Open Access

Check for updates

Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2026.57.2.290-293

DNA Barcoding- A rapid and reliable tool for molecular identification of buffalo flies *(Haematobia exigua)*

Gotakanapura Sanjeevamurthy Mamatha*[1], Nagaraju Gautham[2], Thiruvengadam Venkatesan[3], Shashidhar Bellari[4], Konanur Javaregowda Ananda[5], Jaya Nagappa Lakkundi[5], Shivaraj Murag[1] and Doddamane Rathanamma[1]

[1]Diagnostic Parasitology, Southern Regional Disease Diagnostic Laboratory, Institute of Animal Health and Veterinary Biologicals, KVAFSU, Hebbal, Bengaluru-560024, [2]Senior Veterinary Officer, AHVS, GOK, [3]Division of Entomology, ICAR-NBAIR, Hebbal, Bengaluru, [4]Department of Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bengaluru, [5]Department of Veterinary Parasitology, Veterinary College, KVAFSU, Hebbal, Bengaluru,

Citation: Mamatha, G.S., Gautham, N., Venkatesan, T, Shashidhar, B., Ananda, K. J., Jaya, N. L., Shivaraj, M. & Rathanamma, D. (2026). DNA Barcoding- A Rapid and Reliable tool for molecular identification of buffalo flies (Haematobia exigua). *Journal of Veterinary and Animal Sciences, 57(2),290-293*
https://doi.org/10.51966/jvas.2026.57.2.290-293

Received: 04.02.2026 Accepted: 10.04.2026 Published: 30.06.2026

Abstract

A study was carried out on DNA barcoding and BOLD for the molecular identification of Haematobia exigua flies collected from cattle farms located in the southern part of Karnataka state. The flies collected using aerial trapping net from all the ten cattle farms were transported to the laboratory and were identified based on the morphological characters. The PCR was carried out for the molecular identification and confirmation of these flies using universal primers by targeting mitochondrial COX1 gene. The genomic DNA extracted from the ten fly pools (3 flies/pool/farm) were subjected to PCR analysis and all the fly pools were confirmed as H. exigua by amplifying 658 bp specific DNA fragment. The resulted PCR products were subjected to sequencing and the obtained sequences were submitted to GenBank data base and uploaded in the metadata analysis(BOLD) to generate the Barcodes. In the present study, only one sequence was submitted and obtained an accession number of PV495814 with BOLD Number (AAC7987). The study aimed at correct identification of flies up to the species level by DNA barcoding and also overcomes the morphological similarity among closely related biting muscid flies within the species.

Keywords: DNA Barcoding, cytochrome oxidase c, PCR, *Haematobia exigua*

The flies belonging to the order Diptera are of economic and veterinary importance in livestock sector. Although about 20,000 species of dipeterans have been categorized, only a small proportion are of veterinary importance because of their impact on animal health. (Subaharan & Verghese, 2014). The buffalo fly (*Haematobia exigua*) grouped under biting muscids is a significant insect pest of livestock known for their persistent blood-feeding behaviour. These haematophagous flies possess piercing and sucking mouthparts that helps them to feed multiple times a day, inflicting painful bites causing irritation, stress and blood loss in cattle. The chronic infestations lead to reduced feed efficiency, lower milk production, impaired weight gain and may predispose animals to secondary infections due to open bite wounds. Moreover, this

Corresponding author: drmamathags@gmail.com, Ph. +91 9886611864

biting fly is a highly competent mechanical vector for various pathogens, including protozoans, bacterial, viral and rickketsiales. Unlike other biting muscid flies, *H. exigua* flies remain on the host most of their lives, feeding frequently and often large numbers (Soulsby, 1982). Each fly ingest an average of 14 µL of blood per feeding and may bite 20 to 38 bites per day leading to daily blood loss per fly ranging from 0.28 to 0.53 mL (Torres *et al*., 2011). They typically congregate along the neck, shoulders, belly of cattle and primarily prefer cattle as their main primary host (Scasta and Smith, 2018). Their life cycle is closely associated to fresh cattle dung, where eggs are laid and larvae develop.

Traditionally, fly identification has relied on morphological traits such as wing venation, antennae and body structures using binary keys. This approach which require expert knowledge while also providing insights into their evolutionary relationship (Hebert *et al*., 2003, Kachhawa and Ghanshyama, 2023). Further, molecular tools complement traditional taxonomy and are increasingly applied in surveillance and ecological studies. Additionally, there is insufficient molecular literature available on DNA barcode databases for buffalo flies. Therefore, the present study was undertaken for the molecular identification of buffalo flies by DNA barcoding and Barcode of Life Data Systems (BOLD) to generate DNA barcodes.

Material and methods

Collection of flies and extraction of genomic DNA

The buffalo flies were collected from ten cattle farms located in the central (Bengaluru Urban and Bengaluru Rural districts), eastern (Tumakuru and Chikkaballapur districts) and southern zones (Ramanagara and Mandya districts) (https://waterresources.karnataka.gov.in) of southern Karnataka state during the period from June 2024 to May 2025 using the customised aerial net. All the farms were situated with an average altitude of 600 to 900 meters above the sea level. The collected flies were transported to the laboratory for the morphological studies and were identified upto the species level (Zumpt *et al.,* 1973; Soulsby, 1982). Later, the identified flies were preserved in 70% ethanol for further identification by molecular analysis.

The genomic DNA extraction procedure for flies was carried out as detailed below. The genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's protocol with minor modifications. About, three adult flies per farm (one pool) were selected for DNA extraction. Flies were removed from ethanol and washed in sterile Petri dish using distilled water**,** three washes were performed with freshly replaced distilled water each time. Washed flies were dried on sterile tissue paper and transferred to glass cavity blocks**.** To enhance tissue disruption, specimens were subjected to three freeze thaw cycles**.** Each cycle included 15 minutes of freezing at -20°C followed by 10 minutes of thawing at room temperature**.** After freeze thawing, wings were dissected off to reduce chitinous exoskeleton material. Remaining body tissues along with the legs (chosen for their high mitochondrial content) were crushed using a sterile mortar and pestle. The homogenate was transferred to 2 ml microcentrifuge tube and 180 µl of ATL buffer (from Qiagen kit) was added to each tube. Further, tissue homogenisation was performed using a hand held pellet pestle (Sigma-Aldrich, USA)**,** at the rate of one minute per cycle and repeated three times with intervals**.** The lysate was then processed as per the standard protocol of the DNeasy Blood and Tissue Kit for DNA purification. DNA was eluted by adding 60 µL of Buffer AE and was stored at -20°C for further DNA barcoding studies.

DNA barcoding using polymerase chain reaction

The barcoding studies were carried out by targeting mitochondrial *COX1* or COI gene using the universal primers to amplify 658 bp DNA fragement as described by Hebert *et al*. (2003). The Forward primer LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and Reverse primer HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) were used during the study. The polymerase chain reaction (PCR) was performed in a final volume of 25 µl, containing 12.5 µl of PCR Master Mix, 1 µl each of forward and reverse primers, 3 µl of DNA template, and 7.5 µl of nuclease-free water. The thermocycling conditions included an initial denaturation of 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1 min and final extension at 72 °C for 10 min) with slight modification and the amplification was carried out at National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru using a C1000™ Thermal Cycler.

The amplified products were examined by 1.5% agarose gel electrophoresis, following the protocol as described by Sambrook and Russell (2001). The purified PCR products were subjected to bi-directional Sanger sequencing at Eurofins Genomics India Pvt. Ltd, Bengaluru. The obtained sequences were analysed for homology, insertions, deletions, stop codons, and frameshifts using the NCBI BLAST tool. All verified sequences were submitted to GenBank data base, to obtain accession numbers. Additionally, the sequences and associated metadata were uploaded to the Barcode of Life Data Systems (BOLD) to generate DNA barcodes.

Results and discussion

The molecular identification through DNA barcoding was undertaken as part of an integrated approach to accurately identify buffalo flies. During the present study, though morphological examination initially confirmed the presence of *H. exigua* among the collected specimens. All the flies obtained from various farms (ten fly

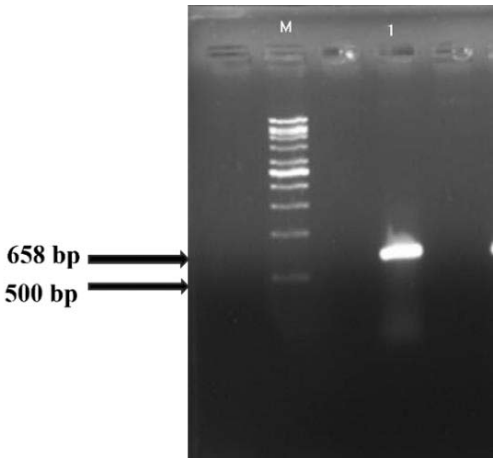


Fig: 1. PCR based amplification of the cytochrome c oxidase subunit I (COI) gene for *Haematobia exigua*

M : 1 kb marker
Lane 1 : *H. exigua*

pools) were subsequently subjected to DNA barcoding for rapid and correct identification by targeting mitochondrial cytochrome c oxidase subunit I (COI) gene with expected amplicon size of 658 bp (Fig. 1). During the study, all the ten fly pools amplified the DNA fragment specific for *H. exigua*. Only one obtained sequences were submitted to GenBank database and submitted sequence was assigned with the accession number PV495814. The identification barcode was obtained through BOLD (Table 1). The positive control was not available during the study, hence the obtained sequences were confirmed by blast analysis. Nuclease free water(NFW) was used as a negative control.

Due to the economic importance and vector potentiality of *H.* exigua flies in cattle farms has influenced necessiated development of more comprehensive and reliable species databases essential for accurate identification. The cytochrome c oxidase subunit(COI) gene has been widely recognised for its high interspecific variability and low intraspecific divergence, making it an ideal barcode region for insect identification, including hematophagous dipterans (Hebert *et al*., 2003; Kachhawa and Ghanshyam, 2023). The present study successfully employed DNA barcoding for molecular identification targeting the COI gene to confirm the identity of H. exigua. Similarly, the previous researchers have also successfully utilised COI-based barcoding for other biting muscids such as stable fly (Stomoxys calcitrans) from different geographical regions, suggesting its utility in distinguishing closely related Stomoxys species (Changbunjong *et al*., 2016a; Sumruayphol *et al*., 2023). Similarly, the barcoding of H. exigua has been employed in studies from South America, Africa, and Australia, but molecular data from the Indian subcontinent remain scarce (Low *et al*., 2014). The current study augments critical sequences to this global dataset, particularly as *H. exigua* records in GenBank are limited. Further, the assignment of BOLD system identification codes for representative isolates enhanced interoperability between genetic repositories, facilitating global accessibility and cross-referencing of these sequences. Similar efforts such as integrating GenBank and BOLD databases have been recommended to improve bio-surveillance and enable the rapid detection of invasive vector species (Ratnasingham , 2007; Renaud *et al*., 2012). The present study is the first report on generation of barcodes againt *H. exigua* in India, particularly in Karnataka state.

Conclusion

To conclude, the combined use of morphological keys and COI barcoding addresses a major challenge in dipteran taxonomy, the morphological similarity among closely related biting muscid flies**,** particularly at immature stages or when specimens are damaged. Similar integrative approaches have been advocated for other biting flies, improving species-level resolution and avoiding misidentification that could bias epidemiological data. Therefore, an integrated taxonomic approach that combines traditional morphology is required with molecular tools for precise species confirmation**,** targeted vector surveillance and control programs.

Conflict of Interest

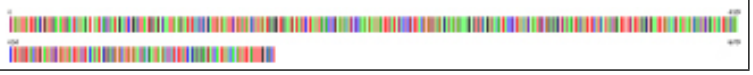
All the authors declare that, there is no conflict of interest.

References

Changbunjong, T., Weluwanarak, T., Samung, Y. and Ruangsittichai, J. 2016a. Molecular identification and genetic variation of hematophagous flies (Diptera: Muscidae: Stomoxyinae) in Thailand based on cox1 barcodes. *Journal of Asia. Pacific Entomol*ogy, **19**(4): 1117-1123. https://doi.org/10.1016/j.aspen.2016.10.006

Hebert, P. D. N., Cywinska, A., Ball, S. L., deWaard, J. R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences,1***270**(1512): 313-321. https://doi.org/10.1098/rspb.2002.2218

Table 1: GenBank and BOLD Identification codes

Species	Accession No.	BOLD ID	Barcode ID
H. exigua	PV495814	BOLD:AAC7987	

Kachhawa and Ghanshyama. 2023. DNA barcoding a review: modern tool for species identification of insects. *CIBTech Journal of Zoology,* **12**(S1): 144-155.

Low, V. L., Tan, T. K., Lim, P. E., Domingues, L. N., Tay, S. T., Lim, Y. A., Goh, T.G., Panchadcharam, C., Bathmanaban, P. and Sofian-Azirun, M. 2014. Use of COI, CytB and ND5 genes for intra- and inter-specific differentiation of *Haematobia irritans* and *Haematobia exigua*. *Veterinary Parasitology* **204**(3-4): 439-42. 10.1016/j.vetpar.2014.05.036

Ratnasingham, S. 2007. Hebert PDN. BOLD: The Barcode of Life Data System. *Molecular Ecology Notes,* **7**(3): 355-364. doi: 10.1111/j.1471-8286.2006.01678.x

Renaud, A. K., Savage, J. and Adamowicz, S. J. 2012 DNA barcoding of northern Nearctic muscid flies. *BMC Ecology,* **12**(6): 1040-1051. https://doi.org/10.1186/1472-6785-12-24

Sambrook, J. and Russell, D. W. 2001. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, pp. 1.1–1.162.

Scasta, J. D. and Smith, T. 2018. Commingled black and white cows (Bos taurus; Angus and Charolais) in high-elevation rangeland are differentially parasitised by Haematobia irritans. *Animal Production Science,* **59** (9): 1727-1738. https://doi.org/10.1071/AN18400

Soulsby, E. J. L. 1982. Helminths, Arthropods and Protozoa of Domesticated Animals. 7th ed. London: Baillière Tindall, pp. 527-537.

Subaharan, K. and Verghese, A. 2014. Dipterans of economic importance on veterinary animals. Insect Environment, 20(3): 64-69.

Sumruayphol, S., Ruangsittichai, J., Phumee, A., Siriyasatien, P., Samung, Y. and Chareonviriyaphap, T. 2023. Molecular characterization and genetic diversity of three Stomoxys flies *S. bengalensis*, *S. calcitrans*, and *S. sitiens* (Diptera: Muscidae). in Central Thailand. *Infection Genetics and Evol*ution, **112**: 105455. https://doi.org/10.1016/j.meegid.2023.105455

Torres, L., Almazan, C., Ayllon, N., Galindo, R.C., Rosario-Cruz, R., Quiroz-Romero, H. and de la Fuente, J. 2011. Functional genomics of the horn fly, Haematobia irritans (Linnaeus, 1758). BMC Genomics, **12**: 105. https://doi.org/10.1186/1471-2164-12-105

Zumpt, F. (1st ed.), 1973. The Stomoxyine Biting Flies of the World. Stuttgart: Gustav Fischer Verlag, 175 pp.

■