



## ***CULICOIDES* SPP. (DIPTERA: CERATOPOGONIDAE) AND THEIR ASSOCIATED BACTERIAL COMMUNITIES, ENDOSYMBIONTS: A REVIEW**

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### **ABSTRACT**

The biting midges, *Culicoides* spp. (Diptera: Ceratopogonidae) are small haematophagous dipterans, demonstrated as vectors of multiple pathogens such as arboviruses, protozoa, fungi, and nematodes of veterinary and public health importance. Bacteria-specific information is far less despite its relevance in spreading arboviral diseases, these flies still need to be addressed. This article deals with the data on the association of bacteria and endosymbionts harboring in different biting midge species belonging to different subgenera. The bacteria influence insects' physiology, such as digestion and oviposition, and their hosts' survivability and vectorial efficiency. Bacterial strain-

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specific information is needed to correlate with these midges. However, few studies recorded pathogenic bacteria's association with vector species' life stages. Along with this, our analysis also corroborates with possible routes of pathogens' transmission and organ-specific localization. Future studies will be required to generate information on developing some putative bio-control strategies by utilizing these bacterial species or strain-specific midge-associated bacteria. The zoonotic potential of bacterial pathogens associated with the vector species requires attention.

**Keywords:** Biting midges, bacteria, endosymbionts, vectors

## INTRODUCTION

Adult individuals belonging to *Culicoides* spp. (Diptera: Ceratopogonidae) are the smallest haematophagous flies<sup>1</sup>. Worldwide, 1347 species have been identified and distributed from the tundra to the tropics and from sea level to 4000 m, except Antarctica and New Zealand<sup>1</sup>. Several species have achieved notoriety for inflicting nasty bites, causing severe arboviral disease outbreaks, and transmitting protozoa and filarial worms affecting livestock, birds, and even humans<sup>1</sup>. More than 50 viruses such as African horse sickness virus (AHSV), Akabane virus (AKAV), Aino virus (AINOV), Chuzan virus (CHUV), D'Aguilar virus (DAGV), Ibaraki virus (IBAV), bluetongue virus (BTV), epizootic haemorrhagic disease virus (EHDV), have been isolated from them<sup>1</sup>. Most notably the BTV, which has gained prime significance in the Indian scenario<sup>1,2</sup>. Of the 29 BTV serotypes, 23 were recorded from this country<sup>2</sup>. The following seven *Culicoides* species vectoring BTV belong to three subgenera, i.e., i) *Avaritia* (*Culicoides actoni* Smith, *Culicoides fulvus* Sen and Das Gupta, *Culicoides brevitarsis* Kieffer, *Culicoides imicola* Kieffer, *Culicoides orientalis* Macfie), ii) *Hoffmania* (*Culicoides peregrinus* Kieffer), and iii) *Remmia* (*Culicoides oxystoma* Kieffer) documented from India<sup>3,4</sup>. Besides BTV, all seven species are also implicated as vectors of other pathogens. A summary table depicting their wide distribution within India and pathogen-specific information are mentioned<sup>5</sup>. Besides that, nematode: *Onchocerca gibsoni*, the causative agent of filaria of cattle, and following protozoa: *Leucocytozoon* sp., an intracellular haemosporidian blood parasite, *Leishmania* (*Mundinia*) *martiniquensis*, *L. (M.) orientalis* and *Crithidia* spp. were detected from various

midges<sup>5</sup>. Data regarding the host ranges of these vector species were also collected, and it was recorded that these species were generally mammalophilic and ornithophilic<sup>5</sup>. Earlier review articles focused primarily on arboviruses, not on the bacteria. Bacteria can impact vectoring efficiency and provide several beneficial services to their hosts, such as nutrition, developmental time, reproduction, blood meal digestion, and egg production in haematophagous vectors<sup>6,7,8,9,10</sup>. These bacterial communities can emanate vertically from their parental origin and congeners or may be acquired via horizontal transmission through larval feeding and via ingestion of blood meal in adult stages<sup>11,12,13</sup>. Few bacteria residing within the development habitat mediate attraction and elicit oviposition responses<sup>14</sup>. In addition, the significance of the bacterial communities during larval development has been well studied in mosquitoes and sandflies. When antibiotics were used, mosquitoes' delayed growth and decreased larval survival were noted; however, these were retrieved by inoculating certain bacteria<sup>9,15</sup>. Likewise, incomplete larval development of *Culicoides stellifer* (Coquillett) was observed when rearing medium containing autoclaved tap water<sup>16</sup>. Antibiotic-treated blood-fed *Aedes aegypti* decreased the lysis of red blood cells and reduced the rate of digestion of blood proteins and production of viable eggs in the first gonotrophic cycle<sup>8</sup>. Blood meal digestion leads to oxidative stress by increasing reactive oxygen species (ROS) levels in mosquitoes, and metabolites or antioxidants synthesized by them play crucial roles in heme-induced redox homeostasis<sup>17</sup>. The gut bacterial composition changes due to antibiotic treatment lead to an increased infection rate of *Culicoides nubeculosus* Meigen with Schmallenberg virus<sup>18</sup>. Arthropod-borne bacteria will constitute an essential reservoir of emerging diseases in the future<sup>19</sup>. So, information regarding the load of pathogenic bacteria within these vectors still needs to be included. Only one study regarding pathogenic bacteria documented the blood-borne pathogen, *Bartonella* sp., within *Culicoides* spp. indicating bacterial zoonosis infecting humans, domestic and wild animals<sup>20</sup>. Horizontal transmission includes regurgitation, salivation, through the cuticle, stercoration, and in rare cases, ingestion of the vector via blood feeding<sup>21</sup>. In addition, endosymbiotic bacteria can reduce the longevity of the vectors and can be used to interrupt onward viral transmission as they may affect vector competence by decreasing host susceptibility to viruses<sup>7,22</sup>. This article combines information on the association of bacteria and endosymbionts with the vector species.

### Subgenus *Avaritia*

The impact of biotic and abiotic factors on bacterial composition was recorded in *C. imicola*<sup>12</sup>. Shared core microbiomes included *Pseudomonas*, *Escherichia*, *Halomonas*, *Candidatus*, and *Propionibacterium* among the populations of this vector species. Unique bacterial genera were found in *C. imicola* trapped from each location, indicating similarities and divergences in microbiome composition between the two populations<sup>12</sup>.

Our study identified bacterial species belonging to two major phyla, i.e., Firmicutes and Proteobacteria, from gut and salivary glands of these vector species: *C. actoni*, *C. fulvus*, *C. imicola*, *C. orientalis*, and *C. jacobsoni* Macfie. Bacterial communities were compared across sexes and physiological ages of females. Within the salivary glands of these midges, the following bacteria: *Bacillus* sp., *Brevibacillus parabrevis*, *Paenibacillus lautus*, *Enterococcus faecium*, *Serratia marcescens*, *Pseudomonas stutzeri* were retrieved, and from the gut, *Bacillus* spp. *Brevibacillus parabrevis*, *Cytobacillus kochii*, *Lysinibacillus* spp., *Paenibacillus lautus*, *S. marcescens*, *Pseudomonas stutzeri* were identified. *Bacillus* spp. was the most abundant, followed by *Lysinibacillus* spp.

The gut microbiota of field-collected adults of four species, i.e., *C. chiopterus* (Meigen), *C. dewulfi* Goetghebuer, *C. obsoletus* (Meigen), and *C. scoticus* Downes and Kettle belonging to *Obsoletus* group from Sweden, Netherlands, and Italy were studied<sup>23</sup>. The gut bacterial communities of *C. chiopterus* differed from the other three species. Bacterial communities were similar for *C. obsoletus* across adults trapped in three countries, while it varied in the case of *C. scoticus*. These bacteria, namely, *Bacillus*, *Pseudomonas*, Enterobacteriaceae, and *Sphingomonas*, were commonly retrieved across the species of this group<sup>23</sup>.

The endosymbionts, *Cardinium*, and *Wolbachia*, were detected from *C. brevitarsis* and *C. imicola*, while only *Cardinium* was identified from *Culicoides wadai* Kitaoka<sup>24,25</sup>. Previously, *Cardinium* spp. was not detected in species of the *obsoletus* group, the primary vector of bluetongue and Schmallenberg viruses in northern Europe<sup>26</sup>. After that, records on occurrences of *Wolbachia* and *Cardinium* in *C. obsoletus*<sup>27</sup>. Furthermore, another endosymbiont, *Rickettsia*, was recently found in field-collected adults of *C. dewulfi*, *C. obsoletus*, and *C. scoticus*. *Cardinium* was found in field-collected adults of *C. scoticus*, and *Wolbachia* was

found in field-collected adults of *C. dewulfi*, *C. obsoletus*, *C. scoticus*, and *C. chiopterus*<sup>23</sup>.

### **Subgenus *Hoffmania***

The first bacterial study was performed in engorged and nulliparous females of *C. peregrinus* to isolate and identify two haemolytic bacterial strains<sup>28</sup>. The strains, CU1A, CU1B of *Bacillus pumilus* and CU2B of *Bacillus licheniformis* were identified. They suggested that the shortening of blood meal digestion time was due to the presence of these haemolytic bacteria. The quantitative enzyme assay recorded that CU1A synthesized protease, and CU1B and CU2B produced amylase and protease<sup>28</sup>. Furthermore, fourteen culturable haemolytic bacterial strains were identified from different life stages (eggs, four larval instars, pupa). They were compared between reared and field-collected adults, including age-graded females (nulliparous, engorged, and parous)<sup>29</sup>. Most of the bacteria belonged broadly to these phyla, Firmicutes and Proteobacteria. Four strains of *Bacillus cereus* (CU2B, CU3G, CU6A, and CU1E) and two strains of *B. licheniformis* (CU7C and CU1A) were isolated from life stages reared in the laboratory. From eggs to lab-emerged adults, *B. cereus* (CU6A, CU1E), *Paenibacillus* sp. (CU9G), *Alcaligenes faecalis* (CU5A), *Brevundimonas* sp. (CU3C), and *Enterococcus faecium* (CU4B) were recovered. Males and nulliparous females shared four bacterial strains, while engorged and parous females also had four strains in common. The most exciting observation was that except for *B. licheniformis* (CU7C), all other isolated bacterial strains were commonly detected from eggs and post-oviposited females. All bacterial strains were beta haemolytic except *Alcaligenes faecalis*, showing alpha haemolysis on blood agar. Besides that, bacterial strains' colony morphology i.e., shape, color, margin, elevation, consistency, and opacity, was recorded. In vitro biochemical characterization tests including catalase, oxidase, citrate utilization test, indole, growth on Triple Sugar Iron (TSI) agar, methyl red, and carbohydrate fermentation test on six sugars (trehalose, sucrose, arabinose, mannitol, mannose, lactose) were carried out. Along with this, antibiotic susceptibility tests of these isolates were done on different wide-range antibiotics, namely, ampicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, and tetracycline. All of the identified bacterial strains could synthesize amylase and protease. Throughout the life history of the vector, two strains of *B. cereus* (CU6A and CU1E) and one strain of *Paenibacillus* sp., i.e., CU9G, were detected, hinting towards their probable role

in ingested blood meal digestion<sup>29</sup>. Similarly, metagenomic analysis of bacterial communities associated with the life stages of this vector species depicted these two predominant phyla, Firmicutes and Proteobacteria<sup>30</sup>.

The bacterial composition was much more diverse in the pupa than in the other life stages of this vector species<sup>30</sup>. *Pseudomonas* spp. was (26-37%) identified throughout all life stages. The abundance of *Bacillus* spp. was slightly increased within the 2nd, 3rd, and 4th instars, with a decrease of *Stenotrophomonas* sp. However, *Stenotrophomonas* and *Pseudomonas* were abundant in this vector species' lab-emerged and field-collected females. High relative abundance of the following bacterial genera, *Stenotrophomonas*, *Pseudomonas*, and *Bacillus*, were seen throughout life stages, which might indicate transstadial transmission<sup>30</sup>. Furthermore, *Alcaligenes faecalis* (CU5A), *Bacillus* sp. (CU5D), *B. cereus* (CU6A, CU3G, CU1E), *B. flexus* (CU8B), *B. licheniformis* (CU1A), *Brevundimonas* sp. (CU3C), *Enterococcus faecium* (CU4B), *Paenibacillus* sp. (CU9G), *Proteus terrae* (CU3B), and *Proteus vulgaris* (CU3A) were isolated throughout the life stages and assumed to be possible transstadial transmission<sup>29</sup>.

*Culicoides ohmorii* Takahashi (synonym of *C. sumatrae* Macfie) and *C. peregrinus* had infections with *Cardinium*<sup>31</sup>. The following endosymbionts *Rickettsia* was noticed in the eggs, 4th instar larva, pupa, and lab-emerged females, while *Wolbachia* was detected only from the 4th instar larva of this vector species<sup>30</sup>.

### Subgenus *Remmia*

*Culicoides oxystoma* is a significant species of this subgenus due to its worldwide distribution, high abundance near livestock, and vectoring of various arboviruses, nematodes, and protozoa<sup>5,32,33</sup>. The first study was performed to identify haemolytic bacterial strains and found two similar strains of *Bacillus pumilus* and one strain of *Bacillus licheniformis* from engorged and nulliparous females of *C. oxystoma*<sup>28</sup>. After that, our unpublished data observed that Firmicutes and Proteobacteria are the predominant Phyla, of which *Bacillus* spp. was the most abundant across the life stages. Across the life history (egg, larval instars, pupa, adults), strains of *B. cereus*, *B. pumilus*, *B. tropicus*, *Lysinibacillus* sp., *Paenibacillus* sp., and *Pseudomonas* sp. were retrieved routinely.<sup>34</sup> Besides that, it was observed that *B. cereus* and

*Alcaligenes faecalis* were shared between natural breeding sites and rearing medium.<sup>34</sup>

Only one endosymbiotic bacteria, *Cardinium* (31.4%), was detected from *C. oxystoma*, and they assumed that the variation of the infection rate of this endosymbiont within *Culicoides* spp. influenced by environmental conditions<sup>24</sup>.

### **Subgenus *Monoculicoides***

The microbial communities of colonized and field-collected pupae, adults and also compared between the microbial communities identified from colonized rearing medium and natural breeding site of *Culicoides variipennis* (Coquillett)<sup>35</sup>. Along with this, bacterial composition of antibiotic treated and untreated midges of two vector species such as *Culicoides nubeculosus* (Meigen) and *Culicoides sonorensis* Wirth and Jones were investigated and it was observed that *Asaia* sp. was dominant among untreated *C. nubeculosus* while relative frequency of *Sphingomonas* sp. was increased within antibiotic treated flies<sup>18</sup>. In contrast the relative frequency of bacteria belonged to family Acetobacteraceae was abundant within untreated *C. sonorensis*, however it was decreased within antibiotic treated flies and *Delftia* sp. was increased<sup>18</sup>. Subsequent changes in the gut bacterial communities within the post antibiotic treated flies of *C. nubeculosus* recorded with increased infection rate of Schmallenberg virus (SBV)<sup>18</sup>.

Two endosymbionts namely *Cardinium* sp. and *Rickettsia* sp. were detected from *C. sonorensis* and *C. nubeculosus*<sup>18,36</sup>. Bacterial communities and endosymbionts identified from other *Culicoides* spp. belonging to other subgenera and species groups were summarized into Table 1, Table 2 and Figure 1<sup>12,18,23,24,25,26,27,29,30,31,34,35,37</sup>.

## **CONCLUSION**

Firmicutes and Proteobacteria are the predominant phyla among these vector species. *Bacillus* spp. in particular several strains of *B. cereus* was the most abundant in all of the species of these subgenera, indicating their close association with these midges and their in vivo role besides blood meal digestion. *Pseudomonas* and *Lysinibacillus* are the important genera identified from species of *Avaritia*, *Remmia*, and *Monoculicoides* indicating their significance within them.

**Table 1.** Bacteria identified from various *Culicoides* spp.

<b>Subgenus</b>	<b>Species</b>	<b>Bacterial families/genera/species</b>
<i>Avaritia</i>	<i>Culicoides actoni</i>	<i>Bacillus cereus</i> , <i>Bacillus paramycoides</i> , <i>Bacillus pumilus</i> , <i>Bacillus flexus</i> , <i>Bacillus megaterium</i> , <i>Lysinibacillus</i> spp., <i>Paenibacillus</i> sp., <i>Serratia marcescens</i>
	<i>Culicoides chiopterus</i>	<i>Pseudomonas</i> sp., Enterobacteriaceae
	<i>Culicoides dewulfi</i>	<i>Asaia</i> sp., Acetobacteraceae, Burkholderiaceae, Enterobacteriaceae, <i>Sphingomonas</i> sp.
	<i>Culicoides fulvus</i>	<i>Bacillus cereus</i> , <i>Bacillus tequilensis</i> , <i>Bacillus paramycoides</i> , <i>Cytobacillus kochii</i> , <i>Enterococcus faecium</i> , <i>Lysinibacillus</i> sp., <i>Pseudomonas stutzeri</i>
	<i>Culicoides imicola</i>	<i>Bacillus cereus</i> , <i>Bacillus haynesii</i> , <i>Bacillus paramycoides</i> , <i>Bacillus tropicus</i> , <i>Escherichia</i> sp., <i>Lysinibacillus mangiferihumi</i> , <i>Brevibacillus parabrevis</i> , <i>Enterococcus faecium</i> , <i>Halomonas</i> , <i>Paenibacillus</i> sp., <i>Propionibacterium</i> sp.
	<i>Culicoides jacobsoni</i>	<i>Bacillus amyloliquefaciens</i> , <i>Bacillus cereus</i> , <i>Bacillus paramycoides</i> , <i>Bacillus velezensis</i> , <i>Enterococcus faecium</i> , <i>Paenibacillus</i> sp.
	<i>Culicoides obsoletus</i>	<i>Asaia</i> sp., Acetobacteraceae, Anaplasmataceae, <i>Aquaspirillum</i> sp., Burkholderiaceae, Enterobacteriaceae, <i>Sphingomonas</i> sp. <i>Bacillus</i> sp., <i>Pseudomonas</i> sp., Enterobacteriaceae, <i>Sphingomonas</i> sp.
	<i>Culicoides orientalis</i>	<i>Bacillus cereus</i> , <i>Bacillus paramycoides</i> , <i>Brevibacillus parabrevis</i> , <i>Enterococcus faecium</i> , <i>Paenibacillus</i> sp., <i>Pseudomonas</i> sp.
	<i>Culicoides scoticus</i>	<i>Asaia</i> sp., Acetobacteraceae, Anaplasmataceae, Burkholderiaceae, Enterobacteriaceae, <i>Sphingomonas</i> sp. <i>Bacillus</i> sp., <i>Pseudomonas</i> sp., Enterobacteriaceae, <i>Sphingomonas</i> sp.

<b>Subgenus</b>	<b>Species</b>	<b>Bacterial families/genera/species</b>
<i>Hoffmania</i>	<i>Culicoides peregrinus</i>	<i>Acinetobacter</i> sp., <i>Alcaligenes faecalis</i> , <i>Arcobacter</i> sp., <i>Bacillus cereus</i> , <i>Bacillus flexus</i> , <i>Bacillus licheniformis</i> , <i>Bacillus pumilus</i> , <i>Bacteroides</i> sp., <i>Bifidobacterium</i> sp., <i>Brevibacillus</i> sp., <i>Brevundimonas</i> sp., <i>Brevifilum</i> sp., <i>Clostridium</i> sp., <i>Chryseobacterium</i> sp., <i>Enterococcus faecium</i> , <i>Flavobacterium</i> sp., <i>Lysinibacillus</i> sp., <i>Lactobacillus</i> sp., <i>Microcystis</i> sp., <i>Paenibacillus</i> sp., <i>Pelolinea</i> sp., <i>Proteus vulgaris</i> , <i>Proteus terrae</i> , <i>Pseudomonas</i> sp., <i>Ralstonia</i> sp., <i>Serratia</i> sp., <i>Stenotrophomonas</i> sp.
<i>Remmia</i>	<i>Culicoides oxystoma</i>	<i>Alcaligenes faecalis</i> , <i>Bacillus cereus</i> , <i>Bacillus flexus</i> , <i>Bacillus licheniformis</i> , <i>Bacillus pumilus</i> , <i>Bacillus paramycoides</i> , <i>Bacillus tropicus</i> , <i>Bacillus thuringiensis</i> , <i>Lysinibacillus</i> sp., <i>Enterococcus faecium</i> , <i>Paenibacillus</i> sp., and <i>Pseudomonas</i> sp.
<i>Monoculicoides</i>	<i>Culicoides nubeculosus</i>	<i>Asaia</i> sp., <i>Brevundimonas</i> sp., <i>Chryseobacterium</i> sp., <i>Delftia</i> sp., <i>Leucobacter</i> sp., <i>Sphingomonas</i> sp., <i>Pseudomonas</i> sp.
	<i>Culicoides sonorensis</i>	<i>Aeromonas hydrophila</i> , <i>Bacillus</i> sp., <i>Bacillus thuringiensis</i> , <i>Bacillus cereus</i> , <i>Chryseobacterium</i> sp., <i>Lysinibacillus</i> sp., <i>Acinetobacter</i> sp., <i>Providencia</i> sp., <i>Brevundimonas</i> sp., <i>Flavobacterium</i> sp., <i>Morganella morganii</i> , <i>Comamonas</i> sp. <i>Acetobacteraceae</i> , <i>Asaia</i> sp., <i>Delftia</i> sp., <i>Leucobacter</i> sp., <i>Sphingomonas</i> sp., <i>Pseudomonas</i> sp.
	<i>Culicoides variipennis</i>	<i>Achromobacter</i> sp., <i>Acinetobacter</i> sp., <i>Aeromonas</i> sp., <i>Bacillus</i> sp., <i>Edwardsiella</i> sp., <i>Enterobacter</i> sp., <i>Escherichia</i> sp., <i>Flavobacterium</i> sp., <i>Micrococcus</i> sp., <i>Proteus</i> sp., <i>Providencia</i> sp., <i>Pseudomonas</i> sp., <i>Zoogloea</i> sp.

**Table 2.** Endosymbionts recorded from various *Culicoides* spp.

<b>Subgenus/Species group</b>	<b>Species</b>	<b>Endosymbionts</b>
<i>Avaritia</i>	<i>Culicoides brevitarsis</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
	<i>Culicoides chiopterus</i>	<i>Wolbachia</i> sp.
	<i>Culicoides dewulfi</i>	<i>Rickettsia</i> sp., <i>Wolbachia</i> sp.
	<i>Culicoides imicola</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
	<i>Culicoides obsoletus</i>	<i>Rickettsia</i> sp., <i>Wolbachia</i> sp.
	<i>Culicoides scoticus</i>	<i>Cardinium</i> sp., <i>Rickettsia</i> sp., <i>Wolbachia</i> sp.
<i>Hoffmania</i>	<i>Culicoides wadai</i>	<i>Cardinium</i> sp.
	<i>Culicoides peregrinus</i>	<i>Cardinium</i> sp., <i>Rickettsia</i> sp., <i>Wolbachia</i> sp.
<i>Remmia</i>	<i>Culicoides sumatrae</i>	<i>Cardinium</i> sp.
<i>Monoculicoides</i>	<i>Culicoides oxystoma</i>	<i>Cardinium</i> sp.
	<i>Culicoides nubeculosus</i>	<i>Rickettsia</i> sp.
	<i>Culicoides sonorensis</i>	<i>Cardinium</i> sp., <i>Rickettsia</i> sp.
<i>Culicoides</i>	<i>Culicoides flavipulicaris</i>	<i>Cardinium</i> sp.
	<i>Culicoides impunctatus</i>	<i>Rickettsia</i> sp.
	<i>Culicoides newsteadi</i>	<i>Cardinium</i> sp.
	<i>Culicoides pulicaris</i>	<i>Cardinium</i> sp.
	<i>Culicoides punctatus</i>	<i>Cardinium</i> sp.
<i>Marksomyia</i>	<i>Culicoides dycei</i>	<i>Cardinium</i> sp.
	<i>Culicoides marksii</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
<i>Oecacta</i>	<i>Culicoides parvimaculatus</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
	<i>Culicoides sahariensis</i>	<i>Cardinium</i> sp.
	<i>Culicoides vexans</i>	<i>Wolbachia</i> sp.
<i>Sensiculicoides</i>	<i>Culicoides festivipennis</i>	<i>Cardinium</i> sp.
	<i>Culicoides haranti</i>	<i>Cardinium</i> sp.
	<i>Culicoides kibunensis</i>	<i>Wolbachia</i> sp.
<i>Trithecoides</i>	<i>Culicoides maritimus</i>	<i>Cardinium</i> sp.
	<i>Culicoides paraflavescens</i>	<i>Wolbachia</i> sp.
<i>Wirthomyia</i>	<i>Culicoides minutissimus</i>	<i>Cardinium</i> sp.
<i>antennalis</i> species group	<i>Culicoides antennalis</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
<i>molestus</i> species group	<i>Culicoides</i> spp.	<i>Cardinium</i> sp.

Subgenus/Species group	Species	Endosymbionts
ornatus species group	<i>Culicoides marmoratus</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
	<i>Culicoides bundyensis</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
victoriae species group	<i>Culicoides henryi</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
	<i>Culicoides victoriae</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
	<i>Culicoides multimaculatus</i>	<i>Cardinium</i> sp.
williwilli species group	<i>Culicoides austropalpalis</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
	<i>Culicoides narrabeenensis</i>	<i>Cardinium</i> sp., <i>Wolbachia</i> sp.
	<i>Culicoides williwilli</i>	<i>Cardinium</i> sp.

<b><i>Culicoides</i> spp.</b>	<b><i>Cardinium</i></b>	<b><i>Wolbachia</i></b>	<b><i>Rickettsia</i></b>
<i>C. brevitarsis</i>	Dark Blue	Dark Blue	Yellow
<i>C. chiopterus</i>	Yellow	Dark Blue	Yellow
<i>C. dewulfi</i>	Yellow	Dark Blue	Dark Blue
<i>C. imicola</i>	Dark Blue	Dark Blue	Yellow
<i>C. obsoletus</i>	Yellow	Dark Blue	Dark Blue
<i>C. scoticus</i>	Dark Blue	Dark Blue	Dark Blue
<i>C. wadai</i>	Dark Blue	Yellow	Yellow
<i>C. peregrinus</i>	Dark Blue	Dark Blue	Dark Blue
<i>C. sumatrae</i>	Dark Blue	Yellow	Yellow
<i>C. oxystoma</i>	Dark Blue	Yellow	Yellow
<i>C. nubeculosus</i>	Yellow	Dark Blue	Dark Blue
<i>C. sonorensis</i>	Dark Blue	Dark Blue	Dark Blue
<i>C. flavipulicaris</i>	Dark Blue	Dark Blue	Yellow
<i>C. impunctatus</i>	Yellow	Dark Blue	Dark Blue
<i>C. newsteadi</i>	Dark Blue	Dark Blue	Yellow
<i>C. pulicaris</i>	Dark Blue	Dark Blue	Yellow
<i>C. punctatus</i>	Dark Blue	Dark Blue	Yellow
<i>C. dycei</i>	Dark Blue	Dark Blue	Yellow
<i>C. marksi</i>	Dark Blue	Dark Blue	Yellow
<i>C. parvimaculatus</i>	Dark Blue	Dark Blue	Yellow
<i>C. sahariensis</i>	Dark Blue	Yellow	Yellow



**Fig. 1.** Heatmap depicting endosymbionts detected in different *Culicoides* spp. (■ present, □ absent)

*Bacillus cereus* causes endophthalmitis, septicemia and food poisoning in humans and other mammals and acute gangrenous mastitis in dairy cows<sup>38,39,40</sup>. Another, ubiquitous bacterium, *Pseudomonas stutzeri* has been reported as a causative agent of some infections, particularly in immune-compromised patients but has rarely been reported as a cause of infective endocarditis<sup>41</sup>. *Lysinibacillus fusiformis* can cause tropical ulcers, severe sepsis, and respiratory illnesses in humans<sup>42</sup>. *Brevibacillus* species are rarely implicated as human pathogens, causative agents of meningitis, and bacteremia<sup>43</sup>. *Serratia marcescens* is an opportunistic nosocomial pathogen that causes a wound, urinary tract, bloodstream, ocular infections, meningitis, endocarditis, pneumonia, and other respiratory diseases. Infection with *S. marcescens* has been associated with mastitis outbreaks in dairy cattle herds in two dairy farms in Finland<sup>44,45,46,47</sup>. The following pathogenic bacteria, *Bacillus cereus*, *Serratia marcescens*, *Pseudomonas stutzeri*, *Brevibacillus parabrevis* were detected in salivary glands and gut of midge species, raising a chance of zoonotic

transmission towards their hosts. The transmissional efficiency of these midges via blood feeding need to be further addressed. Some of these midge-associated strains may be targeted further to develop control strategies by alternating the vector physiology. Some of the endosymbionts, *Wolbachia*, *Cardinium*, and *Rickettsia* were detected. These endosymbionts along with the bacteria such as *Bacillus*, *Pseudomonas*, *Lysinibacillus* may be symbiotically associated with these midges. These bacteria along with endosymbionts may alter competence for disease transmission and affect gene flow by altering cross-compatibility, as reported in other haematophagous vectors. The tripartite interactions among host, vector and pathogens need to be further explored in these vector species of *Culicoides*.

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