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Studies on Molecular Taxonomy and Morphological Characteristics of *Haematobia irritans exigua* (de Meijere, 1903); A New Range Extension in the Bargur Hills of Erode District in Tamil Nadu, India

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Authors' contributions

This work was carried out in collaboration among all authors. Author YS did the insect collection, morphological and molecular analysis and written original manuscripts. Authors VR and GP as the research supervisors and review and edit the manuscript. Author VR does review and edits the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The flies of the genus *Haematobia* (Diptera:Muscidae) are hematophagous ectoparasites of medical and veterinary importance. The morphological identification of these flies is often complicated due to their similarities. This was the first documentation of *Haematobia irritans exigua* in Tamil Nadu which affected free-ranging Bargur Cattle (*Bos indicus*) from Bargur Hills of Tamil Nadu. The aim of this research was to provide the details on the molecular characterization and molecular divergence of the cytochrome c oxidase subunit 1 gene (COI) and 28S Ribosomal RNA of *Haematobia irritans exigua* and to compare them with the morphological features. The results of this study showed that the nucleotide composition of the COI gene of *Haematobia irritans exigua* had a higher AT (69.5%) and a lower GC (30.4%). The rate of transition was higher than that of transversion. The intra-specific distance based on COI gene analysis did not exceed 1.1%, while the inter-specific distance between different species ranged from 0.9% to 14.1%. A neighbor-joining tree and an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) tree were constructed using 1000 bootstrapped samples to analyze the phylogenetic relationship between different muscidae species based on the COI gene as an identification marker. The analysis of 28S Ribosomal RNA showed an intra-specific distance of 1.6% and an inter-specific distance ranging from 1.49% to 8.56%. The 28S rRNA revealed the presence of intra-specific variation among the species. Additionally, this study found notable color variations on the thorax of the flies in the same population, along with a minute phenotypical variation with minimum intraspecific distance among the flies in the same population. This taxonomic data of the hematophagous fly *Haematobia irritans exigua* would be well-intentioned for identification.

Keywords: Bos indicus; DNA barcoding; COI; 28S Ribosomal RNA; Haematobia irritans exigua (de Meijere,1903).

1. INTRODUCTION

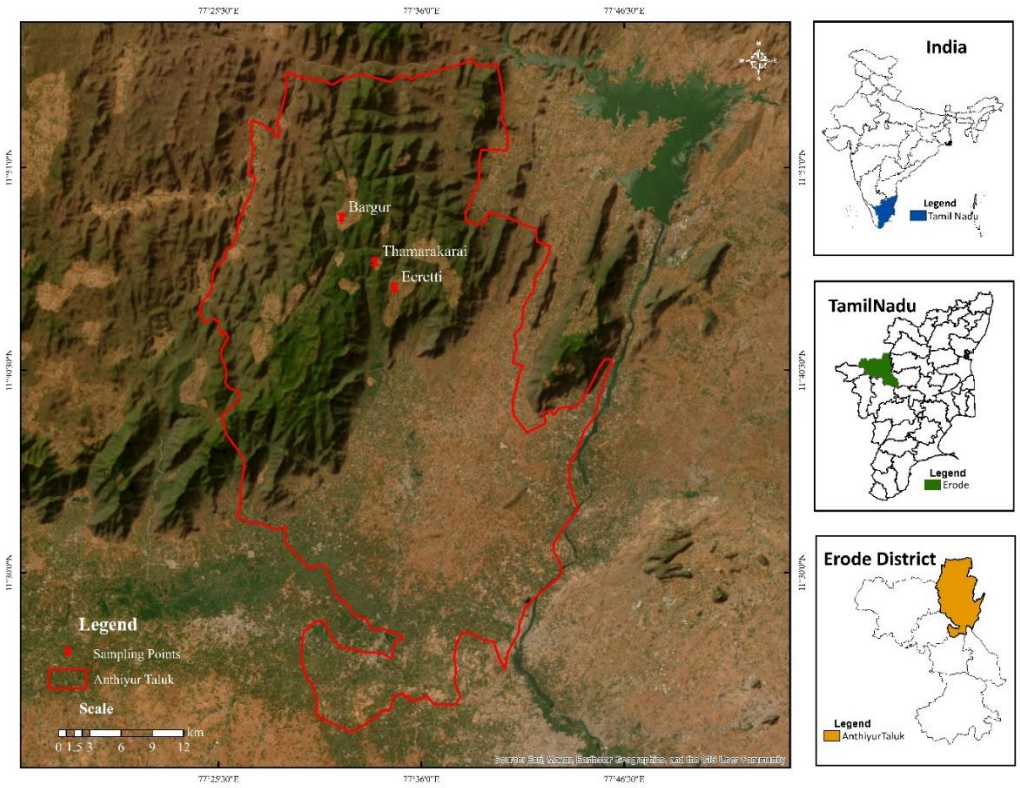
The flies belong to the order Diptera of the cosmopolitan distributed genus *Musca* (Linnaeus,1758) with evolved mouthparts required for blood feeding causing blood loss for the host and acting as the vector for the transmission of pathogens [1,2]. Insect identification is important to understand the interaction among the host [3] and to report the essential queries in evolution, economic and ecological threats, and conservation biology [4]. The morpho-taxonomy has some limitations such as a lack of experts, resolving cryptic species, sexual dimorphism, adaptations, etc. [5,6]. The misidentification of veterinary and medicinal important flies will lead to ineffective control and management [7,8,9]. The proper identification of flies is essential for both epidemiological studies and to formulate control strategies [10]. Molecular identification is a potential tool to identify the species by matching unknown genes with known genes of a species [11]. Molecular gene markers are the more sensitive and accurate method for the identification of parasitic flies affecting livestock farms [12]. DNA barcoding is a complementary tool in the identification process by using some conserved and standardized regions of DNA [13]. The mitochondrial DNA, mitochondrial cytochrome c oxidase subunit I gene (COI gene) is one of the most useful gene markers for the identification of insects and analyses of the phylogenetic relationship [14]. COI marker helps in the identification of cryptic species and provides taxonomic-based diversity estimation [13]. In addition to the Mitochondrial gene, 18S and 28S Ribosomal RNA were used for the molecular identification of the lower taxonomy up to the species level [15]. The taxonomic status of *Haematobia* taxa is still misperception because of the similarity in morphological characteristics [16]. There are variations between the taxa are minor. The Buffalo flies (*Haematobia irritans exigua* de Meijere) and horn flies (*Haematobia irritans* Linnaeus) are the major flies with a minor morphological variation. The *Haematobia irritans exigua* (Buffalo fly) is a dipteran hematophagous fly affecting cattle and buffalo in the oriental region [17]. Both male and female flies feed solely on blood, piercing the animal's skin through their sharp mouthparts only ovipositing on the cattle dung. The heavy infestations of these flies may include several thousand flies per animal, with each feeding up to 40 times daily, irritating cattle and causing economic losses and welfare issues, reducing hide value and making cattle less appealing to consumers [18]. These lesions can range from dry and alopecic or scab-encrusted to severe open ulceration which lead

to secondary bacterial infections [19]. They are most commonly found beneath the cattle's eyes, but can also be found on the neck, dewlap, belly, and flanks [20], and their development and persistence have been linked to a currently unidentified variety of filarial nematode (*Stephanofilaria sp*.), which is transmitted by buffalo flies [21]. Bargur cattle are the indigenous free-ranging cattle endemic to the Bargur hills of Anthiyur taluk of Erode district in Tamil Nadu [22]. The free-ranging Bargur cattle were affected by *Haematobia irritans exigua.* The study aims to identify the molecular and morphological taxonomic status of *Haematobia irritans exigua* affecting the bargur cattle. The present study on identification and characterization of flies by using molecular markers COI and 28S rRNA and compares with the morphological character to provide clear data on the taxonomical identification of *Haematobia irritans exigua.* This was the first report on Bargur hills of Tamil Nadu.

2. MATERIALS AND METHODOLOGY

2.1 Study Area

The Bargur hills is located between latitude of 11.805° and longitude of 77.534° in the Anthiyur taluk of Erode district in the Eastern Ghats region with elevation of about 1000 m above the mean sea level (MSL). The average temperature ranging from 15^0c to 32^0c and rainfall about 350 cm per annum. There are 34 hamlets are located in the Bargur hills namely Bargur, Thamarakarai, Thalakarai, Thurusanampalayam, Bejilpalayam, Oosimalai, Eeretti, Devarmalai, Bejilitti and Ondanai. The highest point is at Thamarakarai with 1078 m above MSL and lowest at Bejiletti with 988m above MSL. These areas receive rainfall during both the south-west and north-east monsoon. The Ragi, Maize, Dry paddy, Tapiocca and other rainfed crops are majorly cultivated agricultural crops. There numerous cattle are being reared in and around the Bargur areas but indigenous Bargur cattle are maintained under zero input with unique '*Patti*' system. These cattle are coexisting with the wild animals especially with elephants which huge existing in this forest so called as semi wild types of cattle. The total area of the Bargur cattle breeding tract is about 34,043 ha and most of which comes under reserve forest category and now Bargur forest is announced a Periyar Wildlife Sanctuary by Tamil Nadu Forest Department.



Map. 1. Study Area Bargur Hills in Tamil Nadu, India

2.2 Sample Collection and Identification

The ectoparasites were collected from all over the body of the Bargur cattle by using the hand-made insect collection net. The collected insect was sprayed with 99% ethanol and then transferred into the glass bottles. The insect samples were taken to the laboratory for further morphological analysis. The parasites were identified with the help of a standard identification key.

2.3 Insect Measurements

The male and female insects were identified based on their morphological characteristic features. The following measurements such as total body length, wing length, and wing breath were taken by using a vernier caliper.

2.4 COI Gene Isolation and Amplification

DNA was extracted from the whole insect sample using a DNeasy Blood and Tissue kit (Qiagen; Cat # 69504) Kit following the manufacturer's instructions. The DNA of each individual was eluted in 50µl and stored at -20°C until further use of DNA amplification. The COI gene was amplified using the primer LCO1490 (5'-CACGACGTTGTAAAACGACGTCAACAAATC ATAAAGATATTGG-3') and HCO2198 (5'GGATAACAATTTCACACAGGTAAACTTCAG GGTGACCAAAAAATCA-3') [23]. The COI genes of insect DNA were amplified in a 20 µl reaction volume containing 1 µl DNA (10–50 ng), 1 µl each of Forward and Reverse primers (10 picomoles µl-1), and 10 µl Emerald Amp GT PCR master mix. The cycling conditions used were as follows: initial denaturation at 95°C for 2 min followed by cycle denaturation at 95°C for 40 sec, annealing at 45°C for 40 sec., extension at 72°C for 1.5 min for a total of 30 cycles, and a final extension for 10 min at 72 °C. The success of the PCR reaction was confirmed by running 5µl PCR product on 1 % agarose gel with ethidium bromide at 120 V; for ~45min in 1X TAE Buffer. The PCR products were further treated with ExoSAP-I. PCR Product Clean-up Reagents were used as a template for sequencing PCR product. Sequencing PCR product was done with ABI PRISM Big Dye terminator ready reaction mix (Life Technologies, USA) available in Enfys Lifesciences Pvt Ltd, Cochin, Kerala.

2.5 28s Ribosomal RNA Isolation and Amplification

The whole sample was picked up and placed in a mortar and homogenized with 1 ml of extraction buffer and the homogenate was transferred to a 2 ml-microfuge tube. The DNA isolation was done by phenol chloroform method and to remove RNA 5 µl of DNAse free RNAse (10 mg/ml) was added to the DNA. 177 ng of extracted DNA is used for amplification along with 10 pM of each primer. The PCR product size is ~0.8 kb using the forward primer sequence (5'-ACTACCCCCTGAATTTAACAT-3') and the reverse primer (5' GACTCCTTGG TCCGTGTTTGAAG-3'). Each reaction mixture for the amplification of the 28S rRNA gene by PCR consists of 1 ul DNA, 2µl 28s Forward Primer, 2µl Reverse Primer, 4 ul dNTPs (2.5mM each), 10 ul 10X Taq DNA polymerase Assay Buffer, 1 ul Taq DNA Polymerase Enzyme (3U/ ml), 30 ul Water. The PCR cycle conditions were as follows: 94°C for 3 minutes; followed by 94°C for 3 minutes, 50°C for 1 minute and 72°C for 2 minutes repeats for 30 cycles, and a final extension of 72°C for 7 minutes. The PCR product was sequenced Bi-directionally. Sequencing was done by using the ABI 3130xl platform in Biokart genomic lab, Bangalore.

2.6 Bioinformatics Analysis

The sequences were analysed, trimmed, and edited by using Bio edit software. The sequences obtained from the present study were compared with already submitted sequences with NCBI to analyse the nucleotide composition, Homogeneity of Substitution, Genetic Distance, and Phylogenetic analysis were done by using Mega 11 software.

3. RESULTS

3.1 Morphological Measurements of Male and Female *Haematobia irritans exigua*

The average body length of the male flies was 4.55 (0.48 ± 0.09) mm, wing length was 2.32 (0.50 ± 0.10) mm and wing breadth was 1.04 (0.25 ± 0.05) mm. The average body length of female flies was 4.09 (0.54 ± 0.10) mm, wing length was 1.95 (0.44 ± 0.08) mm and wing breadth was 1.14(0.3 ± 0.06) mm. The body length and wing length ($r=0.8$) and the wing length and wing breadth ($r=0.7$) show a positive correlation.

3.2 Morphological Characteristics of *Haematobia irritans exigua*

Head: The both male and female adult flies had piercing and sucking mouth part. The maxillary palp was shorter than the proboscis and held

apart. The antenna has six to seven aristae. The eyes were dark red in fresh samples (Fig. 1.). The space between the eyes was the main distinguishable character between males and females and male. The eyes are close together in the male (Holoptic) and wider spaced in female (Dicoptic). A single row of bristles was present on each side of the midline between the eyes (Fig. 7.).

Thorax: Thorax has two dark inconspicuous stripes on the mesothorax region (Scutum) (Fig. 5*.)*. A pair of presultural supra-alar seta and a pair of postsultural supra-alar seta was present. A pair of postalar seta and a pair of intra-alar seta were present at the lateral proximal end of the scutum region. A pair of dorsocentral seta and a pair of acrostichal seta were present in the middle above the scuto-scutellar suture. The scutellum region was dark with a pair of basal scutellar seta and a pair of apical scutellar seta (Fig. 6.).

Abdomen: The abdomen was oval, dark without any markings, attached anteriorly to the thorax. In females, the distal segments were modified to form an ovipositor (Figs. 3 and 4).

Legs: The presence of two long hairs at the distal tibia followed by two long hairs with curled tip on the first segment and 4 to 8 long hairs with curled tip on the second segment of the hind tarsus of male flies while this character was absent in female files (Fig. 2).

Wings: Wing vein M1 is gently curved forward. The wings had a dark hint due to minuscule pubescence (Fig. 8).

3.3 Molecular analysis of *Haematobia irritans exigua*

The NCBI (GenBank) sequences accession numbers are PP335804, PP336418 for the COI gene, PP335205, PP335233 for 28S ribosomal RNA. Also, sequences were recorded in Barcode Index Number Registry BOLD:AAC7987 in the Barcode of Life Data Systems (BOLD).

Molecular Analysis Based on Mitochondrial Cytochrome c Oxidase Subunit I Gene

Nucleotide composition of specimen: The nucleotide sequence and particular nucleotide percent have been evaluated due to their importance in the study of variations among the different species. The nucleotide composition of the sample was analyzed to know the percentage of base content by using Mega 11 software. The average percentage of T (40.5%), A (29.0%), C (14.2%), and G (16.3%). The higher AT percentage (69.5%) and the lower GC percentage (30.4) were found in *Haematobia irritans exigua*. The variations in the A+T content was higher than G+C. The higher A+T-rich region is the distinguishing makeup of insect mtDNA [24].

Maximum composite likelihood estimate of the pattern of nucleotide substitution: The entry shows that probability of substitution (r) from one base (row) to another base (column). The sum of r values was made equal to 100. The nucleotide frequencies are (28.90%) A, (40.14%) T/U, (15.49%) C, and (15.46%) G. The transition/transversion rate ratios are k_1 = 0.741 (purines) and k_2 = 4.515 (pyrimidines). The overall transition/transversion bias was R =1.272, where $R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]$. The most frequent transitions were T to C type. There were 24 nucleotide sequences involved for the analysis. The codon positions included were 1^{st} + 2^{nd} + 3^{rd} + Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were total of 356 positions in the final dataset.

Test of the homogeneity of substitution patterns between sequences: The sequences have evolved with the same pattern of substitutions and the probability of rejecting the null hypothesis that, as judged from the extent of differences in base composition biases between sequences (Disparity Index test). A Monte Carlo test (500 replicates) was used to estimate the *P*-values, which are shown below the diagonal. The *P*-values smaller than 0.05 are considered significant. The estimates of the disparity index per site are shown for each sequence pair above the diagonal. Codon positions include 1st+2nd+3rd+Noncoding for 24 nucleotide sequences.

Genetic distance: The intra-specific genetic distance range of flies varies between 0.3% to 1.1%. Similarly, the interspecific genetic distance between the *Muscidae flies* was 1.1% to 14.1%. The maximum genetic distance was observed with *Musca domestica* 13.0% to 14.1% followed by *Haematobosca sanguinolenta* with 9.4% to 13.8% and *Stomoxys indicus* with 6.3% to 12.9%. The least inter-specific distance was found with *Haematobia irritans* 0.3 % to 1.7%. The minimum genetic distance was noted because the *Haematobia irritans exigua* was a subspecies of *Haematobia irritans*. The *Tabanus biannularis* was taken as the out-group and shows the maximum genetic distance of 20.6% to 22.7%.

Fig. 1 Adult *Haematobia irritans exigua* (female)

Fig. 2. Hind Leg of Male *Haematobia irritans exigua*

Fig. 3. Female *Haematobia irritans exigua*

Fig. 4. Male *Haematobia irritans exigua*

Fig. 5. Dorsal view of Thorax

Fig. 6. Scutellum region

Fig. 7. Head variation

Fig. 8. Wing Venation

Phylogenetic analysis: The evolutionary relationship of the COI gene obtained during the study together with the sequences available from NCBI by using the Neighbor-Joining method (Fig. 9.) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method (Fig. 10.). The percentage of replicate trees in which the associated taxa clustered together in the

bootstrap test with 1000 replicates. The Phytogenic tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and units of the numbers of base substitutions per site. The proportion of sites where at least one unambiguous base was present in at least one sequence for each descendent clade was shown next to each internal nodes of the tree. The phylogenetic analysis revealed that species belonging to the same genus because the same node formed with two branches. The first branch had two clades of *Haematobia irritans exigua*. The first clade was occupied by *Haematobia irritans exigua* from India and Japan and the second clade with Thailand flies. The *Haematobia irritans* was listed in the second branch and formed three clades. The specimen shows 100% similarity with the *Haematobia irritans exigua.* The *Tabanus biannularis* was taken as the outgroup for the phylogenetic analysis.

3.4 Molecular Analysis Based on 28S Ribosomal RNA

Genetic distance: The intra-specific variations showed between the sample was 1.16%. The variation between the same genus was 1.49%, and the results *Haematobia irritans exigua* may be a sub species varies from *Haeamatobia irritans* because inter-specific variation ranges from 3.52% to 8.56%.

Phylogenetic analysis: The neighbor-joining analysis of 28S rRNA with 1000 bootstraps by the Kimura 2-parameter model showed similar topologies. The node-linking sequences of the same *Haematobia* species had high bootstrap values. The tree clustered into four major clades, the first clade consists of *Haematobia irritans exigua*, the second clade consists of *Stomoxys calcitrans,* and the third clade consists of Musca domestica (Fig. 11). The phylogenetic analysis shows that the sample was more similarity with the *Haematobia irritans exigua* with minimum variations.

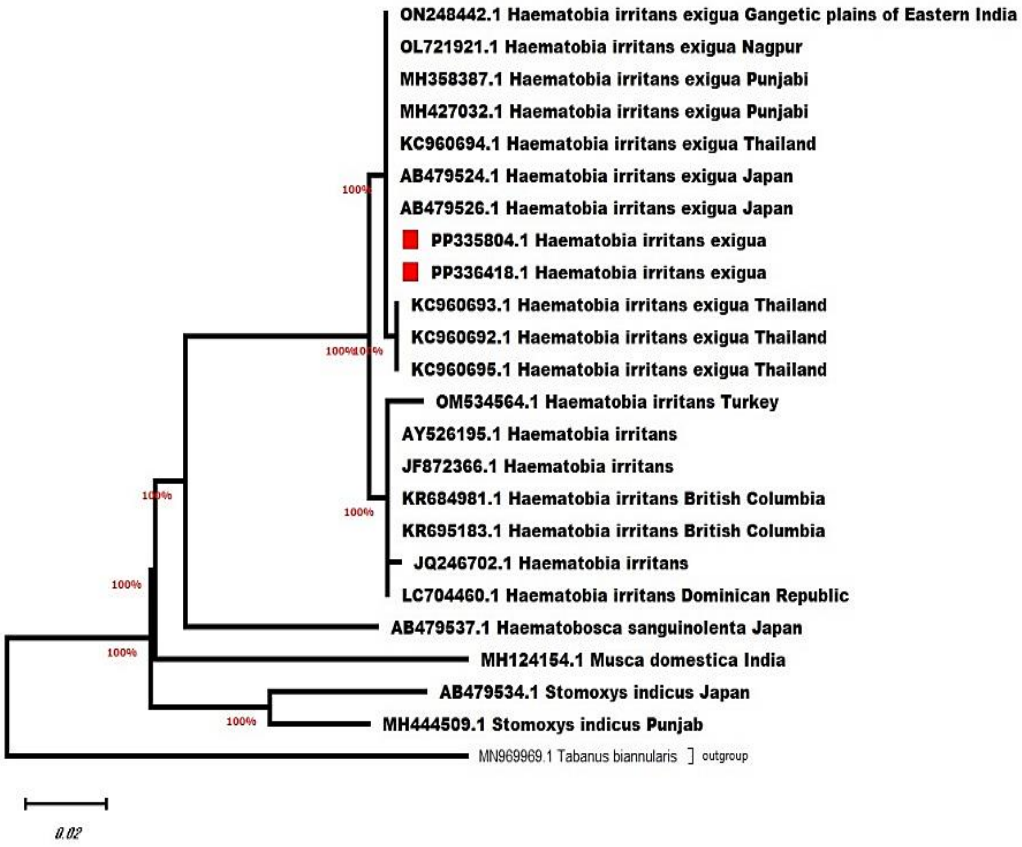


Fig. 9. Neighbour-Joining Tree of COI gene

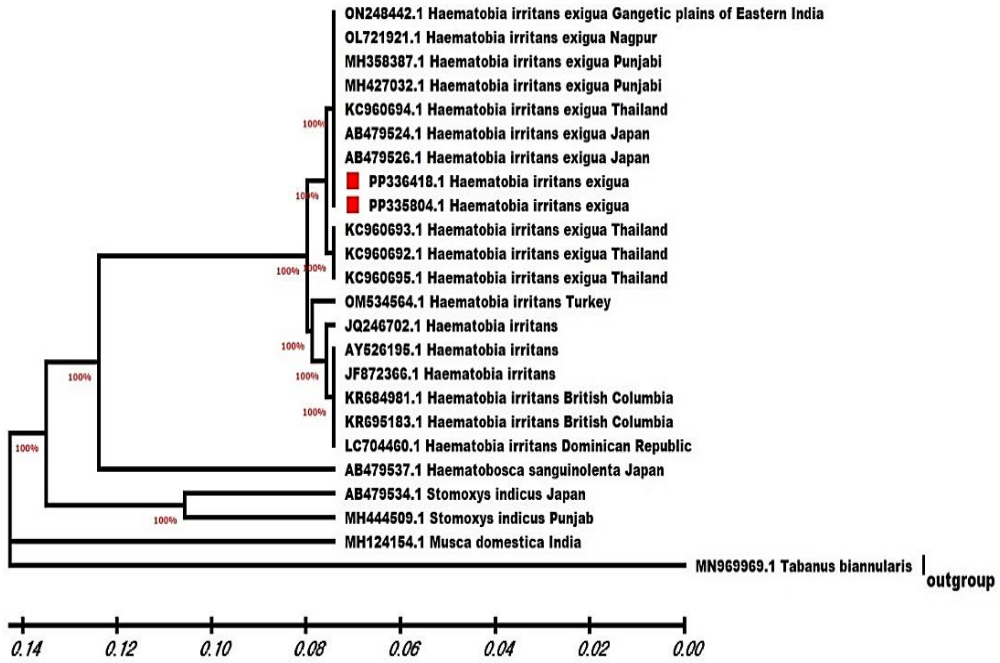


Fig. 10. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Tree of COI gene

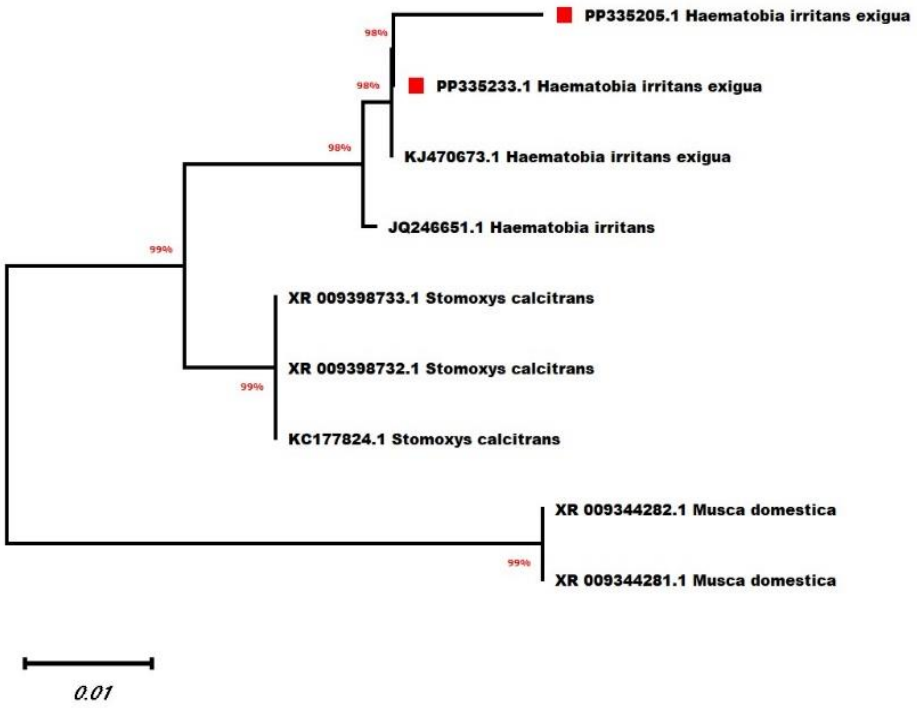


Fig. 11. Neighbour-joining tree of 28S Ribosomal RNA

4. DISCUSSION

The Stomoxyini is the bloodsucking insects under the tribe of Muscinae subfamily of Muscadiae comprising about 50 species in the world [1]. In Stomoxyini, the horn flies *Haematobia irritans* with two closely related subspecies of *Haematobia*, the horn fly, *H. irritans irritans* and the buffalo fly, *H. irritans exigua* (De Meijere), have morphological and genetic differences [25,16]. *H. irritans* is mostly distributed in the Holarctic Region and *Haematobia exigua* is distributed throughout the Oriental and Australasian Regions. Morphological differences between these two species are very minor. *Haematobia Lepeletier & Serville, Haematobia minuta (Bezzi,1872), Haematobia sanguisugens (Austen,1909), Haematobia titillans (Bezzi,1907), Haematobia exigua* were found in India [26]. The body length and number of frontal setulae between the populations of *Haematobia irritans* and *Haematobia exigua* collected from various localities were reported along with the morphological variation based on the latitudinal gradient [27]. The genetic variability in the Brazilian population of *H.irritans* which dispersed throughout the country through cattle trade [28]. In *H. exigua*, variations in male and female body length and number of frontal setulae in males between Taiwan and Vietnam may be due to minor genetic variations (0.3–0.4%) and more variable from Vietnam (0.4%) suggested as the presence of more intraspecies gene diversity in the tropical region [14]. *H.exigua* prefers water buffalo than cattle in the Oriental region [29]. The *H.irritans* and *H.exigua* had adapted based on the different geographical areas [14]. The distinguishable character of *Haematobia exigua* from other *Haematobia* species is the presence of 4 to 6 long hairs with curled tips on the second segments of the male's hind tarsus [27]. The *Haematobia irritans* and *Haematobia exigua* have often been regarded as different species [30,31], or as subspecies [25,31]. There are only a few studies that have looked at the molecular characterization and variability of *Haematobia* species in terms of genetic surveys [32]. The genetic and morphological differences between *Haematobia irritans* and *H. exigua* and molecular phylogeny of Japanese stomoxyini flies were reported [14]. The two species are genetically distinct, as shown by recent studies on molecular differentiation of the two species using the mtDNA COI, cytochrome B (Cytb), NADH dehydrogenase subunit 5 (ND5), nuclear, and 18S and 28S ribosomal RNA regions [16,17]. The most informative genes for differentiating the two species were the COI and Cytb genes. All evidence points to a very close relationship between buffalo and horn flies, regardless of whether they can be classified as distinct species [33]. The transitional substitution frequency is known to be higher than the transversional substitutions in a genome [34]. The high level of sequence divergence with transition saturation indicates saturation in data. Our study shows a higher rate of transversions than the transitions lead to considerable saturation of the sequences. By analyzing these substitutions, the transversion contains a stronger phylogenetic signal. The invertebrate DNA barcoding shows 3 to 3.9% of intra-specific variation [35]. According to Iwasa and Ishiguro (2010), the mt-DNA in the COI to COII genes of buffalo flies from Taiwan and Vietnam and horn flies collected from Japan showed sequence divergence of 1.8% to 1.9% between the two species. Based on the COI gene, the least inter-specific divergence was found with *Haematobia irritans* 0.3 % to 1.7%. The intra-specific genetic divergence range of flies varies between 0.3% to 1.1%. The genetic distance between the haplogroups of *H.exigua* was about 0.12-0.95% was lower than the distances between the *H.irritans* and *H.exigua* [17]. Based on the analysis of 28S rRNA the intra-specific variation between the sample flies was 1.16%. and the variation between the same genus is 1.49%, The inter-specific variation ranges from 3.52% to 8.56%. According to the 10X rule, the percentage of nucleotide divergence between intraspecies should be less than 3%, while interspecies divergence should exceed 3%. The sequence analysed in this study showed significant inter-species variability based on nucleotide sequences and the intraspecific divergence was high enough to distinguish between individuals. The mitochondrial DNA variations provide the phylogenetic relationship of various insect groups at a generic level [36,37].

5. CONCLUSION

This was the first report of *Haematobia irritans exigua* with morphological and molecular identification on Bargur Cattle of Tamil Nadu, India. The molecular analysis shows that *Haematobia irritans exigua* is a subspecies of *Haematobia irritans* with morphological characteristics of *Haematobia exigua.* This study can provide clear morphological and molecular data on the *Haematobia irritans exigua* for identification.

6. SIGNIFICANCE OF THIS WORK

This is the first record of the *Haematobia irritans exigua* infested on Bargur Cattle which is one of the indigenous and endemic cattle found in Bargur Hills of Eastern Ghats of Tamil Nadu, India. This paper can provide clear data on the Morphological and Molecular identification of *Haematobia irritans exigua*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Desquesnes M, Onju S, Chalermwong P, Jittapalapong S, Masmeatathip R. A review and illustrated description of *Musca crassirostris*, one of the most neglected hematophagous livestock flies. Medical and Veterinary Entomology. 2018;33:16-30.
DOI: https://doi.org/10.1111/mve.12339
2. Skidmore P. The biology of the muscidae of the World. Springer Science & Business Media, Dordrecht. 1985;1-550.
3. Karthika P, Natraj Krishnaveni, Chithravel Vadivalagan, Kadarkarai Murugan, Marcello Nicoletti, Giovanni Benelli. DNA barcoding and evolutionary lineage of 15 insect pests of horticultural crops in South India Karbala. International Journal of Modern Science. 2016;2:156 - 168.
DOI:https://doi.org/10.1016/j.kijoms.2016.03.006.
4. Pathour R. Shashank, Nadur L Naveena, Nernakallu N Rajgopal, Tyler A Elliott, Kolla Sreedevi, Sunil Sunil, Naresh M Meshram. DNA barcoding of insects from India: Current status and future perspectives. Molecular Biology Reports. 2022;49:10617–10626.
DOI: https://doi.org/10.1007/s11033-022-07628-2.
5. May RM. Tomorrow's taxonomy: Collecting new species in the field will remain the rate-limiting step. Philos T Roy Soc B. 2004;359:733–734.
6. Packer L, Gibbs J, Sheffield C, Hanner R. DNA barcoding and the mediocrity of morphology. Mol Ecol Resour. 2004;9:42–50.
DOI: 10.1111/j.1755-0998.2009.02631.x
7. Rivera J, Currie DC. Identification of nearctic black flies using DNA BARCODES (Diptera: Simuliidae). Mol. Ecol. Resour. 2009;9:224-236.
DOI: 10.1111/j.1755-0998.2009. 02648.x.
8. Van Lun Low TK, Prakash BK, Vinnie-Siow WY, Tay ST, Masmeatathip R, Hadi UK, Lim YA, Chen CD, Norma-Rashid Y, Sofian-Azirun M. Contrasting evolutionary patterns between two haplogroups of Haematobia exigua (Diptera: Muscidae) from the mainland and islands of Southeast Asia.
9. Changbunjong T, Weluwanarak T, Samung Y, Ruangsittichai J. Molecular identification and genetic variation of hematophagous flies,(Diptera: Muscidae: Stomoxyinae) in Thailand based on cox1 barcodes. Journal of Asia-Pacific Entomology. 2016, Dec 1;19(4):1117-23.
10. Bhakdeenuan P, Siroyasatien P, Payungporn S, Preativatanyou K, Thavara U, Tawatsin A, Sukontason K, Sukontason KL, Choochote W, Sunannayod S, Sasaki H. Molecular analysis of medically and veterinary important muscid flies; 2012.
11. Aslam Abu Faiz Md, Sultana Sharmin Rain, Faria Farhana Das, Sumita Siddika, Ayesha Howlader Abdul. Molecular characterization and identification of three stored grain pests based on mitochondrial cytochrome C oxidase subunit I (COI) gene sequences. Bangladesh Journal of Zoology. 2019;47:1-11.
DOI:10.3329/bjz.v47i1.42016.

12. Salmah Yaakop, Puteri Amira Amiruddin, Muhamad Azmi Mohammed, Aqilah Sakinah Badrulisham, Nadiatur Akmar Zulkifli, Mohd Noor Hisham Mohd Nadzir. Molecular identification and species richness of flies (Diptera) and their associated bovidae hosts at cattle farms in Selangor, Malaysia. Pertanika J. Trop. Agric. Sci. 2022;45(3):611-630. DOI:10.47836/pjtas.45.3.05
13. Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator, Proc. Natl. Acad. Sci. U. S. A. 2004;101:14812-14817. DOI:10.1073/pnas.0406166101
14. Mitsuhiro iwasa, Naotaka Ishiguro. Genetic and morphological differences of *Haematobia irritans* and *H. exigua*, and molecular phylogeny of Japanese Stomoxyini flies (Diptera, Muscidae). Medical Entomology and Zoology. 2010; 61(4):335-344.
15. Rosaline Wanjiru Macharia, Fidelis Levi Ombura, Erick Onyango Aroko. Insects' RNA profiling reveals absence of ''Hidden Break'' in 28S Ribosomal RNA molecule of onion thrips, thrips tabaci. Journal of Nucleic Acids. 2015;8. DOI: 10.1155/2015/965294
16. Low VN, Tan TK, Lim PE, Domingues LN, Tay ST, Lim AL, Goh TZ, Panchadcharam C, Bathmanaban P, Sofian-Azirun M. Use of COI, CytB and ND5 genes for intra and interspecific differentiation of *Haematobia irritans* and *Haematobia exigua.* Veterinary Parasitology. 2014;204:439-442.
17. Low VL, Tan TK, Prakash BK et al. Contrasting evolutionary patterns between two haplogroups of *Haematobia exigua* (Diptera: Muscidae) from the mainland and islands of Southeast Asia. Sci Rep. 2017; 7:1- 5871.
18. Lane J, Jubb T, Shepherd R, Webb-Ware J, Fordyce G. The priority list of endemic diseases for the red meat industries. Final Report Project B.AHE.0010. Meat and Livestock Australia, North Sydney, New South Wales, Australia. 2015;1-260.
19. Johnson SJ. Studies on *Stephano filariasis* in Queensland. PhD thesis. James Cook University. Brisbane, Queensland, Australia. 1989;191. Available:http://eprints.jcu.edu.au/11414
20. Sutherst RW, Bourne AS, Maywald GF, Seifert GW. Prevalence, severity, and heritability of *Stephanofilari*a lesions on cattle in central and southern Queensland, Australia. Australian Journal of Agricultural Research. 2006;57:743–750. DOI:10.1071/AR05265
21. Shaw SA, Sutherland IA. The prevalence of *Stephanofilaria sp*. in buffalo fly, *Haematobia irritans exigua*, in central Queensland. Australian Journal of Entomology. 2006;45:198–201. DOI:10.1111/j.1440-6055.2006. 00545.x
22. Ganapathi P, Kumar V, Rajesh NV. Production and reproduction performance of endangered bargur cattle under the field condition in Tamil Nadu. International Journal of Food, Agriculture and Veterinary Sciences. 2013;3(1):207-209. Available:http://www.cibtech.org/jfav.htm
23. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 1994;3(5):294-9.
24. Ana Carolina M, Junqueira, Ana Cláudia Lessinger, Tatiana Teixeira Torres, Felipe Rodrigues da Silva, André Luiz Vettore, Paulo Arruda, Ana Maria L, Azeredo Espin. The mitochondrial genome of the blowfly *Chrysomya chloropyga* (Diptera: Calliphoridae). Gene. 2004;339;7-15. DOI: https://doi.org/10.1016/j.gene.2004.06.031.
25. Zumpt F. The Stomoxyine biting flies of the world. Diptera: Muscidae. Taxonomy, biology, economic importance, and control measures. Gustav Fischer Verlag, Stuttgart, Germany. 1973;175.
26. Meenakshi Bharti. Current status of family Muscidae (Diptera) from India. Journal of Entomological Research. 1973;32(2):171-176.
27. Kano R, Shinonaga S, Hasegawa T. On the specific name of *Haematobia* (Diptera, Muscidae) from Japan. Japan Journal of Sanitary Zoology. 1972;23: 49–56.
28. Castiglioni L, Bicudo HEMD. Molecular characterization and relatedness of *Haematobia irritans* (horn fly) populations, by RAPD-PCR. Genetica. 2005;124:11-21. DOI: 10.1007/s10709-004-4309-0
29. Handschin E . *Lyperosia exigua* in Java und Nordo australien. Revue suisse Zool. 1933;40:449-528.
30. Snyder FM. Diptera: Muscidae. Insects of Micronesia. 1965;13:191–327.

31. Pont AC. Studies on the Australian Muscidae (Diptera). A revision of the subfamilies Muscinae and Stomoxyinae. Australian Journal of Zoology (Suppl. Series). 1973;21:129–296.
DOI:10.1071/AJZS021
32. Brito LG, Regitano LCA, Huacca MEF, Carrilho E, Borja GEM. Genotype characterization of *Haematobia irritans* from different Brazilian geographic regions based on randomly amplified polymorphic DNA (RAPD) analysis. Pesq. Vet. Bras. 2007;27:1-5.
DOI: 10.1590/s1984-29612008000400002.
33. James PJ, Madhav M, Brown G. Area-wide integrated pest management: Development and field application. CRC Press, Boca Raton, Florida, USA. 2021; 463–482.
Available:https://doi.org/10.1201/9781003169239
34. Wakeley J. The excess of transitions among nucleotide substitutions: new methods of estimating transition bias underscore its significance. Trends Ecol. Evol. 1996;11:158-163.
DOI: 10.1016/0169-5347(96)10009-4
35. Carew ME, Pettigrove V, Cox RL, Hoffmann AA. DNA identification of urban Tanytarsini chironomids (Diptera: Chironomidae). J. North Am. Benthol. Soc. 2007l;26:587-600.
DOI:10.1899/06-120.1
36. Ruo-Yu L, Gong-She Y, Chu-Zhao L. The genetic diversity of mtDNA D-loop and the origin of Chinese goats, Yi Chuan Xue Bao. 2006;33:420-428.
DOI: 10.1016/S0379-4172(06)60069-3
37. Wang JY, Guo JF, Zhao DY, Wang C, Zhang Y, Wang HZ, Wu Y. The genetic diversity and phylogenetic relationship among pig breeds of Shandong Province based on mtDNA CytB gene, Sci. Agric. Sin. 2009;42:1761-1767.
DOI: 10.1109/ICASSP.2009.4959945.

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