Original Article

A REVIEW OF THE BIOLOGY AND ECOLOGY OF *CULICOIDES* VECTORS (DIPTERA: CERATOPOGONIDAE) ABUNDANT IN INDIA

Ankita Sarkar, Paramita Banerjee and Abhijit Mazumdar*

*Entomology Research Unit, Department of Zoology, The University of Burdwan, West Bengal 713104, India

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ABSTRACT

The medico-veterinary importance of the biting midges *Culicoides* (Diptera: Ceratopogonidae) lies in the fact that they vector a multitude of arboviruses, protozoa, and nematodes among livestock, wild ruminants as well as humans. Bluetongue (BT) is a non-contagious viral disease causing morbidity and mortality in affected wild ruminants and livestock. Frequent outbreaks of this disease have caused substantial economic losses, particularly in the southern states of India.

BT's controlling strategy is confined to developing vaccines in disease-prone states and has overlooked these potentially neglected virus-transmitting agents. In India, the majority of studies are seroprevalence-based and largely overlooked the

Corresponding Author:
Dr Abhijit Mazumdar; Email: abhijitbu02@gmail.com

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significance of knowledge about the biology and ecology of these vectors. Among 84 species reported from India, seven are designated as bluetongue virus (BTV) vectors. An information regarding biosystematics and bionomics of these vector species, i.e., *C. peregrinus* Kieffer, *C. oxystoma* Kieffer, *C. actoni* Smith, *C. brevitarsis* Kieffer, *C. fulvus* Sen & Das Gupta, *C. imicola* Kieffer, and *C. orientalis* Macfie will not only provide a better insight for their control but also render a comprehensive idea of their epidemiologically significant vector competence and vectorial capacity. This review stitches together the information generated on biology and ecology of *Culicoides*, the neglected vectors prevalent in India.

Short Title: Bio-ecology of *Culicoides* vectors

Keywords: biology, BTV vectors, *Culicoides*, ecology, taxonomy

INTRODUCTION

Members of the *Culicoides* Latreille, 1809 (Diptera: Ceratopogonidae) are the smallest (1-3 mm) nematoceran haematophagous midges which are implicated as the world players in the epidemiology of more than 50 arboviruses of veterinary and public health importance such as bluetongue virus (BTV), African horse sickness virus (AHSV), epizootic hemorrhagic disease virus (EHDV); protozoa such as *Haemoproteus* sp., *Leucocytozoon* sp., *Hepatocystis* sp., *Leishmania* spp., *Crithidia* sp. and filarial nematodes including *Onchocerca gibsoni*, *O. cervicalis*, *Dipetalonema reconditum*, *Mansonella perstans* and *M. ozzardi* of livestock, wild ruminants, birds as well as humans[1,2,3,4,5,6]. Bluetongue virus (BTV) belonging to the genus *Orbivirus* of the family Reoviridae[7]. In Bluetongue disease (BTD) epidemiology, 23 serotypes of BTV have so far been reported from India, and records of several outbreaks among various union territories, especially in the southern states, have led to substantial economic losses[8]. Species within the subgenera mainly transmitting bluetongue virus (BTV) in the Indian scenario include: *Avaritia* (*C. actoni* Smith, *C. brevitarsis* Kieffer, *C. orientalis* Macfie, *C. fulvus* Sen & Das Gupta and *C. imicola* Kieffer), *Hoffmania* (*C. peregrinus* Kieffer) and *Remmia* (*C. oxystoma* Kieffer)[9,10]. Previous studies suggested the prevalence of those seven putative vectors within BTD-prone areas[9,11]. The prevalence of BTV serotypes in different states of India has been already summarized[7]. Following BTV

serotypes have been isolated from *C. oxystoma* (serotype-1 and 16), while *C. peregrinus* (serotype-23) associated with Indian livestock farms of Gujarat and Tamil Nadu, respectively[12,13,14]. BTV serotype-21 was isolated from *C. fulvus* and *C. orientalis*,[15] while BTV serotype-1, four was detected from *C. imicola*[16, 17]. BTV serotype-1 was also identified from *C. brevitarsis*[18]. Besides, vectoring BTV, *C. peregrinus* is also a vector of the ephemeral fever virus in cattle[2]. *Culicoides oxystoma* are the vectors of various (i) arboviruses viz., *Orbivirus*: AHSV, EHDV, Chuzan virus (CHUV), D'Aguilar virus (DAGV), Ibaraki virus (IBAV); *Orthobunyavirus*: Akabane virus (AKAV), Aino virus (AINOV); (ii) *Onchocerca gibsoni*, the causative agent of filaria of cattle in Malaya, (iii) *Leucocytozoon* sp., an intracellular haemosporidian blood parasite, and (iv) *Leishmania* (*Mundinia*) *martiniquensis*, *L.* (*M.*) *orientalis* and *Crithidia* species[1,5,6,12,19]. *Culicoides imicola* is reported as potential vectors of AHSV, Schmallenberg virus (SBV)[20,21], and *Culicoides brevitarsis* is a significant vector of AKAV, DAGV, ephemeral fever virus, and Ngaingan virus affecting livestock[2]. Microfilariae of *Onchocerca gibsoni* were found in wild-caught *C. actoni* and *C. orientalis* females in Malaysia[2]. *Leucocytozoon* sp. was also detected from *C. fulvus* collected from Phatthalung Province, Southern Thailand[5]. Despite various records of serotypes and disease-causing agents, gathering information regarding the putative vector species prevalent in India is urgently warranted (Fig.1).

This article reviews various aspects of biosystematics and bionomics, especially ecology, taxonomy, and biology of seven neglected vectors of *Culicoides* abundant in India. This baseline information will facilitate the development of effective vector control strategies.

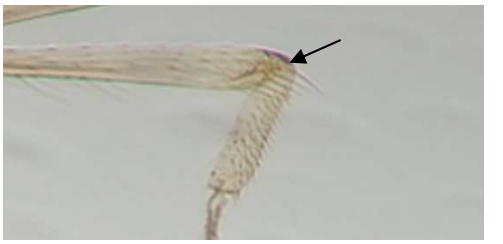
1. Cu fork with proximal pale streak ..... *C. peregrinus*
   Cu fork without proximal pale streak 2

C. peregrinus

C. fulvus

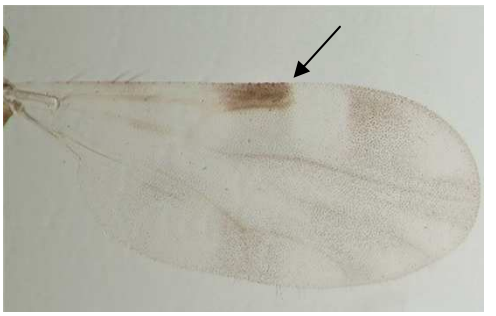
2. Eyes separated; hind tibial comb with 4 spines ..... *C. oxystoma*
   Eyes contiguous; hind tibial comb with 5 spines 3


C. oxystoma; (a) Eyes separated, and (b) hind tibial comb with 4 spines

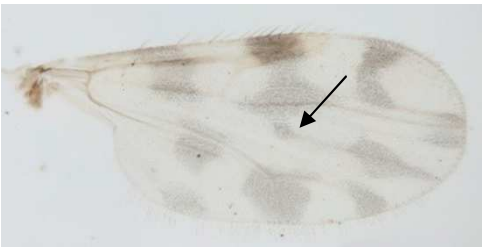
C. fulvus; (a) Eyes contiguous, and (b) hind tibial comb with 5 spines

3. Eyes with interommatidial hairs; poststigmatic pale spot covering 2nd radial cell distally ..... *C. actoni*
   Eyes without interommatidial hairs; poststigmatic pale spot covering distal half of 2nd radial cell 4

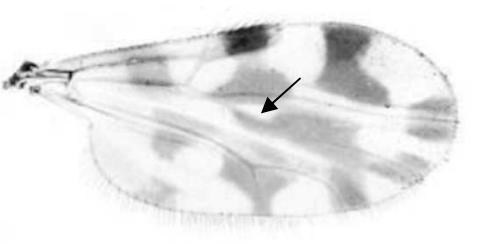



C. actoni *C. fulvus*

4. Proximal pale spot in cell m_1 straddling through vein M_2 5
   Proximal pale spot in cell m_1 not straddling through vein M_2 6

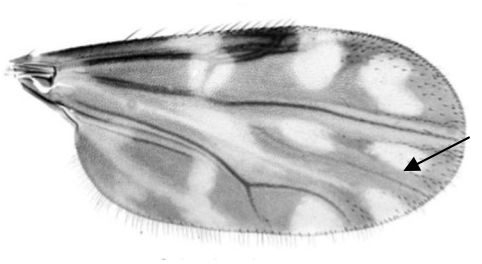


C. fulvus



*C. imicola**

5. Distal dark spot on vein M_1 broad .....*C. orientalis*
   Distal dark spot on vein M_1 narrow ..... *C. fulvus*



C. orientalis

C. fulvus

6. Distal pale spot in cell r_3 quadrate .....*C. imicola*
   Distal pale spot in cell r_3 oval .....*C. brevitarsis*

*C. imicola**

C. brevitarsis

Fig. 1. Key to the adults of vector species of India (*Source:* Economic *Avaritia* key of G. Bellis).

TAXONOMY

Traditional adult *Culicoides* (Figure 1a) taxonomy is phenetic, primarily based on wing spots along with other morphometric characteristics such as antennal ratio, localization of sensilla coeloconica (SCo) on antennal flagellomeres, proboscis/ head ratio, palpal ratio, number of mandibular teeth, hind tibial spines, banding pattern of leg, length and breadth of wing, costal ratio, infuscation of halter and parts of genitalia[2]. Among species, this often leads to misidentification due to minute differences. Many of these require taxonomic validation by comparing with the types in light of modern terminologies. The two vector species, i.e., *C. peregrinus* and *C. oxystoma*, were first recorded from Puri's coastal location and Calcutta, respectively[22]. *Culicoides fulvus*, *C. orientalis*, *C. peregrinus*, *C. alatus* Das Gupta and Ghosh (synonym of *C. oxystoma*), *C. pattoni* Kieffer (synonym of *C. oxystoma*), *C. actoni* and other species were morphologically identified and collected from various regions of India (West Bengal, Assam, Madhya Pradesh, Madras, Coimbatore, Bihar, Bombay, Dharwar, Orissa)[23]. *Culicoides pseudoturgidus* Das Gupta was collected from Calcutta and adjoining areas[24]. Distribution of seven vectors recorded from India is summarized in Table 1.

Table 1. A list of distribution and pathogens transmitted by vectors of India

Subgenus	Vector Species	Distribution	Disease Pathogen*
Avaritia Fox	*Culicoides actoni* Smith	West Bengal, Bihar, Odisha, Assam, Madhya Pradesh, Tamil Nadu, Kerala, Karnataka, Maharashtra	Virus: Bluetongue virus Nematode: *Onchocerca gibsoni*
	Culicoides brevitarsis Kieffer	West Bengal, Tamil Nadu, Karnataka	Virus: Bluetongue virus, epizootic haemorrhagic disease virus, D'Aguilar virus, Aino virus, Akabane virus, ephemeral fever virus, Ngaingan virus
	Culicoides fulvus Sen & Das Gupta	West Bengal, Tamil Nadu	Virus: Bluetongue virus

Subgenus	Vector Species	Distribution	Disease Pathogen*
	Culicoides imicola Kieffer	West Bengal, Tamil Nadu, Karnataka, Kerala, Maharashtra	Virus: Bluetongue virus, African horse sickness virus, Schmallenberg virus
	Culicoides orientalis Macfie	West Bengal, Sikkim, Karnataka	Virus: Bluetongue virus, Nematode: *Onchocerca gibsoni*
Hoffmania Fox	*Culicoides peregrinus* Kieffer	West Bengal, Odisha, Assam, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra	Virus: Bluetongue virus, ephemeral fever virus Protozoa: *Leishmania (Mundinia) martiniquensis*
Remmia Glukhova	*Culicoides oxystoma* Kieffer	West Bengal, Bihar, Odisha, Assam, Gujarat, Himachal Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra	Virus: Bluetongue virus, African horse sickness virus, epizootic hemorrhagic disease virus, Chuzan virus, D'Aguilar virus, Ibaraki virus, Akabane virus, Aino virus Nematode: *Onchocerca gibsoni* Protozoa: *Leucocytozoon* sp., *Leishmania (Mundinia) martiniquensis*, L. (*M.*) *orientalis* and *Crithidia* spp.

*Name of the pathogens transmitted by these seven vectors reported from worldwide

Later on, synonyms of *C. imicola*, *C. actoni*, *C. brevitarsis,* and *C. orientalis* were recognized as follows: *C. pseudoturgidus*, *C. minutes* Sen and Das Gupta*, C. imperceptus* Das Gupta, *C. superfulvus* Das Gupta, *C. nayabazari* Das Gupta respectively[10]. A checklist of 27 species was published initially, which was later increased to 73 species based on an updated annotated checklist[25,26]. The list containing 79 *Culicoides* spp. collected within India, was further taxonomically resolved to 11 subgenera, five species groups, and three unplaced species[27]. Till now, 84 species belonging to 12 subgenera, five species groups (*clavipalpis*, *ornatus*, *saundersi*, *shermani*, *shortti*), and four species belonging to 3 unplaced groups have been documented from India[10,28]. An identification key based on adults

of the Indian *Culicoides* spp. was provided[29]. This article also represented an identification key of seven vector species of India. The present status of the Schultzei group is messy as records suggested most of the Asian literature until 1960 misidentified *C. oxystoma* as *C. schultzei* Enderlein[2]. Specific primers of *C. actoni* and *C. oxystoma* have been developed that may be helpful in rapid identification among pooled samples[30]. The integrative taxonomic approach may address some of the problems faced in morphological taxonomy. So, reinterpretation of phylogenetic relationships will be needed to follow significant discrepancies in the placement and identification of cryptic species.

Adult taxonomy needs to be revised to resolve issues of species delimitation and become more challenging as many vector species belong to complexes of morphologically similar species that may be taken up by studying the immatures of these midges. For this reason, the immature taxonomy of this genus needs to be studied better. The structural elaboration of immature stages, i.e., eggs, larval instars, and pupae of *C. peregrinus*, was elucidated by a Scanning Electron Microscope[31]. In order to develop egg taxonomy and to enumerate species-specific characteristics, a description of eggs of *C. fulvus* and a redescription of eggs of *C. oxystoma*, along with a key based on the structure of eggs, were provided[32]. Likewise, the ultrastructure of the egg surface of *C. actoni*, *C. imicola*, and *C. brevitarsis* was elaborated[33,34,35]. The structure of the larva and pupa of *C. brevitarsis* was also depicted[36]. Notwithstanding these developments, the morphological features of immature *C. orientalis* and *C. oxystoma* are yet to be worked out. Therefore, immature taxonomy of proven vectors and abundant midges leads to more extension of works that may be useful in understanding their feeding habits and breeding habitats.

ECOLOGY

Several biting midges were collected by using UV traps from 11 different livestock farms of cattle, buffalo, sheep, and goats in rural and urban districts of Bangalore. It was noted that *C. imicola* and *C. oxystoma* as the most predominant species[4]. Prevalence of *C. peregrinus, C. actoni*, *C. oxystoma,* and *C. imicola* occurred in the livestock farms of Marathwada[37]. Adult midges were collected by a UV LED light trap fabricated in collaboration with the University Science Instrumentation Center at the University of Burdwan. UV LED traps attracted more

adult midges, followed by blue and green light-based traps[38]. A high relative abundance of *C. oxystoma* followed by *C. peregrinus*, *C. fulvus*, and other non-vector species was recorded from West Bengal[39]. Later, several *Culicoides* spp. were collected from goat and sheep pens of Jharkhand, where *C. peregrinus* was abundant, followed by *C. imicola*[40]. Other midge collection methods included a mouth aspirator, sticky trap, and emergence trap[41].

The preferred time of feeding of *C. peregrinus* and *C. oxystoma* on cattle was found to be early morning, and the preferential landing of these vectors on hosts was mainly restricted to the sites of neck and hump of the cattle. *Culicoides actoni* and *C. fulvus* were observed to prefer landing initially on cattle, followed by sheep and goats in Adisaptagram, West Bengal[42]. *Culicoides orientalis* preferred to feed on the dorsal parts of cattle rather than ventral[2]. Earlier studies identified blood meal sources of these midges by precipitin test; further, a DNA-based approach has been applied to detect this[43]. Reports suggested that *C. peregrinus* is strongly zoophilic and a general feeder[2]. *Culicoides oxystoma* fed the blood of cows, buffalo, sheep, and humans[43]. Blood meal analysis records detected positive *C. actoni* for cow, buffalo, chicken, horse, and human blood, *C. brevitarsis* for red-collared dove and human blood, *C. fulvus* for cow, buffalo, chicken, goat, and sheep blood, *C. imicola* for cow, buffalo, horse, donkey, human blood, and *C. orientalis* for cow blood[5,43,44,45,46,47,48]. This increases the chances of zoonotic pathogen transmission among their hosts. Resting sites of adult *Culicoides* spp. from cattle-sheds were studied in West Bengal[49].

BIOLOGY

Culicoides spp. inhabit a wide range of biotopes, but the breeding habitat of few species has been known. The larval habitat of *C. actoni* was not found despite extensive searching, but later, it was reported that they breed in rotting native fruits[2,50]. *Culicoides orientalis* was reared from 2-3 weeks old manure piles[2]. Breeding sites include banana vegetation, and soil samples of fringes of ponds for *C. alatus*, *C. turgidus* Sen and Das Gupta, and *C. peregrinus*[51]. *Culicoides peregrinus* is also common in ricepaddies and puddles, while *C. oxystoma* was recorded from unspecialized aquatic and semi-aquatic sites, including margins of streams, lakes, drains, ponds, and puddles containing little organic matter and rich in oxygen[2,52]. An earlier attempt was made to rear *C. oxystoma* from exposed mud

on the margins of muddy pools and wells[53,54,55]. Pupae of this species were collected from the margins of small muddy pools, and adult females emerged, but details of the rearing procedure were not mentioned[54]. The breeding habitat of this species was recognized, so the life stages of this species were retrieved from mud and slime taken from the sides of drains or small streamlets and developed into adults[1]. Rearing of this species was done from pupae isolated from the substrate at the margins of water bodies[55]. The screening of larvae and pupae was performed from the soil of the intertidal zone of the Ganga estuary, Sagar Island, and collected adults, followed by rearing[56]. It was reared from their habitat, where it was found along with the larvae of *C. peregrinus* (Figure 1b), *C. guttifer* de Meijere, and *C. huffi* Causey[2]. Previously, many researchers tried to rear *C. oxystoma* frequently, but they have yet to report this vital vector species' life history traits and rearing parameters in laboratory settings.

Laboratory colonies of vector species are essential for a better understanding their vectorial capacity and competence. Standardizing larval food, rearing, captive mating, and artificial blood feeding in laboratory conditions is essential. Globally, only 23 species were attempted to rear under laboratory conditions, of which only two colonies, i.e., *C. sonorensis* Wirth & Jones and *C. nubeculosus* (Meigen), are extant[57,58,59,60,61,62]. Laboratory rearing of *C. peregrinus* and *C. schultzei* were performed[58]. Later on, the biology of *C. peregrinus* was worked on in detail, including fecundity, rearing, and life history parameters. It showed the highest adult emergence possible when inoculating substrate from their habitat into rearing plates[59]. The overwintering of *C. brevitarsis* was noted after the influence of temperature on the development and rearing of this species was also noticed[63,64]. Larvae and pupae were found on the cow pat; this species is anautogenous[2]. Life history parameters of *C. imicola* depending on various temperatures were also observed[65]. The bacterial communities among *C. imicola* populations are shaped by various biotic and abiotic factors[66]. Along with this, metagenomic analysis of microbial communities associated with the life history of *C. peregrinus* and identification of fungal communities from the fourth instar of this vector species were recorded[67]. The haemolytic bacteria, i.e., *Bacillus pumilus* (CU1A and CU1B) and one blood-utilizing bacterium, *Bacillus licheniformis* (CU2B) were isolated and identified from wild-caught *C. peregrinus* and *C. oxystoma* and suggested a possible role in shortening of blood digestion period[68]. Further isolation, biochemical characterization, and antibiotic sensitivity of haemolytic bacterial

strains across life history documented that 13 bacterial strains were beta haemolytic while only one was alpha haemolytic bacteria[69]. Only specific strains of culturable bacteria and effective antibiotics can be used for further applications in managing vector species by paratransgenesis techniques[69]. It was noticed that adults and juveniles of *Menemerus bivittatus* and juveniles of *Marpissa* sp. also feed on engorged adults of *C. peregrinus* and *C. oxystoma* and suggested that the spiders may serve as biological control agents of these vector species[70].

CONCLUSION

Studying intraspecific variation among vector species is urgently needed because of Chitradurga's (Karnataka) recent BT disease outbreak. For this reason, proper identification and documentation of Indian *Culicoides* spp., an entomological survey covering our country's physiographic regions, and an effective dry and wet repository are urgently required. Along with this, the study of type specimens and validation of existing *C. schultzei* were recorded by several researchers from India. Species complexes and associated knowledge gaps may be taken up by practicing integrative taxonomy. Despite several host-specific observations, vector-centric dispersal, host range expansion and biology-based study will be needed to develop effective management strategies. Besides categorizing and identifying larval microhabitats, standardizing larval food for rearing, blood feeding, and captive mating are pivotal to developing a thriving laboratory colony and further strengthening vector research.

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