





# OPEN Identifications, spatial distribution, and seasonal occurrence of Culicoides in selected districts of Northwest Ethiopia

Bimrew Asmare Ayele<sup>1</sup>, Abrham Ayele<sup>2</sup>, Wassie Molla<sup>3</sup>, Adugna Berju Molla<sup>3</sup>, Mastewal Birhan<sup>2</sup>, Saddam Mohammed Ibrahim<sup>2</sup>, Bereket Dessalegn<sup>2</sup>, Ambaye Kenubih<sup>2</sup>, Abebe Tesfaye Gessese<sup>4</sup>, Mebrie Zemene Kinde<sup>4</sup>, Gashaw Getaneh Dagnaw<sup>4</sup>, Melkie Dagnaw Fenta<sup>5</sup>, Tesfaye Mulatu<sup>6</sup>, Hana Tesfaye<sup>7</sup>, Molalegne Bitew<sup>8</sup>, Zewdu Seyoum Tarekegn<sup>2</sup> & Haileyesus Dejene<sup>3</sup>  

Culicoides, among the tiniest and most abundant hematophagous insects globally, serve as vectors for a variety of pathogens such as viruses, bacteria, parasites, protozoa, and nematodes. This study aimed to identify Culicoides species and assess their spatial distribution and seasonal occurrence in selected districts of the Central, South, and West Gondar zones, Northwest Ethiopia. A cross-sectional study was conducted between January to July 2023. A total of 44 UV light- onderstepoort traps were deployed in the study districts near specific areas. The traps were operational from dusk (6:00 PM) until dawn (6:00 AM) and were suspended at a height of 1.5 to 2 m above the ground. Poisson regression was used to assess associations, the Shannon diversity index to measure diversity, and QGIS 3.22.6 to create maps. In this study, 8,857 Culicoides were captured across the 44 trapping sites. Of the total flies captured, 8,838 were identified as belonging to 12 distinct species, while the classification of the remaining 19 flies remained unclear. Notably, *C. kingi* (54.01%) was the most prevalent species, followed by *C. imicola* (44.55%). The abundance of Culicoides observed from January to late April (3505) was significantly lower compared to the wet season (5355), with a marked increase in the capture of *C. kingi* (2499) from May to late July. A statistically significant association ( $p < 0.05$ ) was observed between the occurrence of Culicoides and factors such as district, sampling point, and season. Spatial analysis revealed that *C. kingi* had a broader range of suitability than other Culicoides species, with high suitability observed in East Dembia. The diversity index analysis indicated that Culicoides species diversity was higher in samples from animal pens ( $H = 0.73$ ) and during the wet season ( $H = 0.75$ ). Additionally, this study documented the presence of eight Culicoides species namely *C. corsicus*, *C. kibunensis*, *C. reioxi*, *C. kiouxi*, *C. sahariennes*, *C. desertorum*, *C. reithi*, and *C. festivipennis*, which have not been previously documented in Ethiopia. In conclusion, the study highlighted that the occurrence of Culicoides species was higher in East Dembia, with moderate presence in Wegera and West Armacho. Further research is needed to assess the impact of various Culicoides species on animal and human health, as well as their economic implications, and to develop corresponding control strategies based on these findings.

**Keywords** Culicoides, Seasonal occurrence, Identification, Spatial distribution, Northwest, Ethiopia

<sup>1</sup>Central Gondar Zone Livestock Resource Development Office, Gondar, Ethiopia. <sup>2</sup>Department of Veterinary Pathobiology, College of Veterinary Medicine and Animal Sciences, University of Gondar, Gondar, Ethiopia. <sup>3</sup>Department of Veterinary Epidemiology and Public Health, College of Veterinary Medicine and Animal Sciences, University of Gondar, Gondar, Ethiopia. <sup>4</sup>Department of Veterinary Biomedical Science, College of Veterinary Medicine and Animal Sciences, University of Gondar, Gondar, Ethiopia. <sup>5</sup>Department of Veterinary Clinical Medicine, College of Veterinary Medicine and Animal Sciences, University of Gondar, Gondar, Ethiopia. <sup>6</sup>Animal Health Institute, Sebeta, Ethiopia. <sup>7</sup>Department of Veterinary Pharmacy, College of Veterinary Medicine and Animal Sciences, University of Gondar, Gondar, Ethiopia. <sup>8</sup>Bio and Emerging Technology Institute, Addis Ababa, Ethiopia. ✉email: haileyesus.dejene@uog.edu.et

## Abbreviations

AHI	Animal Health Institute
BT	Bluetongue
BTV	Bluetongue virus
GPS	Global positioning system
IKC	Interactive Identification Key for Culicoides
IPM/IVM	Integrated Pest/Vector Management
OVI	Onderstepoort Veterinary Institute
QGIS	Quantum Geographic Information System
UV	ultraviolet

The genus *Culicoides*, comprising over 1,000 species, is highly significant within the family Ceratopogonidae. Commonly known as biting midges, members of the Ceratopogonidae family are dipteran flies belonging to the suborder Nematocera. They are among the smallest and most abundant blood-feeding flies globally, with sizes reaching up to 3 mm<sup>1,2</sup>. The Ceratopogonidae family includes approximately 78 genera and over 4,000 documented species. This family is divided into four subfamilies: Leptoconopinae, Forcipomyiinae, Dasyheleinae, and Ceratopogoninae. With the exception of Dasyheleinae, all subfamilies include species that feed on vertebrate blood. Only four genera are known to prey on both humans and animals. *Culicoides* includes the majority of globally problematic species and serves as a primary vector for animal-borne disease agents<sup>3,4</sup>.

Globally, 1,368 species of *Culicoides* have been identified. The ability of these vectors to transmit viruses can be influenced by various environmental factors, including climate change<sup>5–7</sup>. This is further compounded by the necessity for these species to primarily feed on ruminants and be present in large numbers. Consequently, only a few species have been identified as potential or confirmed carriers of the blue-tongue virus (BTV) in different geographical areas. For instance, in Africa, species such as *C. imicola* and *C. bolitinos*; in Asia, *C. imicola* and *C. fulvus*; *C. brevitarsis* and *C. fulvus* in Australia; *C. sonorensis* in North America; and *C. insignis* and *C. pusillus* in Central and South America have been implicated<sup>8</sup>. In Northern Europe, *C. obsoletus* and *C. pulicaris* complexes are associated with BTV transmission<sup>9</sup>. Female *Culicoides* are hematophagous and serve as vectors for various pathogens. They bite hosts such as amphibians, birds, mammals, and both wild and domestic animals to obtain blood, which is necessary for egg maturation<sup>10–12</sup>.

*Culicoides* is both a hematophagous insect and a vector for various viruses, filarial nematodes, and protozoa, making it globally significant in veterinary health<sup>1,3</sup>. Their role in transmitting viruses, bacteria, protozoa, and nematodes is crucial<sup>13,14</sup>. Over 50 arboviruses, classified under the Bunyaviridae, Reoviridae, and Rhabdoviridae families, have been identified in various *Culicoides* species<sup>15</sup>. After ingesting infected blood from animals, these viruses multiply in the midgut cells of female *Culicoides*, then migrate to the salivary glands and are transmitted through subsequent biting episodes in animal hosts<sup>1</sup>. The propagation of BTV infection relies on the presence of *Culicoides*, with factors such as species, population density, virus strain<sup>16</sup>, and external conditions like temperature<sup>17</sup> influencing infection likelihood. Although reliable data on *Culicoides* in Northwest Ethiopia is lacking, existing research on the Ceratopogonidae family has predominantly focused on its role in transmitting viruses such as African horse sickness, Bluetongue virus, and Schmallenberg virus<sup>1,3,15</sup>. Numerous studies have also explored the presence, species composition, and geographical distribution of *Culicoides*<sup>18–23</sup>.

In Ethiopia, several *Culicoides* species have been documented, including *C. milnei*, *C. zuluensis*, *C. imicola*, *C. neavei*, *C. fulvithorax*, and *C. isiolensis*<sup>24</sup>, *C. fuscicaudae*<sup>25</sup>, and *C. kingi*, *C. imicola*, *C. milnei*, *C. schultzei*, *C. zuluensis*, and *C. pycnostictus*<sup>26</sup>. The country faces recurrent and severe outbreaks of *Culicoides*-borne diseases. Between 2007 and 2010, 737 outbreaks of African horse sickness (AHS) were reported<sup>27</sup>. Serological evidence for bluetongue (BT) exist<sup>28,29</sup> but there is no molecular confirmation or isolation of Schmallenberg virus (SBV), despite a high apparent seroprevalence of 56.6%<sup>30</sup>. The lack of data on BT and SBV in Northwest Ethiopia may be due to misdiagnoses of other prevalent ruminant illnesses, such as foot and mouth disease (FMD), peste des petits ruminants (PPR), lumpy skin disease, and sheep and goat pox, which present similar clinical symptoms<sup>31</sup>. Understanding the species composition and population abundance of *Culicoides* in a given area is crucial for effective risk mitigation. Therefore, this study aimed to identify *Culicoides* species, map their spatial distribution, and document their seasonal occurrences in selected districts within the Central, South, and West Gondar zones of Northwest Ethiopia.

## Materials and methods

### Description of sample collection area

Samples were collected from six selected districts in the three Zones of Northwest Ethiopia: Central Gondar (Chilga, Wegera, and East Dembia), West Gondar (Metema and West Armacho), and South Gondar (Fogera). These districts were chosen for their unique geographical locations bordering neighboring countries and their large animal populations. Chilga District is situated at latitude 12°32'52" N and longitude 37°3'48" E, at an elevation of 2248 meters above sea level (m.a.s.l.). It features a mild climate with temperatures ranging from temperate to warm, averaging 20°C year-round, and receives 1175 mm of annual rainfall<sup>32</sup>. Wegera District is located at latitude 12°52'5" N and longitude 37°39'47" E, with elevations ranging from 1100 to 3040 m.a.s.l. The district experiences annual rainfall between 1000 to 1200 mm, with temperatures ranging from 14°C to 33°C. The rainy season extends from June to September, with peak rainfall occurring in July and August<sup>33</sup>. East Dembia District, at latitude 12°40' N and longitude 37°10' E, has an average elevation of 2053 m.a.s.l., an average annual temperature of 28 °C, and receives annual rainfall ranging from 995 to 1175 mm<sup>32</sup>.

Metema is located at latitude 12°58' N and longitude 36°12' E, with an elevation of 712 m above sea level. Bordered by Sudan to the west, Chilga to the east, Tach Armacho to the northeast, and West Armacho to the north, Metema is a key development corridor in the Amhara region. The area is characterized by fertile farms

and large-scale cultivation of cash crops such as sesame, maize, cotton, and sorghum. The district has a total population of 115,285, residing in both urban and rural areas<sup>34,35</sup>. West Armacho is situated at latitude 13°13'33" N and longitude 36°26'21" E, with an elevation of 667 m above sea level. It experiences high temperatures, averaging 38 °C, and high humidity at 78%. Daytime temperatures can soar up to 43 °C from March to May. West Armacho is an agricultural region known for extensive farming, with a population of 34,473. It is bordered by Metema to the south, Sudan to the west, Tigray Region to the north, and Central Gondar to the east<sup>35,36</sup>.

Fogera District is located at latitude 11°56'59" N and longitude 37°35'9" E. It is bordered by Dera to the south, Lake Tana to the west, Reb to the north, Ebenat to the northeast, and Farta to the east. The district's altitude ranges from 1774 to 2410 m above sea level, providing favorable conditions for extensive crop production and livestock rearing. Fogera has an average annual temperature of 27 °C<sup>37</sup> (Fig. 1).

### Description of laboratory processing areas

Culicoides were identified at the Veterinary Parasitology Laboratory of the University of Gondar. Species identification and enumeration were performed at the Animal Health Institute (AHI) in Sebeta, Ethiopia.

### Study design

A cross-sectional study design was employed between January and July 2023 to collect, classify, and ascertain the occurrence and spatial dispersion of *Culicoides* in the study area.

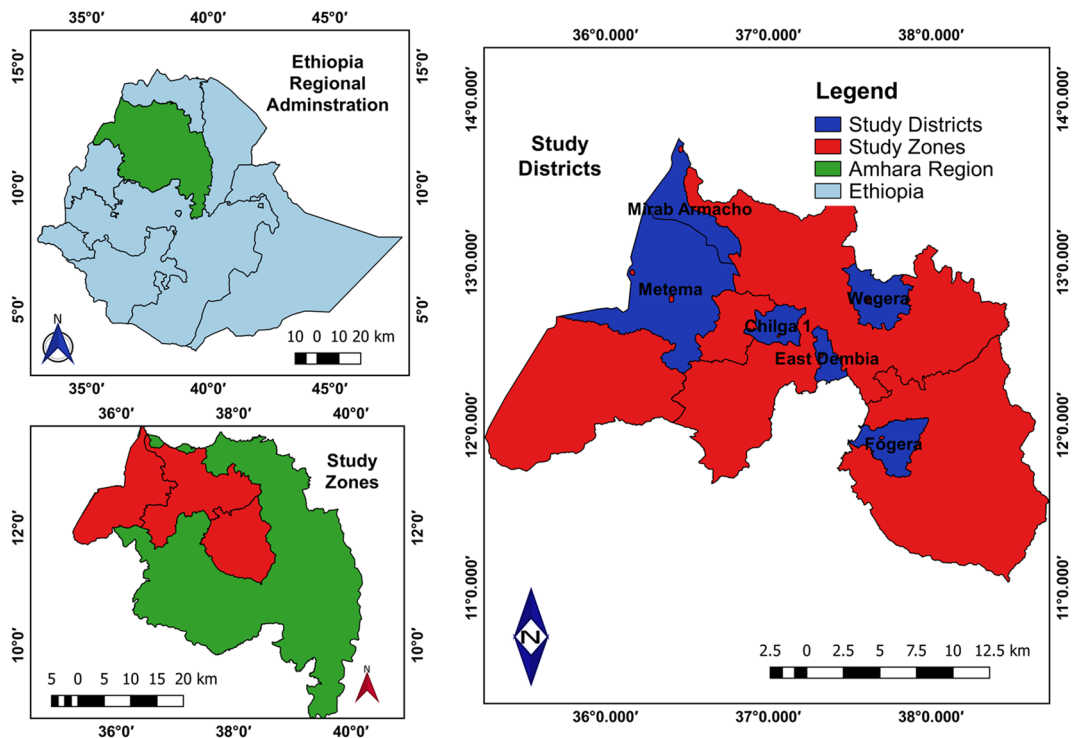
### Culicoides collection and identification

#### *Culicoides* collection

*Culicoides* trapping was conducted in two distinct phases between January and July 2023. The initial sampling round, from January to April 2023, covered the dry season, while the second round, from May to July 2023, corresponded with the wet season following the rainy period. Ultraviolet (UV) light/suction traps, manufactured by the Onderstepoort Veterinary Institute (OVI) in South Africa and powered by a 12 V car battery, were used. The OVI trap has been recognized as an effective method for capturing a diverse range and large quantity of *Culicoides* species<sup>38</sup>. These traps are equipped with UV lights to attract insects, gauze, fans, and collection beakers. Trap deployment sites were carefully chosen to ensure that they were conducive to *Culicoides* breeding, including locations near animal pens, indoors, outdoors, wetlands, and livestock farms. *Culicoides* and other insects were collected in 500 ml beakers containing 250 ml of phosphate-buffered saline. After collection, samples were stored at -4 °C for 15 min to immobilize the flies. Subsequently, the identified *Culicoides* were preserved in cryovial tubes containing cotton and 70% ethanol to maintain their integrity for species identification.

#### Identification of *Culicoides* spp

*Culicoides* species were initially distinguished from other insects by using a stereomicroscope at the Veterinary Parasitology Laboratory of the University of Gondar. The identified *Culicoides*, preserved in cryovial tubes, were



**Fig. 1.** Map of *Culicoides* collection sites (QGIS 3.22.6 software was used to draw).

then transferred to the Animal Health Institute (AHI) for species classification and quantification. This was done by examining their morphological characteristics under a stereomicroscope. Key distinguishing features of *Culicoides* from other fly species include the size and shape of the antennae, wing pigmentation patterns, vein alignment with the wings, and the arrangement of wing macrotrichia at both distal and proximal ends. Additionally, the presence of grey and white spots in the anal region helps in identification. These distinctive patterns are specific to each species and are readily observable using dissection microscopy. Specimens were also mounted on slides and examined under a light microscope to assess morphological attributes such as the shape, size, position, and number of female spermathecae (as male *Culicoides* lack spermathecae). Male *Culicoides* exhibit hairy protrusions on the antennae, and particular attention was paid to ocular features, with the compound eye and inter-eye distance serving as crucial identification markers. This included determining whether the eyes were fused or separated by one or two facets. Furthermore, the ratio of antennae length, specifically segment XI divided by segment X, was calculated. The maxillary palps, consisting of five segments, were examined for variations in the shape of the third segment, which can be cylindrical, triangular, swollen, moderately swollen, or slender. Finally, all observed characteristics were compared with images in the Interactive Identification Key for *Culicoides* (IIKC) database developed by Mathieu et al.<sup>39</sup>.

### Spatial distribution of *Culicoides*

For spatial distribution analysis, geographic coordinates were recorded using a global positioning system (GPS) or smartphones, specifying latitude and longitude. A table joint technique was used to present the collected data on *Culicoides* species from both the dry and wet seasons. The spatial distribution model was developed using QGIS software, version 3.26.2.

### Data management and analysis

The data collected from each location were documented using Microsoft Excel (2019). Descriptive statistics, expressed as percentages, were used to present the number of species obtained at each sampling location and during different seasons. The Shannon diversity index and Shannon equitability index were employed to measure the diversity and evenness of *Culicoides* species at sampling points and across seasons. Statistical significance was assessed using Poisson regression, with a  $p$ -value  $< 0.05$  considered significant. A spatial distribution map was created using QGIS software, version 3.26.2.

## Results

### Entomological survey

In the present study, 8,857 *Culicoides* were collected from 44 collection locations. A total of twelve *Culicoides* species were identified, encompassing 8,838 individuals. Additionally, nine *Culicoides* specimens were of unknown classification. Ten of the identified *Culicoides* showed similarity with *C. pole* and *C. festivipennis*. Notably, *Culicoides kingi* was the most prevalent species, accounting for 4,784 (54.01%), followed by *C. imicola* (3946; 44.55%). The abundances of the remaining species were as follows: *C. reithi* (60; 0.78%); *C. desestrum* (23; 0.26%); *C. corsicus* (14; 0.158%); *C. festivipennis* (5; 0.056%); *C. reioxi* (3; 0.034%); *C. kibunensi* (1; 0.011%); *C. kiouxi* (1; 0.011%); and *C. sahariennes* (1; 0.011%). Additionally, the distribution of *Culicoides* across different districts revealed that East Dembia yielded the highest number of *Culicoides*, with 5,968 (67.38%), followed by Fogera with 822 (9.28%), Metema with 687 (7.76%), West Armacho with 592 (6.69%), Wegera with 447 (5.04%), and Chilga with 341 (3.85%). There was a statistically significant association ( $p < 0.05$ ) between the distribution of *Culicoides* and districts. The results also indicated that the diversity of *Culicoides* species was higher and more evenly distributed in samples collected from East Dembia ( $EH = 0.32$ ) (Table 1).

The results in Table 2 show that the majority of *Culicoides* (6,790; 76.7%) were collected in the vicinity of animal pens. A statistically significant association was observed ( $p < 0.05$ ) between the number of *Culicoides* collected and the sampling points. The results also indicated that the diversity of *Culicoides* species was higher in samples collected from animal pens ( $H = 0.73$ ) (Table 2).

### Seasonal occurrence and spatial distribution of *Culicoides*

During this investigation, all 12 *Culicoides* species were observed during the dry season. However, the quantity of *Culicoides* collected during the dry season (3,505) was significantly lower than that collected during the wet season (5,355), with a statistically significant association ( $p < 0.05$ ). *C. kingi* emerged as the most prevalent species captured during the dry season. In addition, five *Culicoides* species were identified during the wet season, with *C. imicola* emerging as the predominant species (52.25%), followed by *C. kingi* (46.67%). The diversity of *Culicoides* species was higher in the wet season ( $H = 0.77$ ) compared to the dry season ( $H = 0.75$ ) (Table 3).

The findings presented in Fig. 2 illustrate the spatial distribution of *C. kingi* during both the wet and dry seasons. During the dry season, *C. kingi* was most predominant in East Dembia, followed by Metema and Mirab Armacho. In the wet season, *C. kingi* showed a preference for East Dembia, followed by Fogera and Wegera. Similarly, Fig. 3 shows that *C. imicola* had a higher presence in East Dembia during the dry season, followed by Metema and Chilga. However, in the wet season, *C. imicola* was most prevalent in East Dembia, followed by Fogera and Metema.

The results presented in Fig. 4 show that during the dry season, *C. desertorum* was most prevalent in West Armacho, followed by East Dembia. However, during the wet season, the presence of *C. desertorum* favored East Dembia.

The findings presented in Fig. 5 demonstrate that *C. reithi* was most prevalent in Mirab Armacho, followed by East Dembia during the dry season. Additionally, the presence of *C. reithi* was more pronounced in Wegera, followed by East Dembia, during the wet season.

Culicoides species	Districts						Total (%)
	East Dembia	Metema	West Armacho	Chilga	Wegera	Fogera	
<i>C. kingi</i>	3,096	409	341	177	287	474	4,784 (54.01%)
<i>C. imicola</i>	2,815	273	238	153	130	337	3,946 (44.55%)
<i>C. corsicus</i>	14	0	0	0	0	0	14 (0.158%)
<i>C. kibunensis</i>	1	0	0	0	0	0	1 (0.011%)
<i>C. reioxi</i>	3	0	0	0	0	0	3 (0.034%)
<i>C. kiouxi</i>	1	0	0	0	0	0	1 (0.011%)
<i>C. sahariennes</i>	1	0	0	0	0	0	1 (0.011%)
<i>C. desertorum</i>	10	0	13	0	0	0	23 (0.26%)
<i>C. reithi</i>	14	5	0	11	25	5	60 (0.78%)
<i>C. festipennis</i>	0	0	0	0	5	0	5 (0.056%)
<i>C. undifiend spp</i>	3	0	0	0	0	6	9 (0.101%)
Similarity with <i>C. paole</i> & <i>C. festipennis</i>	10	0	0	0	0	0	10 (0.112%)
Total (%)	5968 <sup>#</sup> (67.38%)	687 <sup>#</sup> (7.76%)	592 <sup>#</sup> (6.69%)	341 (3.85%)	447 <sup>#</sup> (5.04%)	822 <sup>#</sup> (9.28%)	8,857 (100%)

**Table 1.** Culicoides species collected across the study sites. Analysis was performed using Poisson regression; compared with the districts, <sup>#</sup> $p \leq 0.001$  Shannon diversity index value; (East Dembia = 0.76; Metema = 0.71; West Armacho = 0.77; Chilga = 0.81; Wegera = 0.86; Fogera = 0.75) Shannon equitability index value; (East Dembia = 0.32; Metema = 0.65; West Armacho = 0.70; Chilga = 0.74; Wegera = 0.62; Fogera = 0.54)

Culicoides species	Sampling point			Total count
	Indoor	Animal pen	Outdoor on field	
<i>C. kingi</i>	287	3,570	927	4,784
<i>C. imicola</i>	130	3,152	664	3,946
<i>C. corsicus</i>	0	14	0	14
<i>C. kibunensis</i>	0	1	0	1
<i>C. reioxi</i>	0	3	0	3
<i>C. kiouxi</i>	0	1	0	1
<i>C. sahariennes</i>	0	1	0	1
<i>C. desertorum</i>	0	10	13	23
<i>C. reithi</i>	25	19	16	60
<i>C. festipennis</i>	5	0	0	5
<i>C. undifiend spp</i>	0	9	0	9
Similarity with <i>C. paole</i> & <i>C. festipennis</i>	10	0	0	10
Total	447 <sup>#</sup>	6,790	1,620 <sup>#</sup>	8,857

**Table 2.** Culicoides species collected at different sampling points. Analysis was performed using Poisson regression; compared with the sampling points, <sup>#</sup> $p \leq 0.001$  Shannon diversity index value; (Indoor = 0.94; Animal pen = 0.75; Outdoor = 0.77) Shannon equitability index value; (Indoor = 0.58; Animal pen = 0.33; Outdoor = 0.56)

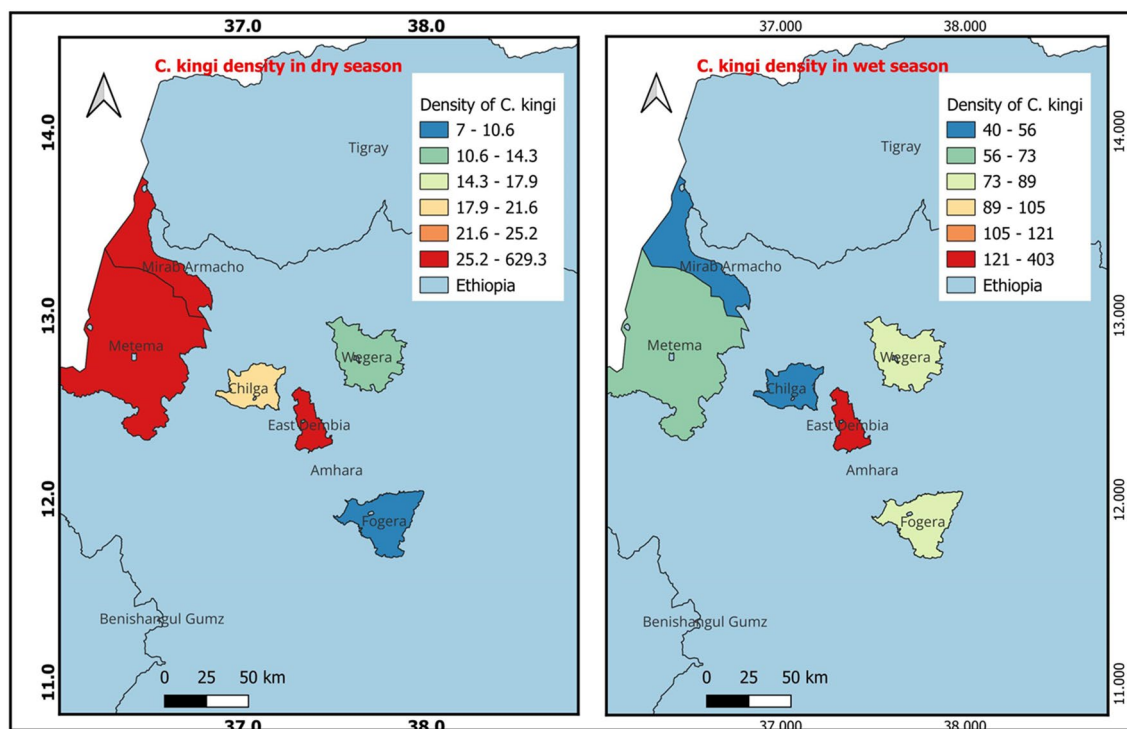
Furthermore, the distribution of *C. corsicus* was most prevalent in East Dembia, as shown in Supplementary Fig. 1. Additionally, *C. festipennis* was most prevalent in Wegera (Supplementary Fig. 2). An unknown Culicoides species was most predominant in Fogera during the dry season (Supplementary Fig. 3). Moreover, *C. riouxi* was exclusively detected in East Dembia during the wet season (Supplementary Fig. 4).

## Discussion

During the study period, a total of 8,857 Culicoides were captured across 44 trapping sites. Of these, 8,838 were classified into 12 distinct species, while the remaining nine Culicoides could not be identified to species level. Among the identified species, ten exhibited similarities to *C. paole* and *C. festipennis*, with *C. kingi* being the most prevalent at 54.01%. This contrasts with an investigation by Fetene et al<sup>26</sup>, which found *C. imicola* to be more abundant than *C. kingi*. Morphological assessment in the present study confirmed the presence of 12 Culicoides species in the study areas. Notably, *C. kingi* was the most frequently collected species, accounting for 4,784 individuals (54.01%). This finding diverges from previous studies in sub-Saharan Africa by Sghaier et al<sup>21</sup>, Gordon et al<sup>22</sup>, and Guichard et al<sup>40</sup>, which identified *C. imicola* as the predominant species. Additionally, Mulatu and Hailu<sup>24</sup> documented the presence of *C. imicola*, *C. milnei*, *C. neavei*, *C. zuluensis*, *C. fulvithorax*, and *C. isoloensis* in Western Ethiopia, while Khamala and Kettle<sup>25</sup> reported *C. fuscicaudae* in West Africa. Fetene et

Culicoides species	Dry season	Wet season
	Total number (%)	Total number (%)
<i>C. kingi</i>	2,284 (65.2%)	2,499 (46.67%)
<i>C. imicola</i>	1,148 (32.8%)	2,798 (52.25%)
<i>C. corsicus</i>	14 (0.4%)	0
<i>C. kibunensis</i>	1 (0.028%)	0
<i>C. reioxi</i>	1 (0.028%)	2 (0.037%)
<i>C. kiouxi</i>	1 (0.028%)	0
<i>C. sahariennes</i>	1 (0.028%)	0
<i>C. desertorum</i>	19 (0.54%)	4 (0.074%)
<i>C. reithi</i>	12 (0.34%)	52 (0.97%)
<i>C. festipennis</i>	5 (0.143%)	0
<i>C. undifend spp</i>	9 (0.26%)	0
Similarity with <i>C. paole</i> & <i>C. festipennis</i>	10 (0.285)	0
Total	3,505 (100%)	5,355 (100%)*

**Table 3.** Culicoides species collected during the different seasons. Analysis was performed using Poisson regression; compared with the season, \* $p \leq 0.001$  Shannon diversity index value; (Dry = 0.77; Wet = 0.75) Shannon equitability index value; (Dry = 0.31; Wet = 0.47)



**Fig. 2.** Density of *C. kingi* occurrence during the dry and wet seasons (QGIS 3.22.6 software was used).

al<sup>26</sup>, also identified *C. kingi*, *C. schultzei*, and *C. pycnostictus*, in various Ethiopian regions. However, the current study revealed eight Culicoides species not previously documented in Ethiopia: *C. corsicus*, *C. kibunensis*, *C. reioxi*, *C. kiouxi*, *C. sahariennes*, *C. desertorum*, *C. reithi*, and *C. festipennis*.

The current investigation identified East Dembia as the most favorable area for the presence of Culicoides species. This finding of ecological preference aligns with research conducted by Blanda et al<sup>10</sup> in Italy. Our study presents the results of a spatial distribution methodology used to assess the abundance of key Culicoides vector species by analyzing a substantial number of collections across both dry and wet seasons. Culicoides were significantly more abundant in the East Dembia, Fogera, and Metema districts, while lower levels were observed in Mirab Armacho, Wegera, and Chilga. Various factors potentially influence the distribution of Culicoides species, including biotic elements (such as vegetation, human activities, and the presence of other animals) and abiotic factors (such as light, soil composition, water sources, air quality, and climatic conditions), along with other variables that may vary between macrohabitats and microhabitats.

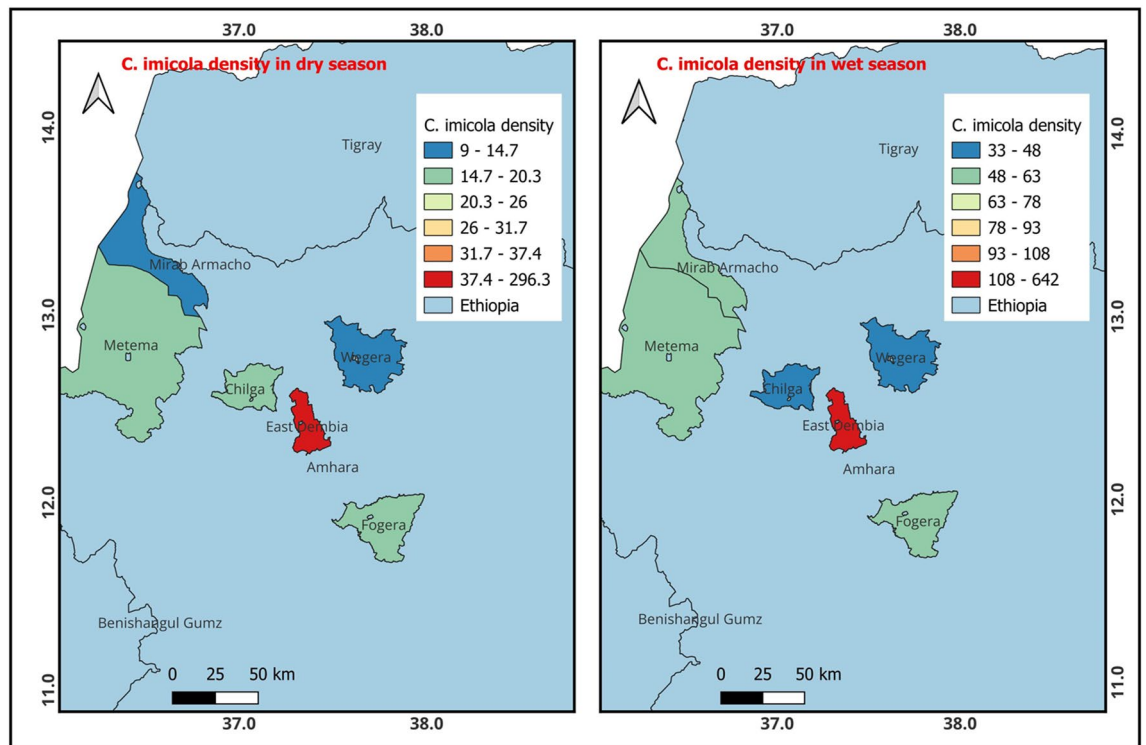


Fig. 3. Density of *C. imicola* occurrence during the dry and wet seasons (QGIS 3.22.6 software was used).

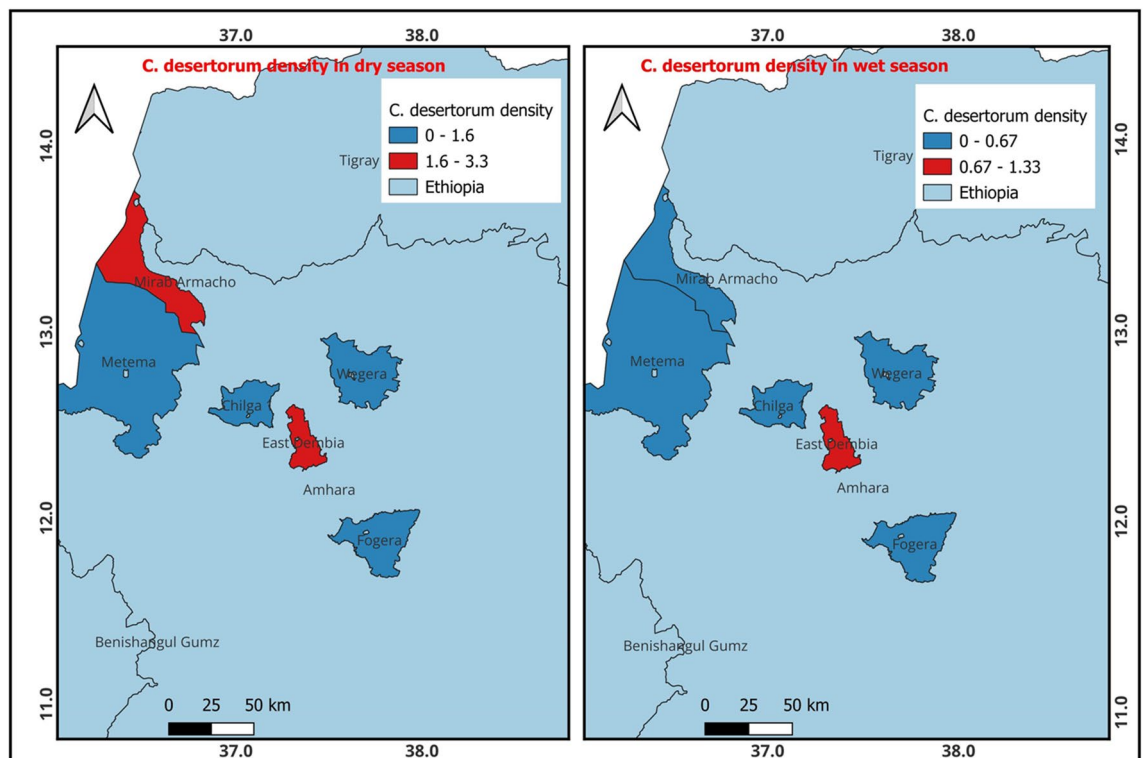
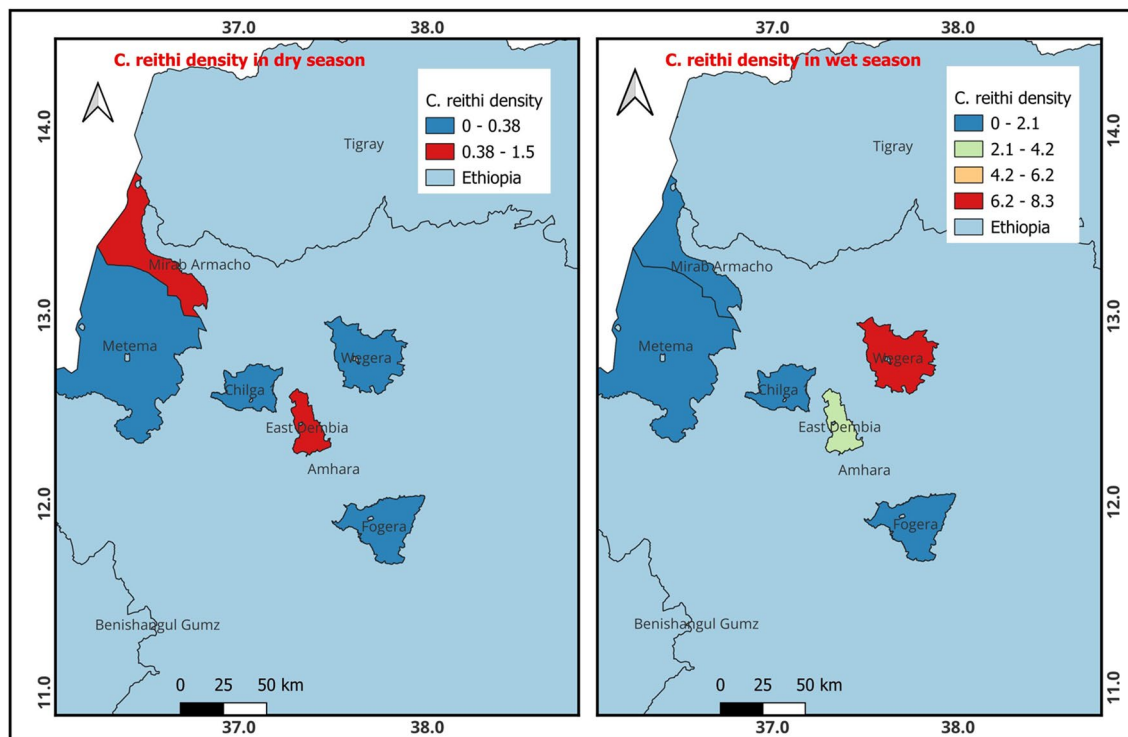


Fig. 4. Density of *C. desertorum* occurrence during the dry and wet seasons (QGIS 3.22.6 software was used).



**Fig. 5.** Density of *C. reithi* occurrence during the dry and wet seasons (QGIS 3.22.6 software was used).

In the current study, during the dry season in East Dembia, *C. kingi* had an average occurrence ranging from 39.3 to 629.3, followed by *C. imicola* with an average range of 17.5 to 296.3. During the wet season, *C. imicola* had an average occurrence ranging from 52.4 to 642, while *C. kingi* ranged from 87.8 to 402.7. The study found that the occurrence of Culicoides was higher during the wet season compared to the dry season, with the association being statistically significant ( $p < 0.05$ ). *C. kingi* was the most prevalent species captured during the dry season, while *C. imicola* emerged as the predominant species during the wet season. The observed seasonal discrepancies might be attributed to the presence of favorable conditions that support the existence and development of Culicoides. Additionally, soil type appears to be crucial in determining the distribution and abundance of *C. imicola*, and climatic variables such as mean annual minimum and maximum temperatures and mean annual rainfall were found to influence its occurrence. Similar findings have been reported by Ander et al<sup>41</sup>., Gordon et al<sup>22</sup>., Fetene et al<sup>26</sup>., and Carpenter et al<sup>17</sup>.. *C. imicola* has a broad global distribution, extending from South Africa to regions including the Mediterranean, Middle East, and Far East. However, certain areas show low densities or complete absence of *C. imicola*<sup>42</sup>, indicating a patchy distribution pattern for this species.

The spatial distribution analysis identified East Dembia as the most favorable area for the presence of Culicoides species. This ecological preference aligns with findings from Blanda et al<sup>10</sup>., conducted in Italy, suggesting a correlation with specific biotic or abiotic factors in different districts. The geographical location of East Dembia, particularly its proximity to Lake Tana, likely contributes to its suitability for most Culicoides species. Additionally, the Metema and Mirab Armacho districts were identified as favorable sites for some Culicoides species, potentially due to their high temperatures and proximity to the Sudan border. Other factors influencing the distribution patterns of Culicoides include vegetation, animal presence, and microclimatic conditions such as altitude, temperature, and precipitation. Previous research has shown that the spatial distribution of Culicoides species. can be affected by diverse climatic zones<sup>43</sup> and local factors like livestock management practices. Additionally, studies have explored the impact of seasonal and meteorological parameters on Culicoides activity<sup>41,43–45</sup> and generated risk maps based on habitat attributes<sup>46–48</sup>. For example, a correlation has been identified between the spread of bluetongue disease and specific landscape characteristics such as woodlands and open grasslands<sup>49</sup>.

### Conclusion and recommendations

In the present study, *C. kingi* and *C. imicola* emerged as the predominant Culicoides species. The occurrence of Culicoides species was found to be higher during the wet season. There was a statistically significant association between Culicoides occurrence and season, sampling point, and districts. Additionally, East Dembia was identified as the most favorable area for the occurrence of Culicoides species. The study also revealed that the diversity of Culicoides species was higher in samples collected from animal pens and during the wet season. Further research is needed to explore the impact of various Culicoides species on both animal and human health, as well as their economic implications. Corresponding control strategies should be developed based on these findings.



## Data availability

All data generated or analyzed during this study are available upon request from the corresponding author.

Received: 17 May 2024; Accepted: 26 September 2024

Published online: 07 October 2024

## 6. References

- Mellor, P. S., Boorman, J. & Baylis, M. Culicoides biting midges: Their role as arbovirus vectors. *Annu. Rev. Entomol.* **45**, 307–40. <https://doi.org/10.1146/annurev.ento.45.1.307> (2000).
- Mullen, G. R. & Murphree, C. S. Biting midges (ceratopogonidae). In: *Medical and Veterinary Entomology*. Elsevier; 213–236. (2018).
- Carpenter, S., Groschup, M. H., Garros, C., Felipe-Bauer, M. L. & Purse, B. V. Culicoides biting midges, arboviruses and public health in Europe. *Antiviral Res.* **100**, 102–113 (2013).
- Shults, P., Borkent, A. & Gold, R. The pupa of *Culicoides Sonorensis* Wirth and Jones (Diptera: Ceratopogonidae) - first detailed description of this stage of the bluetongue virus vector. *Ann. Entomol. Soc. Am.* **109**, 280–318. <https://doi.org/10.1093/aesa/sav119> (2016).
- Paweska, J. T., Venter, G. J. & Mellor, P. S. Vector competence of South African *Culicoides* species for bluetongue virus serotype 1 (BTV-1) with special reference to the effect of temperature on the rate of virus replication in *C. Imicola* and *C. Bolitinos*. *Med. Vet. Entomol.* **16**, 10–21. <https://doi.org/10.1046/j.1365-2915.2002.00334.x> (2002).
- Mellor, P. Environmental influences on arbovirus infections and vectors. *Microbe-vector Interact. vector-borne Dis.* 181–197. (2004).
- Wilson, A. J. & Mellor, P. S. Bluetongue in Europe: past, present and future. *Philosophical Trans. Royal Soc. B: Biol. Sci.* **364**, 2669–2681. <https://doi.org/10.1098/rstb.2009.0091> (2009).
- Baldet, T., Mathieu, B., Delecolle, J., Gerbier, G. & Roger, F. Emergence de la fièvre catarrhale ovine dans le Bassin méditerranéen Et surveillance entomologique en France. *Rev. d'élevage et médecine vétérinaire des pays Trop.* **58**, 125–132 (2005).
- Bessell, P. R. et al. Assessing the potential for bluetongue virus 8 to spread and vaccination strategies in Scotland. *Sci. Rep.* **6**. <https://doi.org/10.1038/srep38940> (2016).
- Blanda, V. et al. Geo-statistical analysis of *Culicoides* spp. distribution and abundance in Sicily, Italy. *Parasites Vectors.* **11**. <https://doi.org/10.1186/s13071-018-2658-2> (2018).
- Black, W. et al. *Biological of Disease Vectors*. 2nd edition. Burlington, USA: Elsevier Academic Press; (2004).
- Meiswinkel, R., Gomulski, L., Delécolle, J., Goffredo, M. & Gasperi, G. The taxonomy of *Culicoides* vector complexes-unfinished business. *Vet. Ital.* **40**, 151–159 (2004).
- Wirth, W., Dyce, A. & Spinelli, G. An atlas of wing photographs, with a summary of the numerical characters of the Neotropical species of *Culicoides* (Diptera: Ceratopogonidae). *Contrib Am Entomol Inst.* (1988). <https://www.cabidigitallibrary.org/doi/full/10.5555/19920509805>. Accessed 9 Aug 2024.
- Atkinson, C. T. Parasitic diseases of Wild Birds. In: (eds Atkinson, C. T., Thomas, N. J. & Hunter, D. B.) *Parasitic diseases of Wild Birds*. New York, America: John Wiley & Sons, Ltd; 13–35. doi:<https://doi.org/10.1002/9780813804620>. (2009).
- Sick, F., Beer, M., Kampen, H. & Wernike, K. *Culicoides* biting midges—underestimated vectors for arboviruses of public health and veterinary importance. *Viruses.* **11**, 376. <https://doi.org/10.3390/v11040376> (2019).
- Tabachnick, W. J. *Culicoides variipennis* and bluetongue-virus epidemiology in the United States. *Annu. Rev. Entomol.* **41**, 23–43. <https://doi.org/10.1146/annurev.en.41.010196.000323> (1996).
- Carpenter, S. et al. Temperature dependence of the extrinsic incubation period of Orbiviruses in *Culicoides* Biting Midges. *PLoS ONE.* **6**, e27987. <https://doi.org/10.1371/journal.pone.0027987> (2011).
- Vasić, A. et al. Species diversity, host preference and arbovirus detection of *Culicoides* (Diptera: Ceratopogonidae) in south-eastern Serbia. *Parasites Vectors.* **12**, 1–9. <https://doi.org/10.1186/s13071-019-3292-3> (2019).
- Martin, E. et al. *Culicoides* species community composition and infection status with parasites in an urban environment of east central Texas, USA. *Parasit. Vectors.* **12**, 39. <https://doi.org/10.1186/s13071-018-3283-9> (2019).
- Gusmão, G. M. C., Brito, G. A., Moraes, L. S., Bandeira, M. D. C. A. & Rebêlo, J. M. M. Temporal variation in species abundance and richness of *Culicoides* (Diptera: Ceratopogonidae) in a Tropical Equatorial Area. *J. Med. Entomol.* **56**, 1013–1018. <https://doi.org/10.1093/jme/tjz015> (2019).
- Sghaier, S. et al. Specie Del genere *Culicoides* (Diptera Ceratopogonidae) e presenza del virus della Bluetonge in Tunisia. *Vet. Ital.* **53**, 357–366 (2017).
- Gordon, S. J. G. et al. The occurrence of *Culicoides* species, the vectors of arboviruses, at selected trap sites in Zimbabwe. *Onderstepoort J. Vet. Res.* **82**. <https://doi.org/10.4102/ojvr.v82i1.900> (2015).
- Villard, P. et al. Modeling *Culicoides* abundance in mainland France: implications for surveillance. *Parasites Vectors.* **12**, 391. <https://doi.org/10.1186/s13071-019-3642-1> (2019).
- Mulatu, T. & Hailu, A. The occurrence and identification of *Culicoides* species in the Western Ethiopia. *Acad. J. Entomol.* **12**, 40–43 (2019).
- Khamala, C. & Kettle, D. The *Culicoides* Latreille (Diptera: Ceratopogonidae) of East Africa. (1971). <https://www.cabidigitallibrary.org/doi/full/10.5555/19711001710>. Accessed 9 Aug 2024.
- Fetene, E. et al. Modeling the spatial distribution of *Culicoides* species (Diptera: Ceratopogonidae) as vectors of animal diseases in Ethiopia. *Sci. Rep.* **12**, 12904. <https://doi.org/10.1038/s41598-022-16911-y> (2022).
- Aklilu, N. et al. African horse sickness outbreaks caused by multiple virus types in Ethiopia. *Transbound. Emerg. Dis.* **61**, 185–192. <https://doi.org/10.1111/tbed.12024> (2014).
- Gizaw, D., Sibhat, D., Ayalew, B. & Sehal, M. Sero-prevalence study of bluetongue infection in sheep and goats in selected areas of Ethiopia. *Ethiop. Vet. J.* **20**, 105. <https://doi.org/10.4314/evj.v20i1.8> (2016).
- Abera, T. et al. Bluetongue disease in small ruminants in south western Ethiopia: cross-sectional sero-epidemiological study. *BMC Res. Notes.* **11**, 112. <https://doi.org/10.1186/s13104-018-3222-z> (2018).
- Sibhat, B., Ayelet, G., Gebremedhin, E. Z., Skjerve, E. & Asmare, K. Seroprevalence of Schmallenberg virus in dairy cattle in Ethiopia. *Acta Trop.* **178**, 61–67 (2018).
- Rojas, J. M., Rodríguez-Martín, D., Martín, V. & Sevilla, N. Diagnosing bluetongue virus in domestic ruminants: current perspectives. *Vet. Med. Res. Rep.* **10**, 17–27. <https://doi.org/10.2147/vmrr.s163804> (2019).
- Melese, A. T., Ayele, D. T., Aljerf, L., Al-Fekaiki, D. F. & Akele, M. L. Investigating the phytoavailability of metals in roots of *Croton macrostachyus* and *Phytolacca dodecandra*: induced rhizosphere processes. *BioMetals.* **36**, 1347–1359. <https://doi.org/10.1007/s10534-023-00522-9> (2023).
- Dessie, A. B., Abteaw, A. A. & Koye, A. D. Analysis of Smallholder Farmers' Cooperation in Eucalyptus Woodlot production in Wegera District, Northern Ethiopia. *Small-scale for.* **18**, 291–308. <https://doi.org/10.1007/s11842-019-09418-4> (2019).
- Gelaye, K. A. et al. Low knowledge and attitude towards visceral Leishmaniasis among migrants and Seasonal Farm workers in Northwest Ethiopia. *Res. Rep. Trop. Med.* **11**, 159–168. <https://doi.org/10.2147/rrtm.s286212> (2020).

35. Lemma, W. Impact of high malaria incidence in seasonal migrant and permanent adult male laborers in mechanized agricultural farms in Metema-Humera lowlands on malaria elimination program in Ethiopia. *BMC Public Health*. **20**. <https://doi.org/10.1186/s12889-020-8415-4> (2020).
36. Aschale, Y., Mengist, A., Bitew, A., Kassie, B. & Talie, A. Prevalence of malaria and associated risk factors among asymptomatic migrant laborers in West Armachiho District, Northwest Ethiopia. *Res. Rep. Trop. Med.* **9**, 95–101. <https://doi.org/10.2147/rrtm.s165260> (2018).
37. Fikadu, A. A. & Gebre, G. G. Evidence from Fogera district in Ethiopia on configuration of farmer's information literacy conditions that explain better productivity performance of the horticultural crops. *Agric. Food Secur.* **10**, 1–13. <https://doi.org/10.1186/s40066-021-00299-5> (2021).
38. Venter, G. J. et al. Comparison of the efficiency of five suction light traps under field conditions in South Africa for the collection of Culicoides species. *Vet. Parasitol.* **166**, 299–307 (2009).
39. Mathieu, B. et al. Development and validation of IIKC: an interactive identification key for Culicoides (Diptera: Ceratopogonidae) females from the Western Palaearctic region. *Parasites Vectors*. **5**, 137. <https://doi.org/10.1186/1756-3305-5-137> (2012).
40. Guichard, S. et al. Worldwide niche and future potential distribution of Culicoides Imicola, a major vector of bluetongue and African horse sickness viruses. *PLoS ONE*. **9**, e112491. <https://doi.org/10.1371/journal.pone.0112491> (2014).
41. Ander, M., Meiswinkel, R. & Chirico, J. Seasonal dynamics of biting midges (Diptera: Ceratopogonidae: Culicoides), the potential vectors of bluetongue virus, in Sweden. *Vet. Parasitol.* **184**, 59–67. <https://doi.org/10.1016/j.vetpar.2011.08.009> (2012).
42. Venter, G. J. & Meiswinkel, R. The virtual absence of Culicoides imicola (Diptera: Ceratopogonidae) in a light-trap survey of the colder, high-lying area of the eastern Orange Free State, South Africa, and implications for the transmission of arboviruses. (1994). <https://scholar.archive.org/work/xjpvusiafa3njhwri6zf3ki7e/access/wayback/http://repository.up.ac.za/bitstream/handle/2263/33050/43venter1994.pdf?sequence=1>. Accessed 9 Aug 2024.
43. Brugger, K. & Rubel, F. Characterizing the species composition of European Culicoides vectors by means of the Köppen-Geiger climate classification. *Parasites Vectors*. **6**, 333. <https://doi.org/10.1186/1756-3305-6-333> (2013).
44. Sanders, C. J. et al. Influence of season and meteorological parameters on flight activity of Culicoides biting midges. *J. Appl. Ecol.* **48**, 1355–1364. <https://doi.org/10.1111/j.1365-2664.2011.02051.x> (2011).
45. Raclouz, V., Venter, G., Griot, C. & Stärk, K. D. C. Estimating the temporal and spatial risk of bluetongue related to the incursion of infected vectors into Switzerland. *BMC Vet. Res.* **4**, 42. <https://doi.org/10.1186/1746-6148-4-42> (2008).
46. Conte, A. et al. Culicoides Imicola. *Vet. Ital.* **40**, 311 (2004).
47. Takken, W. et al. The phenology and population dynamics of Culicoides spp. in different ecosystems in the Netherlands. *Prev. Vet. Med.* **87**, 41–54 (2008).
48. Caligiuri, V. et al. Bluetongue surveillance in the Campania region of Italy using a geographic information system to create risk maps. *Vet. Ital.* **40**, 385–389 (2004).
49. Guis, H. et al. Use of high spatial resolution satellite imagery to characterize landscapes at risk for bluetongue. *Vet. Res.* **38**, 669–683. <https://doi.org/10.1051/vetres:2007025> (2007).

## Acknowledgements

The authors acknowledge the University of Gondar, Office of the Vice President for Research and Community Service, for their financial support during this work. The authors also thank the Animal Health Institute, Ethiopia (AHI) for their cooperation during vector collection and identification.

## Author contributions

The contribution of the authors to the manuscript as follows, Conceptualization and Methodology: WM, SMI, GGD, ZST, and HD; Data curation and Formal analysis: BAA, ZST and HD; Software, Supervision and Validation: AA, ZST and HD; Writing- Original draft preparation: BAA; and Funding acquisition, Investigation, Project administration, Resources, Visualization and Writing- Review and Editing: WM, ABM, MB, SMI, BD, AK, ATG, MZK, GGD, MDF, TM, HT, MB, ZST, and HD. All authors read and approved the final manuscript.

## Funding

This study was funded by the Office of the Vice President for Research and Community Services at the University of Gondar, Gondar, Ethiopia. However, the funding body played no role in the design and execution of the study, including the data collection and analysis.

## Declarations

## Ethics statement

This study was reviewed and approved by the Institutional Review Board of the College of Veterinary Medicine and Animal Sciences of the University of Gondar, Ethiopia (reference number: CVMAS/11/127/2022).

## Consent for publication

All the authors have read and approved the final manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1038/s41598-024-74524-z>.

**Correspondence** and requests for materials should be addressed to H.D.

**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2024