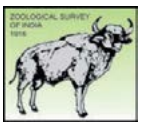
ISSN (Online) : 2581-8686
ISSN (Print) : 0375-1511

Rec. zool. Surv. India: Vol. 123(i2S)/253-260, 2023
DOI: 10.26515/rzsi/v123/i2S/2023/172521



Morpho-taxonomy and seasonal prevalence of *Culicoides* Latreille, 1809 (Diptera: Ceratopogonidae) in Sonamukhi protected forest, Bankura, West Bengal

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Abstract

Culicoides (Diptera: Ceratopogonidae), popularly known as 'biting midges' play a significant role in transmission of pathogens to vertebrate animals- particularly livestock animals, humans, and birds causing severe diseases like Bluetongue (BT), Epizootic haemorrhagic disease (EHD), African horse sickness (AHS), Equine encephalitis (EE), etc. The present research focuses on morpho-taxonomy, ecological diversity, seasonal prevalence, and species abundance of *Culicoides* in different cattle farms and pastoral regions of Sonamukhi protected area, Bankura, West Bengal for three seasons (pre-monsoon, monsoon, and post-monsoon) from 2013 to 2015 in sixteen sampling sites. Taxonomic studies show four species under three subgenera - *Avaritia*: *Culicoides actoni, C. imicola; Remmia*: *C. oxystoma*; *Hoffmania*: *C. peregrinus* of genus *Culicoides* for the first time from Sonamukhi area. Ecological analyses show the highest species diversity (H =0.881) and species dominance maximum (D= 0.5179) in the post-monsoon, and species richness-maximum in monsoon (D_{mg}=0.6). Species relative abundance (p_i=0.664), rank abundance curve and seasonal prevalence pattern shows-*C. oxystoma* as the most dominant species. This study provides a brief idea of the taxonomy, ecology, and seasonal prevalence of *Culicoides* species in the Sonamukhi Protected area, Bankura. The moderate rainfall and moist weather play an essential role in the development of this vector fly. Despite the area showing extreme weather conditions, the pre- and post-monsoon seasons are the ideal time and the species abundance to reach its highest seasonal peak. This research is a preliminary step which delivers insights into the taxonomy, ecological role, species diversity, seasonal abundance, and factors affecting the growth and survival of *Culicoides,* for its scientific direction in vector control strategies as well as conservation and management of livestock via proper monitoring and surveillance programs.

Keywords: *Culicoides*, vector, ecology, prevalence, post-monsoon

Introduction

Genus *Culicoides* or biting midges belong to the family Ceratopogonidae which comprises 1347 species globally (Borkent, 2020). They are hematophagous, attacking mammals (mostly livestock) and birds, found in most continents (Mellor *et al.*, 2000). 30 *Culicoides* species in the world, including 13 species from India, are reported vectors of disease pathogens like Bluetongue virus (BTV), Epizootic hemorrhagic disease virus (EHDV), African horse sickness (AHSV), Akabane, Aino, Equine encephalitis virus (EEV), Schmallenberg virus, etc. (Wirth *et al.*, 1989; Mellor *et al.*, 2000; Mullen, 2009; Lassen *et al.*, 2012; Harrup *et al.*, 2015; Mukhopadhyay *et al.*, 2016). They live in the moist environment near cattle farms; females feed on blood, while males on plant juices (Birley and Boorman, 1982; Mellor *et al.*, 2000; Meiswinkel *et al.*, 2004).

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Article Received on: 10.04.2023 Accepted on: 15.06.2023

Earth encompasses different biomes and abiotic factors determine its native flora and fauna. The physiography of West Bengal provides huge scope to study ecology, distribution, vector biology, and seasonal dynamics of various harmful insects. Our study was based on Sonamukhi protected forest area of dry deciduous vegetation, average rainfall of 1740mm, temperature range of 6°C to 41°C, laterite soil with drought, heat waves, floods, soil erosion, and deforestation (State Forest Report, West Bengal, 2006-07). The present research focuses on the discovery of *Culicoides* fauna from different cattle farms, their ecology and seasonal prevalence in this tropical dry deciduous ecosystem.

Materials and methods

Insect samples were collected from sixteen livestock farms in Sonamukhi forest area, Bankura for three seasons of pre-monsoon, monsoon, and post-monsoon from 2013 to 2015 by sweep net in the early morning, afternoon, and before sunset. Collections were preserved in 70% ethanol and identified by mounting different parts of the specimen in a phenol-balsam mixture on glass slides. Ecological data analyses on species' relative abundance were calculated to check the seasonal peak and highest abundance of the species, species diversity, and dominance were evaluated by using Shannon's diversity index (H) and Simpson's index of dominance (D) respectively, species richness per total sampling area was calculated by using Margalef's index of richness (D_{mg}).

Abbreviations: Cell r_2-second radial cell, cell r_5- fifth radial cell, cell m_2- second medial cell, cell m_4- fourth medial cell, R-M cross vein- Radio-medial cross vein, vein M_2- second medial vein, PRM-pre-monsoon, MON-monsoon, PST-post-monsoon, RA- relative abundance.

Systematic account

Superfamily **Chironomoidea**
Family **Ceratopogonidae**
Subfamily **Ceratopogoninae**
Tribe **Culicoidini**
Genus ***Culicoides***
Subgenus ***Avaritia***
Culicoides actoni Smith, 1929
Culicoides imicola Kieffer,1913
Subgenus ***Remmia***
Culicoides oxystoma Kieffer, 1910
Subgenus ***Hoffmania***
Culicoides peregrinus Kieffer, 1910

Results and Discussion

A. Taxonomy

Genus ***Culicoides*** Latreille, 1809
1809. *Culicoides* Latreille, *Paris and Strasbourg* 4: 399 pp.
Type species: *Culicoides punctatus* Latreille 1809 (= *Ceratopogon punctatus* Meigen 1804)
Pictorial key to subgenera shown in **Figure 1**

Subgenus ***Avaritia*** Fox, 1955
1955. *Avaritia* Fox, *Journal of Agriculture of the University of Puerto Rico* 39:214-285.
Type species: *Ceratopogon obsoletus* Meigen 1818

Key to species

1. One pale spot in cell m_1 at wing margin ..... *C. actoni.*
   More than one pale spot in cell m_1 ..... 2.
2. Cell m_1 with pale area continued to wing margin expanded posteriorly nearly to vein M_2 ..... *C. imicola.*

Culicoides actoni Smith, 1929

1929. *Culicoides actoni* Smith, *Indian J. Med. Res.* 17:255-257.

1962. *Culicoides imperceptus* Das Gupta, *Science and Culture* 28:537-539.

Material examined. 7♂♂ and 5♀♀ Sonamukhi forest area, Bankura district, 23°10'29.24"N, 87°25'26.76"E, 24.ix.2013, coll. D. Banerjee & party. 4♂♂ and 1♀ Bandarhati, Bankura district, 23°13'16.9"N, 86°51'59.2"E, 19.ix.2013, coll. D. Banerjee & party. 2♀♀ Dihipara, Bankura district, 23°24'16.98"N, 87°24'57.47"E, 22.ix.2013, coll. D. Banerjee & party. 3♂♂ and 3♀♀ Pachal, Bankura district, 23°13'35.71"N, 87°17'16.59"E,15.ix.2013, coll. D. Banerjee & party. 1♂ and 5♀♀, Nachanhati, Bankura district, 23°20'52.08"N, 87°25'10.24"E, 12.i.2014, coll. D. Banerjee & party. 1♂, Rampur, Bankura district, 23°14'29.33"N, 87° 4'11.29"E, 2.i.2014, coll. D. Banerjee & party. 1♂ and 1♀,Radhamohanpur, Bankura district, 23°0'15.26"N, 86°50'54.19"E, 27.i.2014, coll. D. Banerjee & party. 2♂♂ and 3♀♀, Palsora, Bankura district, 23°27'49.6"N, 86°62'35.3"E, 24.vi.2014, coll. D. Banerjee & party. 4♂♂ and 5♀♀, Churamanipur, Bankura district, 23° 4'40.42"N, 87°17'34.49"E,11.xi.2014, coll. D. Banerjee & party. 3♂♂ and 5♀♀,Muslo, Bankura district, 23°18'16.6"N, 86°54'03.1"E, 22.xi.2014, coll. D. Banerjee & party. 1 ♂, Krishtobati, Bankura district, 23°22'47.9"N, 86°'59.8"E, 15.xi.2014, coll. D. Banerjee & party. 1♂ and 4♀♀, Kalyanpur, Bankura district, 23°16'6.71"N, 87°23'36.51"E, 4.xi.2014, coll. D. Banerjee & party.

Diagnosis. Pictorial diagnosis shown in **Figure 2**

Present distribution: India: West Bengal: Bankura: Bandarhati, Bondalhati, Churamanipur, Dihipara, Kalyanpur, Krishtobati, Muslo, Nachanhati, Pachal, Palsora, Radhamohanpur, Rampur, Sonamukhi forest area.

Culicoides imicola Kieffer, 1913

1313. *Culicoides imicola* Kieffer, *Resultats scientifiques. Diptera* (5): 1-43.

1959. *Culicoides minutus* Sen and Das Gupta, *Annals of the Entomological Society of America* 52:617-630.

1962a. *Culicoides pseudoturgidus* Das Gupta, *Science and Culture* 28:537-539.

Material examined. 9♀♀, Sonamukhi forest area, Bankura district, 23°10'29.24"N, 87°25'26.76"E, 24.ix.2013, coll. D. Banerjee & party. 5♀♀, Hamirhati, Bankura district, 23°17'55.21"N, 87°21'46.60"E, 2.ix.2013, coll. D. Banerjee & party. 2♀♀, Dihipara, Bankura district, 23°24'16.98"N, 87°24'57.47"E, 22.ix.2013, coll. D. Banerjee & party. 3♀♀, Pachal, Bankura district, 23°13'35.71"N, 87°17'16.59"E, 15.ix.2013, coll. D. Banerjee & party. 2♀♀, Nachanhati, Bankura district, 23°20'52.08"N, 87°25'10.24"E, 12.i.2014, coll. D. Banerjee & party.7♀♀, Rampur, Bankura district, 23°14'29.33"N, 87°4'11.29"E, 2.i.2014, coll. D. Banerjee & party.1♀, Radhamohanpur, Bankura district, 23°0'15.26"N, 86°50'54.19"E, 27.i.2014, coll. D. Banerjee & party. 6♀♀, Balarampur, Bankura district, 23°15'49.67"N, 87°4'49.91"E, 20.vi.2014, coll. D. Banerjee & party. 1♀, Krishtobati, Bankura district, 23°22'47.9"N, 86°'59.8"E,15.vi.2014, coll. D. Banerjee & party. 2♀♀, Churamanipur, Bankura district, 23°4'40.42"N, 87°17'34.49"E, 11.xi.2014, coll. D. Banerjee & party. 9♀♀, Muslo, Bankura district, 23°18'16.6"N, 86°54'03.1"E, 22.xi.2014, coll. D. Banerjee & party. 11♀♀, Bandarhati, Bankura district, 23°13'16.9"N, 86°51'59.2"E, 3.xi.2014, coll. D. Banerjee & party.

Diagnosis. Pictorial diagnosis shown in **Figure 3.**

Present distribution: India: West Bengal: Bankura: Balarampur, Bandarhati, Bondalhati, Churamanipur, Dihipara, Hamirhati, Krishtobati, Muslo, Nachanhati, Pachal, Radhamohanpur, Rampur, Sonamukhi forest area.

Subgenus ***Remmia*** Glukhova, 1977

1977. *Remmia* Glukhova, *Parazitologicheskii Sbornik* 27:112-118.

Type species: *Ceratopogon schultzei* Enderlein, 1908

Culicoides oxystoma Kieffer, 1910

1910. *Culicoides oxystoma* Kieffer, *Mem Ind. Mus*. 2:181-242.

1913. *Culicoides kiefferi* Patton, *Indian J. Med. Res.* 1:336-338, pl. 18.

1921. *Culicoides pattoni* Kieffer, *Bull. Soc. Entomol. Fr.* 1921:7.

1956. *Culicoides alatus* Dasgupta and Ghosh, *Bull. Calcutta Sch. Trop. Med.* 4:162-163.

Material examined. 25♂♂ and 70♀♀, Sonamukhi forest area, Bankura district, 23°10'29.24"N, 87°25'26.76"E, 24.ix.2013, coll. D. Banerjee & party. 35♂♂ and 32♀♀, Hamirhati, Bankura district, 23°17'55.21"N, 87°21'46.60"E, 2.ix.2013, coll. D. Banerjee & party. 3♂♂ and 40♀♀,

Bandarhati, Bankura district, 23°13'16.9"N, 86°51'59.2"E, 19.ix.2013, coll. D. Banerjee & party. 19♂♂ and 70♀♀, Dihipara, Bankura district, 23°24'16.98"N, 87°24'57.47"E, 22.ix.2013, coll. D. Banerjee & party. 40♂♂ and 16♀♀, Pachal, Bankura district, 23°13'35.71"N, 87°17'16.59"E, 15.ix.2013, coll. D. Banerjee & party. 17♂♂ and 71♀♀, Churamanipur, Bankura district, 23°4'40.42"N, 87°17'34.49"E, 6.i.2014, coll. D. Banerjee & party. 11♂♂ and 21♀♀, Nachanhati, Bankura district, 23°20'52.08"N, 87°25'10.24"E, 12.i.2014, coll. D. Banerjee & party. 9♂♂ and 18♀♀, Rampur, Bankura district, 23°14'29.33"N, 87°4'11.29"E, 2.i.2014, coll. D. Banerjee & party. 11♂♂ and 20♀♀, Radhamohanpur, Bankura district, 23°0'15.26"N, 86°50'54.19"E, 27.i.2014, coll. D. Banerjee & party. 19♂♂ and 14♀♀, Balarampur, Bankura district, 23°15'49.67"N, 87°4'49.91"E, 20.vi.2014, coll. D. Banerjee & party. 20♂♂ and 9♀♀, Krishtobati, Bankura district, 23°22'47.9"N,86°'59.8"E, 15.vi.2014, coll. D. Banerjee & party. 6♂♂ and 30♀♀, Palsora, Bankura district, 23°27'49.6"N, 86°62'35.3"E, 24.vi.2014, coll. D. Banerjee & party. 12♂♂ and 29♀♀, Muslo, Bankura district, 23°18'16.6"N, 86°54'03.1"E, 22.xi.2014, coll. D. Banerjee & party. 11♂♂ and 30♀♀, Kalyanpur, Bankura district, 23°16'6.71"N, 87°23'36.51"E, 4.xi.2014, coll. D. Banerjee & party.

Diagnosis. Pictorial diagnosis shown in **Figure 4.**

Present distribution: India: West Bengal: Bankura: Balarampur, Bandarhati, Bondalhati, Churamanipur, Dihipara, Hamirhati, Kalyanpur, Krishtobati, Muslo, Nachanhati, Pachal, Palsora, Radhamohanpur, Rampur, Sonamukhi forest area.

Subgenus ***Hoffmania*** Fox 1948

1948. *Hoffmania* Fox, *Proceedings of the Biological Society of Washington* 61:21-28.

Type species: *Culicoides inamollae* Fox and Hoffman 1944 (= *Culicoides insignis* Lutz 1913).

Culicoides peregrinus Kieffer, 1910

1910. *Culicoides peregrinus* Kieffer, *Memoirs of the Indian Museum* 2:181-242.

1932. *Culicoides assamensis* Smith and Swaminath, *Memoirs of Indian Medical Research* 25:182-186.

Material examined. 15♂♂ and 7♀♀, Sonamukhi forest area, Bankura district, 23°10'29.24"N, 87°25'26.76"E, 24.ix.2013, coll. D. Banerjee & party. 5♂♂ and 15♀♀, Hamirhati, Bankura district, 23°17'55.21"N, 87°21'46.60"E, 2.ix.2013, coll. D. Banerjee & party. 5♂♂ and 4♀♀, Bandarhati, Bankura district, 23°13'16.9"N, 86°51'59.2"E, 19.ix.2013, coll. D. Banerjee & party. 13♂♂ and 7♀♀, Dihipara, Bankura district, 23°24'16.98"N, 87°24'57.47"E, 22.ix.2013, coll. D. Banerjee & party. 9♂♂ and 21♀♀, Pachal, Bankura district, 23°13'35.71"N, 87°17'16.59"E,15.ix.2013, coll. D. Banerjee & party. 4♂♂ and 19♀♀, Churamanipur, Bankura district, 23°4'40.42"N, 87°17'34.49"E, 6.i.2014, coll. D. Banerjee & party. 9♂♂ and 15♀♀, Nachanhati, Bankura district, 23°20'52.08"N, 87°25'10.24"E, 12.i.2014, coll. D. Banerjee & party. 1♀, Rampur, Bankura district, 23°14'29.33"N, 87° 4'11.29"E,2.i.2014, coll. D. Banerjee & party. 1♂ and 3♀♀, Radhamohanpur, Bankura district, 23°0'15.26"N, 86°50'54.19"E, 27.i.2014, coll. D. Banerjee & party. 5♂♂ and 12♀♀, Balarampur, Bankura district, 23°15'49.67"N, 87°4'49.91"E, 20.vi.2014, coll. D. Banerjee & party. 6♂♂ and 15♀♀, Krishtobati, Bankura district, 23°22'47.9"N, 86°'59.8"E, 15.vi.2014, coll. D. Banerjee & party. 12♂♂ and 17♀♀, Muslo, Bankura district, 23°18'16.6"N, 86°54'03.1"E, 17.ix.2015, coll. D. Banerjee & party.

Diagnosis. Pictorial diagnosis shown in **Figure 5**

Present distribution: India: West Bengal: Bankura: Balarampur, Bandarhati, Bondalhati, Churamanipur, Dihipara, Hamirhati, Kalyanpur, Krishtobati, Nachanhati, Pachal, Radhamohanpur, Rampur, Sonamukhi forest area.

The present research reports four species under three subgenera of genus *Culicoides -Avaritia*: *C. actoni, C. imicola; Remmia*: *C. oxystoma*; *Hoffmania*: *C. peregrinus* for the first time from Sonamukhi protected forest area.

C. imicola and *C. oxystoma* are distributed across the Afrotropical, Saharo-Arabian and Oriental regions while, *C. actoni* and *C. peregrinus* are found in the Australian, Oceanian, and Oriental region (Borkent, 2020). All these species are reported as vectors from India (Mukhopadhyay *et al.*, 2016; Maheshwari *et al.* 2012).

B. Seasonal prevalence

Ecological data analyses on species diversity, dominance and species richness are shown in the **Figure 6.**

The Shannon Index (H) shows the diversity of *Culicoides* species in the livestock community- post monsoon > pre monsoon > monsoon, collective species dominance pattern

(D) was post-monsoon > pre-monsoon >monsoon, species richness (D_{mg}) was highest in the monsoon > post monsoon > pre monsoon for all the sites. Relative abundance values (RA), seasonal prevalence, and species rank abundance calculated from each sample site based on the four species show prevalence -*Culicoides oxystoma*> *C.peregrinus*>*C. actoni*>*C.imicola* shown in **Figures 7-9.**

The highest species diversity is obtained in the post monsoon because humid conditions with moderate temperature favor vector flies but they do not survive on severe climatic situations or very high rainfall. Similarly, post –monsoon is the dominant period in regulating species diversity. But, the species richness value (D_{mg}) was highest in the monsoon, because species richness of an area depends on the collection per sampling site and not on the number of samples. Relative abundance pattern of four species reveal that the most prevalent species was *C. oxystoma* with highest seasonal prevalence in the post monsoon.

Our research delivers a brief example on the veterinary significance, taxonomy, ecology, and seasonal prevalence of *C. actoni, C. imicola, C. oxystoma, and C. peregrinus* in the tropical dry deciduous forests of Sonamukhi protected area, Bankura. From the seasonal effect studies, it is established that moist weather plays an essential role in the development of this vector fly. Despite the area showing extreme weather conditions, the pre- and post-monsoon seasons are the ideal time and the species abundance to reach its highest seasonal peak. Other factors like rural conditions with many cattle farms having poor sanitization, illiteracy on livestock management, and much more which still needs to be revealed. Therefore, following can be added as a wish list for future years: (i) a complete study on the habitats, breeding sites, and life cycle of genus *Culicoides* in the drought prone Sonamukhi and other parts of Bankura, and West Bengal, (ii) to authorize reports of diseases vectored by biting midges, and (iii) undergo proper investigations to conserve the livestock in various rural and urban areas of West Bengal.

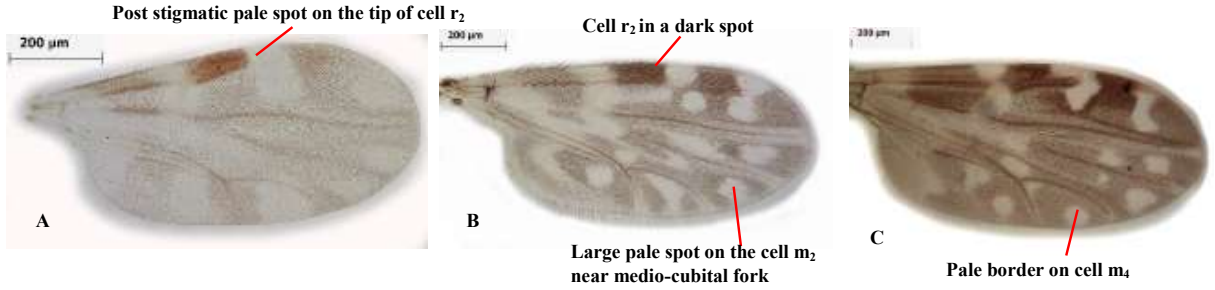

Figure 1. Pictorial key to subgenera (A. *Avaritia*, B-*Remmia*, C-*Hoffmania*) of genus *Culicoides*

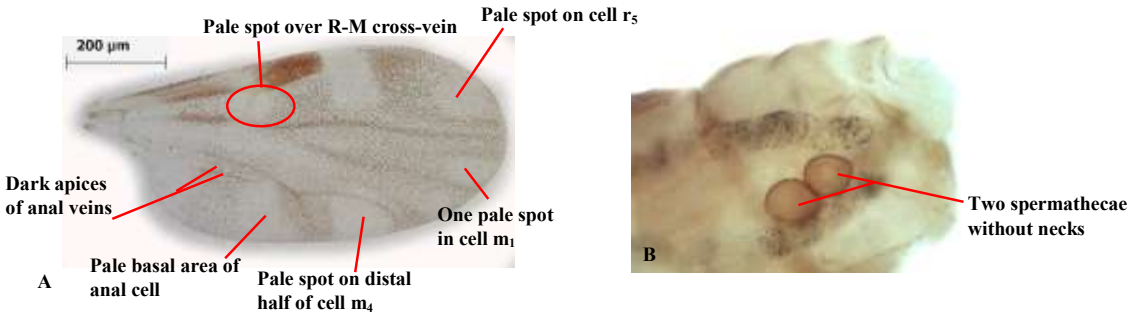

Figure 2. Pictorial diagnosis (A. Wing, B. Female genitalia) of *Culicoides actoni* Smith, 1929

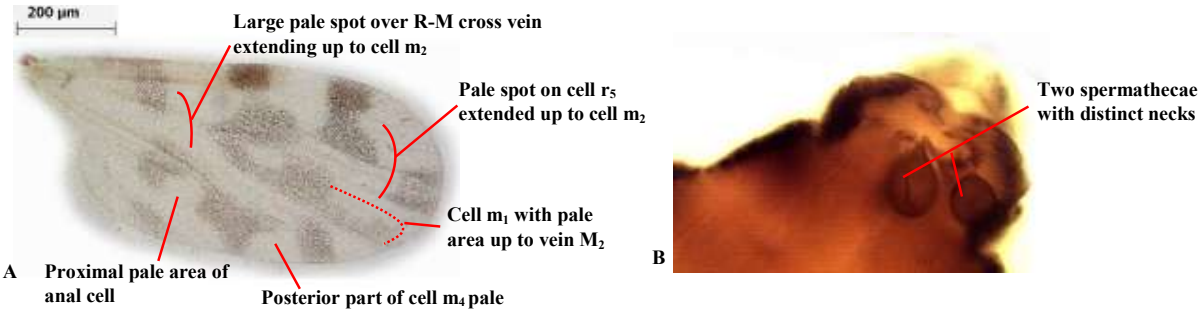

Figure 3. Pictorial diagnosis (A. Wing, B. Female genitalia) of *Culicoides imicola* Kieffer, 1913

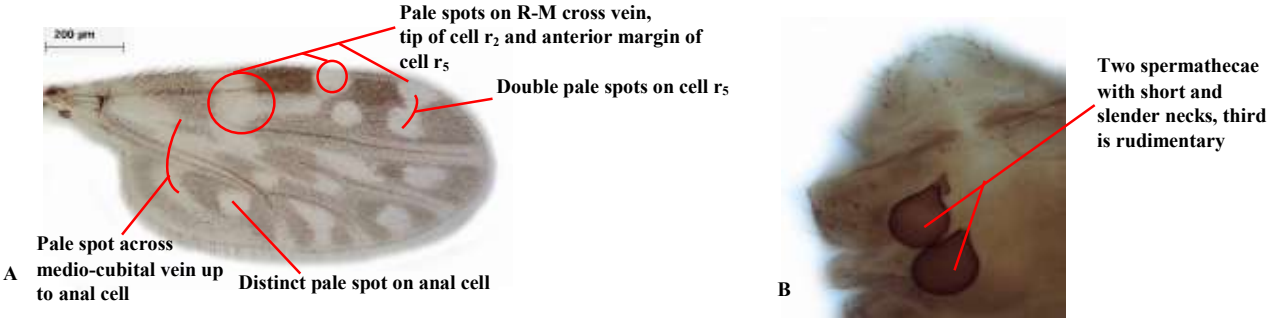

Figure 4. Pictorial diagnosis (A. Wing, B. Female genitalia) of *Culicoides oxystoma* Kieffer, 1910

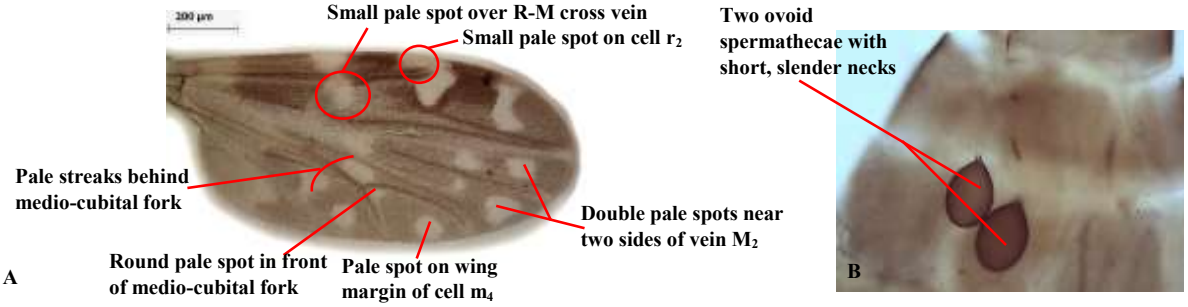

Figure 5. Pictorial diagnosis (A. Wing, B. Female genitalia) of *Culicoides peregrinus* Kieffer, 1910

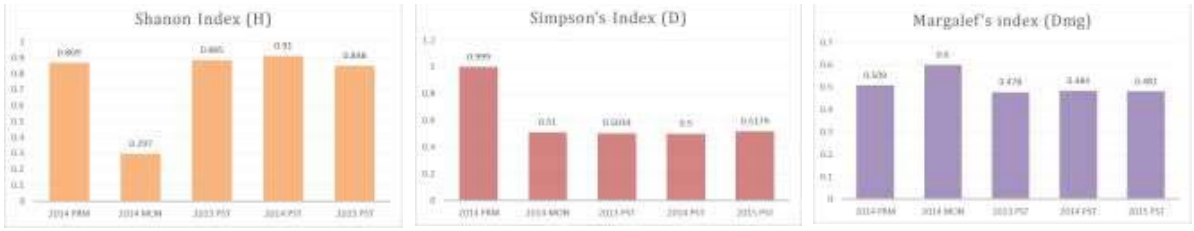

Figure 6. Ecological indices (A. Diversity, B. Dominance, C. Richness) of *Culicoides* species in different seasons.

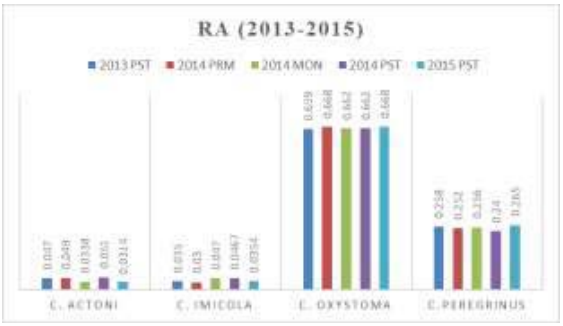

Figure 7. Species relative abundance values showing prevalence of *Culicoides* species in different seasons.

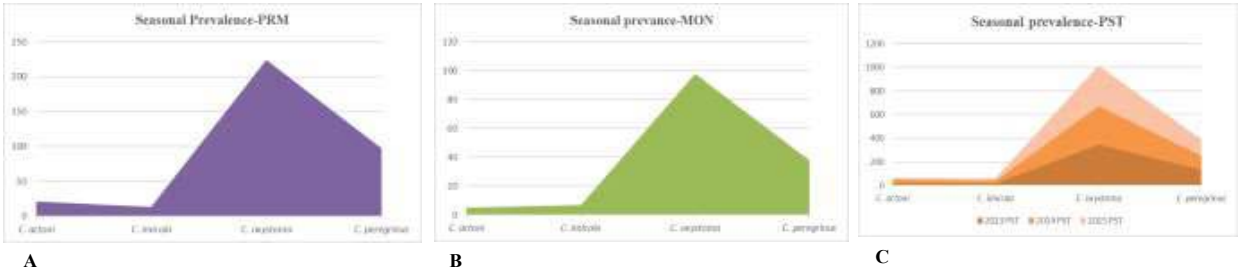

Figure 8. Seasonal prevalence (A. Pre-monsoon, B. Monsoon, C. Post-monsoon) of *Culicoides* species in different seasons.

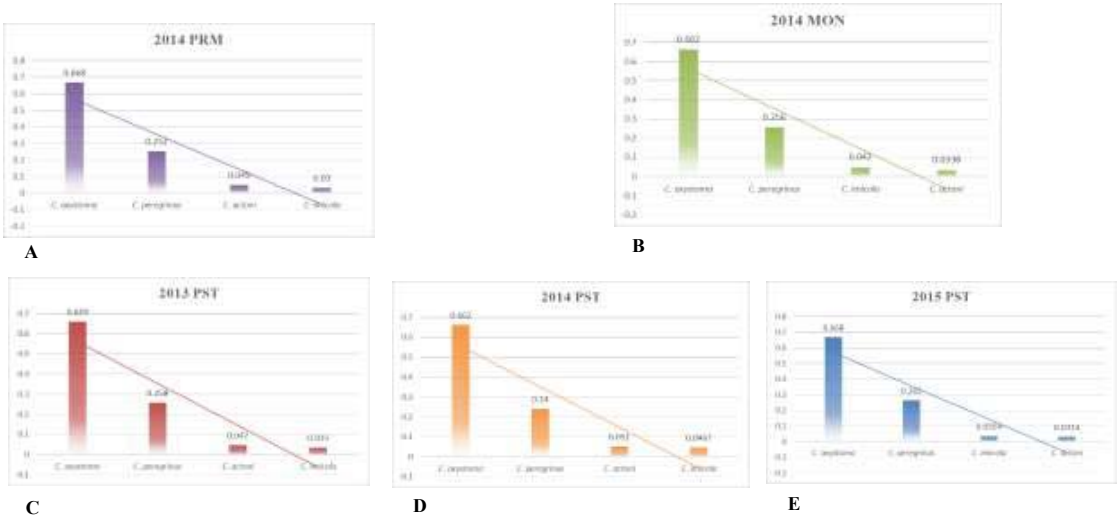

Figure 9. Species rank abundance curve (A. Pre-monsoon, B. Monsoon, C, D, E. Post-monsoon) of *Culicoides* species in different seasons.

Acknowledgement

We are gratified to the Bankura Forest Department for permitting us to survey the district. Further, we convey our heartfelt thanks to all the affiliates of the Diptera section who had contributed with their knowledge and technical support.

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