



A Report on the Tabanus Species *Tabanus rufofrater* in Mithun (*Bos frontalis*)

D. Borkotoky¹, S.K. Mukhopadhyaya², J.K. Chamuah³, R. Devi²

10.18805/IJAR.B-5225

ABSTRACT

Background: Mithun (*Bos frontalis*) experience infestations from various ectoparasites, including several types of flies that cause painful bites, stress, blood loss and transmit a range of microorganisms. In the Mithun-inhabited forested regions of Phek, Nagaland, the Tabanus fly is particularly prevalent.

Methods: Researchers studied Tabanus flies infesting free-range mithun in Phek District, Nagaland. The flies were collected and processed at the ICAR-NRC on Mithun in Nagaland. Both morphological (using stereoscopic microscopes) and molecular methods (employing ribosomal and mitochondrial gene markers) were utilized for identification. Additionally, DNA barcoding was done at Eurofins Genomics India Pvt. Ltd. to further ascertain the flies' identities. Climate attributes (Temperature, Rainfall and Humidity) was recorded during the study.

Result: Through morphological and molecular investigations, it was conclusively determined to be *Tabanus rufofrater*. The highest occurrence of *Tabanus rufofrater* encounters takes place between May and August, aligning with the pre-monsoon and monsoon seasons, before gradually declining thereafter. The prevalence of *Tabanus rufofrater* attacks peaks from May to August, coinciding with the pre-monsoon and monsoon periods and subsequently decreases.

Key words: DNA barcoding, Incidence, Morphology, Phylogenetic, Relationship, Tabanus.

INTRODUCTION

Mithun (*Bos frontalis*), a majestic bovine species, is found exclusively in the hilly regions of Northeast India, especially in the states of Arunachal Pradesh, Nagaland, Manipur, and Mizoram. Also referred to as the "cattle of the mountains," this semi-wild species inhabits free-range environments within tropical rainforest ecosystems (Perumal *et al.*, 2016; Rout *et al.*, 2017; Choudhary *et al.*, 2020). The external parasitic infections including tick, lice and leech and infections with helminths and protozoans commonly occur in the mithun (Chamuah *et al.*, 2020a). Tabanids usually known as horse flies, gadflies, stouts, elephant flies, buffalo flies, moos flies, deer flies, clegs, and green head flies are true flies, with medical and veterinary importance. Family Tabanidae belongs to the order Diptera and suborder Brachycera with cosmopolitan distribution in all continents except Antarctica (Pechuman, 1973; Pechuman *et al.*, 1983; Mullens, 2009; Al-Talafha *et al.*, 2018).

The genus Tabanus is the richest group of species, with about 1,350 described species (Pape and Thompson, 2021; Henriques *et al.*, 2022). The genus Tabanus is the richest group of species, with about 1,350 described species (Pape and Thompson, 2021). The genus Tabanus is the richest group of species, with about 1,350 described species (Pape and Thompson, 2021). These flies are bloodsucking insects that attack animals and human and can act as mechanical vectors for various pathogens. High populations have a significant economic impact on outdoor activities, tourism, and livestock production (Baldacchino *et al.*, 2014). They are known to spread diseases such as

¹Krishi Vigyan Kendra, North 24 Parganas (Addl.), ICAR-Central Research Institute for Jute and Allied Fibers, Barrackpore-700 121, West Bengal, India.

²West Bengal University of Animal and Fishery Sciences, Kolkata-700037, West Bengal, India.

³ICAR-National Research Centre on Mithun, Medziphema-797 106, Nagaland, India.

Corresponding Author: D. Borkotoky, Krishi Vigyan Kendra, North 24 Parganas (Addl.), ICAR-Central Research Institute for Jute and Allied Fibers, Barrackpore-700 121, West Bengal, India. Email: debojyoti.borkotoky@gmail.com

How to cite this article: Borkotoky, D., Mukhopadhyaya, S.K., Chamuah, J.K. and Devi, R. (2024). A Report on the Tabanus Species *Tabanus rufofrater* in Mithun (*Bos frontalis*). Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5225.

Submitted: 28-08-2023 **Accepted:** 05-08-2024 **Online:** 21-10-2024

anthrax, equine infectious anemia (swamp fever), tularemia and various other bacterial and protozoal infections.

Tabanus fly bites can cause painful wounds, which can become sites for secondary infections. Pathak and Chhabra (2012) opined that knowledge of parasites and parasitic diseases of marginal livestock as exemplified by elephant, mithun and yak, is still in its infancy and only baseline data are available so far. Molecular phylogeny using highly polymorphic mitochondrial genes such as cytochrome oxidase 1 (Cox1) elucidates the origin and source of infection besides the identification of species of the parasite (Chamuah *et al.*, 2020b). The aim of this paper

is to investigate and identify species of the genus *Tabanus* affecting Mithun in its habitat.

MATERIALS AND METHODS

The material studied comes from recent captures from various free range mithun in the villages for Phek District on Nagaland. Animals in these village are reared under free range system of management in a vast hilly pasture at an altitude above 6000 ft. The summers are moderately warm with the average temperature being 27°C without exceeding 32°C. Winters are cold with the temperature dropping to 0°C in the coldest months of January and February. Flies were collected using a hand net/insect net. The available animals were assessed round the year for incidence of Tananus files. The specimens were kept in separate plastic containers with ventilated cap. The material was deposited to the division of Parasitology, ICAR-NRC on Mithun, Nagaland. The tabanus flies were first examined under stereoscopic microscope and wing venation were studied for the morphological features for taxonomic identification of fly, with the help of available keys (Soulsby, 1982). For molecular identification, marker sequences of ribosomal and mitochondrial genes (ITS-2 and mitochondrial enzyme COX1), in addition to restriction fragment length polymorphism (RFLP) analysis of other marker genes were carried out as per Protocol of Sambrook (2001). Phylogenetic analysis of these sequences was carried out to find out their evolutionary relationship with similar parasites of counterpart domesticated animals. The samples were sent to Eurofins Genomics India Pvt. Ltd. for DNA barcoding for the identity of flies. Meteorological data on temperature (Max. and Min.), humidity and rainfall recorded from Automatic Weather Station (AWS) at ICAR-NRC on Mithun, Porba, Phek.

RESULTS AND DISCUSSION

The flies were mostly observed on the soft part of the body in the dewlap, perpetual flap, udder and peri-anal regions. Hard cutaneous nodules formation in the various body parts of 2-3 cm size were noted during the study. Similar findings of extreme annoyance and blood loss (up to 0.5 ml per fly) and dermal nodules (local reactions to bites) were recorded by Foil and Hogsette (1994). The *Tabanus* flies (Fig 1) recorded from body surface of mithun were prominent size (17-18 mm), proboscis was stout, pointed downwardly projecting with large eyes and hyline wings. Thorax black with yellowish tomentum, black pubescence. Abdomen reddish yellow on first 3 segments with large black median spot on 2nd and 3rd segment, 4th abdominal tergite blackish at its posterior border (Fig 2). Legs are black and fore pair of tibiae with black apex. Wing venation was prominent and R5 cell was open. Wing vein R4 and R5 were widely splayed either side of the wing tip with R5 converging on vein M1 (Fig 3).



Fig 1: Gross view of adult tabanus flies.



Fig 2: Abdominal segment after removing appendages.



Fig 3: Wing vein R4 and R5 were widely splayed either side of the wing tip. 4X.

Tabanus flies' infestation increased during May to August (premonsoon and monsoon) and moderately declines thereafter which may be due to the breeding season of the flies (Table 1). These periods are also characterized by higher humidity and rainfall, which create favorable breeding conditions for *Tabanus* flies. As the seasons progress and weather conditions change, the incidence of infestations usually declines. In a study in Himachal Pradesh on cattle animals, Maity *et al.* (2015) also recorded abundant *Tabanidae* mainly throughout the year with a little decline in mid to higher elevations during winter. Barros (2001) noted the tabanid peak, in October,

While Lucus *et al.* (2020) observed horse fly season in Tacuarembó, Uruguay started in September and ended in May.

Based on morphological findings followed by DNA barcoding with the PCR amplification of marker genes: ITS-2 and mitochondrial enzyme COX1 (Fig 4), species were identified as *Tabanus rufofrater*. The sequence analysis confirmed 99% similarity with *Tabanus rufofrater*

(DQ631993.1) and 98% similarity with *Tabanus atratus* (KU568511).

Phylogenetic analysis

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model as per Kimura M. (1980). The tree with the highest log likelihood (-2316.98) is shown (Fig 5). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 11 nucleotide sequences. Codon position included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 648 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 as described by Kumar *et al.* (2015).

Distance matrix

The number of base substitutions per site from between sequences are shown (Table 2). Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (Felsenstein, 1985) (1000 replicates). Analyses were conducted using the Kimura 2-parameter

Table 1: Month incidence of Tabanus in mithun in Phek, Nagaland (n=1778).

Month	No. of animal	Tabanus (%)
March	336	42(12.5)
April	198	24(12.12)
May	136	48(35.29)
June	126	41(32.54)
July	73	28(38.36)
August	150	53(35.33)
September	108	15(13.89)
October	132	32(24.24)
November	174	35(20.11)
December	98	21(21.43)
January	127	32(25.20)
February	120	33(27.5)
	1778	404(24.88)

Table 2: Estimates of evolutionary divergence between sequences.

FLY	0.011	0.012	0.012	0.010	0.010	0.012	0.010	0.011	0.011	0.011
DQ631993.1	0.080	0.011	0.011	0.012	0.010	0.012	0.010	0.010	0.010	0.013
KM243507.1	0.080	0.070	0.011	0.012	0.008	0.013	0.008	0.009	0.008	0.013
KM243513.1	0.087	0.077	0.064	0.011	0.010	0.012	0.011	0.011	0.010	0.013
KM243537.1	0.076	0.085	0.083	0.085	0.012	0.012	0.011	0.012	0.012	0.012
KJ088765.1	0.073	0.058	0.040	0.065	0.090	0.011	0.008	0.009	0.008	0.012
KM243527.1	0.087	0.089	0.092	0.089	0.101	0.078	0.011	0.011	0.012	0.013
KU875229.1	0.075	0.061	0.045	0.068	0.076	0.046	0.080	0.006	0.008	0.012
KM570780.1	0.075	0.063	0.053	0.073	0.087	0.048	0.075	0.028	0.009	0.012
KM285564.1	0.073	0.061	0.045	0.065	0.095	0.041	0.088	0.038	0.045	0.012
KM243501.1	0.087	0.096	0.097	0.101	0.092	0.095	0.102	0.090	0.092	0.088

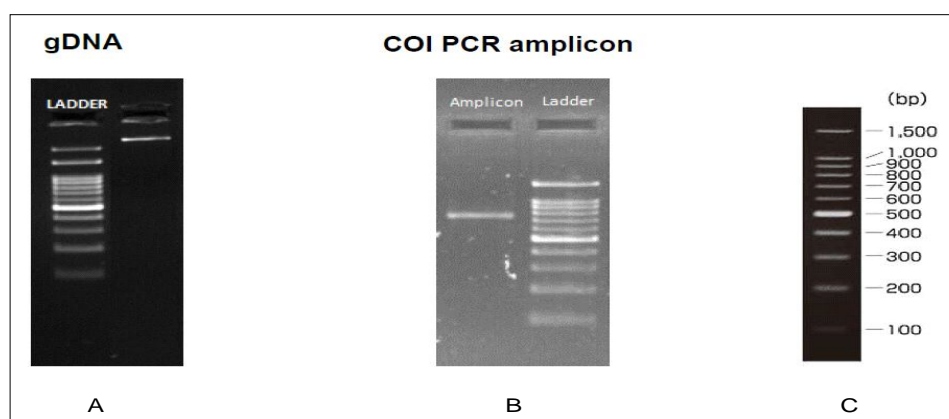


Fig 4: PCR amplification of CO1 gene of Tabanus species.

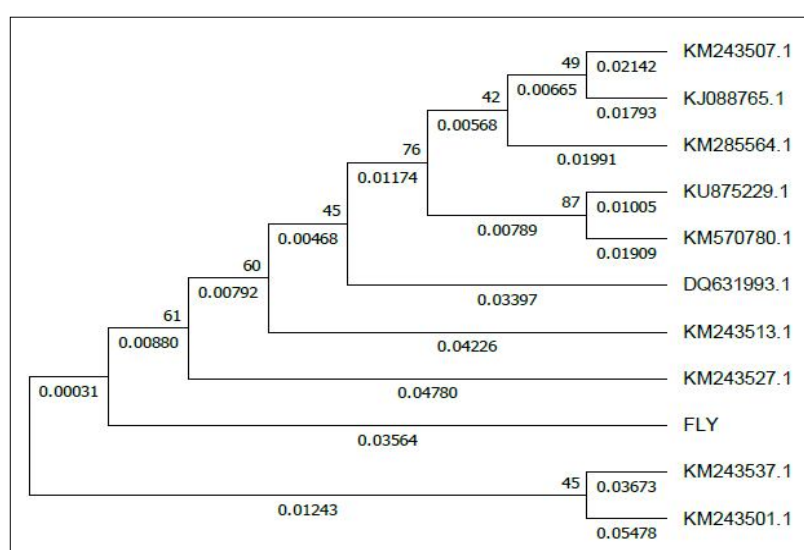


Fig 5: Molecular phylogenetic analysis by maximum likelihood method.

model. The analysis involved 11 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 648 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 as per Kumar *et al.* (2016). There were a total of 648 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

CONCLUSION

Mithun (*Bos frontalis*) plays an important role in the local economies of hill states of North East India. There are concerns about the decline in mithun populations due to various reasons such as habitat loss, inbreeding, and diseases. Tabanids affecting mithun have received little attention. Within the mithun inhabited regions of Phek district, the *Tabanus* fly is notably abundant causing irritation, stress, blood loss and affecting animal production. Utilizing a combination of morphological and molecular investigations, it was conclusively determined to be *Tabanus rufofrater*. The highest occurrence of *Tabanus rufofrater* encounters takes place between May and August, aligning with the pre-monsoon and monsoon seasons, before gradually declining thereafter. Using updated molecular methods to identify ectoparasites and gaining insights into their prevalence and role in disease transmission is crucial for the health and protection of valuable animals.

ACKNOWLEDGEMENT

The authors are thankful to the Director, NRC on Mithun Jharnapani Nagaland for providing necessary facilities for the present research work.

Conflict of interest

The authors declare no conflicts of interest.

REFERENCES

- Al-Talafha, H., Yaakop, S. and Abd Latip, N.F. (2018). New species of the genus *Tabanus* LINNAEUS, 1758 (Diptera:Tabanidae) from Malaysia. *Serangga*. 23: 110-116.
- Baldacchino, F., Desquesnes, M., Mihok, S., Foil, L.D., Duvallet, G., and Jittapalpong, S., (2014). Tabanids: Neglected Subjects of Research, but Important Vectors of Disease Agents. *Infection, Genetics and Evolution*. 28: 596-615.
- Barros, A.T.M. (2001). Seasonality and relative abundance of tabanidae (Diptera) captured on horses in the Pantanal, Brazil. *Memórias do Instituto Oswaldo Cruz*. 96(7): 917-923.
- Chamuah, J.K., Raina, O.K. and Amenti (2020a). Parasites of mithun (*Bos frontalis*) in North Eastern hilly region of India- A review. *Indian Journal of Animal Research*. 54(2): 133-137. doi: 10.18805/ijar.B-3761.
- Chamuah, J.K., Borkotoky, D., Amenti, Khate, K., Jacob, S.S., Lalchamliani, Raina, O.K., Khan, M.H. and Mitra, A. (2020b). Molecular characterization and histopathological studies on *Fasciola gigantica* in Mithun (*Bos frontalis*). *Indian Journal of Animal Research*. 54(8): 1012-1017. doi: 10.18805/ijar.B-3856.
- Choudhary, O.P., Priyanka, Kalita, P.C., Dalga, S., Kalita, A., Doley, P.J. and Keneisenuo (2022). Morphological Studies on the Skull bones of Indian Mithun (*Bos frontalis*). *Indian Journal of Animal Research*. 56(1): 40-45. DOI: 10.18805/IJAR.B-4279.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*. 39: 783-791.
- Foil, L.D. and Hogsette, J.A. (1994). Biology and control of tabanids, stable flies and horn flies. *Revue Scientifique et Technique*. 13(4): 1125-1158.
- Henriques, A.L., Krolow, T.K., Zamarchi, T.B.O. and Camargo, L.M.A. (2022). Description of *Tabanus rondoniensis* (Diptera: Tabanidae), a new species of horsefly from the State of Rondônia, Brazil. *Biodiversity Data Journal*. 10: e76904.

- Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*. 16: 111-120.
- Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*. 33(7): 1870-1874.
- Lucas, M., Krolow, T.K., Riet-Correa, F., Barros, A.T.M., Krüger, R.F., Saravia, A. and Miraballes, C. (2020). Diversity and seasonality of horse flies (Diptera: Tabanidae) in Uruguay. *Science Reporter*. 10: 401.
- Maity, A., Naskar, A., Mukhopadhyay, E., Hazra, S., Sengupta, J. and Ghosh, S. (2015). Taxonomic studies on Tabanidae (Insecta: Diptera) from Himachal Pradesh, India. *International Journal of Fauna and Biological Studies*. 43: 43-52.
- Mullens, B.A. (2009). Horse Flies and Deer Flies. In Mullen, G.R. and Durden, L.A. (Eds.), *Medical and Veterinary Entomology*. Cambridge: Academic Press. pp 264-276.
- Pape, T. and Thompson, F.C. (2021). *Systema Dipteriorum*. [URL: www.catalogueoflife.org/annual_checklist/].
- Pathak, K.M.L. and Chhabra, M.B. (2012). Parasites and parasitic diseases of the Indian elephant, mithun and yak: An overview. *Indian Journal of Animal Sciences*. 82(12): 1459-1467.
- Pechuman, L.L. (1973). Horse flies and deer flies of Virginia (Diptera: Tabanidae). *Virginia Polytechnic Institute and State University*. 6: 1-96.
- Pechuman, L.L., Webb, D.W. and Teskey, H.J. (1983). The Diptera, or true flies, of Illinois. I. Tabanidae. *Illinois. Natural History Survey Bulletin*. 33(1): 1-118.
- Perumal, P., Srivastava, S.K., Baruah K.K., Ghosh, S.K. (2016). Study on the *in vitro* fertilizing potential of mithun (*Bos frontalis*) semen. *Indian Journal of Animal Research*. 50(6): 909-914. doi: 10.18805/ijar.11323.
- Rout, M., Subramaniam, S., Mohapatra, J.K., Sanyal, A., Dash, B.B., Pattnaik, B. (2017). Phylogenetic characterization of foot-and-mouth disease virus recovered from mithuns and yaks in India. *Indian Journal of Animal Research*. 51(6): 1113-1119. doi: 10.18805/ijar.v0iOF.9138.
- Sambrook J. and Russell D.W., (2001). *Molecular Cloning: A Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Soulsby, E.J.L. (1982). *Helminths, Arthropods and Protozoa of Domesticated Animals*. London: English Language Book Society, Baillere Tindall.