

Culicoides species: The Biting Midges

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Abstract:

The haematophagous flies known as *Culicoides* are important carriers of veterinary and human arboviruses. Since 1998, dramatic changes took place in epidemiology as number of global disease cases associated with vector *Culicoides* was increasing. In temperate locations, this epidemiological shift also contributes to the appearance of exotic viruses. These epidemiological changes are the result of changes in the climate, land use, and animal husbandry. This haematophagous dipteran is a vector for numerous arboviruses that are significant for both veterinary and public health. Although there are important gaps in our knowledge of geography, biology, and taxonomy, recent advances in genomics, molecular biology tools, and methodologies will help us fill those gaps. We explore their life cycle, ecology, phylogeny, and classification in addition to their role as carriers.

Introduction:

Small biting midges in the family Ceratopogonidae are flies of the genus *Culicoides*. These flies have significant role in medicine, veterinary medicine, and economics because they stress their hosts and spread disease through their blood-feeding behaviour. Numerous species in the genus serve as biological carriers of important pathogens for human and animal health (Bagchi & Saradar, 2021; Ivanišová et al., 2022). This role garners the genus substantial attention. Over 50 arboviruses have been isolated from *Culicoides* species in addition to various protozoan and nematode species, and their role in the transmission of veterinary (Borkent, 2004; Meiswinkel et al., 2004b; Mellor et al., 2000) and human (Carpenter et al., 2013; Linley, 1985; Debnath, 2020; De & Dey, 2019.) pathogens has been reviewed. Tourism, forestry, and agricultural industries may be negatively impacted by *Culicoides* feeding opportunistically on humans (Mellor et al., 2000).

Morphology:

About 1-3 mm long, adult insects are tiny and black. In males, the antennae are longer (15 segments) and more thickly haired than in females. The two wings have thick hairs that provide

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distinct colouring patterns. Biologists utilise these wing patterns to distinguish between different species. The bases of the 15 segmented antennae are more or less continuous with the enormous compound eyes. The Johnston's organ is located in the pedicel of the males' antennae. While both men and females consume nectar, only females consume blood, which is necessary for the maturation of fertilised eggs. In general, females bite during twilight or dawn, frequently in large swarms, and usually close to water, marshes, or decaying vegetation.

Adult head:

The head bears enormous compound eyes. They are barely separated, and the degree of separation can serve as a species-level distinguishing characteristic. The arched supraorbital suture, which is present between the eyes in some species (like the subgenus *Avaritia*), is lacking in other species (Battle and Turner, 1971). *Culicoides* have significant antennae that include taxonomic, phylogenetic, and ecological data. On the antennae of *Culicoides*, there are five different types of sensilla that serve as chemoreceptors and mechanoreceptors (Wirth and Navai, 1978).

Flagellomeres having Sensilla coeloconica (functions as mechanoreceptor and chemoreceptor) have been extensively employed in the development of the *Culicoides* classification and are important from a taxonomic perspective. Sensilla like these are signs of host association. The majority of species with Sensilla coeloconica on 4-6 flagellomeres are mammophilic, while those with sensilla on 8-13 flagellomeres are typically ornithophilic (Jamnback, 1965; Chu-Wang et al., 1975; Braverman and Hulley, 1979; Felipe-Bauer et al., 1989; Blackwell et al., 1992). These sensilla coeloconica reacts to carbon dioxide and humidity, according to Blackwell et al. (1992). The maxillary palps on either side of the proboscis are an important source of ecological and taxonomic data. The palps of *Culicoides* have five segments. There are several sensilla basiconica on the third segment (referred to as capitates sensilla in some of the literature, e.g., Borkent, 1995). Females have palps with larger, more developed third segments due to sexual dimorphism.

The third segment's length to width ratio, as well as the size and depth of the sensory pit or region, provide taxonomically useful information. It was established that the sensilla basiconica are sensitive to changes in carbon dioxide concentration, an essential cue in host location (Grant and Kline, 2003). Sensilla count is correlated with host size and can indicate host relationships (Rowley and Conford, 1972). Adult mouth components are extended into a proboscis that is made up of the labrum, mandibles, hypopharynx, maxillary laciniae, and labium from anterior to posterior. Information on the ecology can be gleaned from the structure of the mouth parts. Typically, non-biting species lack lacinial, labral, mandibular, and hypopharyngeal teeth (Borkent, 1995).

Thorax:

The legs, wings, and halters of the pro meso and meta thorax, as well as the sclerites, form the thorax. The prominent pre scutal pits have been employed as a diagnostic characteristic for

the genus, according to Downes and Wirth (1981), but it was discovered that this property is difficult to discern and found in other genera. For species diagnosis, the colour pattern of the scutum, scutellum, and post-scutellum are employed. The characteristic of important taxonomic relevance in *Culicoides* is the pattern of bright and dark spots on the wings. The length and colouring of the macrotrichia on the wing surface produce these patterns (Blanton and Wirth, 1979). These patterns offer the current *Culicoides* subgeneric classification scheme. A leg is composed of six segments i.e. coxa, trochanter, femur, tibia, basitarsus, tarsus.

Abdomen:

Segments II through VII of the abdomen's ten segments contain spiracles (Downes and Wirth, 1981). Male terminalia were reported to possess a variety of taxonomic and phylogenetic characteristics.

Life cycle:

Culicoides are holometabolous. For completing their life cycle they need two to six weeks, depending on the species and the surrounding circumstances. Typically, male *Culicoides* emerge before the females do, and they are prepared to mate when the females leave the pupal stage. Mating occurs during flying.

Egg:

Males and females consume nectar, the females need blood for the development of their eggs. Depending on the amount of the blood meal, different species lay different numbers of eggs.

Larvae:

Larvae are not exclusively aquatic and terrestrial; they need air and water. Moisture is necessary for their development. The larvae can be found in and near muddy substrates, mangrove swamps, and salt marshes. The larval habitat in the tropics is found in rotting fruit, bromeliads and other water-holding plants, cattle waste, and pond edges. The larval stage can last between two weeks and a year, depending on the species, local climate, and other factors. Some larvae can grow in damp, manure-contaminated areas, but they do not grow inside the animal (Mullen, 2002).

Pupae:

Normally, the pupal stage lasts between two to three days.

Public health and veterinary impact of *Culicoides*:

Culicoides is an important human and animal pest with major economic impact and is a key vector for the development of various diseases. The saliva of some *Culicoides* species can cause horses to have severe allergic reactions, causing painful sores (Van der Riit et al., 2008). It was discovered that their significance for veterinary or public health stems from their role in

the transmission of pathogens, particularly viruses, but also protozoans and filarial parasites like avian hamosporidians (Veiga et al., 2018; Chagas et al., 2018) and *Tetrapetalonema* sp. (Carpenter et al., 2013 ; Yates et al., 1982; Lowrie et al., 1978; Linley et al., 1985).

They are the most prevalent hematophagous vectors for 66 viruses, including the Blue tongue virus (BTV), the epizootic hemorrhagic disease virus (EHDV), the African horse sickness virus (AHSV), and the equine encephalitis virus (EEV), as well as 15 species of protozoans and 26 filarial nematodes, and they are also in charge of causing allergic reactions in hosts all over the world (Borkent, 2004; Mullen, 2009). Monitoring of diseases spread by biting midges is being done in numerous nations across several continents. In the Indian states of Andhra Pradesh, Assam, Gujrat, Haryana, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Nagaland, Punjab, Rajasthan, Sikkim, Tamilnadu, Tripura, Uttar Pradesh, and West Bengal, it was discovered that they are in charge of spreading livestock diseases (Mehrotra et al., 1991; Kulkarni et al., 1992; Prasad et al., 1992; Joardar et al., 2016; Chand et al., 2015).

Diseases caused by *Culicoides* are closely related to climate and weather. The seasonal pattern of virus transmission in temperate areas occurs during the hot, humid summer and fall months. High rates of vertebrate infection have been observed in tropical and subtropical locations during wet summers, but transmission rates decline during dry seasons (Braveman et al., 1996). Several articles claimed that though midges may only fly a few hundred metres, the wind can spread them passively over a very large area (Carpenter et al., 2001; Hunt et al., 1994). High humidity of more than 80% aids adult midges in their reproduction, and this climatic condition affects their vector competence, according to Halder et al. (2016). According to research by Joardar et al. (2016), *C. oxystoma* and *C. schultzei* were shown to be possible carriers of the bluetongue virus and the epizootic haemorrhagic sickness virus among the *Culicoides schultzei* complex that had been gathered from various agroclimatic zones of West Bengal. A thorough description of the ecology, breeding locations, and biology of immature and adult organisms is still missing.

Ecology:

The *Culicoides* genus is found all over the world. Each species of biting midge needs a different ecological environment. Many different species of biting midges are mostly produced in areas with significant salt marsh habitat. Highly organic soil with significant manure loads from swine, sheep, and cow farming activities are additional supplies for some species. These insects don't establish themselves inside of buildings, houses, or inside of people or other animals. Under a range of environments, including damp, muddy regions, faeces, and plant waste, midges breed in moist conditions. The removal of organic debris from these places and drainage of muddy areas are crucial components of the *Culicoides* control approach. High lignin content in silage residue was favourably correlated with the development of *C. obsoletus* and *C. scoticus* larvae (Zimmer et al., 2013b). The two main areas of interest in studies on the ecology of adult midges are (1) seasonality and (2) activity and blood feeding. Though certain

species exhibit bimodal activity, one at dawn and the other at night, the majority of *Culicoides* are active around dusk (Blanton & Writh, 1979). (Kline and Roberts, 1982). In temperate locations, the population of many species peaks in the spring, with some species persisting throughout the summer (Blanton and Writh, 1979). A secondary peak in some species' populations occurs in the fall after their spring peak (Kline and Axtell, 1979). To find hosts, biting midges use a variety of indicators. Carbon dioxide is one of the most significant indicators. Carbon dioxide is released during vertebrate exhalation, which prompts female midges to fly upwind in the direction of the source (Bhasin et al., 2000). The midges react by acting erratically and failing to fly upwind if the concentrations above a certain level (Bhasin et al., 2000). Even after feeding on vertebrate hosts, blood-fed females were recovered more frequently at 10m above ground than at ground level (D.A. Swanson unpublished data). The distribution of adult midges is strongly determined by the larval habitat because they rarely move far from these areas. *Culicoides* larvae can be found in a range of aquatic and semiaquatic environments. Higher organic loading is a favourable indicator of the habitat for *C. variipennis*, *C. sonorensis*, and *C. nubeculosus* larvae (high phosphate, % organic matter, and nitrate) (Meigen, 1830, Schmidtmann et al., 2000; Uslu and Dik, 2010). Members of the *C. variipennis* complex are distributed in areas with high salt ion concentration (Schmidtmann et al., 2000).

Phylogeny:

Within the genus *Culicoides*, the phylogenetic relationship has to be revised. The genus has been classified into subgenera and species groups by numerous writers (Root and Hoffman, 1937; Edward et al., 1939; Fox, 1948, 1955; Vagus, 1953; Khalaf, 1954). 35 subgenera of *Culicoides* were recognised by Borkent and Wirth in 1997. The way that species are now classified is based on their overall similarity. The relationship between the subgenera was first examined by Khalaf (1954), however these connections were based on phenetic similarities rather than cladistic synapomorphies. Recent research has concentrated on using molecular traits to infer phylogenetic relationships, such as nuclear ribosomal DNA and mitochondrial DNA (Dallas et al., 2003; Nolan et al., 2007; Pages et al., 2009 ; Gomulski et al., 2005; Perrin et al., 2006; Matsumoto et al., 2009; Schwenkenbecher et al., 2009).

There have been some attempts to break up this vast genus into smaller subgenera. These groupings have been identified by adult morphological characteristics, while Glukhova (1977) also employed larval characteristics. The initial division theory was put forth by Root and Hoffmann (1937) and Edward et al. (1939) using characteristics of male genitalia and female spermathecae. The phylogeny of *Culicoides* has recently been tested using a variety of markers. Studies employing internal transcribed spacer 1 (Mathieu et al., 2007), 2 (Gomulski et al., 2005), or in combination (Matsumoto et al., 2009), as well as studies combining data from *cox1* and *cox2* analyses, are also successfully employed to study the phylogenetic relationship. Insightful information about taxonomic investigations is being provided by the combined use of

molecular markers and mitochondrial DNA, and this information is offering guidance for future research.

Taxonomy:

More than 1400 species of the genus *Culicoides* are found globally (Borkent, 2012a). It was discovered that the requirement to identify females and medicinal and veterinary concerns have spurred the taxonomic investigations of the genus. As a result, even though males offer more diagnostic features to identify species, many identification keys are written for females. The taxonomy is further complicated by the lack of complete identification keys for all biogeographical zones and the numerous species that still require descriptions. One must check numerous regional keys, subgeneric keys, and species group keys in order to identify the specimen.

It is difficult to pinpoint immaturity down to the genus level. The only family of nematocerous Diptera lacking a key to genera for the larvae or pupae is Ceratopogonidae (Borkent and Grogan, 2009). For young *Culicoides*, there is currently no complete species key. It was discovered that research on the immature stages can help with ecological studies, disclose cryptic species, provide phylogenetic features, and provide information for vector management and surveillance. Similar to the adults, pupal *Culicoides* have not been the subject of extensive investigations that include a wide variety of species and geographies. Although the pupal stage is the most well-known of the juvenile stages, there is still much to learn about the biology and taxonomy of many *Culicoides* species. The taxonomy of larvae still requires a lot of investigation. We need to increase our understanding of larval taxonomy in order to comprehend larval ecology and potential phylogenetic traits.

Kieffer (1910) conducted the first taxonomic investigation on *Culicoides*, which was followed by Patton (1913), Dover (1921), Edwards (1922), Smith (1929), Mukherji (1931), Smith and Swaminathan (1932), and Macfie (1933). Sen and Dasgupta (1959); Dasgupta (1962) examined the Indian *Culicoides* fauna in and around Kolkata after a protracted hiatus. In several regions of West Bengal, taxonomy research on numerous *Culicoides* species has recently been ongoing.

In order to identify and describe new species, Dasgupta and colleagues researched the taxonomy, biology, and ecology of *Culicoides* species in India (Sen and Dasgupta, 1958, 1959; Dasgupta, 1961, 1995). Sen and Dasgupta conducted a survey at Presidency College in Kolkata (West Bengal). Although there is no information on their biology in this area, *Culicoides imicola*, *C. actoni*, *C. fulvus*, and *C. brevetaris* have also been identified in India (Dasgupta, 1995). 6 new species of *Culicoides* were discovered on a cattle farm in the coastal saline districts of Howrah, North, and South 24 Parganas in West Bengal, according to Mukhopadhyay et al. (Mukhopadhyay et al., 2017).

Although a relatively small number of studies have taken into account characteristics of juvenile stages in their evaluations, Borkent (2014a) emphasised that the subgeneric classification of *Culicoides* is almost totally phenetic, generally of adult specimen (Glukhova,

1977; Nevill and Dyce, 1944; Nevill et al., 2009). The vast bulk of *Culicoides* taxonomy investigations relies on morphological examinations. Many people believe that the introduction of molecular entomology for systematics offers a quick substitute for the development of traditional taxonomic competence (Tautz et al., 2003).

The small size of the specimen, a poorly defined subgeneric classification, the absence of descriptions for con-specific life stages and sexes, the lack of phylogenetic data, intraspecific variation in diagnostic morphological characters, the identification of potentially synonymous species, and a lack of agreement on defining appropriate intraspecific genetic distances are some of the major difficulties the *Culicoides* taxonomists are facing. Without finding a solution to these issues, it will be impossible to precisely define the geographic range of many species, and we will only have scant knowledge of their regional distribution and seasonal patterns of abundance. Understanding the association between species richness and climate, latitude, landcover, terrain, host availability, and seasonality would benefit from proper specimen identification (Andrew et al., 2013).

DNA characterisation and other developments in molecular entomology offer fresh perspectives and motivation for comprehending morphological and functional disparities within the genus *Culicoides*. Molecular and morphological analysis have already been shown to be coherent (Gomulski et al., 2005; Pages et al., 2009; Pages et al., 2005), demonstrating that both techniques can aid in the investigation of phenotypic plasticity. Their usage in investigations into morphologically related species, such as those in the subgenus *Avaritia*, has begun (Bellis et al., 2014a; Mathieu et al., 2007; Pages et al., 2005).

Key knowledge gaps:

Because of their tiny size, the scarcity of principle vector species' colonies, and the relatively minor impact of *Culicoides*-borne animal disease agents in affluent nations, research on *Culicoides* has lagged.

Geographical gaps:

The most recent waves of arbovirus transmission in Europe have reignited interest in *Culicoides* across the globe. However, there is still a dearth of fundamental survey data in huge regions, especially the tropics (Africa, Asia, and South America). *Culicoides* arbovirus transmission is likely steady and endemic in tropical areas. Rare reports of outbreaks may be due to local animals' mutual adaption to these viruses. The ability to comprehend epidemiology is severely hindered in endemic tropical zones due to a lack of epidemiological data.

Parameter gaps:

Researchers can characterise parameter gaps using vectorial capacity. It's important to look at host biting behaviours. The host biting rates of immature midges are ultimately influenced by environmental factors, and further research is required to identify the factors that regulate the

density of these populations at different stages. To understand transmission dynamics, complex and opposite interactions between vectorial capacity parameters are need to be studied.

Ecological knowledge gap:

The inability to comprehend *Culicoides*' distribution and the mechanisms controlling their abundance is still hampered by a lack of knowledge about their biology. Various moist microhabitats are used by *Culicoides* for oviposition and larval development. Although the value of more natural habitats is less researched in relation to virus vectors, many of these habitats are located in close proximity to live cattle. Except for a few new morphological descriptions, there is little current research on *Culicoides* pupae (Ronderos et al., 2013). Pupal ecology has largely been disregarded. We need to learn more about the *Culicoides* larvae, including their eating habits, winter behaviour, and interactions with other environmental factors. Then we will be better able to comprehend their population dynamics and distribution, their control methods. Predicting the possible ranges of vector species may be aided by research into how eggs, larvae, and pupae react to environmental conditions.

Taxonomy:

Taxonomy of the embryonic stages of *Culicoides* lags much behind than that of the adults. Only 3% of *Culicoides* species are known as eggs, 13% as larvae, and 17% as pupae, according to Borkent's (2014) analysis. While the immature stages of a few more *Culicoides* species are still being described (Ronderos et al., 2010), the majority of species lack even the most fundamental morphological descriptions.

Genetics:

Culicoides are still difficult to describe on a global scale. Expert morphological identification is being used to identify the fauna of South and Central America, India, North and Central America, and Russia. Although some of the most esteemed taxonomists in the history of *Culicoides* research have been sponsored by these areas, it is obvious that fundamental revisions utilising genetic and morphological methodologies will be necessary. As it has recently been perceived, the advent of molecular marker sequencing also permits routine study of the source of blood meals in *Culicoides* as a means of inferring host-preference (Martinez-de la Puente et al., 2015).

Genomics:

The interactions between vectors, viruses, and hosts have been revealed by emerging genomic technologies. The recent application of approaches based on genomics are now exclusively applied to the North American vector *C. sonorensis*, has been a significant advancement since 2003. Innate immunity and developmental biology in *C. sonorensis* have already been studied using transcriptomic studies (Nayduch et al., 2014b) (Nayduch et al., 2014c). Additionally, work on *Culicoides*' first complete genome has begun. Despite the fact that the majority of current research focuses on *C. sonorensis*, it is expected that in the coming

decades similar techniques will be applied to other species and that entomologists, geneticists, and bioinformaticians will collaborate more frequently.

Conclusion:

Uneven research on *Culicoides* has led to information gaps. The biology of adult midges has received more research, while several elements of survival, resting site choice, and dispersal strategy have received less attention. The immatures of *Culicoides* require additional work (taxonomy and detail ecology) to be done. A comprehensive review of the *Culicoides* fauna of the Indian subcontinent was not included in the authoritative taxonomic review of the *Culicoides* fauna of the oriental region (Wirth WW, Hubert AA. 1989), and the *Culicoides* fauna of India has only occasionally been the subject of morphological studies. West Bengal is the home of 62 of the 78 *Culicoides* species that have been recognised as existing in India based on morphology (Sen and Dasgupta, 1958, 1959), Kolkata and the surrounding areas (Sen and Dasgupta, 1962), Assam and Bengal and other parts of India (Sen and Fletcher, 1962), Chennai (Jayalakshmi, 1966), Marathwada region of Maharashtra (Narladkar et al., 2009). But the majority of India has very little information available. Additionally, only a single DNA barcode from a single place can be used for molecular DNA analysis of the Indian *Culicoides* fauna. DNA barcoding is a popular genetic technique used to research biodiversity and identify species (Hebert et al., 2003). To comprehend the morphological and biological studies of the Indian *Culicoides* fauna, additional molecular research is required.

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