Parasitology Research (2025) 124:27
https://doi.org/10.1007/s00436-025-08471-7

RESEARCH

Host blood meal analysis of *Culicoides oxystoma* (Diptera: Ceratopogonidae) in Tunisia

Darine Slama[1] · Rania Essid[1] · Soufien Sghaier[2] · Hamouda Babba[1,3]

Received: 7 June 2024 / Accepted: 18 February 2025

Abstract

Culicoides are small hematophagous biting midges belonging to the family Ceratopogonidae. The genus is distributed worldwide yet remains poorly studied. This study investigated the vector and host specificity of *Culicoides oxystoma*, a species of significant relevance to the surveillance of vector-borne diseases in Tunisia and globally. The research was conducted in two Tunisian governments: Tozeur and Kairouan. A total of 24,366 adult midges were collected using two types of suction traps: the Center for Disease Control trap and the Onderstepoort Veterinary Institute trap. Females of *Culicoides oxystoma* were isolated, carefully dissected, and slide mounted in a phenol alcohol balsam mixture. A portion of the abdomen was excised for total DNA extraction to identify the origin of the blood meal. A total of 108 engorged females were analyzed using polymerase chain reaction (PCR) to amplify specific fragments of the cytochrome b gene, followed by sequencing and sequence analysis. However, DNA sequences were successfully obtained for only 56 individuals. Sequence analysis revealed that the midges fed on a variety of mammalian hosts, including humans, with a prevalence of *Mus musculus* and *Bos taurus*. This represents the first study aiming to identify a wide range of hosts in Tunisia and North Africa, providing valuable insights into the hosts utilized by *Culicoides oxystoma* for blood feeding.

Keywords Blood meal analysis · *Culicoides oxystoma* · PCR · Sequencing · Tunisia

Introduction

The genus *Culicoides* (Diptera: Ceratopogonidae) comprises small hematophagous dipterans, significant due to their ability to transmit viral pathogens. These include Bluetongue virus (BTV), African horse sickness virus (AHS), epizootic hemorrhagic disease virus (EHD), equine encephalitis virus (EEV), Akabane virus (AKAV), Schmallenberg virus (SBV), bovine ephemeral fever virus (BEFV), Palyam virus, and Oropouche virus (OROV) (MacLachlan and Guthrie 2010). Notably, AHSV and BTV are classified as World Organization for Animal Health (WOAH) list A pathogens due to their substantial global impact (WOAH 2021). In addition, certain *Culicoides* species also transmit microbial and parasitic pathogens, such as *Leishmania* spp. (Slama et al. 2014) and *Hemoproteus* spp. in birds (Martínez-de la Puente et al. 2011; Santiago-Alarcon et al. 2012). Only female *Culicoides* feed on blood, which provides protein for egg production. Tunisia is considered endemic for BTV, with numerous strains circulating, including serotypes 1, 2, 3, 4, and 26, as well as two novel strains, BTV-Y TUN2022 and BTV-W TUN2022 (Thabet et al. 2024).

To date, more than 1347 species of biting midges have been described globally (Borkent and Dominiak 2020), with 35 confirmed species in Tunisia (Slama et al. 2016). Among these species, *Culicoides imicola* is recognized as the primary vector of Bluetongue virus (BTV) in the Mediterranean Basin, playing a central role in the epidemiology of the disease. Its ability to adapt to various environmental conditions and its high abundance in areas with significant

Section Editor: Van Lun Low

Clinical trial number Not applicable.

✉ Hamouda Babba
hamouda.babba@rns.tn

[1] Faculté de Pharmacie, Laboratory of Parasitology-Mycology Medical and Molecular, Université de Monastir, LR12ES085000 Monastir, Tunisia

[2] Food and Agriculture Organization (FAO), Subregional Office for North Africa, Les Berges du Lac 1, 1053 Tunis, Tunisia

[3] Laboratory of the Maternity, Neonatology Center of Monastir, Monastir, Tunisia

Published online: 05 March 2025

livestock populations contribute to its effectiveness in transmitting the virus. However, it is important to note that other *Culicoides* species are also implicated in the transmission of a variety of pathogens in the Mediterranean region. For instance, species such as *Culicoides newsteadi*, *Culicoides paolae*, *Culicoides circumscriptus* (Foxi et al. 2019), and *Culicoides oxystoma* (Thabet et al. 2023) have been identified as vectors of other important viruses, including BTV and EHDV. These species, although secondary in terms of their role in BTV transmission, contribute to the overall vectorial capacity in the region, complicating control measures and surveillance efforts.

Culicoides oxystoma is recognized as a potential arbovirus vector in various geographic regions, including the Palearctic, Saharan-Arabic, Oriental, Austral, and Senegal (Mullen and Murphree 2019). In the Afrotropical region, it is notably abundant in the Niayes region, showing aggressive feeding behavior toward horses (Diarra et al. 2014). Previous studies have reported its role as a BTV vector in India (Dadawala et al. 2012) and Thailand (Fujisawa et al. 2021), and its involvement in the transmission of Akabane virus in Japan (Kurogi et al. 1987; Yanase et al. 2005). Furthermore, *C. oxystoma* is suspected of transmitting African horse sickness in conjunction with *C. imicola* (Riddin et al. 2019). Recent research in southern Thailand has also detected *Leishmania (Mundinia) martiniquensis* and *Leishmania (Mundinia) orientalis* in *C. oxystoma* populations (Songumpai et al. 2022).

From an epidemiological perspective, understanding the spread dynamics of these diseases and the re-emergence of outbreaks in Tunisia requires investigating the interaction between pathogens, vectors, and susceptible hosts. Research in Tunisia has primarily focused on faunal surveillance (Slama et al. 2016; Sghaier et al. 2017), but the host preferences of species like *C. oxystoma* are not yet well understood.

To better understand vector−host relationships, it is crucial to identify the vertebrate hosts on which *Culicoides* feed. Environmental factors influence the activity patterns of *Culicoides*, leading to variations in feeding frequency depending on species and conditions (Feitoza et al. 2023). Host availability plays a critical role in the feeding behavior of biting midges, with most *Culicoides* species being mammalian and/or ornithophiles, though some also feed on reptiles (Borkent 2005).

Studying the dynamics of pathogen transmission by *Culicoides* necessitates understanding their interactions with vertebrate hosts through molecular methods that allow identification of the blood meal's taxonomic origin. Genes commonly used in these molecular analyses include cytochrome c oxidase subunit I (COI), cytochrome b (Cyt-b) (Townzen et al. 2008; Reeves et al. 2018), 16S ribosomal RNA (16S rRNA) (Tomazatos et al. 2020), and the PNOC gene (Kamyingkird et al. 2023).

In Europe, molecular identification of vertebrate hosts for *Culicoides* species has revealed a diverse range of hosts for 31 *Culicoides* species, with a total of 33 avian species and 12 mammal species identified (Martínez-de la Puente et al. 2015). Similarly, a study by Slama et al. (2015) on the trophic preferences of *Culicoides* species in Tunisia also indicated that these midges feed on a wide variety of hosts.

This study aims to (i) investigate the epidemiological role of *C. oxystoma* in Tunisia and (ii) specifically identify the blood meals of engorged female *C. oxystoma*, a species recently recorded for the first time in Tunisia, to enhance understanding and assist with managing future epidemics.

Materials and methods

Study sites and field sampling

Culicoides midges were collected in two distinct regions of Tunisia (Fig. 1). The first site Kairouan, located in central Tunisia, was sampled in May 2014 and November 2015. This region covers an area of 6712 km^2 and has a population of 570,559 people. It has an annual rainfall ranging from 250 to 400 mm, with a semiarid climate characterized by hot, dry summers and cold, humid winters. The second region, Tozeur, located in the southwest of the country along the Tunisian–Algerian border, was sampled in October 2021. The area spans 5600 km^2 and is characterized by a desert-like climate: the average temperature is 23 °C, with precipitation varying between 0.7 and 10.7 mm, depending on the specific location.

The population is predominantly rural, and all collections were made in human-inhabited areas with domestic animals (such as cattle, horses, dogs, goats, and chickens) and in muddy environments formed around livestock watering points.

Two types of light traps were employed: a custom-made miniature CDC (Centre of Disease Control, Atlanta, USA) trap in Kairouan, and an OVI (Onderstepoort Veterinary Institute, South Africa) light/suction trap in Tozeur. The traps were set up at sunset and placed outdoors near sheep and cattle herds and irrigation systems. The samples were collected at sunrise the following day. The captured insects were then preserved in 70° ethanol in the dark to maintain the morphological details required for identification.

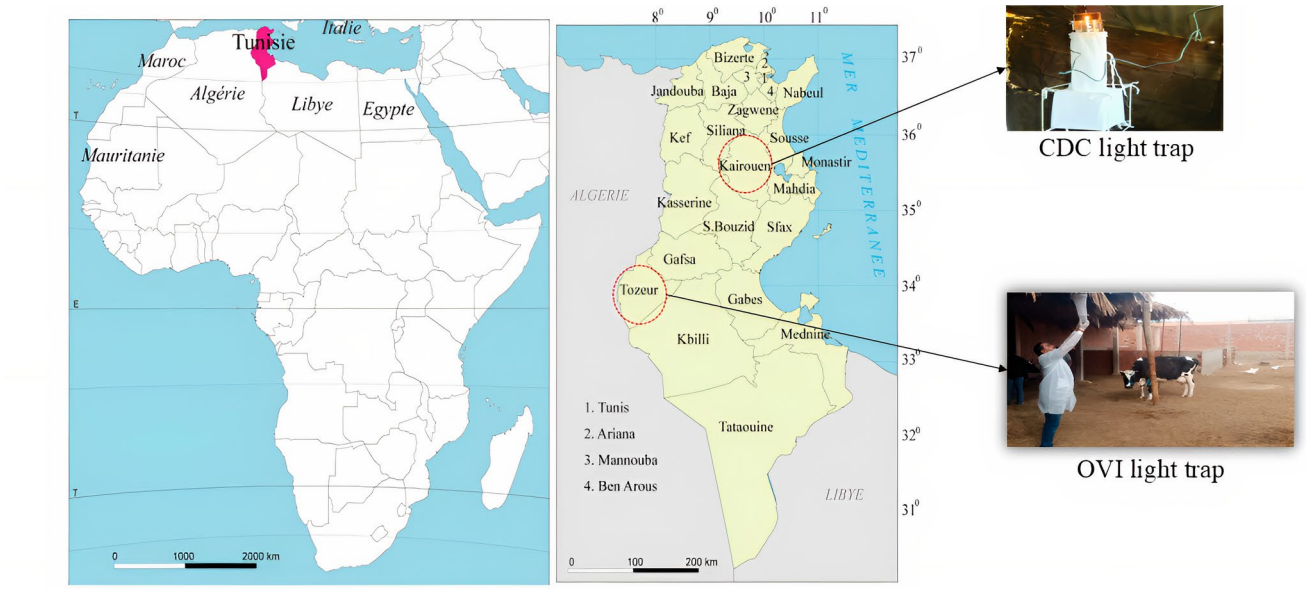

Fig. 1 Map of the study area, including the regions of Kairouan and Tozeur

Morphological identification

Morphological identification was performed according to the identification keys described by Delecolle (1985) and Mathieu et al. (2012). In addition, for the identification of *C. oxystoma*, the criteria from previous studies were applied (Bakhoum et al. 2013; Slama et al. 2021). *Culicoides* individuals were dissected for morphological identification under a stereomicroscope. The head, wings, and genitalia were slide-mounted in a phenol alcohol balsam mixture for observation. Some *Culicoides* species, such as *Culicoides circumscriptus*, *C. paolae*, *C. saevus*, and *C. puncticollis*, do not require dissection, as they can be easily identified under a stereomicroscope. The females were age graded according to Dyce (1969). All engorged female abdomens were stored individually at − 20 °C in sterile microcentrifuge tubes (Eppendorf, Hamburg, Germany) prior to DNA extraction.

Molecular analysis

DNA extraction

DNA extraction from the abdomens of engorged female *C. oxystoma* was performed using the Qiamp DNA mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. A manual grinding step was carried out using a sterile pestle. The extracted DNA was eluted in a final volume of 150 µl of AE buffer.

Molecular analysis of blood meal

All PCR tests were performed in a final volume of 50 µl, with 15 to 20 ng of extracted DNA (or control DNA from male *Culicoides* and sterile distilled water), 0.2 mM dNTPs (dATP, dCTP, dGTP, dTTP), 1 × buffer containing MgCl2, 0.4 pmol/µl of each primer, and 2 U of DreamTaq DNA polymerase (Thermo Fisher Scientific). The primer sequences used for detecting mitochondrial DNA (mtDNA) were described previously (Kocher et al. 1989). Amplification was carried out in a Mini Amp thermal cycler with the following program: 95 °C for 10 min; 40 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 45 s; and, finally, 72 °C for 5 min (Kocher et al. 1989; Slama et al. 2015).

Five microliters of each amplified product was analyzed in a 1.5% agarose gel stained with 5 µl of ethidium bromide. The target DNA was observed using an ultraviolet transilluminator. The PCR product of the Cyt-b gene was approximately 359 bp in length.

Fig. 2 Wings of female *Culicoides kingi* (left) and *Culicoides oxystoma* (right) collected from Tunisia

Sequence analysis

The Cyt-b gene amplicons were subjected to direct sequencing using the same primers of the PCR assay (CarthaGenomics Advanced Technology, Tunisia). The obtained sequences were edited using Chromas software version 2.33 (http://www.technelysium.com.au/chromas.html) and identified by comparing them with the sequences available in GenBank through the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/blast/). A species was considered correctly identified if the sequence matched at least 98% identity with those in GenBank.

Statistical analysis

The proportion of engorged females associated with each host species was calculated using the following proportion formula:

$$\text{Proportion of Host Species A} = \frac{\text{Number of } C.oxystoma \text{ engorged on host species A}}{\text{Total Number of engorged } C.oxystoma \text{ collected}}$$

Data visualization was performed using RAW Graphs (https://rawgraphs.io/) which allowed for the creation of a circular layout (chord diagram) to effectively illustrate the relationships between sites and hosts.

Results

Morphological identification

The catches confirmed the presence of *C. oxystoma* at both study sites (Tozeur and Kairouan). In Tozeur, a total of 23,176 *Culicoides* were collected in October 2021, with 95% of them belonging to the *Schultzei* group, predominantly *C. kingi* and *C. oxystoma* (Fig. 2). Notably, *C. imicola*, the main vector of Bluetongue virus (BTV) in Tunisia, was not detected in this region. In contrast, in Kairouan, a total of 1190 *Culicoides* were collected between May 2014 and November 2015, with the most common species being *C. imicola* (35%) and *C. kingi* (30%), and with *C. oxystoma* representing 7% of collected midges.

During this study, additional *Culicoides* species, which are not included in the current results, were collected (65% from Tozeur and 5% from Kairouan). These specimens will undergo comprehensive morphological and molecular analysis to contribute to the development of a more comprehensive and updated checklist of *Culicoides* species in Tunisia.

The overall parity rate was relatively low, with an average of 18.9% (Fig. 3), suggesting a predominantly young population of *Culicoides*. A conspicuous spike in the number of nulliparous females (Fig. 3) indicates that they have recently emerged, which means that the larval habitats are likely close to the traps.

Molecular identification

Among the 24,366 *Culicoides* captured in this study, 1345 were engorged females. This group included 108 engorged *C. oxystoma* (103 from Tozeur and 5 from Kairouan). Amplification of the Cyt-b gene from DNA extracts of these engorged females resulted in successful amplification for 56 individuals (51.85%), including 52 from Tozeur and 4 from Kairouan, as indicated by a single band of 359 bp. All PCR products that tested positive for Cyt-b amplification were subjected to sequencing. The resulting chromatograms showed a single signal, indicating that these insects had fed on a single host. To identify the host species, the sequences obtained were compared with those available in GenBank using BLAST (Basic Local Alignment Search Tool). The

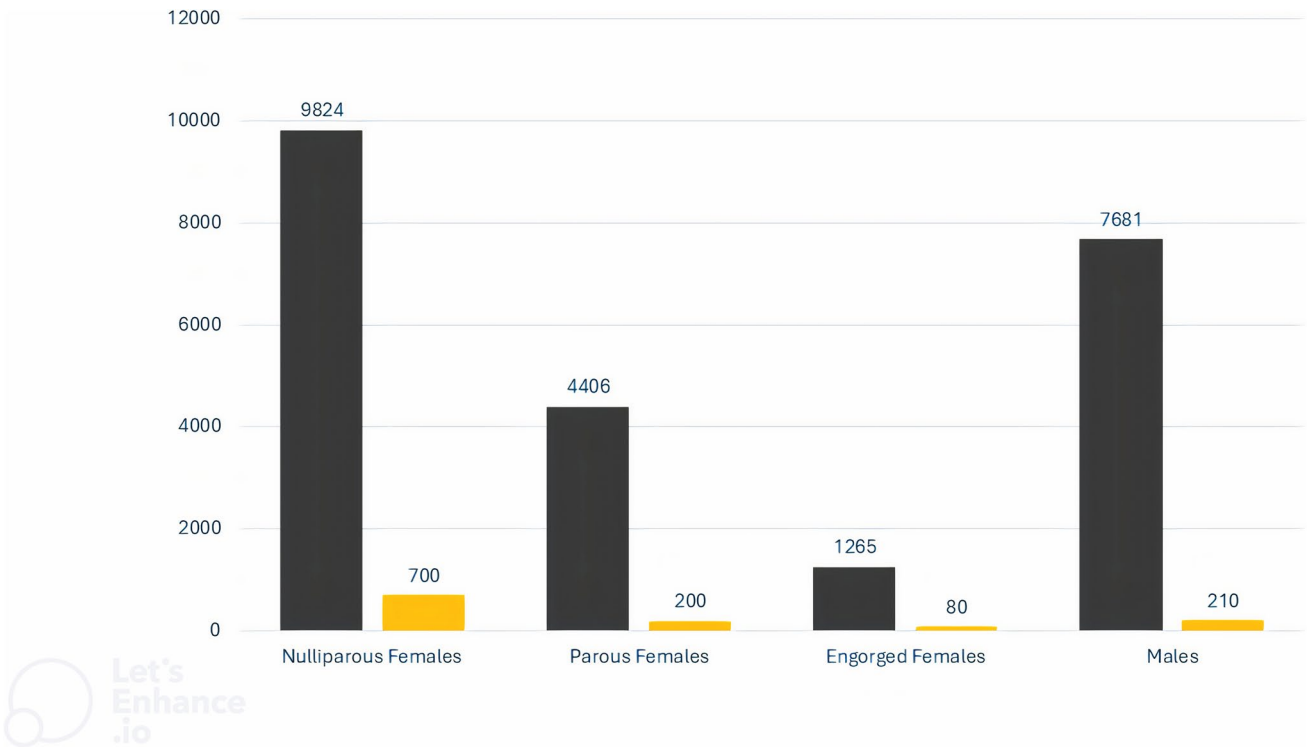

Fig. 3 Parity rate of *Culicoides* females, indicating the proportion of age-grading of *Culicoides* collected in Tunisia

comparison revealed three vertebrate species as hosts: *Mus musculus*, *Bos taurus*, and *Homo sapiens*.

In Tozeur, *Mus musculus* was the most common host species, accounting for 71% ($n=37$) of the total number, followed by *Bos taurus* at 25% ($n=13$), and *Homo sapiens* at 4% ($n=2$). The engorged females of *C. oxystoma* collected in the Kairouan showed a preference for *Bos taurus* (100%; $n=4$) (Fig. 4).

Discussion

The identification of species composition and the detection of host blood are important steps in controlling the spread of emerging *Culicoides* species and the pathogens they transmit. Monitoring *Culicoides* species is crucial to preventing the introduction of new pathogens into regions where they were previously absent.

This study provides valuable insights into the feeding behavior of *C. oxystoma* in Tunisia, though certain limitations should be considered when interpreting the results. Sampling biases related to the small sample size, specific sampling methods, and sampling periods may influence the accuracy of our findings. The limited sample size may not fully reflect the diversity and feeding preferences of *C. oxystoma* populations under various environmental conditions. Additionally, the use of light traps, particularly OVI and CDC traps, may selectively attract certain species or individuals, potentially underrepresenting species or physiological states that are less responsive to these traps. For example, the low capture rate of engorged females (3%) is consistent with findings from France (Garros et al. 2011), Germany (Bartsch et al. 2009), and Denmark (Lassen et al. 2010), but contrasts with the higher capture rates observed when alternative methods, such as oiled paper in nests (VotÝpka et al. 2009), are employed. This highlights the need for a diverse range of complementary sampling methods in future studies to minimize bias and improve representativeness. The timing and duration of sampling also play a crucial role. Our study was conducted within a limited timeframe, which may not account for seasonal fluctuations in *Culicoides* populations and their feeding behavior. Extending sampling periods covering different seasons and climatic conditions could reveal shifts in host preferences and abundance, enhancing our understanding of *C. oxystoma* ecology.

Environmental conditions play a critical role in the distribution, abundance, and feeding behavior of *Culicoides* species. Factors such as temperature, humidity, vegetation, and water availability significantly influence their breeding and feeding activity. In this study, the dominance of *C. oxystoma* and *C. kingi* in the arid Tozeur region highlights their adaptability to dry environments, whereas the presence of *C. imicola* in Kairouan suggests a preference for wetter conditions.

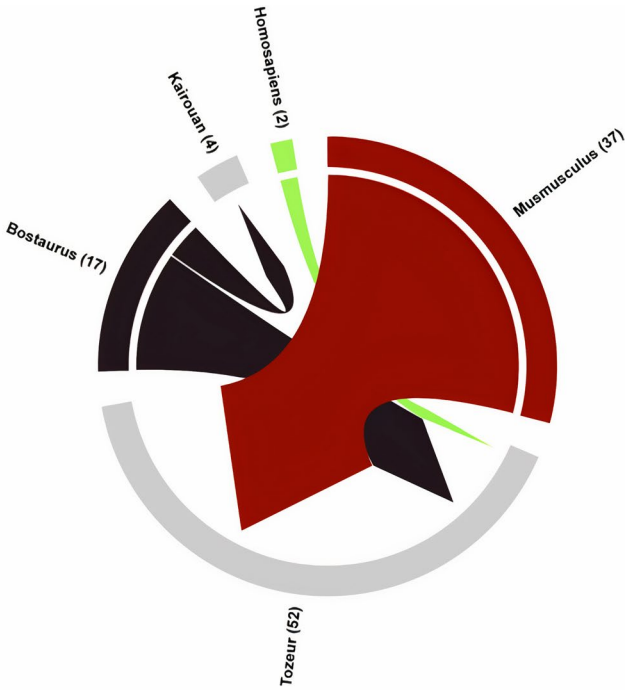

Fig. 4 Origin of blood meals in *Culicoides oxystoma*, identifying the animal hosts from which the females obtain their blood meals

Host availability and density are also decisive factors in shaping feeding habits. In Tozeur, the trap was set close to the ground, probably explaining the high prevalence of the small mammal *Mus musculus* from blood meal analyses. This finding is consistent with previous studies based on traps set at various altitude (VotÝpka et al. 2009; Bobeva et al. 2015). Although we observed only two instances of *C. oxystoma* feeding on humans, this observation aligns with results from Thailand (Gomontean et al. 2023), suggesting that while *C. oxystoma* does not prefer primarily human hosts, it cannot be excluded as a potential vector of human pathogens, such as the Oropouche virus. Further research into the vector competence of *C. oxystoma* for pathogens such as epizootic hemorrhagic disease virus (EHDV) and others is warranted, especially considering that 95% of midges collected in Tozeur belonged to *C. kingi* and *C. oxystoma* (Thabet et al. 2023). In contrast, the detection of *C. imicola* feeding on livestock in Kairouan reinforces its established role as the primary vector of Bluetongue virus in Tunisia. Future studies should focus on quantifying host densities and analyzing their spatial distribution to establish clearer links between host availability and feeding preferences. In the Kairouan region, a low number of samples were collected via CDC traps, a method that may be less efficient than OVI traps for capturing *C. oxystoma*. Conversely, *C. imicola* accounted for 35% of the total midges collected. In terms of trapping efficiency, the OVI light trap was more effective than the CDC trap, capturing 77.18% of all the samples. This finding is consistent with previous studies (Viennet et al. 2011) which confirms that OVI traps are the most effective for collecting *Culicoides* species in the Palearctic region.

The successful amplification of vertebrate DNA in 51.85% of engorged females via the Cyt-b gene highlights the utility of this marker for blood meal analysis. The Cyt-b gene is widely used to identify blood meals due to its high sensitivity for PCR detection and the substantial interspecific genetic variation at this locus (Boakye et al. 1999; Ngo and Kramer 2003; Townzen et al. 2008; Bartsch et al. 2009; Ernieenor et al. 2012; Kamyingkird et al. 2023). Furthermore, the availability of over 8000 Cyt-b gene sequences in the GenBank database (Parson et al. 2000) increases the reliability of this method. The blood meal preferences of *Culicoides* species vary depending on the species, host availability (Bakhoum et al. 2016), geographical region, and specific primers used for identification (Hadj-Henni et al. 2015). Understanding host preferences is crucial for identifying potential hosts susceptible to pathogens transmitted by *Culicoides*. Several previous studies on host preferences have been conducted, for example, in Spain (Martínez-De La Puente et al. 2012), France (Ninio et al. 2011), Germany (Bartsch et al. 2009), Tunisia (Slama et al. 2015), Romania

(Townzen et al. 2008), Serbia (Vasić et al. 2019), Brazil (Carvalho et al. 2021), Slovakia (Kasičová et al. 2021), Thailand (Kamyingkird et al. 2023), and South Africa (Snyman et al. 2021). Host availability plays a pivotal role in shaping the blood-feeding behavior of *Culicoides* species, which in turn influences pathogen transmission. As shown in previous researches, factors such as environmental conditions, anthropogenic activities, and host density can lead to shifts in host preference and blood-feeding behavior (Pettersson et al. 2013; Santiago-Alarcon et al. 2013; Jacquot et al. 2017; Farias et al. 2020).

Using blood meal DNA for host identification may be affected by its possible degradation and by the small amount of blood in insect abdomens. In our study, 48.15% of the 108 individuals analyzed did not yield identifiable blood meal sources. However, the inability to identify the origin of the blood meals underscores potential limitations, such as DNA degradation during storage (Ninio et al. 2011), insufficient representation of certain hosts in genetic databases, or ingestion of vertebrate hemolymph. These challenges highlight the need for standardized protocols for sample storage and the expansion of genetic reference databases to improve the accuracy of blood meal identification.

This study is the first in Tunisia to identify the diverse host range of *C. oxystoma*, contributing to the growing knowledge on the feeding ecology of *Culicoides* in North Africa. These findings are crucial for understanding the role of *C. oxystoma* in pathogen transmission and for developing targeted vector control strategies. Identifying the most important hosts and understanding their spatial distribution can provide insights for measures such as targeted insecticide use or habitat modification to reduce vector−host interactions.

Conclusion

A comprehensive understanding of the biology and ecology of *Culicoides* species is essential for predicting and controlling the spread of pathogens. This study provides valuable insights into *C. oxystoma*, a species recently incriminated as a vector of EHDV in Tunisia, showing significant variations in its geographic distribution.

For the first time in Tunisia, we have highlighted the preference of *C. oxystoma* for rodents (*Mus musculus*), ruminants (*Bos taurus*), and humans. However, these findings also suggest that *C. oxystoma* may exhibit an opportunistic feeding behavior, though further studies are needed to clarify these patterns across different environments. Furthermore, we acknowledge that host preferences may vary depending on geographic location, seasonality, and environmental conditions which could influence pathogen transmission dynamics. While our results offer valuable insights, we acknowledge the limitations of the study, including the relatively small sample size and the need for broader geographic and temporal data to validate the observed patterns. Additionally, the relationship between host preferences and pathogen transmission remains complex and warrants further investigation.

This study lays the groundwork for future research that could refine predictive models of pathogen transmission, contributing to the development of more effective veterinary emergency plans and vector control strategies. By adopting a holistic approach, we hope to provide a framework for ongoing studies aimed at further exploring the diversity of *Culicoides* species, their ecological drivers, and their role in the transmission of vector-borne diseases.

Acknowledgements We sincerely acknowledge Dr Maria Goffredo for her invaluable contributions to this manuscript. We gratefully acknowledge the valuable input of the anonymous reviewers on this paper. Additionally, we thank The Veterinary Research Institute (VRI) for assisting with specimen collection.

Authors' contributions Soufien Sghaier undertook sampling, Darine Slama and Hamouda Babba conceived the project, Darine Slama and Rania Essid wrote the main manuscript text, Darine Slama prepared Figs. 1–4, and Hamouda Babba and Soufien Sghaier revised the manuscript for submission. All the authors reviewed the manuscript.

Funding This work was supported by finances from the Tunisian Ministry of Scientific Research, Technology and Competences Development. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval No ethical approval was needed, as this study did not involve clinical trials. The traps were placed on private property. All landowners were contacted before the field experiment, and all traps were set up with permission from the landowners to conduct the study on their properties. The fieldwork did not involve any endangered or protected species. The materials used in the experiment posed no health risk to researchers or farmers, and no vertebrate animals were harmed.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Bakhoum MT, Fall M, Fall AG et al (2013) First record of Culicoides oxystoma Kieffer and diversity of species within the Schultzei group of Culicoides Latreille (Diptera : Ceratopogonidae) biting midges in Senegal. 8. https://doi.org/10.1371/journal.pone.0084316

Bakhoum MT, Fall M, Seck MT et al (2016) Foraging range of arthropods with veterinary interest: new insights for Afrotropical Culicoides biting midges (Diptera: Ceratopogonidae) using the ring method. Acta Trop 157:59–67. https://doi.org/10.1016/j.actatropica.2016.01.023

Bartsch S, Bauer B, Wiemann A et al (2009) Feeding patterns of biting midges of the Culicoides obsoletus and Culicoides pulicaris groups on selected farms in Brandenburg, Germany. Parasitol Res 105:373–380. https://doi.org/10.1007/s00436-009-1408-y

Boakye DA, Tang J, Truc P et al (1999) Identification of bloodmeals in haematophagous Diptera by cytochrome B heteroduplex analysis. Med Vet Entomol 13:282–287. https://doi.org/10.1046/j.1365-2915.1999.00193.x

Bobeva A, Zehtindjiev P, Ilieva M et al (2015) Host preferences of ornithophilic biting midges of the genus Culicoides in the Eastern Balkans. Med Vet Entomol 29:290–296. https://doi.org/10.1111/mve.12108

Borkent A (2005) The biting midges, the Ceratopogonidae (Diptera). In: Marquardt WC, Black IV, Higgs S, Freier JE, James AA, HH Hagedorn B, Kondratieff J, Hemingway CGM (eds) Biology of disease vectors. Elsevier Academic Press, p 785

Borkent A, Dominiak P (2020) Catalog of the biting midges of the world (Diptera: Ceratopogonidae). Zootaxa 4787:1–377. https://doi.org/10.11646/zootaxa.4787.1.1

Carvalho LPC, Pereira Júnior AM, de Paulo PFM, et al (2021) DNA-based blood meal analysis of Culicoides (Diptera: Ceratopogonidae) species from Jamari National Forest, Southwestern Amazon, Brazil. Acta Trop 221. https://doi.org/10.1016/j.actatropica.2021.106025

Dadawala A, Biswas S, Rehman W et al (2012) Isolation of Bluetongue virus serotype 1 from Culicoides vector captured in livestock farms and sequence analysis of the viral genome segment-2. Transbound Emerg Dis 59:361–368. https://doi.org/10.1111/j.1865-1682.2011.01279.x

de Farias ES, Almeida JF, Pereira-Silva JW et al (2020) Diversity of biting midges culicoides (Diptera: Ceratopogonidae), potential vectors of disease, in different environments in an amazonian rural settlement, Brazil. Rev Soc Bras Med Trop 53:1–8. https://doi.org/10.1590/0037-8682-0067-2020

Delecolle J-C (1985) Nouvelle contribution à l'étude systématique et iconographique des espèces du genre Culicoides, (Diptéra) : (Cératopogonidae) du Nord-Est de la France. ULP Strasbourg

Diarra M, Fall M, Fall AG et al (2014) Seasonal dynamics of Culicoides (Diptera: Ceratopogonidae) biting midges, potential vectors of African horse sickness and Bluetongue viruses in the Niayes area of Senegal. Parasit Vectors 7:1–11. https://doi.org/10.1186/1756-3305-7-147

Dyce AL (1969) The recognition of nulliparous and parous Culicoides (Diptera: Ceratopogonidae) without dissection. Aust J Entomol 8:11–15. https://doi.org/10.1111/j.1440-6055.1969.tb00727.x

Ernieenor FCL, Ahamad M, Haron MS, Ming HT (2012) Establishment of a molecular tool for blood meal identification in Malaysia. Asian Pac J Trop Biomed 2:223–227. https://doi.org/10.1016/S2221-1691(12)60046-X

Feitoza LHM, de Carvalho LPC, da Silva LR et al (2023) Influence of meteorological and seasonal parameters on the activity of Culicoides paraensis (Diptera: Ceratopogonidae), an annoying anthropophilic biting midge and putative vector of Oropouche Virus in Rondônia, Brazilian. Amazon Acta Trop 243:106928

Foxi C, Meloni G, Puggioni G et al (2019) Bluetongue virus detection in new Culicoides species in Sardinia, Italy. Vet Rec 184:621. https://doi.org/10.1136/vr.105118

Fujisawa Y, Homat T, Thepparat A, Changbunjong T (2021) Acta Tropica DNA barcode identification and molecular detection of Bluetongue virus in Culicoides biting midges (Diptera : Ceratopogonidae) from western Thailand. Acta Trop 224:106147. https://doi.org/10.1016/j.actatropica.2021.106147

Garros C, Gardès L, Allène X et al (2011) Adaptation of a species-specific multiplex PCR assay for the identification of blood meal source in Culicoides (Ceratopogonidae: Diptera): applications on Palaearctic biting midge species, vectors of Orbiviruses. Infect Genet Evol 11:1103–1110. https://doi.org/10.1016/j.meegid.2011.04.002

Gomontean B, Vaisusuk K, Chatan W et al (2023) Diversity, abundance and host blood meal analysis of Culicoides Latreille (Diptera: Ceratopogonidae) from cattle pens in different land use types from Thailand. Insects 14:574. https://doi.org/10.3390/insects14070574

Hadj-Henni L, De Meulemeester T, Depaquit J et al (2015) Comparison of vertebrate cytochrome b and prepronociceptin for blood meal analyses in Culicoides. Front Vet Sci 2:1–8. https://doi.org/10.3389/fvets.2015.00015

Jacquot M, Nomikou K, Palmarini M et al (2017) Bluetongue virus spread in Europe is a consequence of climatic, landscape and vertebrate host factors as revealed by phylogeographic inference. Proc R Soc B Biol Sci 284:1–10. https://doi.org/10.1098/rspb.2017.0919

Kamyingkird K, Choocherd S, Chimnoi W et al (2023) Molecular identification of Culicoides species and host preference blood meal in the African horse sickness outbreak-affected area in Hua Hin district, Prachuap Khiri Khan province, Thailand. Insects 14:1–11. https://doi.org/10.3390/insects14040369

Kasičová Z, Schreiberová A, Kimáková A, Kočišová A (2021) Blood meal analysis: host-feeding patterns of biting midges (Diptera, Ceratopogonidae, Culicoides Latreille) in Slovakia. Parasite 28. https://doi.org/10.1051/parasite/2021058

Kocher TD, Thomas WK, Meyer A et al (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A 86:6196–6200. https://doi.org/10.1073/pnas.86.16.6196

Kurogi H, Akiba K, Inaba Y, Matumoto M (1987) Isolation of Akabane virus from the biting midge Culicoides oxystoma in Japan. Vet Microbiol 15:243–248

Lassen SB, Nielsen SA, Skovgård H, Kristensen M (2010) Molecular identification of bloodmeals from biting midges (Diptera: Ceratopogonidae: Culicoides Latreille) in Denmark. Parasitol Res 108:823–829. https://doi.org/10.1007/s00436-010-2123-4

MacLachlan NJ, Guthrie AJ (2010) Re-emergence of Bluetongue, African horse sickness, and other Orbivirus diseases. Vet Res 41. https://doi.org/10.1051/vetres/2010007

Martínez-de la Puente J, Martínez J, Rivero-de Aguilar J et al (2011) On the specificity of avian blood parasites: revealing specific and generalist relationships between haemosporidians and biting

midges. Mol Ecol 20:3275–3287. https://doi.org/10.1111/j.1365-294X.2011.05136.x
Martínez-de la Puente J, Figuerola J, Soriguer R (2015) Fur or feather? Feeding preferences of species of Culicoides biting midges in Europe. Trends Parasitol 31:16–22. https://doi.org/10.1016/j.pt.2014.11.002
Martínez-De La Puente J, Martínez J, Ferraguti M et al (2012) Genetic characterization and molecular identification of the bloodmeal sources of the potential Bluetongue vector Culicoides obsoletus in the Canary Islands, Spain. Parasit Vectors 5:1–7. https://doi.org/10.1186/1756-3305-5-147
Mathieu B, Cêtre-Sossah C, Garros C et al (2012) Development and validation of IIKC: An interactive identification key for Culicoides (Diptera: Ceratopogonidae) females from the Western Palaearctic region. Parasit Vectors 5:1–11. https://doi.org/10.1186/1756-3305-5-137
Mullen GR, Murphree CS (2019) Biting midges (Ceratopogonidae). In: Gary R, Mullen LAD (eds) Biting midges (Ceratopogonidae), Medical an. Academic Press, pp 213–236
Ngo KA, Kramer LD (2003) Identification of mosquito bloodmeals using polymerase chain reaction (PCR) with order-specific primers. J Med Entomol 40:215–222. https://doi.org/10.1603/0022-2585-40.2.215
Ninio C, Augot D, Delecolle JC et al (2011) Contribution to the knowledge of Culicoides (Diptera: Ceratopogonidae) host preferences in France. Parasitol Res 108:657–663. https://doi.org/10.1007/s00436-010-2110-9
Parson W, Pegoraro K, Niederstätter H et al (2000) Species identification by means of the cytochrome b gene. Int J Legal Med 114:23–28. https://doi.org/10.1007/s004140000134
Pettersson E, Bensch S, Ander M et al (2013) Molecular identification of bloodmeals and species composition in Culicoides biting midges. Med Vet Entomol 27:104–112. https://doi.org/10.1111/j.1365-2915.2012.01038.x
Reeves LE, Gillett-kaufman JL, Kawahara AY, Kaufman E (2018) Barcoding blood meals : new vertebrate- specific primer sets for assigning taxonomic identities to host DNA from mosquito blood meals. PLoS Negl Trop Dis 12:e0006767
Riddin M, Venter G, Labuschagne K, Villet MH (2019) Culicoides species as potential vectors of African horse sickness virus in the southern regions of South Africa. Med Vet Entomol 33:498–511
Santiago-Alarcon D, Havelka P, Pineda E et al (2013) Urban forests as hubs for novel zoonosis: blood meal analysis, seasonal variation in Culicoides (Diptera: Ceratopogonidae) vectors, and avian haemosporidians. Parasitology 140:1799–1810. https://doi.org/10.1017/S0031182013001285
Santiago-Alarcon D, Havelka P, Schaefer HM, Segelbacher G (2012) Bloodmeal analysis reveals avian plasmodium infections and broad host preferences of culicoides (Diptera: Ceratopogonidae) vectors. PLoS One 7. https://doi.org/10.1371/journal.pone.0031098
Sghaier S, Lorusso A, Portanti O et al (2017) A novel Bluetongue virus serotype 3 strain in Tunisia, November 2016. Transbound Emerg Dis 64:709–715. https://doi.org/10.1111/tbed.12640
Slama D, Haouas N, Mezhoud H et al (2015) Blood meal analysis of Culicoides (Diptera : Ceratopogonidae) in Central Tunisia. PLoS ONE 10(3):1–14. https://doi.org/10.1371/journal.pone.0120528
Slama D, Baraket R, Remadi L et al (2021) Morphological and molecular differentiation between Culicoides oxystoma and Culicoides kingi (Diptera: Ceratopogonidae) in Tunisia. Parasit Vectors 14:1–11. https://doi.org/10.1186/s13071-021-05084-8
Slama D, Haouas N, Remadi L et al (2014) First detection of Leishmania infantum (Kinetoplastida: Trypanosomatidae) in Culicoides spp. (Diptera: Ceratopogonidae). Parasites and Vectors 7. https://doi.org/10.1186/1756-3305-7-51
Slama D, Chaker E, Zrelli S et al (2016) Culicoides (Diptera: Ceratopogonidae) fauna in Central Tunisia. Entomol Ornithol Herpetol Curr Res 5. https://doi.org/10.4172/2161-0983.1000184
Snyman J, Venter GJ, Venter M (2021) An investigation of Culicoides (Diptera: Ceratopogonidae) as potential vectors of medically and veterinary important arboviruses in South Africa. Viruses 13. https://doi.org/10.3390/v13101978
Songumpai N, Promrangsee C, Noopetch P, Siriyasatien P (2022) First evidence of co-circulation of emerging Leishmania martiniquensis, Leishmania orientalis, and Crithidia sp. in Culicoides biting midges (Diptera: Ceratopogonidae), the putative vectors for autochthonous transmission in southern Thailand. Trop Med Infect Dis 11:379
Thabet S, Sghaier S, Ben HT et al (2023) Characterization of epizootic hemorrhagic disease virus serotype. Viruses 15:1–10
Thabet S, Sghaier S, Valentina C et al (2024) Identification and characterization of two atypical strains of bluetongue virus in sheep, Tunisia. Acta Trop 29:260. https://doi.org/10.1016/j.actatropica.2024.107416
Tomazatos A, Jöst H, Schulze J et al (2020) Blood - meal analysis of Culicoides (Diptera : Ceratopogonidae) reveals a broad host range and new species records for Romania. Parasit Vectors 1–12. https://doi.org/10.1186/s13071-020-3938-1
Townzen JS, Brower AVZ, Judd DD (2008) Identification of mosquito bloodmeals using mitochondrial cytochrome oxidase subunit I and cytochrome b gene sequences. Med Vet Entomol 22:386–393
Vasić A, Zdravković N, Anită D et al (2019) Species diversity, host preference and arbovirus detection of Culicoides (Diptera: Ceratopogonidae) in south-eastern Serbia. Parasit Vectors 12:1–9. https://doi.org/10.1186/s13071-019-3292-3
Viennet E, Garros C, Lancelot R et al (2011) Assessment of vector/host contact: comparison of animal-baited traps and UV-light/suction trap for collecting Culicoides biting midges (Diptera: Ceratopogonidae), vectors of Orbiviruses. Parasit Vectors 4:119. https://doi.org/10.1186/1756-3305-4-119
VotÝpka J, Synek P, SvobodovÁ M (2009) Endophagy of biting midges attacking cavity-nesting birds. Med Vet Entomol 23:277–280. https://doi.org/10.1111/j.1365-2915.2009.00800.x
WOAH (2021) Old Classification of Diseases Notifiable to the OIE – List A. https://www.woah.org/en/what-we-do/animal-health-and-welfare/animal-diseases/old-classification-of-diseases-notifiable-to-the-oie-list-a/
Yanase T, Kato T, Kubo T et al (2005) Isolation of bovine arboviruses from Culicoides biting midges (Diptera : Ceratopogonidae) in southern Japan : 1985–2002. Med Entomol 42(1):63–67