropolis by human bait method from December – to April and DNA was extracted from morphologically identified Culex species, the COI and ITS2 regions of the mosquitoes were amplified and their sequences analyzed by comparison with other GenBank entries of BOLD and NCBI. These sequences together with GenBank sequences were used in phylogenetic tree construction and molecular characterization of the mosquitoes. According to morphological identification, the field-collected adult mosquitoes belong to the Culex species, Maximum likelihood trees from COI suggest that the Culex species has 99%-100% similarity to *Culex quinquefaciatus*, C.*pipiens Cpipien pipiens*, C.*molestus*, and C.*terzii* and C piping pipiens an indication that the variability in the Culex species on this gene is slow and cannot distinguish between the various species and that of ITS 2 suggests an evolutionary relationship to C. *quinquefaciatus,* C.*pipiens*, C. *pipiens molestus,* C.*modestus,* and C. piping pipiens at maximum identity 83% shows that the species of Culex mosquitoes present in Bauchi is different from all the Culex species from those available in the NCBI and BOLD databases. Phylogeny from the sequences of all the specimens collected from locations in Bauchi with morphology characteristics of *Culex* form a new molecular type with close resemblance to C.*quinquefaciatus*, C.*pipiens*, and C.*molestus*, it also reflects the importance and feasibility of COI and ITS2 genetic markers in identifying mosquitoes and their sibling species and the significance of integrated systematic approach in mosquito taxonomy.

Keywords: cytochrome oxidase subunit I (COI), internal transcribed spacer 2 (ITS2), Culex mosquito, barcode, phylogeny

lymphatic filariasis, West Nile viruses, Zika viruses, etc. and more than 1 billion cases of such are reported yearly (1,3) (Famakinde, 2018; WHO, 2013).

The Southern House Mosquito Culex *quinquefasciatus* is a well-known vector, which plays a prime role in filariasis and the incidences have been seen in different parts of the world (4) (Daravath, et al, 2015). Nearly 1.2 billion people are at risk of developing lymphatic filariasis (LF) and more than 180 million people are estimated to have circulating microfilariae or one of the various clinical conditions associated with filarial infection (5,6) (Abdullahi, 2015; Goodman, et al, 2003). It is an ancient disease with economic and social consequences for affected individuals, families, and communities (7) (Ojiako & Onyeze, 2009). Currently, 25 million people suffer from genital swelling and 15 million people suffer from severe lymphedema or elephantiasis (5) (Abdullahi, 2015). These diseases are transmitted by members of the Anopheles, Culex, Momsonia, and Aedes genera of mosquitoes (1,8) (Famakinde, 2018; Okorie et al., 2016), depending on the geographical location and biological peculiarity of each species (1) (Famakinde, 2018)

More than 1.3 million people in 83 countries and territories approximately 18% of the world live in areas at risk of infection (9) (Nwoke et al, 2010). Nigeria is among the most highly endemic countries (3) (WHO, 2013). Nigeria is ranked third in the world and has the highest number of cases in Africa (8,10) (Dogara et al., 2012; Okorie et al., 2016). Lymphatic filariasis is prevalent in all states and geopolitical zones of Nigeria and a total of 241 lymphodemas and 205 hydrocele cases have been reported from mapping surveys conducted in the country (8) (Okorie et al, 2016). The highest rate of hydrocele is found in the northeast region of Nigeria, including Adamawa, Gombe, Bauchi, Taraba, and Southern Borno (2,11), (Motayo et al., 2016; Okorie,

2011). A study of the available literature shows that local government areas in the Bauchi State of Nigeria seemed to have been overlooked in these surveys (12) (Anosike, 2005). As a result, many endemic communities still need to be identified and unstudied. There is a need to identify the culprit vector which is most likely to transmit the filarial parasite.

The abundance of mosquitoes throughout the year round in Bauchi local government which are well-known vectors of various diseases such as Malaria, Lymphatic filariasis, etc. has become so disturbing that there has come the need to determine which species of the mosquito is present throughout the said period to provide empirical data on the mosquito species by DNA characterization.

Since the discovery of DNA and the recognition of its role in inheritance, genetic variation has played a major role in distinguishing the diversity of life (13) (Adl et al, 2015). Traditional morphology-based taxonomic procedures are time-consuming and not always sufficient for identification at the species level (Krzywinski & Besansky, 2003). Another important marker is the second internal transcribed spacer *(ITS2)* of nuclear ribosomal ribonucleic acid (rRNA) genes. rRNA genes of mosquitoes are represented in the genome as a set of tandem repeats. Each transcriptional unit consists of the genes encoding three ribosomal RNAs (18S, 5.8S, and 28S) separated by internal transcribed spacers *ITSl* and *ITS2* (Schocha et al 2012). It is known that the structure of ITS2 varies among different mosquito species (16) (Porter & Collins, 1991) The rRNA genes are conserved, whereas internal transcribed spacers are variable and useful for comparing species

Several workers have used COI as the only marker in mosquito species recognition and in investigating their molecular evolution (Foley et al.

(2007); Laurito et al,2013; Cywinska et al., 2003; Banerjee et al., 2007; Batovska et al (2016), Daravath et al. (2015). Even if the evolutionary rate of COI is fairly rapid it does not seem to be fast enough (Banerjee et al., 2007; Yao et al., 2010). However, some studies have shown that mosquito identification through COI alone is not always sufficient to make precise conclusions and the need for genetic examination using analysis of faster-evolving regions, such as ITS2, has been emphasized (Kumar et al, 2013; Banerjee et al., 2007). While mitochondrial genes are highly variable and easier to amplify due to their high copy number, nuclear genes exhibit variable rates of substitution that can provide greater resolving power (Batovska et al, 2017). ITS2 regions alone have been used to distinguish closely related mosquito species from various genera such as Anopheles (Banerjee et al., 2007), *Culex*, and *Aedes.* Moreover, it has been indicated that more reliable information about species could be obtained if several molecular markers are used simultaneously) (Garros et al, 2005; Sharpe et al, 2000; Gutiérrez et al., 2010; Hernández-triana et al., 2017; Lopez et al, 2012).

Methods

THE STUDY AREA

The area of coverage during this investigation is Bauchi local government area, Bauchi state, Nigeria. It has in 2006 a projected population of 493,810 people. Bauchi state, however, is located in the northeastern part of Nigeria. It is located at coordinates of 10°30'N and 10°00'E latitude. The climate is tropical. Temperature ranges between 12°C and 30°C and relative humidity between 10-43%, which is similar to that of Jos in Plateau state. The vegetation is pure savannah. Most of

the sampled populations are nomads and subsistence farmers who cultivate millet, maize, sugar cane, rice, beans, and groundnuts.

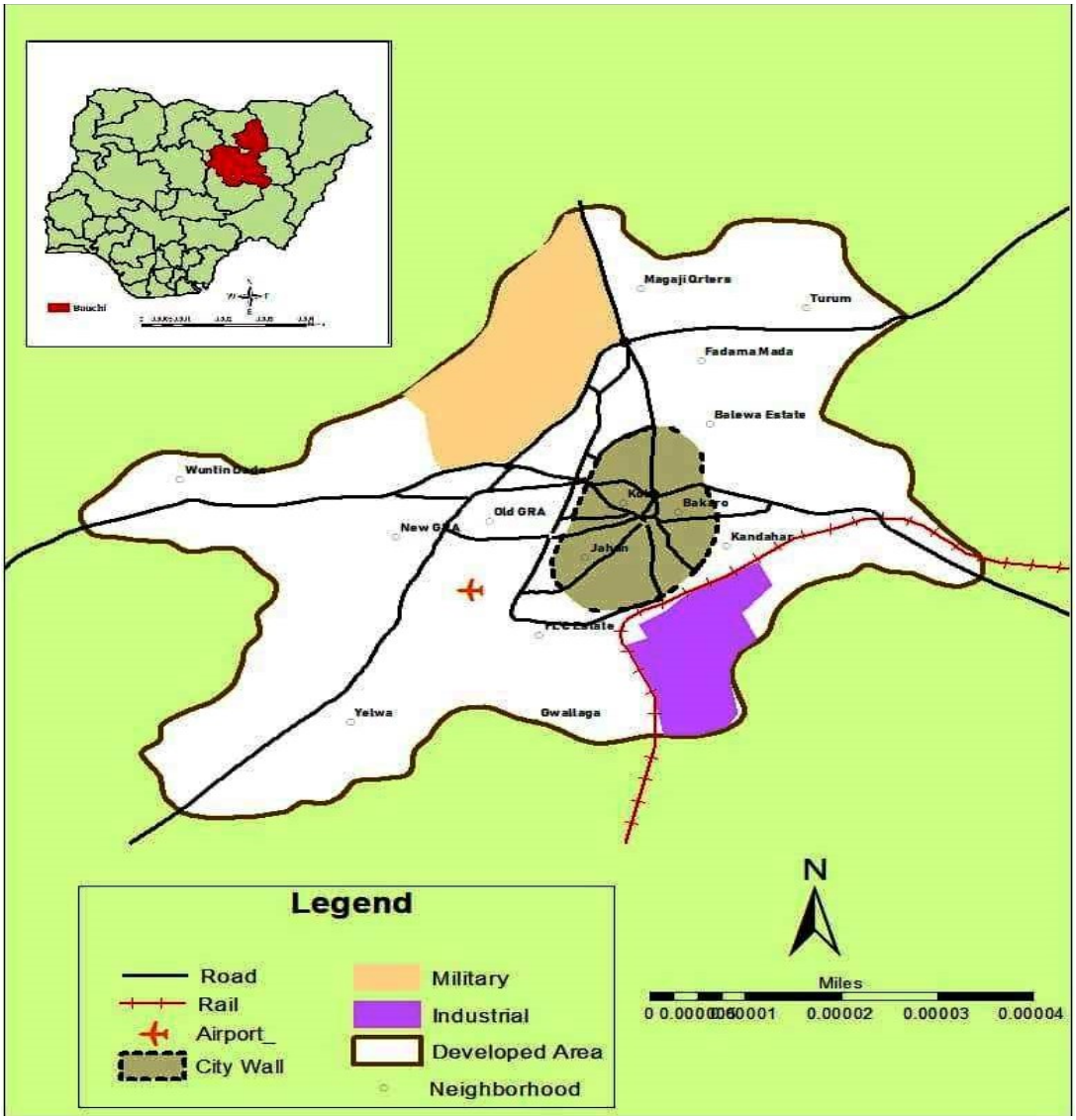

Fig 1: Map of Bauchi showing collection sites from which mosquitoes were collected for DNA barcoding study

MOSQUITO CAPTURE AND IDENTIFICATION

Adult mosquitoes were captured using the human baiting method in sterile sample bottles by trained volunteers in four randomly selected

spots around each selected study location. Volunteers for sample collection were put on Malaria prophylactic treatment throughout mosquito field collection. Mosquito capture was done between the hours of 8 pm and 11 pm. Upon capture were sorted out by species from other insects using morphological keys. The Culex mosquitoes were morphologically identified by their genitalia, plumose (bushy) antennae, and also their palpi under the microscope. Male adult mosquitoes were discarded and only identified females were selected for the study.

DNA EXTRACTION

Individual Culex mosquitoes previously identified into individual species by morphometric methods were selected and sorted out according to their physical features and ground in a sterile porcelain mortar and pestle in serum-free, minimum essential medium (MEM). Genomic DNA was extracted from the homogenate using a DNA extraction kit (ZR Quick-gDNA™ Mini-prep Extraction kit (ZYMO RESEARCH USA) according to the manufacturer's protocol. DNA was extracted from a maximum of ten individuals from each morphologically identified species, depending on the availability.

PCR PROTOCOL FOR COI and ITS2

The PCR reaction was carried out with a program of 35 cycles of denaturation at 94 OC for 3 min, annealing at 56 OC for 1 min, and extension at 72 OC for 2 min. A region of the gene was amplified using forward primer COI -F (5′-GGAGGATTTGGAAAATTAGTTCC- 3′) and reverse primer COI -R (5′-CCCGGTAAAATTAAAATATAAACTTC- 3′) (31) (weeraratne et al,2017). 5 µL from the PCR products was mixed

with 5 µL of loading dye, electrophoresed through a 1.5 % agarose gel, and stained with ethidium bromide stain (Promega USA). Amplicons were visualized on 1.5% agarose gel to check the integrity of the fragments and thereafter PCR products with the expected band size (approximately 450 base pair) Purified PCR products were sent for bidirectional sequencing using the same primers.

The PCR reaction for ITS-2 was carried out with a program of 35 cycles of denaturation at 94OC for 3 min, annealing at 57OC for 30 seconds, and extension at 72OC for 2 min. After the PCR was completed, 5 µL from the PCR products were mixed with 5 µL of loading dye, electrophoresed through a 1.5 % agarose gel, and stained with ethidium bromide stain (Promega USA). Amplicons were visualized on 1.5% agarose gel to check the integrity of the fragments and thereafter PCR products with the expected band size (approximately 475 base pair) Purified PCR products were sent for bidirectional sequencing using the same primers.

Data analysis

The COI and ITS2 nucleotide sequences of the mosquito species were compared with sequences previously deposited in the National Centre for Biotechnology Information (NCBI) using the nucleotide Basic Local Alignment Search Tool (BLASTn) (http://www.ncbi.nlm.nih.gov/BLAST).

The sequences were also compared with reference sequences of barcode-specific databases namely Barcode of Life Database version 4 (BOLD) (www.barcodingoflife.com) for species identification using the "Identification Request" function.

Multiple sequence alignment (MSA) was performed in ClustalW for both COI and ITS2 gene sequences with the default parameters from the

molecular evolutionary and genetic analysis tool (MEGA) version X. For phylogeny, the available nucleotide sequences from the National Center for Biotechnology Information (NCBI) most closely matched with sample sequences were collected and similarities between sequences were attained by maximum likelihood method with the deletion of gaps and missing data. Furthermore, Boot-strap replication (500 numbers) was used to validate the tree.

Result

According to morphological identification, the wild mosquito samples collected from all ten study sites belonged to *Culex* species. The number of *Culex* species collected from all the study sites is given in Table 1

Table 1. The distributions of the number of mosquitoes collected among the study sites.

SN	Location	number of mosquitoes collected
1	Yelwa	53
2	Wunti Dada	42
3	Old GRA	84
4	Jahun	90
5	Gwallaga	72
6	Fadama mada	54
7	Kandahar	44
8	New GRA	42
9	Balewa Estate	36
10	Kobi	87
	Total	604

The mosquitoes were separated into groups based on features which include dark unbanded legs, half-moon-shaped underside, blunt end of the abdomen, brittleness, and post-post-spirited thorax. The thickness

of the half-moon-shaped, basal bands on the abdominal terga, the level of darkness of the tarsa, and the white underside of the abdomen (sterna) and Bristles.

Plate 1: The picture shows the thorax, head, and legs of the mosquitoes

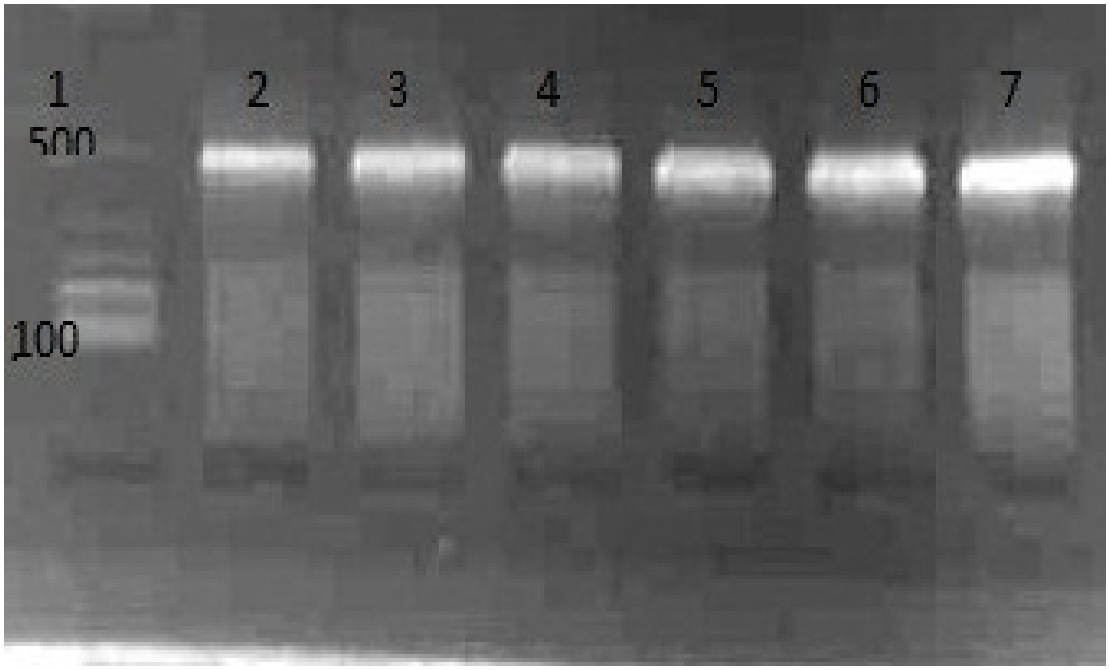

Plate 2: Gel electrophoresis of mitochondrial COI gene isolated from Culex mosquito. Lane 1: 100 bp DNA ladder; lane 2-7: PCR products of

CO I gene. The amplified product of 498 bp was corresponded between 400 bp and 500 bp.

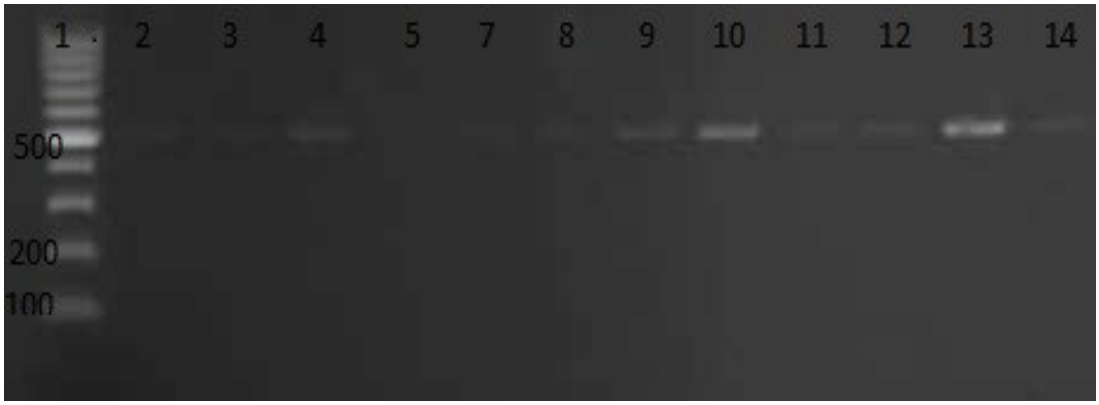

Plate 3: Gel electrophoresis of mitochondrial ITS 2 gene isolated from Bauchi Culex mosquito. Lane 1: 100 bp DNA ladder; lane 2-14: PCR products of ITS 2 gene. The amplified product of 483 1bp was corresponded between 400 bp and 600 bp

From Table 2, the blast result shows the similarities between the sample sequence and those on the NCBI and BOLD. Mosquito Species were confirmed by comparing the sequences with the sequences already available in the GenBank, all the sequences of COI had a maximum identity value between 99 and 100%. The mosquitoes are evolutionary close to Culex quinquefasciatus, Culex pipiens,, Culex pipiens molestus,, Culex pipiens pipiens,, Culex pipiens pallens and on the BOLD system all had a 100% identity with Culex pipiens molestus,Culex pipiens, Culex quinquefasciatus.

Table 2: summary of BLAST result of CO 1 sequences from NCBI and BOLD system.

Gene	Query Cover	Species NCBI	% Similarity	and Accession Nunmber	Species (BOLD)	Similarity
COI	92	*Culex*	99%	MG646675.1	*Culexpipiensmolestus*	100
	92	*quinquefasciatus,*	100%	KM233150.1	*Culexpipiens*	100

	92	*Culex pipiens, Culex quinquefasciatus, Culex pipiensmolestus, Culex pipienspipiens, Culex pipienspallens,*	100%	JQ716525.1	*Culexquinquefasciatus*	100
	91		99%	KU495005.1		
	91		100%	HQ724614.1		
	90		99%	KT851543.1		

Multiple Sequence Alignment

Mitochondrial COI genes were subjected to multiple sequence alignments on crustal W to speculate the sequence conservations among Culex species from voucher specimens. From the multiple sequence alignment (MSA) results, similarities, variations, insertions, and deletions were observed. However, the Bauchi Culex species sequence of ITS2 seems more evolutionary and close to *Culex australis, Culex tritaeniorhynchus, Culex Bitaeniorhynchus, Culex annulirostris, Culex cylindricus, Culex modestus, Culex palpalis, Culex torrentium* on the BOLD system.

The result from the alignment of ITS2 conducted using the NCBI database as shown in Table 4 shows *Culex quinquefasciatus* having a maximum identity of 92% and *Culex molestus* with an identity of 92%, Culex pipien pipiens with an identity of 91%. The BOLD system has a percentage identity match ranging from 77.94-88.74. *Culex torrentium* has the highest match with 88.74 % and a score of 110 but *Culex quinquefasciatus* has a score of 197 which is the highest score and a similarity of 78.96% Table 3.

Table 3: Summary of BLAST result of ITS 2 sequences from NCBI and BOLD system.

GENE	QUERY CC	SPECIES NCBI	% SIMILARITY	AND ACC NUMBER(NCBI)	SPP (BOLD)	% SIMILARITY	SCORE
ITS2	58	*Culex quinquefascia*	**92%**	FJ416058.	*Culex pipiens molest*	77.94	193
	58	*Culex pipiens,*	92%	U22131.1	*Culex pipiens*	76.99	175
	58	*Culex pipiens moles*	92%	KU175324.1	*Culex quinquefasciat*	**78.96**	**197**
	58	*Culex pipiens pipien*	91%	EF539854.	*Culex australicus*	78.04	159
	58	*Culex pipiens pallen*	91%	U33025.1	*Culex Tritaeniorhync*	84.92	129
					Culex Bitaeniorhynch	84.38	125
					Culex Annulirostris	82.27	123
					Culex cylindricus	83.25	118
					Culex modestus	88.74	105
					Culexpalpalis	80.86	119
					Culex torrentium	88.97	110

Figure 2 shows the evolutionary history of the sequences using the Neighbor-Joining method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. This analysis involved 19 nucleotide sequences. The phylogeny tree clearly shows that the mosquitoes have the closest relationship with *Culex torii* and

Culex pipiens. The mosquitoes present in Bauchi clustered separately away from the GenBank sequences.

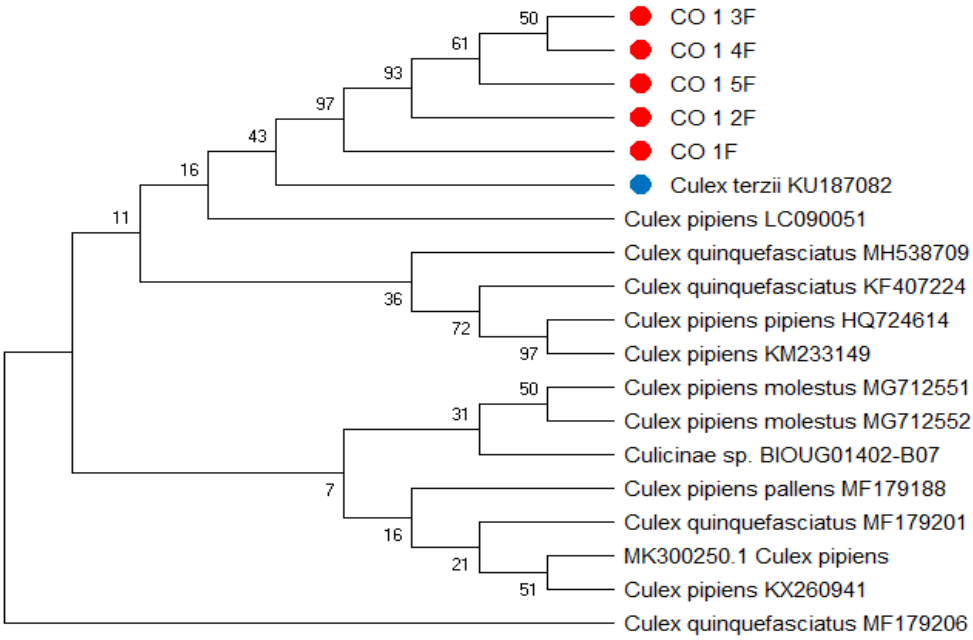

Figure 2: Neighbor-joining phylogenetic tree (based on Kimura 2-parameter genetic distance model) (red label) of COI sequences, the closest relative of the query sequences is in blue label with other sequences retrieved from the GenBank.

Figure 3 shows the evolutionary history using the Neighbor-Joining method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and are in the

units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA X software.

The tree shows that the mosquitoes have the closest relationship with *Culex pipiens*. Among the sequences of sampled mosquitoes, it can be seen that there are two separate *Culexes* that exist in Bauchi based on their clusters. Two sequences ITS2 3F and ITS2 4F are related but are different from the other sequences.

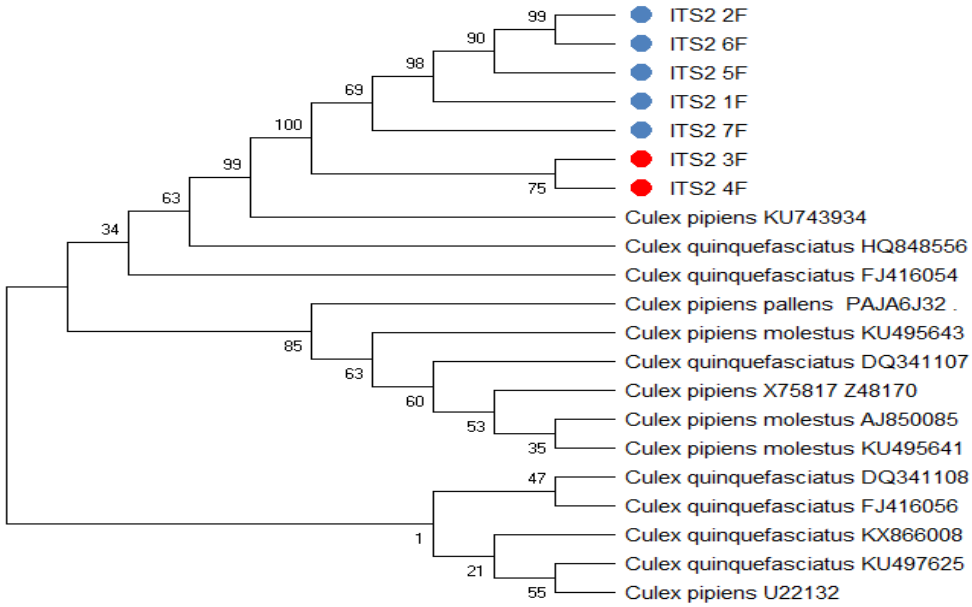

Figure 3: Neighbor-joining phylogenetic tree (based on Kimura 2-parameter genetic distance model) (red and blue label) of ITS 2 sequences and the sequences retrieved from the GenBank.

Discussion

The presence of the major and potential vectors of Filarial throughout the country regardless of environmental conditions shows the potential threat of filarial re-emerging in the country where it has at present been almost eliminated. As mosquitoes are vectors of many human diseases,

accurate identification is essential in implementing vector control programs. Culex mosquito species, including anophelines, have shown significant seasonal and site-related density variation and the weather has been identified as the most responsible macro-ecological factor (32) (Oyewole, et al.,2005). During the present study, *Culex* was common to all the study sites from the morphological identification, which may be due to the time of collection of the samples. As was stated earlier, the samples were collected during the dry season from December to April.
The mitochondrial gene and nuclear ribosomal region ITS2 were used to confirm the identity of morphologically identified species. As was previously mentioned, all the species were confirmed by comparing the COI sequences with the sequences already available in the GenBank and BOLD system and all the sequences had a maximum identity value between 99 and 100%. The BOLD similarity search has a 100% identity for all *Culex* hits. A final confirmation cannot be made as a 99% and 100% match was attained for all the *Culex* suggesting that there is not enough variation in the COI gene to bring about the successful identification of the *Culex* species. The COI is a poor tool for the identification of *Culex* as the evolutionary rate of the gene is slow (Cywinska et al., 2006) as opposed to that of Daravath et al. (2015) who claimed the successful use of the gene in the identification of Culex from Hyderabad.

Phylogeny

Based on the sequence similarity of COI, mosquitoes from Bauchi clustered separately into distinct clades from the others retrieved from the NCBI database, with strong bootstrap support in the NJ phylogenetic tree. This is an indication that the mosquitoes from Bauchi are different molecularly from others around the globe.

There is a strong bootstrap support of 99% with *Culex pipiens* as the closest relationship which is the in NJ topology, within the subgenus, *Culex* formed two distinct but closely related groups with bootstrap value of 100%. This proves that there are two separate groups of *Culex* mosquitoes from the ITS2 samples from Bauchi.

It is suggested that a single barcode is not effective in resolving all species. A combined approach such as coxI-ITS2 and barcodes should be used to reduce the extent of overlap (Bourke et al., 2013). Traditional (morphology) and modern (molecular) techniques should be used collectively for integrative study of DNA barcode sequences (Dayrat, 2005)

Conclusion

In the present study, the predominant species during the dry season, which corresponds to the time of sample collection, was *Culex*. Mitochondrial DNA sequence, COI, and nuclear DNA, ITS2 were successfully isolated and sequenced. Sequence similarity with other species from voucher specimens was used to check for similarity and multiple sequence analysis, MSA, was accomplished. Furthermore, a phylogeny tree was constructed to confirm the identity of the mosquitoes. Results from this research show that the *Culex* species from Bauchi is different molecularly from other species on the NCBI and BOLD databases and can be used as a DNA barcode to identify the organism. Genetic markers COI was unsuccessful in characterizing *Culex* species complexes but ITS2 phylogeny proved that there are two separate species of Culex from the mosquitoes from Bauchi and their molecular form is different from voucher specimens.

REFERENCES

Famakinde. (2018). Mosquitoes and the Lymphatic Filarial Parasites : Research Trends and Budding Roadmaps to Future Disease Eradication. https://doi.org/10.3390/tropicalmed3010004

Motayo, B. O., Onoja, B. A., Faneye, A. O., & Adeniji, J. A. (2016). Seasonal abundance and molecular identification of West Nile virus vectors , Culex pipens and Culex quinquefasciatus (diptera : culicidae) in Abeokuta , South-West , Nigeria ., 16(1), 135–140.

WHO. (2013). Global Programme to Eliminate Lymphatic Filariasis: Managing Morbidity and Preventing Disability. World Health Organization, 1–69. https://doi.org/10.1371/jour

Daravath, S. S., Siddaiah, M., & Reddyanaik, B. (2015). Molecular Characterization and Phylogenetic Analysis of Culex quinquefasciatus by DNA Barcoding, (July), 118–124.

Abdullahi, A. G. (2015). Epidemiology of Malaria and Lymphatic Filariasis ; Prevention and Control under Single Disease Control Program in Rural, 5(2), 55–58.

Goodman, D. S., Orelus, J., Roberts, J. M., Lammie, J., & Streit, T. G. (2003). Levels following mass treatment, 9, 1–9.

Ojiako, O. A., & Onyeze, G. O. C. (2009). Epidemiological and biochemical studies of human lymphatic filariasis and associated parasitoses in Oguta, South-Eastern, Nigeria. African Journal of Biochemistry Research, 3(4), 114–119. https://doi.org/10.5580/7e2

Okorie, P. N., Ademowo, G. O., Saka, Y., Davies, E., Okoronkwo, C., Bockarie, M. J., … Kelly-hope, L. A. (2016). Lymphatic Filariasis in Nigeria ; Micro-stratification Overlap Mapping (MOM) as a Prerequisite for Cost-Effective Resource Utilization in Control and Surveillance, 7(9). https://doi.org/10.1371/journal.pntd.0002416

Nwoke, B. E. B., Nwoke, E. A., Ukaga, C. N., & Nwachukwu, M. I. (2010). Epidemiological characteristics of Bancroftian filariasis and the Nigerian environment, 2(September), 113–117.

Dogara, M., Nock, H., Agbede, R., Ndams, S., & Joseph, K. (2012). Prevalence Of Lymphatic Filariasis In Three Villages In, 10259(January)

Patricia Nkem Okorie, &, Emmanuel Davies, Olushola Omoniyi Ogunmola, Olusola Ojurongbe, Yisa Saka, Bridget Okoeguale, Ekanem Ikpi Braide.(2015) .Lymphatic filariasis baseline survey in two sentinel sites of Ogun state, Nigeria. 8688, 3–8. https://doi.org/10.11604/pamj.2015.20.397.5686

Okorie Anosike JC, Nwoke BEB, Ajayi EG, Onwurili CO, Okoro OU, et al. (2005) Lymphatic Filariasis among the Ezza people of Ebonyi State, Eastern Nigeria. Ann Agric Env Med 12: 181–186

Adl, S.M., Leander, B.S., Simpson, A.G., Archibald, J.M., Anderson, O.R., Bass, D., Bowser, S.S., Brugerolle, G., Farmer, M.A., Karpov, S., 2007, Diversity, nomenclature, and taxonomy of protists. Syst. Biol. 56, 684-689.

Krzywinski, J . & Besansky, N . J . (2003) Molecular systematics of Anopheles: from subgenera to subpopulations. Annual Review of Entomology, 48, 111 – 139

Conrad L. Schocha, Keith A. Seifertb, Sabine Huhndorfc, Vincent Robertd, John L. Spougea, C. André Levesqueb, Wen Chen and Fungal Barcoding Consortium(2012).Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi

Porter, C. H., and F. H. Collins. 1991. Species-diagnostic differences in a ribosomal DNA internal transcribed spacer from the sibling species Anopheles freeborni and Anopheles hermsi (Diptera: Culicidae). Am. J. Trop. Med. Hyg. 45: 271Ð279.

Foley DH, Wilkerson RC, Cooper RD, Volovsek ME, Bryan JH. (2007). A molecular phylogeny of Anopheles annulipes (Diptera: Culicidae) sensu lato: The most species-rich anopheline complex. Mol Phylogenet Evol 43:283–97.

Laurito, M., Oliveira, T. M. P. De, Almirón, W. R., Anice, M., & Sallum, M. (2013). barcode versus morphological identification of Culex (Culex) (Diptera : Culicidae) species : a case study using samples from Argentina and Brazil, 108, 110–122. https://doi.org/10.1590/0074-0276130457

Cywinska, A., Hebert, P. D. N., Cywinska, A., Ball, S. L., & Jeremy, R. (2003). Biological identification through DNA barcodes Biological identifications through DNA barcodes, (January). https://doi.org/10.1098/rspb.2002.2218

Banerjee, A. K., Arora, N., & Murty, U. S. N. (2007). How Far is ITS2 Reliable as a Phylogenetic Marker for the Mosquito genera ?, 3(3), 61–68.

, J., Blacket, M. J., Brown, K., & Lynch, S. E. (2016). Molecular identification of mosquitoes (Diptera : Culicidae) in southeastern Australia. https://doi.org/10.1002/ece3.2095

Daravath, S. S., Siddaiah, M., & Reddyanaik, B. (2015). Molecular Characterization and Phylogenetic Analysis of Culex quinquefasciatus by DNA Barcoding, (July), 118–124.

Yao, H., Song, J., Liu, C., Luo, K., Han, J., Li, Y., Pang, X., Xu, H., Zhu, Y., Xiao, P., Chen, S., 2010. Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS ONE 5 (10), e13102. Yoccoz,

Kumar, N. P., Krishnamoorthy, N., Sahu, S. S., Rajavel, A. R., & Sabesan, S. (2013). DNA Barcodes indicate members of the Anopheles fluviatilis (Diptera : Culicidae) species complex to be conspecific in India. https://doi.org/10.1111/1755-0998.12076

Jana Batovska, Noel O. I. Cogan, Stacey E. Lynch, and Mark J. Blacket.(2017). Using Next-Generation Sequencing for DNA Barcoding: Capturing Allelic Variation in ITS2. www.g3journal.org/lookup/suppl/ doi:10.1534/g3.116.036145/-/DC1

Garros, C . , Harbach, R . E . & Manguin, S . (2005) Systematics and bio-geographical implications of the phylogenetic relationships between members of the funestus and minimus groups of Anopheles (Diptera: Culicidae). Journal of Medical Entomology, 42, 7 – 18.

Sharpe RG et al. PCR-based methods for identification of species of the Anopheles group: allele-specific 63. Committee Vector Medical amplification and single-strand conformation polymorphism. resistance and on Veterinary to Biology pesticides. minimus Fifteenth, 1999, 13: 265–273

Lina A Gutiérrez1 , Lina M Orrego1 , Giovan F Gómez , Andrés López, Shirley Luckhart , Jan E Conn, Margarita M Correa(2010). A new mtDNA gene lineage closely related to Anopheles antennae of the Albitarsis complex in the Caribbean region of Colombia. Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. 105(8), December 2010.

Hernández-Triana, L.M., Crainey, J.L., Hall, A., Fatih, F., Mackenzie-Dodds, J., Shelley, A.J., Zhou, X., Post, R.J., Gregory, R.T. & Hebert, P.D.N. (2012) The utility of DNA barcoding for species identification within the blackfly subgenus Trichodagmia Enderlein (Diptera: Simuliidae: Simulium) and related taxa in the New World. Zootaxa, 3514, 43–69

Lopez L, Morales G, Ursic R, Wolff M, Lowenberger C (2003). Isolation and characterization of a novel insect defensin from Rhodnius prolixus, a vector of Chagas disease. Insect Biochem. Mol. Biol., 33: 439-447.

Thilini C. Weeraratne, Sinnathambi N. Surendran, Lisa J. Reimer3, Charles S. Wondji, M. Devika B. Perera, Culicidae) mosquitoes from eight geographical locations in Sri Lanka. Malaria Journal, DOI 10.1186/s12936-017-1876-y Catherine Walton5 and S. H. P. Parakrama Karunaratne(2007).Molecular characterization of Anopheline (Diptera:

Oyewole IO, Ibidapo CA, Oduola AO, Awolola TS, Obansa JA. Molecular identification and population dynamics of the major malaria vectors in a rainforest zone of Nigeria. Biochem. 2005;17(20): 171-78

Cywinska, A., Hunter, F.F. & Hebert, P.D.N. (2006) Identifying Canadian mosquito species through DNA barcodes. Medical Veterinary Entomology, 20, 413–424. https://doi.org/10.1111/j.1365-2915.2006.00653.x

Bourke BP, Oliveira TP, Suesdek L, Bergo ES, Sallum MA. (2013). A multi-locus approach to barcoding in the Anopheles strodei subgroup (Diptera: Culicidae). Parasit Vectors 6:111

Dayrat B. (2005). Towards integrative taxonomy. Biol J Linn Soc 85: 407–15

Appendix

Query sequence cox 1 and ITS2 genes (both forward and reverse) from *Culex* mosquitoes from Bauchi

ITS-1F

CMTAKCGYAGAATGTGACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACATCGTACA
ACAGTACGATGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTGTGCRCACACACRCMCGCAA
AAGGGTGTTTTGTTGCCTTCGGGGGTTGGCAAAACATMCAAGACYCTCAGCGGTTCGGGTTTTTCTTT
CGGGMGACGGCCCCCTTGTGGCCCCCCCAGCCAATTGAAGGACCAAAAASRAGARAAYAAATCCCTCC
AACACCCCACCTTGGTTYGGGSACGAATTTTSTCTCTCTACCCCCACKCCSCCCACACACGTTCGTTCTC
CCTTACGMCGTGG

ITS-1R

CATCGAGCGTATGGGGGGGTTTTTTTACYCYTTCCATCSCCTTGCTYGGGGTTGGGTTTTTTTTTTTGG
GGGAACSGGRACCCCSAAAAACCCCGAGAACCCAAAAAAATGTGGACAAAAARKGGGGRGAGAAATTT
CTTCTTCGGCCCCCMSMCGGGGGGGGGGGGGGGGTTTTTTTTCCCCCSYCCCCCCCCCCCCKTTCSGGG
GGGGGGGCCCCCCCGGGGGCGCCCCCCCACAAAAACCCCCCCCCCMYYMWTWWTKTTTTTTTTTTTTT
TTCCCCCCCACRCARGAAAAAAAACCTTCTTTTTTGTGTTTGYGGGGGCMMRAAAAAATAAATAATA
AAACCKWCCAAAAAGTGTGCTTTTCTTTTGTTAAAAAGAATGAATTKCTTGCTTTTTTTGGGGGTTT

TTTTKSMAMMTAAATTTTTTTTGCGGGGGTTWTTKKGGGGGGGTTTTTTTCRWCMAKSAAGGAGG
GTTKGAAAT

ITS-2F

TTTYRGTAGAATGTGAACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACATCGTACA
ACAGTACGATGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTGTGCACGCACWGRCMCMMA
AATGGGGGTTTGTTTGCCTYCGGGGGTGGRAAAACATTTAAAAAGCCTCRGGGTCTCGGGTTTTCSGT
TCGGGGGCCGCCACCGTGGGGCMCACGCACGRCTGGGGACRGGACAARACGGAGAAAAAATCCACATC
ACCCACCTGGCTSGGGGGGGGGGGCGAAATTRCTTTCTCTCACCCCCCYTCYCARCCTCYCTTTCGTTT
GCGGYTCCGGCGCGGKRCCCCWWCKGCKKAAACGAAAAAAACAACCCAACAACACAGCRATGRRAW
AAATAAAAAAAAAATAAACCCCTCCGATAARGGGRAAAAATGCCCTACACCCACACTTAAAAWAAAA
AAA

ITS-2R

TTTYCRGGCTTATGGAGGGGGTCTTTTCTCTCTTGTCSATCTCTGGTGTYGGGTTGKGSTGTTCTGTGT
TCGGGGMACCGSGACCCCSCCACACCCCCTKARCCCACMGASACGTGKACACGACGTGGGAKAAAAAC
KCTYCCTCCCCSCCCAMGCCGGGGTGGGGGGTGGGATGTYTTTTCCCCCKCCCGTTCCSCTGCGTGCGM
GCGCGGGGGYGCGGCCGGGCGCCGCCCAACCCCAAAACCCCCCCACCKTSTTGARTGTTTTGTTRGTCT
CCTCACCCAACGASACAAAAAAAAAACCCCCYTTGGGGYGGGGGGGMAYAGAAMAAAAAAAAAATTTA
AAATCMGCAATTTAAAATTTGTATATTGTGGTGATYAAACATTTAAATTTTCCGTTCTMKTTGGYGC
TTGTGWTGAGYTCCTCACTTWATATTCCMAWTGGTTTTTAGCTGTCCCCKTCCACACACACCGAGSA
AKATAATA

ITS-3F

CWAMSGTYAGATGTGAACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACATCGTAC
AACAGTACGATGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTGTGCGCRCACACGCMCGM
ARAAGGGKGGTTTGTTGCCTTCGGTGGTTGGAAAAAATTTCAAAACGTTCAGTGGCTGGGGGTTTTCG
TTCGYGGACGGGCACACTGGGGYGSACGCACGCSACTGAACGGACCACSACCGMGAGAATACATCCTTC
CATCCCACCTGCCTGGGTGGCGGATGKATRATCTCTCTCTCTCCCMCGYCCTCACACWCTCTTTCGGT
CTTCCGTCCKCGKCGGGGKMCCGRCCCCMCMMAAAAAAAAAAACCAAACCCACRARCAMGGATSGRA
RAGSGATAAAAAAAACCCCCCCCCCMSGGSKCARAAAAWGWGGGRGTCCCCCCTCACCTTAAAAAAA
AAAAAAA

ITS-3R

TTTCAGGCTYATGGGGGGKTTTYYTATYSCYTGTCGATCSCTGSTCSTGKGKTGGGGTGKTCTGKTCTG
KGGGCSCSGKACCCCCACCACCCCCGATGACCCAACSAACSTGTGACCACRACSTGGGGKGAAAAATGY
TACMTCGGSCCCCMASCCSGGGGGGGGGGGGGGATGTWTTTTCCCCSYCCYCSTCCCCTCTKTCGCGKG
GGGGGGCACCACTGGGGGGGCCCCCCAACAAAAAACCCCCCCCSCTGAKSGTYTTGTTTGTTTTTTTYC
CCCCCCAAMGRAGMAAAAAACCATTCTTTTTGGGTGGGGGGGGGCRGKKGAATAAAAAAATATARG
WWCTCMRAAATGTGTACTTTGTACTGTTGTACGATGTGCAATATGCATTWAACTTGTCGGTGGTCG
TGGGTCCTGCSKYTCMCYTTCTGACGCSCATTTATYTGKGGGCTTCYTCCWTCCCCCACCCMAGAGAT
AAAAAAAA

ITS-4F

CTTWARSGTASGTAGAATGTGAACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACAT
CGTACAACAGTACGATGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTGTGCRCRCACACGC
MCSMAAAAGGGKGTTTTGYTGCCTTSGGGGGKTGGMAAAACTTTCAAAACGTTCAGSGGTTCGGGGT
TTTCGTTCGGGGACGGCCCCCCTGGGGGGCCCSCCCSCSACTGAACGGACCACCACCGMGAGAAAAATT
CCCCCCCCCCACCCTGGTTGGGTGGGCGACGKAGMATCTTTCTCCCCCCCCCGGCGTCACCCCCTCTTT
CGTTCATCCTGCCGSGKCGSGGYACCGCCCCCMCAAAAAAAAAAAAACCCCCCAACCSARCAGGRRTSYA
MRAAMRAWAAAAAAAACCCCCCCCCSGGGCSTSMCAWAARGGGGGGCYRCCCCCCCCCTTAAAAAAA
AAAAAA

ITS-4R

CATCGAGCGACTGGGGGGKTTATYATATCYCTTGTCATCGCTGCTCGTGKGKTGGG

ITS-5F

GTACGTAGAATGTGACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACATCGTACAAC
AGTACGATGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTGTGCRCRCACYGMMMCSAAAA
WGGRGKTTTGSTTSCTTSSGKGGGTGGGAAAAMATTYAAAAASSTCAGCGGKTCGGGGTTTTCKTTTC
GGGGAGGGCCCACCTGGGGGGMCCCSCCCGRACGGAACGGAACACAACSRSAGGAAAAATTCCTTCCA
CCCCCCCARCTTGYGTGGCSMGCSAKTWTWTTTCTCCCCACSCACGCCCGCCACACCCRTCGGTTCTCC
CWSGCGGGGSGGGGRCGGSRCCCCSCCAACAAAAAAACAAMAMACAACMCARGRGTGGRWRRRARA
AAAAAAAAACMCCCCCCSSSKGKSSGRMRARGKGGGGSGCCCCCCACWWATAAAAAWAAAAAA

ITS-5R

CTTTKGGGCTTATGGGGGGGTTTTTTTYTCTTGTTTTCTCTGTGTTGGGGTGGGGTGTTCTGTGTTCG
GGGACCGGKACCACGCCSACTGCCGAGGACCAAACGASACGAGGACAAGACGTGGGAGAAAAAATSTC
TYCCCCCSCCCCARGRCGGGGGGGGGGGGGGGTTYTTTTTYYCCCCYTCCCCCCCCCCYSTTCKTGGGGG
GTGGGCCCCGCCGGGGGCCCCCCCCCAAAAAAAACCACCCRMTYTTTTGTTTKTTTTTTTTTTTTTCCC
CWCCCCAGAAGAAGAAAMAYTCCTGTTTTTGGGGTGGGGGGGGGGGWTTGAATAAATATTTTGGTT
RGMCGGGGTRTTTGGTTTTTGTTTTTTTTGAAAAAAAGAGAAAATTTGGRTRGGGGGGGGGGTGGGTG
GGGGGGGGGGGSGTGTTCTTTTTTTTTTTTTTTTTTTGGTGGGTGTGTCCTCCCCCCCCCAASAAAAAAA
AAAAAAAAAA

ITS-6F

GYACGGTYAGAATGTGAACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACATCGTAC
AACAGTACGATGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTGTGCRCACACACGCACGMA
AAAGGGGGTTTTGTTGCCTTCGGGGGTGGRAAAAAMTTTAAAAACGCTCAGCGGTTCGGGGTTTTCT
TTCGGAGACGGGCACCGGTGGGCRCCCCCCCCCMCATTTAACGGGACAACAACGAGAGTKAAATACCCT
CCCAAACACGWGTTGGGGGGGGGGGTAGATTTTCTCTTTCCCCCGCCCCGTCWCACACTCATTTTCGT
TTTTKGTTCGGGSGCGGKRSACCRRCCCCMYAAAAAAAAAAACACACCACCAACAAMCAAGSATGGRA
MAAAAAWAAAAAAAAWCCCCCCCCCCGGKYSRMSWRRAGGGRGGCGCCCCCCCCCCCYWAAAATAA
AAAAAAA

ITS-6R

CTTTYSGGCTACTGGGGGGGTTTTTTTTCTCTTTTCGTCCCKRTGCTCGGGTGGGGGTTTTTTTTTTTK
GGGGMACSSSMCCCCCCCCCCCCCCCGAAYCCCCAAAAAAAACGTGGGACCACCCCGGGGGGAGAAAKTT
TCCCCCCCCGCCCCCCSMCCGGGGGGGGGGGGGGGGKTTTTTTCCCCCCKYCKYCCYYYYYYKTTCRGGG
GGGGGGGCGCCCMSGGGGGCGCCCCCCCCCAAAAAACCACCCCCCSRYYYKKTTTTTTTTTTTTTTTTT
TCCCCCCCASRRAAAAAAAACAACACTCTTTTGGGGGGGGGGGGGSGSRMAAATTAAAAAAATAAAATA
TARAAAAAAAAAATTTTTTCATTTTTTTTTGGAGGGAAATATAAAATTCWCTTTWRTTTGGGGGGTT
GTTGTGGGCCCCACATTCCAATTCCAAYTTTKTTTTGGTGTGCTTTCCCCCCCCCCCCCAAAAAKAAAA
AAAA

ITS-7F

TWRRSSGTYAGAATTGTGAACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACATCGT
ACAACAGTACGATGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTGTGCRCACACACGCACG
CARAAKGGKGTTTTGYTGCYTTSGGGGGKTGGMAAAACTTTMAARACGCTCAGGGGCTCGGGGTTTT
CGTTCGGGGGAGGGCCCCATTGGGGGCCCCCACCGCAATTGAAGGGACGACGACGACGWTGAATTTCC
TTCCCACAAACCACCTTGGSTTGGGGGCCAATGTASTTTCTTTACCCCCACTTCGCRGTAACACGTTCT
TTCGTCCTTCCGGGGTGGTGGCGTYCCGCGTCCMAGAAACAGACCACCCCAAAACACAAGRATGSGAT
RAAAARGAAAAAAAAAAACCCCCCCCSGTGWGCCCCAAGAAAAGTGKWRATTCCCCTTTAATTWAAA
AAAT

ITS-7R

CTTTGGGGCTTATGGGGGGGKTTTTTTTATCTTTATCAATCCCATGCTCGKGTGTTGGKTTGTTCTGT
TCTGGGGACSCGGWCCCSCMACRACSCCGGATGACCRAACAAACGTGKGACRACMACGKGGSGTGAA
AAATGYTACYTCGSCCCCCAACCCGGGGGGGKGTGGGGRATGTTTTCTCCCCKYCGTCSYCCYCCGTTC
AGKCGCGGGGGKGCGCACCAGGGGGGCCSYCCMCCAAAAAAAACCCCCSACCCKYTRAGYKTTTTRAW
TGTTTTCCCMCCCCCCRAAGGMAAAAAAACMCCTTTTTGGGGGSGTGKGKGCGCAMAKTAAAATAAA
TAAATATRGGCACTCAAAAGTGTGTACYTCRTATTGTTGAAAAATGYACAATATGCKTTCAWTTTGK

GGGTGTTCKTGTGYCCTGMATTTCATTTTCTGACGCRTTTTTATCTGSGKTCTTCCCCAACCCAAGAS
MSAAGAKAAA

CO1F

AGGGRCTCAGATATKKCCYTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTCCTTCATTGAC
ACTACTACTTTCAAGTAGTTTAGTAGAAAATGGGGCTGGGACTGGATGAACAGTGTATCCCCCTCTTT
CATCTGGAACAGCTCATGCTGGAGCTTCAGTAGACTTAGCTATTTTTTCTTTACATTTAGCAGGAATT
TCATCAATTTTAGGTGCAGTAAATTTTATTACAACAGTAATTAATATACGATCTTCAGGAATTACTCT
TGATCGAATACCTTTATTTGTTTGATCAGTAGTAATTACTGSAGTTTTATTACTTCTTTCTTTACCTG
TTTTAGCTGGTGCTATTACTATGTTATTAACAGATCGAAATTTAAATACTTCATTCTTTGATCCAATT
GGAGGAGGAGATCCAATTTTATATCAACATTTATTTTGATTCTTTGGACATCCAGAAGTTTATATTTT
AATTTTACCGGGA

CO1R

GRKACAAGGKTCATAATMAMTWWYCYATMYAMAMYATGGATCTCCTCCTCCAATTGGATCAAAGA
ATGAAGTATTTAAATTTCGATCTGTTAATAACATAGTAATAGCACCAGCTAAAACAGGTAAAGAAAG
AAGTAATAAAACTGCAGTAATTACTACTGATCAAACAAATAAAGGTATTCGATCAAGAGTAATTCCT
GAAGATCGTATATTAATTACTGTTGTAATAAAATTTACTGCACCTAAAATTGATGAAATTCCTGCTAA
ATGTAAAGAAAAAATAGCTAAGTCTACTGAAGCTCCAGCATGAGCTGTTCCAGATGAAAGAGGGGGA
TACACTGTTCATCCAGTCCCAGCCCCATTTTCTACTAAACTACTTGAAAGTAGTAGTGTCAATGAAGG
AGGTAGTATTCAAAAACTTATATTATTTATTCGAGGAAAGGCCATATCTGGAGCTCCTAACATTAAAG
GAACTAATCAATTTCMAAATCCTCCAATT

CO2F

ARRRKTRRYMWTYYWRTWTATKKWACYTGTMSMSGARTAAATAATATAAGTTTTTGAATACTACC
TCCTTCATTGACACTACTACTTTCAAGTAGTTTAGTAGAAAATGGGGCTGGGACTGGATGAACAGTGT
ATCCCCCTCTTTCATCTGGAACAGCTCATGCTGGAGCTTCAGTAGACTTAGCTATTTTTTCTTTACATT
TAGCAGGAATTTCATCAATTTTAGGTGCAGTAAATTTTATTACAACAGTAATTAATATACGATCTTCA
GGAATTACTCTTGATCGAATACCTTTATTTGTTTGATCAGTAGTAATTACTGCAGTTTTATTACTTCT
TTCTTTACCTGTTTTAGCTGGTGCTATTACTATGTTATTAACAGATCGAAATTTAAATACTTCATTCT
TTGATCCAATTGGAGGAGGAGATCCAATTTTATATCAACATTTATTTTGATTCTTTGGACATCCAGAA
GTTTATATTTTAATTTTACCGGGA

CO2R

GKGCGASKTCTAATMWMYWWYCYAYMYAMWMYATGGATCTCCTCCTCCAATTGGATCAAAGAAT
GAAGTATTTAAATTTCGATCTGTTAATAACATAGTAATAGCACCAGCTAAAACAGGTAAAGAAAGAA
GTAATAAAACTGCAGTAATTACTACTGATCAAACAAATAAAGGTATTCGATCAAGAGTAATTCCTGA
AGATCGTATATTAATTACTGTTGTAATAAAATTTACTGCACCTAAAATTGATGAAATTCCTGCTAAAT
GTAAAGAAAAAATAGCTAAGTCTACTGAAGCTCCAGCATGAGCTGTTCCAGATGAAAGAGGGGGATA
CACTGTTCATCCAGTCCCAGCCCCATTTTCTACTAAACTACTTGAAAGTAGTAGTGTCAATGAAGGAG
GTAGTATTCAAAAACTTATATTATTTATTCGAGGAAAGGCCATATCTGGAGCTCCTAACATTAAAGGA
ACTAATCAATTTCCAAATCYTCCKACA

CO3F

TSGTARKTWWWWYYWKWAWTKTWMKSTTMCWSGRTRAATAATATAAGTTTTTGAATACTACCT
CCTTCATTGACACTACTACTTTCAAGTAGTTTAGTAGAAAATGGGGCTGGGACTGGATGAACAGTGTA
TCCCCCTCTTTCATCTGGAACAGCTCATGCTGGAGCTTCAGTAGACTTAGCTATTTTTTCTTTACATTT
AGCAGGAATTTCATCAATTTTAGGTGCAGTAAATTTTATTACAACAGTAATTAATATACGATCTTCAG
GAATTACTCTTGATCGAATACCTTTATTTGTTTGATCAGTAGTAATTACTGCAGTTTTATTACTTCTT
TCTTTACCTGTTTTAGCTGGTGCTATTACTATGTTATTAACAGATCGAAATTTAAATACTTCATTCTT
TGATCCAATTGGAGGAGGAGATCCAATTTTATATCAACATTTATTTTGATTCTTTGGACATCCAGAAG
TTTATATTTTAATTTTACCGGG

CO3R

GSKRCGRRCKTYTWWTAYMYMKTYCKAYCYAMWMYATGGATCTCCTCCTCCAATTGGATCAAAGA
ATGAAGKATTTAAATTTCGATCTGTTAATAACATAGTAATAGCACCAGCTAAAACAGGTAAAGAAAG

AAGTAATAAAACTGCAGTAATTACTACTGATCAAACAAATAAAGGTATTCGATCAAGAGTAATTCCT
GAAGATCGTATATTAATTACTGTTGTAATAAAATTTACTGCACCTAAAATTGATGAAATTCCTGCTAA
ATGTAAAGAAAAAATAGCTAAGTCTACTGAAGCTCCAGCATGAGCTGTTCCAGATGAAAGAGGGGGA
TACACTGTTCATCCAGTCCCAGCCCCATTTTCTACTAAACTACTTGAAAGTAGTAGTGTCAATGAAGG
AGGTAGTATTCAAAAACTTATATTATTTATTCGAGGAAAGGCCATATCTGGAGCTCCTAACATTAAAG
GAACTAATCAATTTCCAAATCCTCC

CO4F

GRKGKWRGKWKMWYYWWKATATKTWMCYKTSSMSGARTAAATAATATAAGTTTTTGAATACTAC
CTCCTTCATTGACACTACTACTTTCAAGTAGTTTAGTAGAAAATGGGGCTGGGACTGGATGAACAGTG
TATCCCCCTCTTTCATCTGGAACAGCTCATGCTGGAGCTTCAGTAGACTTAGCTATTTTTTCTTTACAT
TTAGCAGGAATTTCATCAATTTTAGGTGCAGTAAATTTTATTACAACAGTAATTAATATACGATCTTC
AGGAATTACTCTTGATCGAATACCTTTATTTGTTTGATCAGTAGTAATTACTGCAGTTTTATTACTTC
TTTCTTTACCTGTTTTAGCTGGTGCTATTACTATGTTATTAACAGATCGAAATTTAAATACTTCATTC
TTTGATCCAATTGGAGGAGGAGATCCAATTTTATATCAACATTTATTTTGATTCTTTGGACATCCAGA
AGTTTATATTTTAATTTTACCGGGA

CO4R

GGKRSARMKTCATAATMWMYWATTCSATMYACAWMYATGGATCTCCTCCTCCAATTGGATCAAAG
AATGAAGTATTTAAATTTCGATCTGTTAATAACATAGTAATAGCACCAGCTAAAACAGGTAAAGAAA
GAAGTAATAAAACTGCAGTAATTACTACTGATCAAACAAATAAAGGTATTCGATCAAGAGTAATTCC
TGAAGATCGTATATTAATTACTGTTGTAATAAAATTTACTGCACCTAAAATTGATGAAATTCCTGCTA
AATGTAAAGAAAAAATAGCTAAGTCTACTGAAGCTCCAGCATGAGCTGTTCCAGATGAAAGAGGGGG
ATACACTGTTCATCCAGTCCCAGCCCCATTTTCTACTAAACTACTTGAAAGTAGTAGTGTCAATGAAG
GAGGTAGTATTCAAAAACTTATATTATTTATTCGAGGAAAGGCCATATCTGGAGCTCCTAACATTAAA
GGAACTAATCAATTTCAAAATCCTCCAATAGA

CO5F

GSGWRRKKWWMWYWKWTTKTWMTTTTMCMSGARTAAATAATATAAGTTTTTGAATACTACCTC
CTTCATTGACACTACTACTTTCAAGTAGTTTAGTAGAAAATGGGGCTGGGACTGGATGAACAGTGTAT
CCCCCTCTTTCATCTGGAACAGCTCATGCTGGAGCTTCAGTAGACTTAGCTATTTTTTCTTTACATTTA
GCAGGAATTTCATCAATTTTAGGTGCAGTAAATTTTATTACAACAGTAATTAATATACGATCTTCAGG
AATTACTCTTGATCGAATACCTTTATTTGTTTGATCAGTAGTAATTACTGCAGTTTTATTACTTCTTT
CTTTACCTGTTTTAGCTGGTGCTATTACTATGTTATTAACAGATCGAAATTTAAATACTTCATTCTTT
GATCCAATTGGAGGAGGAGATCCAATTTTATATCAACATTTATTTTGATTCTTTGGACATCCAGAAGT
TTATATTTTAATTTTACCGGG

CO5R

GGGSARAGSKATCAWCTMWMYWMTYCWAMMYAMWMYATGGATCTCCTCCTCCAATTGGATCAA
AGAATGAAGYATTTAAATTTCGATCTGTTAATAACATAGTAATAGCACCAGCTAAAACAGGTAAAGA
AAGAAGTAATAAAACTGCAGTAATTACTACTGATCAAACAAATAAAGGTATTCGATCAAGAGTAATT
CCTGAAGATCGTATATTAATTACTGTTGTAATAAAATTTACTGCACCTAAAATTGATGAAATTCCTGC
TAAATGTAAAGAAAAAATAGCTAAGTCTACTGAAGCTCCAGCATGAGCTGTTCCAGATGAAAGAGGG
GGATACACTGTTCATCCAGTCCCAGCCCCATTTTCTACTAAACTACTTGAAAGTAGTAGTGTCAATGA
AGGAGGTAGTATTCAAAAACTTATATTATTTATTCGAGGAAAGGCCATATCTGGAGCTCCTAACATTA
AAGGAACTAATCAATTTCCAAATCCTCCA