Major Article

Diversity of biting midges *Culicoides* (Diptera: Ceratopogonidae), potential vectors of disease, in different environments in an Amazonian rural settlement, Brazil

**Emanuelle de Sousa Farias**[1],[2], **Jessica Feijó Almeida**[1],[3], **Jordam William Pereira-Silva**[1],[4],[5], **Luiz de Souza Coelho**[6], **Sérgio Luiz Bessa Luz**[1], **Claudia María Ríos-Velásquez**[1] and **Felipe Arley Costa Pessoa**[1]

[1]. Fundação Oswaldo Cruz, Instituto Leônidas e Maria Deane, Laboratório de Ecologia e Doenças Transmissíveis na Amazônia, Manaus, AM, Brasil.  
[2]. Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Programa de Pós-Graduação em Biodiversidade e Saúde, Rio de Janeiro, RJ, Brasil.  
[3]. Instituto Nacional de Pesquisas da Amazônia, Programa de Pós-Graduação em Entomologia, Manaus, AM, Brasil.  
[4]. Universidade do Estado do Amazonas, Escola Superior de Ciências da Saúde, Programa de Pós-Graduação em Medicina Tropical, Manaus, AM, Brasil.  
[5]. Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Departamento de Ensino e Pesquisa, Manaus, AM, Brasil.  
[6]. Instituto Nacional de Pesquisas da Amazônia, Laboratório de Inventário Florístico e Botânica Econômica, Coordenação de Biodiversidade, Manaus, AM, Brasil.

**Abstract**

Introduction: The *Culicoides* transmit a variety of pathogens. Our aim was to survey the *Culicoides* species occurring in an Amazonian rural settlement, comparing abundance, richness, and diversity in different environments. **Methods:** *Culicoides* were captured using CDC light traps. The Shannon-Wiener (H’) and Rényi indices were used to compare species diversity and evenness between environments, the equitability (J’) index was used to calculate the uniformity of distribution among species, and similarity was estimated using the Jaccard similarity index. A permutational multivariate analysis of variance was applied to assess the influence of environment on species composition. A non-metric dimensional scale was used to represent the diversity profiles of each environment in a multidimensional space. **Results:** 6,078 *Culicoides* were captured, representing 84 species (45 valid species/39 morphotypes). H’ values showed the following gradient: forest > capoeira > peridomicile > forest edge. The equitability J’ was greater in capoeira and forests compared to peridomiciles and the forest edge. The population compositions of each environment differed statistically, but rarefaction estimates indicate that environments of the same type possessed similar levels of richness. Species of medical and veterinary importance were found primarily in peridomiciles: *C. paraensis*, vector of Oropouche virus; *C. insignis* and *C. pusillus*, vectors of Bluetongue virus; *C. filariferus*, *C. flavivenula*, *C. foxi*, and *C. ignacioi*, found carrying Leishmania DNA. **Conclusions:** This study indicates that diversity was higher in natural environments than in anthropized environments, while abundance and richness were highest in the most anthropized environment. These findings suggest that strictly wild *Culicoides* can adapt to anthropized environments.

**Keywords:** *Culicoides*. Diversity. Abundance. Anthropized environments.

INTRODUCTION

Tropical forest ecosystems host two thirds of the Earth’s terrestrial biodiversity and provide significant benefits to the biosphere and the global economy1. According to Steege et al. (2015)2, approximately 40% of the original Amazon forest will be lost by 2050 if historical rates of deforestation continue. Anthropization is the primary cause of environmental change and the degradation of tropical ecosystems.

Environmental changes caused by anthropogenic interference are associated with increased health risks. Vectors and pathogens previously found only in forests have been observed in human settlements. According to Gottdenker et al. (2014)3, disease transmission is affected land-use changes that include: deforestation, forest and habitat fragmentation, agricultural development, irrigation, urbanization, and suburbanization. In the Amazon, these changes may be responsible for the increased incidence of
endemic diseases such as Malaria, Yellow fever, Dengue, Zika, Mayaro, Oropouche, Chikungunya, and several other arboviruses and protozoa that cause leishmaniasis.

Hematophagous biting midge populations are affected by anthropogenic interference. In the Brazilian Amazon, Castellón (1990) observed a greater abundance of biting midges in capoeira and clearings than in primary forest. In urban and rural areas in Maranhão State, Silva and Carvalho (2013) found both a greater richness and abundance of biting midges in peridomiciles. According to Cazorla and Campos (2018), anthropization impacts ceratopogonid communities by decreasing biodiversity and favoring species that best adapt to altered environments.

This study was conducted in the settlement of Rio Pardo, Presidente Figueiredo Municipality, Amazonas State, Brazil. Until recently, human activity in Rio Pardo was limited to subsistence farming, raising livestock, hunting, fishing, and gathering wood for local use, but newly expanded branches of forestry and fish farming have had a significant impact on the natural environment. Disease transmission dynamics in the region may have been altered by the degradation of natural habitats causing increased human exposure to forests, and to disease vectors and their hosts. The increased supply of blood meal sources around peridomiciles and a lack of basic sanitation offer favorable conditions for insect vectors to flourish.

The genus Culicoides is comprised of 1,368 species worldwide; 299 of these species occur in the Neotropics and 122 species occur in the Brazilian Amazon Basin. Certain Culicoides species transmit viruses such as African horse sickness virus (AHSV), Bluetongue virus (BTV) which infects domestic and wild ruminants, and Oropouche virus (OROV) which infect humans. Oropouche virus is one of the most common arboviruses in Brazil; it has affected an estimated 500,000 people since it was first isolated in 1955. Biting midges and simulids have been implicated in the transmission of some species of filariae to humans, including: Mansonella ozzardi, M. perstans and M. streptocerca. Culicoides may also be involved in the transmission of Leishmania.

Identifying possible disease vectors is therefore of significant epidemiological importance. The aim of this study was to survey the Culicoides species that occur in a typical Amazonian rural settlement, and to compare the abundance, richness, and diversity of Culicoides populations present in different environments.

METHODS

The rural settlement of Rio Pardo (1°49’02.3’’S 60°19’03.5’’W), Presidente Figueiredo Municipality, Amazonas State, Brazil (Figure 1), was founded in 1996 and has approximately 700 inhabitants (ILMD/FIOCRUZ). In 2002, approximately 95% of its total area, about 28,000 hectares, was composed of preserved

---

**FIGURE 1:** Location of the study area where Culicoides were captured: (A) Brazil; (B) Location of Presidente Figueiredo Municipality in the Central Amazon; (C) The rural settlement of Rio Pardo, numbers indicate specific dwellings where collections were made; (D) Diagram of a single collection area, a collection area was defined as the area within 300 m of a dwelling (Adapted by Ramos et al. 2015).
forest. From 1996-2002, the rate of deforestation was estimated to be about 150 ha/year, while land was developed for agricultural and community use at a rate of about 220 ha/year.

*Table 1:* Culicoides species collected in forests and anthropized environments in the rural settlement of Rio Pardo, Municipality of Presidente Figueiredo, Amazonas, Brazil.

| Environment | Total | % |
|--------------|-------|---|
| C. aldomani  | 1     | 0,016 |
| C. baniwa    | 0     | 0,049 |
| C. batesi    | 2     | 0,165 |
| C. benarrochi* | 0 | 0,016 |
| C. bricenoi  | 0     | 0,016 |
| C. brownie   | 0     | 0,016 |
| C. castelloni | 1   | 0,016 |
| C. coutinhoi | 31    | 1,333 |
| C. dasyophrus| 7     | 0,165 |
| C. debilipalpis* | 17 | 0,577 |
| C. diabolicus| 2     | 0,099 |
| C. efferus   | 0     | 0,066 |
| C. euplepharus| 0  | 0,082 |
| C. filarifer | 13    | 0,520 |
| C. flavivenula* | 1  | 0,049 |
| C. fluvialis | 0     | 0,016 |
| C. fluvialis*| 0     | 0,016 |
| C. foxi*     | 12    | 0,380 |
| C. franklini | 3     | 0,099 |
| C. fusipalpis* | 87 | 35,84 |
| C. glabellus*| 7     | 0,214 |
| C. glabior   | 3     | 0,230 |
| C. guamai    | 1     | 0,033 |
| C. hylas     | 54    | 1,859 |
| C. ignacii*  | 0     | 1,218 |
| C. insignis* | 6     | 3,768 |
| C. irreguralis| 0   | 0,016 |

*Results*

A total of 6,078 Culicoides (96.38% females and 3.62% males) were collected, representing 84 species, comprised of 45 valid species and 39 morphotypes.

*Culicoides fusipalpis* exhibited the highest abundance (2.178-35.84%), followed by *C. dasyophrus* (919-15.12%), *C. pseudodiabolicus* (459-7.55%), *C. diabolicus* (426-7.01%), *C. filarifer* (319-5.25%), *C. ocumarensi* (290-4.77%), *C. foxi* (231-3.80%), and *C. insignis* (229-3.77%). The remaining species represented less than 3% of the collected specimens (Table 1). The most abundant species in peridomiciles were *C. fusipalpis* and treated as valid species in the diversity and richness analyses. The Shannon-Wiener (H’) and Rényi indexes were used to compare species diversity and evenness between the four environments. The Pielou equitability index (J’) was used to calculate the uniformity of distribution of individuals among species, and similarity was calculated using the Jaccard similarity index (Cj), which qualitatively compares species similarity along an environmental gradient. A permutational multivariate analysis of variance (PERMANOVA) was applied to assess the influence of each environment on species composition. A non-metric dimensional scale (NMDS) was used to represent the diversity profiles of each environment in a multidimensional space. All analyses were carried using the statistical package R version 3.4.2. The level of significance considered for all tests was 95%.

**Table 1:** Culicoides species collected in forests and anthropized environments in the rural settlement of Rio Pardo, Municipality of Presidente Figueiredo, Amazonas, Brazil.
The abundance, richness, diversity indexes ($H'$) and equitability ($J'$) calculated for the Culicoides species collected in environments of forest (fo), forest edge (fe), capoeira (ca) and peridomicile (pe). *species known to exhibit anthropophilic behavior.
C. diabolicus (20-23.53%); the most abundant species in capoeira were C. pseudodiabolicus (27-31.77%) and C. fusipalpis (430-27.59%); and the most abundant species in forests were C. pseudodiabolicus (246-34.75%) and C. fusipalpis (87-12.29%) (Table 1).

Of the incriminated and putative vectors recorded (Table 1): C. paraensis was found primarily in peridomiciles; C. insignis was most abundant in peridomiciles near corrals and chicken coops; a single specimen of C. pusillus was collected in a peridomicile near a corral; C. filariferus was found in all environments except capoeira; C. flavivenula exhibited low abundance in forests and peridomiciles; C. foxi occurred in all environments; and C. ignacioi occurred in all environments except forests.

Richness between environments varied from 22 to 58 species/morphotypes. Abundance varied significantly between environments, with values ranging from 85 to 3.725 individuals (Table 1). Species diversity was highest in forests (H' = 2.55) and lowest at the forest edge (H' = 1.71). H' values showed the following gradient: forest > capoeira > peridomicile > forest edge. The equitability (J') index was greater in capoeira and forest than in peridomicile and the forest edge (Table 1), which indicates that Culicoides individuals are more equitably distributed among different species in capoeira and forest environments.

Similarity was greater between the forest and capoeira than between capoeira and peridomiciles. The population compositions of each environment differed statistically (PERMANOVA S.S = 1.61, PSEUDO-F = 1.96, p = 0.04) (Figure 2 and Figure 3). Richness differed between environments, but rarefaction estimates indicate that environments of the same type possessed similar levels of richness (Table 1 and Figure 4).

FIGURE 2: Rényi diversity profiles of Culicoides for the four environments investigated: capoeira (successional vegetation), forest, forest edge, and peridomicile in the rural settlement of Rio Pardo, Presidente Figueiredo Municipality, Amazonas, Brazil. Circles show the values for each site, and dashed lines show the median and extreme values.

DISCUSSION

Eighty-four Culicoides species/morphotypes were collected in the rural settlement of Rio Pardo, representing 69% of the known Culicoides species found in the Brazilian Amazon Basin. Previous studies conducted in Rio Pardo recorded five new species of Culicoides and nine new occurrences. Until now, the highest levels of richness in Brazil have been recorded in the municipality of Belém in Pará State, with 50 species; followed by Porto Velho in Rondônia, with 40 species; Alto Alegre in Roraima, with 38 species; and Manaus and Tefé in Amazonas, with 35 species and 19 species, respectively.

Species of medical and veterinary importance were found primarily in peridomiciles, these include: Culicoides paraensis, which is a vector of OROV in humans; C. insignis and C. pusillus, which are vectors of BTV in domestic and wild ruminants; C. filariferus and C. flavivenula, which have been found carrying Leishmania amazonensis DNA; and C. foxi and C. ignacioi which have been found carrying Le. braziliensis DNA. All of these species were found in this study, but some in low abundance. This is probably due to the collection method. In entomological inventories, it is common for some species to appear with low frequency because bait type, capture effort, collection environment, and the presence of animals favors the capture of other species. This low frequency may also be related to the fact that some species are diurnal while others are nocturnal.

Few studies conducted in the Amazon have examined Culicoides anthropophily. Of the species identified in this study, the following are known to exhibit anthropophilic behavior: Culicoides batesi; C. benarrochei; C. debilipalpis; C. flavivenula; C. flaviatilis; C. foxi; C. fusipalpis; C. glabellus; C. ignacioi; C. insignis; C. leopondoi; C. limai; C. lutzi; C. paraensis; C. paraignacioi; C. paramaruim;
FIGURE 3: Non-metric multidimensional scaling (NMDS) showing *Culicoides* diversity profiles in capoeira, forest, forest edge, and peridomicile environments in the rural settlement of Rio Pardo, Presidente Figueiredo Municipality, Amazonas, Brazil.

FIGURE 4: Rarefaction curves representing the species richness of *Culicoides* in capoeira, forest, forest edge, and peridomicile environments in the rural settlement of Rio Pardo, Presidente Figueiredo Municipality, Amazonas, Brazil (95% CI).
C. pseudodiabaticus and C. pusillus. Of these, all except C. glabellus were found in peridomiciles. Santiago-Alarcon et al. (2013) argue that humans may serve as blood meal sources for dominant Culicoides species in peridomicile environments.

Abundance was greatest in peridomiciles, followed by the forest edge, forest, and capoeira. Culicoides fusipalpis has been observed in high abundance in anthropic environments where it feeds on a variety of blood meal sources, including humans, other mammals and birds. Species richness was highest in peridomiciles, followed by forest, forest edge, and capoeira. The concentration of a variety of blood meal sources and the presence of suitable breeding sites may be attracting Culicoides to peridomiciles. Santiago-Alarcon et al. (2013) surveyed Culicoides feeding behavior in households near an urban forest in Germany where the dominant Culicoides species were known to be ornithophilous and found that blood ingested by these midges contained the mitochondrial DNA of mammals such as cows and humans. Generalist species are able to tolerate a broad set of environmental conditions and make use of a wide range of resources, which allows them to become both widespread and locally abundant (Brown, 1984).

In Rio Pardo, species diversity was highest in forests, while richness and abundance was highest in peridomiciles. In a study conducted in Rondônia, Carvalho et al. (2016) found that species diversity was higher in forests than in pasture. In a study conducted in rural and urban areas in Maranhão, Silva and Carvalho (2013) found that species diversity was highest in the Cerrado (savanna) and in gallery forests, while richness and abundance were highest in peridomiciles.

Equitability was highest in capoeira environments, where the number of individuals per species ranged from 1 to 27, and equitability was lowest in the forest edge environments, where the number of individuals per species ranged from 1 to 745 (Table 1). The high equitability observed in capoeira environments is the result of low abundance and a relatively homogeneous distribution of species. The low equitability observed in forest edge environments is likely due to an uneven distribution of species abundance caused by the predominance of C. dasyophorus, which comprised 47.76% of all individuals collected at the forest edge. These findings demonstrate that the local diversity index may decrease in environments where one species is highly dominant.

Species similarity between environments was low (> 50%), which is likely due to the different degrees of anthropic interference present in each environment. Capoeira–forest edge and forest–forest edge exhibited the highest similarity. These findings suggest that strictly wild Culicoides fauna tends to adapt to anthropized environments.

We observed that richness differed between environments, but rarefaction estimates indicate that environments of the same type possessed similar levels of richness. This is probably due to the three-month capture effort occurring during the dry season, a period that the abundance of general hematophagous dipteria are low, which may have interfered with the results, indicating the need for more captures and in different seasons.

The data obtained in this study indicate that diversity was higher in natural environments (forest) than in anthropized environments (capoeira), while abundance and richness were both highest in the most anthropized environment (peridomiciles). It is likely that the concentration of a variety of food sources and the presence of suitable breeding grounds in peridomiciles favors the establishment of certain species.

In settled areas, the presence of domestic and wild animals provides vectors with a rich variety of food sources and this fosters their adaptation to new environments. This behavior may alter pathogen transmission dynamics and increase the risk of disease transmission by Culicoides.

ACKNOWLEDGMENTS

To the CT-Amazônia CNPq/FAPEAM project, “Risk of vector-borne diseases in the central Amazon: effect of deforestation and human population density”. To the Brazilian Council for Scientific and Technological Development (CNPq) for FAC Pessoa research scholarship, and JA Feijó research scholarship number 141323/2019-1. To Dr. Maria Luiza Felippe Bauer-FIOCRUZ/JOC, for the Culicoides taxonomy training.

FINANCIAL SUPPORT

Função de Amparo à Pesquisa do Estado do Amazonas-FAPEAM-Project number 062.01844/2014 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES-process number 001.

AUTHORS’ CONTRIBUTION

EF designed the study, slide-mounted and identified specimens, and wrote the manuscript; JAF slide-mounted specimens and wrote the manuscript; JWPS and LSC performed the statistical analysis and wrote the manuscript; SLBL, CMRV and FACP: conceived and designed the study, and wrote the manuscript; all authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Gardner TA, Barlow J, Chazdon R, Ewers RM, Harvey CA, Peres CA, Sodhi NS. Prospects for tropical forest biodiversity in a human-modified world. Ecol Lett. 2009;12(6):561–82. doi:10.1111/j.1461-0248.2009.01294.x
2. Steege H, Pitman NCA, Killeen TJ, Laurance WF, Peres CA, Guevara JE, et al. Estimating the global conservation status of more than 15,000 Amazonian tree species. Sci Adv. 2015;1(10):e1500936. doi: 10.1126/sciadv.1500936.
3. Gottendenker NL, Streicker DG, Faust CL, Carroll CR. Anthropogenic land use change and infectious diseases: a review of the evidence. Eco Health. 2014;11(4):619–32. doi:10.1007/s10393-014-0941-z
4. Castellón EG. Culicoides (Diptera: Ceratopogonidae) na Amazônia Brasileira. II. Espécies coletadas na Reserva Florestal Ducke, aspectos ecológicos e distribuição geográfica. Act Amaz. 1990;20:83–93. http://dx.doi.org/10.1590/1389-43921990201093
5. Silva FS, Carvalho LPC. A Population Study of the Culicoides Biting Midge (Diptera: Ceratopogonidae) in Urban, Rural, and Forested Sites in a Cerrado Area of Northeastern Brazil. Ann Entomol Soc Am. 2013;106(4):463–70. doi:10.1603/AN12047
6. Cazorla CG, Campos RE. Synanthropy and Community Structure of Ceratopogonidae from the Northeast of Buenos Aires Province,
Argentine. J Med Entomol. 2018;56(1):129–36. doi.org/10.1093/jme/jty165

7. Borkent A. Numbers of Extant and Fossil Species of Ceratopogonidae. 2016. Available from: http://www.inhs.illinois.edu/files/8514/2385/5055/CulicoidesSubgenera.pdf

8. Santarém M, Felippe-Bauer M. Brazilian species of biting midges. Fundação Oswaldo Cruz. 2019. Available from: https://portal.fiocruz.br/sites/portal.fiocruz.br/files/documentos/brazilian_species_of_biting_midges_2019.pdf

9. Mellor PS, Boorman J, Baylis M. Culicoides biting midges: their role as arbovirus vectors. Annu Rev Entomol. 2000;45:307–40. doi: 10.1146/annurev.ento.45.1.307

10. Travassos da Rosa JF, Souza WM, Pinheiro FP, Figueiredo ML, Cardoso JF, Acrani GO, et al. Oropouche Virus: Clinical, Epidemiological, and Molecular Aspects of a Neglected Orthobunyavirus. Am J Trop Med Hyg. 2017;96(5):1019–30. doi: 10.4269/ajtmh.16-0672

11. Linley JR, Hoch AL, Pinheiro FP. Biting midges (Diptera: Ceratopogonidae) and human health. J Med Entomol. 1983;20(4):347–64. doi: 10.1093/jmedent/20.4.347

12. Sebola V, Sadlova J, Vojtikova B, Votyoka J, Carpenter S, Bates PA, et al. The Biting Midge Culicoides sonorensis (Diptera: Ceratopogonidae) is Capable of Developing Late Stage Infections of Leishmania enriettii. PLoS Negl Trop Dis. 2015;9(9),e0004060. https://doi.org/10.1371/journal.pntl.0004060

13. Ramos WR, Medeiros JF, Julião GR, Ríos-Velásquez CM, Marialva EF, Wirth WW, Blanton FS. Biting Midges of the Genus Culicoides (Diptera: Ceratopogonidae). Contrib Amer Entomol Inst. 1993;27:1–91.

14. Trindade RL, Gorayeb IS. Culicoides (Diptera: Ceratopogonidae) and hospedeiros potenciais em área de ecoturismo do Parque Nacional dos Lençóis Maranhenses, Brasil. Rev Pan-amaz Saúde. 2013;4(3):11–8. doi.org/10.5123/S2176-622320130000300002

15. Wirth W, Roberts DR, Pinheiro FP. Host-seeking behavior and seasonal abundance of Culicoides paraensis (Diptera: Ceratopogonidae) in Brazil. J Am Mosq Control Assoc. 1990;6(1):110–4.

16. Koch HG, Axtell RC. Attraction of Culicoides fusipes and C. hollensis (Diptera: Ceratopogonidae) to animal hosts in a salt marsh habitat. J Med Entomol. 1979;15(5–6):494–9. https://doi.org/10.1093/jmedent/15.5-6.494

17. Aitken THG, Wirth WW, Williams RW, Davies JB, Tikasingh ES. A review of the bloodsucking midges of Trinidad and Tobago, West Indies (Diptera: Ceratopogonidae). J Entomol Ser B Taxon. 1975;44(2):101–44. doi:10.1111/j.1365-3113.1975.tb00007.x

18. Trindade RL, Gorayeb IS, Maruins (Ceratopogonidae: Diptera) of the estuarine area of the River Pará and of the litoral of the Estado do Pará, Brasil, Entomol Vect. 2005;12(1):61–74. http://dx.doi.org/10.1590/S038120050000100005.

19. Trindade RL, Gorayeb IS. Maruins (Diptera: Ceratopogonidae), após a estação chuvosa, na Reserva de Desenvolvimento Sustentável Itapuá-Baquitã, Gurupá, Pará, Brasil. Rev Pan-Amaz Saúde. 2010;1(2):121–30. http://dx.doi.org/10.5123/S2176-62232010000200015

20. Santiago-Alarcon D, Havelka P, Pineda E, Segelbacher G, Schaefer HM. Urban florests as hubs for novel zoonosis: blood meal analysis, seasonal variation in Culicoides (Diptera: Ceratopogonidae) vectors, and avian haemosporidians. Parasitol. 2013;140(14):1799–810. doi: 10.1017/S0031182013001285

21. Santarém MCA, Confalonieri UEC, Felippe-Bauer ML. Diversity of Culicoides (Diptera: Ceratopogonidae) in the National Forest of Caxiuanã, Melgaço, Pará State, Brazil. Rev Pan-Amaz Saúde. 2010;14(10):22–33. http://dx.doi.org/10.5123/S2176-6223201000400005t

22. Brown JH. On the Relationship between Abundance and Distribution of Species. Am Nat. 1984;124(2):255–79. doi: 10.1086/284267

23. Carvalho LPC, Pereira Júnior AM, Farias ES, Almeida JF, Rodrigues MS, Resadore F, Pessoa, FAC, Medeiros JF. A study of Culicoides in Rondônia, in the Brazilian Amazon: species composition, relative abundance and potential vectors. Med Vet Entomol. 2016;31(1):117–22. doi.org/10.1111/mve.12208.