ADSUJSR 14 (1) (2026) 1 - 6
Adamawa State University Journal of Scientific Research (ADSUJSR)
ISSN: 2705-1900 (Online); ISSN: 2251-0702 (Print)
https://www.adsujsr.adsu.edu.ng

PCR-AMPLIFIED AND AGAROSE GEL ELECTROPHORESIS ANALYSIS OF THE MITOCHONDRIAL COI GENE OF *EPOMOPHORUS GAMBIANUS* IN MUBI, NIGERIA

Gadzama I. M. K.[1]*, Okoye E.[1], Adam M.[1], Alhassan A.B.[2], Shaaba R.[2] and Yerima R.[3]
[1]*Departmentt of Biology, Federal University of Agriculture Mubi, Adamawa State.*
[2]*Departmentt of Biology, Ahmadu Bello University Zaria.*
[3]*Department of Biological Sciences, Gombe State University Gombe.*
**Corresponding author email: ibskatsallah@gmail.com.*

Received in April 2026
Accepted May 2026

Abstract
This study was aimed to provide an initial step toward molecular characterization and phylogenetic analysis of *Epomophorus gambianus*, a fruit bat in the family Pterpodidae, in Mubi, Nigeria. Genomic DNA was extracted from the bat's membrane tissue in the two colonies, using standard protocols. The amplified products were resolved on 1.5% Agarose Gel Electrophoresis and visualized under ultraviolet illumination. The PCR amplification of cytochrome c oxidase subunit I (COI) Gene, as the target gene, was successfully achieved by using bat-specific COI primer sequence according to Irwin et al. (1991). This was confirmed by the presence of distinct bands at approximately 650 base pairs, consistent with the expected size for mammalian COI fragments. The clear and well-resolved banding patterns from this study indicate high-quality DNA extraction and efficient PCR amplification. This study also has shown high quality DNA, with the expected PCR fragment at ~650 bp, as the correct COI barcode fragment for sequencing and phylogenetic analysis of *E. gambianus* bats and validates the suitability of the COI gene as a reliable molecular marker for the identification and genetic studies of *E. gambianus*. Additionally, the data generated contributes to the growing database of mitochondrial sequences useful for biodiversity conservation, species identification, and future evolutionary studies of fruit bat populations in the region.

Keywords: PCR, Cytochrome c Oxidase I, Agarose Gel Electrophoresis, Molecular Identification, *Epomophorus gambianus*.

Introduction

Bats belong to the taxonomic order: Chiroptera, a group of mammals capable of actual and sustained flight (Sorn, 2022), and with unique anatomy and physiology, which predispose them to fulfill a variety of ecological functions. Bats are the second most diverse among mammalian class. This implies great physiological and ecological diversity and has evolved incredibly rich diversity of roosting and feeding habits (Goodman et al*.,* 2008). They form one of the largest aggregations and the most abundant groups of mammals when measured in numbers of individuals (Goodman *et al.,* 2008; Amponsah-Mensah et al. 2024). Numerous studies have defined the impact of bats as reservoirs of zoonotic viruses (Hayman, 2016), particularly the bat-borne zoonotic, the bat rabies. However, bats perform vital ecological role by pollinating flowers and serve an important role in seed dispersal and perform many ecological services, including invasive pest surveillance (Liu, et al. 2023).

Bats occur universally except in Antarctica (Mansour, et al. 2016; Prasad et al., 2025). In Nigeria, bats are found in all the vegetation zones and are represented by nine families (Happold, 1987). *Epomophorus gambianus* and *Tadarida nigeriae* are savanna woodland bat species in Nigeria that are relatively resistant to human impacts (Willis, et al., 2002). *Epomophorus gambianus* is a common fruit bat that belongs to the family Pteropodidae that is widely distributed in all the savannah zones of Nigeria (Happold, 2013; Gadzama and Bala, 2014). *Epomophorus gambianus* roost singly, in small groups of 12 to 20, and in large groups of greater than fifty individuals (Marshall and Mcwilliam, 1982) and is of least concern according to IUCN Red List. This bat can be considered as part of urban wildlife in most parts of Northeast Nigeria. The increasing ease and efficiency of molecular technique have facilitated the studies on its prevalence, phylogenetic origins (Megali, et al, 2011) and the discovery of new species (Witsenburg, et al., 2012). There are a growing number of cases in which DNA-based identification systems have been applied to higher organisms (Brown *et al.* 1999; Bucklin *et al.* 1999; Trewick 2000; Vincent *et al.* 2000).Mitochondrial DNA (mtDNA) markers are routinely used for phylogenetic studies of bat family, Vespertilionidae (Simmons 2005; Park et al. 2019; Kruskop et al. 2020). The most frequently used mtDNA markers were NADH dehydrogenase subunit 1 (ND1), cytochrome b (Cytb), 12S rRNA, and 16S rRNA (Demos, et al., 2019; Jargalsaikhan, et al., 2022). The mtDNA marker cytochrome c oxidase subunit 1 (COI).was used to reconstruct phylogenetic trees to clarify evolutionary relationships and evaluate genetic diversity parameters of Mongolia vesper bats (Jargalsaikhan, et al., 2022). Therefore, this study investigates the application of mitochondrial DNA amplification for identification of *E. gambianus*, using band pattern analysis.

MATERIALS AND METHODS

Study Sites

The survey of bat roost sites was carried out. Two roost sites in Mubi North in Adamawa State were mapped for this study, The coordinate of each bat roost was taken using a GPS (eTrex™).

Capture of Bats

Bats were captured using mist-nets. Mist-nets (12 × 2.4 m) were hoisted in the tree canopy at the two identified bat roost sites just before twilight (dusk), according to the method described by Hudt (2012). A total of 15 individual megachiropteran bats were trapped during the dry seasons. For conservation reasons, the trap and release method was used to reduce negative effects on the structure and functionality of the bat's roosts.

Bat Identification

Morphological identification of bats was carried according to taxonomic features by Happold (1987) of the wings, the tail and tail membrane, the face, tragus, nose leaf and the palatal ridges.

Collection of Bat Tissue Samples

Samples of the wing membrane of each trapped bat were collected using a puncher and preserved in labeled sterilized collection bottle containing 80% absolute ethanol. Preserved samples were refrigerated until DNA extraction.

DNA Extraction and Electrophoresis

The extraction of genomic DNA was carried out using commercial genomic DNA extraction kits (Inqaba Biotec West Africa Ltd.), following the standard protocols. The quantity of the DNA obtained was checked using spectrophotometry and the quality of PCR products using agarose gel electrophoresis. The PCR amplification of cytochrome c oxidase subunit I (COI) Gene, as the target gene, was achieved by using bat-specific COI primer sequence (Irwin et al., 1991):

Forward primer:

CGAAGCTTGATATGAAAAACCATCGTTG

Reverse primer:

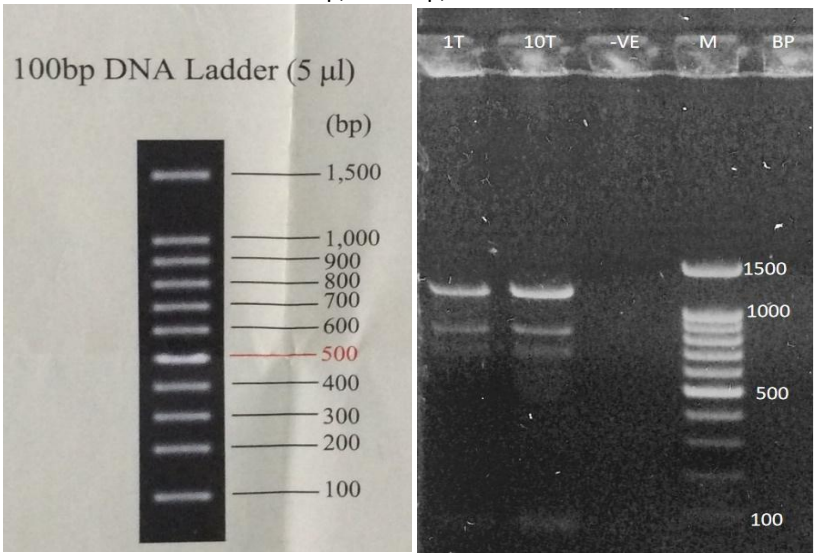
AACTGCAGTCATCTCCGGTTTACAAGAC

The PCR set up constituted the following reaction components: Nucleated-free water dH_2O (6 µl); Forward Primer (1 µM); Reverse Primer (1µM);

2^{x}EasyTaq^{R} PCR SuperMix. For the PCR reaction set-up, the templates, specific primers and water were added to the premix. PCR Conditions (Thermal cycler PTC 100, MJ Research): Pre- Denaturation: 5 minutes at 94 ºC, Denaturation: 30 seconds at 94 ºC, Annealing: 30 seconds at 56 ºC, Extension: 1 minute: 30 seconds at 72 ºC, 35 cycles; Final extension: 5 minutes at 72 ºC. The PCR products were separated on a prepared 1.5% agarose gel, on a casting tray sealed at the ends of the gel chamber with tape and stained with 5 µl Ethidium bromide. This was placed in electrophoresis chamber and covered with Tris-acetate-EDTA (TAE) buffer and visualized under UV illumination. The molecular analysis was carried out at DNA LABS Limited® (RC1027690).

RESULTS AND DISCUSSION

The result of the molecular marker shows that there were clear reference bands around 1500 bp, 1000 bp, 500 bp, and 100 bp. The band size and migration distance in the DNA ladder (M) indicated the top and strongest band is at 1500 bp (Figure 1). The estimated upper and lower band sizes for bat colony 1(10º 26' 49' N: 13º 27' 18' E) (Lane 1T) lie approximately at 680 bp and 470 bp respectively, while that of the bat colony 2 (10º 27' 49' N: 13º 26' 18' E) (Lane 10T) showed respective values of ~670 bp and ~460 bp for *E. gambianus*. This result shows that the ~680 bp and ~670 bp are the likely targeted DNA fragments in the sampled *E. gambianus* bats from the first and second colonies, respectively. Gu et al. (2008) used partial sequences of mitochondrial 12S and 16S rRNA (gene sequences at 2400bp) to investigate intra- and interfamily relationships of three families of bats, with species from the same 3 families retrieved from GenBank along with those of Pteropodidae and Molossidae species.



A **B**

Figure 1: A: 100 bp DNA ladder (5 µl) showing fragment sizes. **B**: Agarose Gel Electrophoresis Showing PCR Amplification of Mitochondrial COI Gene from Bat Tissue Samples. (1T and 10T = Amplified COI products ~ 1300 pb: -VE = control: M: DNA ladder: BP = Base pair scale).

The lower fragments of ~470 bp and ~460 bp can be considered as non-specific products of the first and second (Lane 1T and 10T) bat colonies for the mitochondrial barcode gene cytochrome c oxidase submit I (COI). Genomic approaches to taxon diagnosis exploit diversity among DNA sequences to identify organisms (Kurtzman, 1994; Wilson, 1995). Jargalsaikhan, et al. (2022) reported using a 598 bp long mitochondrial DNA cytochrome c oxidase subunit 1 gene to investigate the genetic

characteristics of 14 species of bats from six genera in Mangolia. Demons et al. (2019) sequenced mitochondrial cytochrome-b (1140 bp) and four independent and informative nuclear introns (2611 bp) for 213 individuals of African Horseshoe bats. The lower bands could suggest non-specific amplification or secondary amplification products. The absence of band on the negative lane (negative control) suggests non-contamination in the PCR reagents in this study. Hebert et al. (2003) evaluated the potential of COI as a taxonomic tool in the analysis and assigning of newly analyzed taxa to their proper phylum and other taxa. Demos et al. (2019) used sequenced mitochondrial cytochrome-b (1140 bp) to investigate phylogenetic relationships and intraspecific genetic variation in the Afro-Palearctic clade of Rhinolophidae. Singh et al. (2018) reported that DNA extraction and successful PCR amplification, are requisites for DNA sequencing and analysis, and emphasized at the quality of DNA, without which the PCR amplification will not be optimal. This study has shown high quality DNA, with the expected PCR fragment at ~650 bp, as the correct COI barcode fragment for sequencing and molecular phylogeny of *E. gambianus* bats. The slightly higher positioning of T1 and T10 amplified COI products which could be indication of possible species variation or morphologically cryptic or difficult to distinguish in the two colonies of bats. Therefore, this study provides fundamental step towards molecular characterization and further phylogenetic analysis of fruits bats in Nigeria.

Ethical Approval

Ethical approval to conduct the research was obtained from Animal Health Care and Ethical Committee of the Adamawa State University Mubi.

Funding acknowledgement: This work was supported by Tetfund IBR grant (Grant No: TETF/DR&D/CE/UNI/MUBI/IBR/2025/VOL.1)

Declaration of competing interest: The authors hereby declare that there are no known competing interest or personal relationships that could have appeared to influence the work reported in this publication.

REFERENCES

Amponsah-mensah, K., Hudson, M.A., Cummingham, A.A. Wood, J,L,N. and Ntiamoa-Baidu, Y. (2024). Demography of the Gambian Epauletted Fruit Bat (Epomophorus gambianus) in Ghana. *Journal of Mammalogy*, 106 (1):168-177.

Brown, B., Emberson, R.M. & Paterson, A.M. (1999). Mitochondrial COI and II provide useful markers for *Weiseana* (Lepidoptera, Hepialidae) species identification. *Bulletin of Entomology Research,* 89, 287–294.

Bucklin, A., Guarnieri, M., Hill, R. S., Bentley, A. M. & Kaartvedt, S. (1999). Taxonomic and systematic assessment of planktonic copepods using mitochondrial COI sequence variation and competitive, species-specific PCR. *Hydrobiology,* 401, 239–254.

Demos, T.C., Webala, P.W., Goodman, S.M., Kerbis, J.C. Peterhans Bartonjo, M. & Patterson, B.C. (2009). Molecular phylogenetics of the African horseshoe bats (Chiroptera: Rhinolophidae): expanded geographic and taxonomic sampling of the Afrotropics. *Evolutionary Biology*, 1 – 14.

Gadzama, I.M.K. & Bala. A.A. (2014). Comparative assessment of the parasitic host-status of *Epomorphorus gambianus* (Ogilby, 1835) and *Tadarida nigeriae* (Thormas, 1913) in Zaria, Nigeria. *Nigeria Journal of Scientific Research* 13 (1) 35-39**.**

Goodman, S. M., Ratrimomanarivo, F. H., Ranivo, J. and Cardiff, S. G. (2008). The hunting of microchiropteran bats in different portions

of Madagascar**.** *African Bat Conservation News*, 16*: 4-7.*

Gu, X., He, S. & Ao, L. (2008). Molecular Phylogenetics among Three Families of Bats (Chiroptera: Rhinolophidae, Hipposideridae, and Vespertilionidae) Based on Partial Sequences of the Mitochondrial 12S and 16S rRNA Genes. *Zoological Studies,* 47(3): 368-378.

Happold, D. C. D. (1987). The Mammals of Nigeria. Clarendon Press, Oxford, 124-1025pp.

Happold, M. (2013).Epomophorus gambianus Gambian Epauletted Fruit Bat. In: Happold, M. Happold, D.C.D. (1987). Edition: Mammals of Africa. Vol. 4. (Hedgehogs, Shrews and Bats). London (UK): Bloomsbury Publishing: p. 242 – 244.

Hayman, D.T.S. (2016). Bats as viral reservoirs. Annual Rev Virology 3(1):77-99. Doi: 10. 1146/annrev-virology-110615-042203.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & deWaard, J.R.
(2003). Biological identifications through DNA barcodes *Proc. R. Soc. Lond.* B 270, 313–321 313 DOI 10.1098/rspb.2002.2218.

Hudt, L. (2012).*Bat Surveys: Good PracGuidelines,2nd edition*. Bat Conservation Trust. Pp. 50 – 67.

Irwin, D.M., Kacher, T.D. & Wilson, A.C. (1991).
Evolution of the cytochrome b gene of mammals. *Journal of Molecular Evolution*, 32(2):128-144. https://doi:org/10.1007/BF02515385.

Jargalsaikhan, A., Bilguun, A., Munkhnast, D.,
Erdenetushig, P., Woon, K. P., Khongorzul, T. & Onolragchaa, G. (2022). Molecular phylogenetic analysis of bats in the family Vespertilionidae (Order: Superfamily) in Mongolia. *Journal of Asia-Pacific Biodiversity,* https://doi.org/10.1016/j.japb.2022.04.006.

Kurtzman, C. P. (1994). Molecular taxonomy of the yeasts. *Yeast,* 10, 1727–1740. In: Hebert, P.D.N., Cywinska, A., Ball, S.L. and deWaard, J.R. (2003). Biological identifications through DNA barcodes *Proc. R. Soc. Lond.* B 270, 313–321 313 Ó 2003. The Royal Society. DOI 10.1098/rspb.2002.2218.

Singh, S., Arya, M. & Vishwakarma, S.K. (2018).
DNA Barcoding: A Significant Molecular Approach for Identification up to Species Level. P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-3 ISSUE-3 E: ISSN NO.: 2455-0817**.**

Kruskop, S.V., Artyushin, I.V. & Yuzefovich, .A.P.(2020). Genetic diversity of Mongolian long-eared bats (*Plecotus*; Vespertilionidae; Chiroptera). *Acta Chiropterologica,* 22:243e255.

Liu. Y,. Si. Y., Huang. Z, & Feng, J. (2023). Bats are sentinels for invasive pest surveillance based on DNA metabarcoding. *Ecologicl Indicators*: 152 (2023) 110354. www.elsevier.com/locate/ecolind.

Mansour, S.A., Soliman, S.S. & Soliman, K.M. (2016).
Monitoring of heavy metals in the environment using bats as bioindicators: first study in Egypt. *Vespertilio,* 18: 61–78. ISSN 1213-6123.

Marshall, A.G., & Mcwilliam, A.N. (1982). Ecological observations epomophorine fruitbats (Megachiroptera) in West African Savannah woodland. *Journal of Zoology*, 198: 53-67.

Megali, A., Yannic, G. & Christ, P. (2011). Disease in the dark: molecular characterization of *Polychromophilus murinus* in temperate zone bats revealed a worldwide distribution

of this malaria-like disease. *Journal of Molecular Ecology*, 20: 1039 - 1048.

Park, S., Noh, P. & Choi, Y.S. (2019). Population genetic structure based on mitochondrial DNA analysis of Ikonnikov's whiskered bat (*Myotis ikonnikovid*) Chiroptera: Vespertilionidae) from Korea. *Journal of Ecology and Environment,* 43:1e8.

Prasad, S.H., Bignell, G., Copeland, R.G., Garg, V., Chitikineni, A., Henry, R. J.,Varshney, R.K.(2025).Integrating multiomics and modern breeding tools for accelerating genetic improvement in Annonas. *Functional and Integrative Genomic,* 25(1):155.

Simmons, N.B. (2005). Order Chiroptera. In: Wilson, D.E, & Reeder, D.M. editors. Mammal species of the World: a taxonomic and geographic reference. 3rd edition. The Johns Hopkins University Press. pp. 312e529.

Sorn, K. (2022). Phylogeny and Taxonomy of Bat. Research & Reviews: *Journal of Zoological Sciences,* Volume 10, Issue 4. eISSN:2321-6190

Vincent, S., Vian, J. M. & Carlotti, M. P. (2000). Partial sequencing of the cytochrome oxidase-b subunit gene. I. A tool for the identification of European species of blow flies for postmortem interval estimation. *Journal of Forensic Science,* 45, 820–823.

Willis, C.K.R., Jennifer, M. & Darren, J.H.S. (2002). "*Chaerephon nigeriae*". *Mammalian Species*: No. 710, pp. 1-3.

Wilson, K. H. (1995). Molecular biology as a tool for taxonomy. *Clin. Infect. Dis.* 20(Suppl.), 192–208. In: Hebert, P.D.N., Cywinska, A., Ball, S.L. & deWaard, J.R. (2003). Biological identifications through DNA barcodes *Proeeding of Royal Society of London,* B 270, 313–321 313 Ó 2003 The Royal Society. DOI 10.1098/rspb.2002.2218.

Wilson, D.E. & Reeder, D.M. (1993). *Mammal species of the world: A taxonomic and geographic reference*.Smithsonian Institution Press, Washington DC.

Wilson, K. H. (1995). Molecular biology as a tool for taxonomy. *Clin. Infect. Dis.* 20(Suppl.), 192–208. In: Hebert, P.D.N., Cywinska, A., Ball, S.L. and deWaard, J.R. (2003). Biological identifications through DNA barcodes *Proc. R. Soc. Lond.* B 270, 313–321 313 Ó 2003 The Royal Society.DOI 10.1098/rspb.2002.2218.

Witsenburg, F., Salamin, N., & Christe, P. (2012). The evolutionary host switches of *Polychromophilus*: A multi-gene phylogeny of the bat malaria genus suggests a second invasion of mammals by a haemosporidian parasite. *Malaria Journal,* 11:53 - 55.