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Aspects of the blood meal of mosquitoes (Diptera: culicidae) during the crepuscular period in Atlantic Forest remnants of the state of Rio de Janeiro, Brazil

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Identifying their food sources provides insights into mosquito foraging behaviors and directly impacts the epidemiology of mosquito-borne pathogens such as dengue, Zika, and chikungunya. Understanding these feeding patterns becomes even more relevant in ecosystems like the Atlantic Forest, where biodiversity is rich and complex. The present study was conducted in Atlantic Forest remnants of the Guapiaçu Ecological Reserve (REGUA) and the Sítio Recanto Preservar, both located in the state of Rio de Janeiro, Brazil. We aimed to identify the food sources of mosquitoes present in these areas, contributing to a better understanding of ecological and epidemiological dynamics. Molecular techniques, such as Sanger DNA sequencing of cytochrome b (Cytb), allows for precise identification of food sources, which is fundamental for designing control and monitoring strategies for mosquito populations. A total of 1,714 mosquitoes were captured, of which only 145 females (6.98%) were engorged. The results revealed a clear tendency for the captured mosquito species to feed predominantly on humans. Additionally, we emphasize the need to continuously improve techniques to identify food sources, aiming to better understand interactions between mosquitoes and their environment. This information is crucial for developing effective policies and strategies to control vector-borne pathogens.

KEYWORDS

mosquito ecology, blood meal analysis, Atlantic Forest, anthropophily, vector-host interaction

Introduction

Identifying the food sources of mosquitoes allows researchers to investigate their feeding patterns and preferences for different food sources. This aids in understanding their life history and the factors that may influence their search and choice of vertebrate hosts. Additionally, it reveals the impact of these habits on their survival and reproductive success (Lyimo and Ferguson, 2009; Takken and Verhulst, 2013). Understanding the feeding habits of hematophagous mosquitoes is essential to determine their role as vectors in the maintenance and transmission of pathogens among vertebrates (Faraji et al., 2014). Furthermore, it provides better insight into their ecological in-teractions and coevolutionary processes (Kent, 2009).

The availability of hosts in an area is one of the factors influencing mosquito blood feeding (Francisco and Da Silva, 2019). The Atlantic Forest biome is home to approximately 850 species of birds, 370 amphibians, 200 reptiles, 270 mammals, and 350 fishes, contributing to the maintenance of culicid populations. Originally, the Atlantic Forest covered more than 1.3 million km² across 17 states in Brazil, and was present along much of the country's coast. However, due to human occupation and activities in the region, as expansion of agriculture with the planting of guava trees, pepper plants, ornamental grass, horse breeding and residential development only about 29% of its original coverage remains today (Ministério do Meio Ambiente, 2025). Deforestation reduces local biodiversity, causing mosquitoes, including vectors of pathogenic agents, to disperse and seek alternative food sources.

Vector-borne pathogens pose an increasing threat to the global human population, with more than 700,000 people dying annually due to infection (World Health Organization, 2017). In 2022, 249 million cases of malaria were reported, resulting in 608,000 deaths (World Health Organization, 2023). The WHO reported 4.2 million dengue cases in 2019, with an estimated 3 billion people at infection risk (Gangula et al., 2023).

The heterogeneity of vertebrate hosts can significantly impact the transmission of pathogens by vectors, as many hosts are sources of infection for a single pathogen (Marm Kilpatrick et al., 2006). However, given the diversity of animals, some pathogens can adapt to specific hosts (Kenney and Brault, 2014). Since mosquitoes exhibit generalist or specialist feeding behaviors, this set of factors demonstrates the complexity and importance of identifying animal species that serve as reservoir hosts for various pathogens (Haydon et al., 2002).

The present study aimed to investigate the food sources of mosquitoes captured during the crepuscular period at Sitio Recanto Preservar in the municipality of Silva Jardim and REGUA (Guapiaçu River Ecological Reserve) in the municipality of Cachoeiras de Macacu, both remnants of the Atlantic Forest in the state of Rio de Janeiro, Brazil.

Materials and methods

Study area

Sampling was conducted at the Sítio Recanto Preservar (SRP), Silva Jardim (22°37'10.7"S 42°18'59.5"W). This sampling point is part of the Environmental Protection Area of the São João River Basin (APA Bacia Hidrográfica do Rio São João). We also collected samples at the Guapiaçu River Ecological Reserve (REGUA), Cachoeiras de Macacu (22° 27′ 10.309 S and 42° 46′ 13.011 W). Both sampling points are located in the state of Rio de Janeiro (Figure 1).

The Environmental Protection Area of the São João River Basin experiences high rainfall levels from November to April, with an annual average of 2,400 mm in Silva Jardim. The dry season spans from May to September (Takizawa, 1995). It is part of the ecological region of dense ombrophilous forests, which includes montane and submontane areas, as well as lowland forests (Veloso et al., 1991). Montane forest occurs at altitudes from 500 to 1500 meters, and submontane forest is present on slopes up to 500 meters between the lowlands and montane forest. Lowland forests are found in alluvial plains and are one of the most threatened landscapes in the Atlantic Forest due to fragmentation.

REGUA is a remnant of the Atlantic Forest, located in the Guanabara Bay sub-basin, with part of the reserve situated within the Três Picos State Park, which, together with the Serra dos Órgãos National Park and the Paraíso State Ecological Station, protects a large continuous area of Atlantic Forest, covering much of the Serra dos Órgãos region, one of the areas with the greatest biodiversity in the state (Almeida-Gomes et al., 2014).

The reserve covers an area of 7,400 hectares and features dense ombrophilous forest vegetation, with the subtypes floodplain forest in gently undulating terrain, submontane in highland undulating terrain, and alluvial in flat terrain (Pires et al., 2022). The regional climate includes rainy and hot summers (October to March) and cold, dry winters (April to September), with an annual average temperature of 23°C, peaking in January and February and dipping in June. The annual average precipitation is around 2,560mm, with December and January as the wettest months and June and July as the driest (Kurtz and De Araujo, 2000; Alvares et al., 2013).

The fauna includes a high species diversity, with approximately 67 species of am-phibians, 455 species of birds, 45 species of reptiles (including the broad-snouted caiman, *Caiman latirostris*), and 61 species of mammals (including the southern muriqui, *Brachyteles arachnoides*) (Viana G. et al., 2016).

Specimen capture, storage, and identification of engorged females

Captures were conducted over two consecutive days using CDC light traps, installed at a height of 2 meters and distributed randomly. The traps were exposed from 6:00 PM to 8:00 AM the following morning. Sampling at the Sítio Recanto Preservar site was conducted from February to April and August to October 2023, while captures at REGUA occurred from March 2023 to February 2024.

Two sampling points were established at REGUA (geographic coordinates (S) 22° 27'10.309 (W) 42°4613.011): Point 1 (yellow trail), located near the reserve headquarters, features a very low

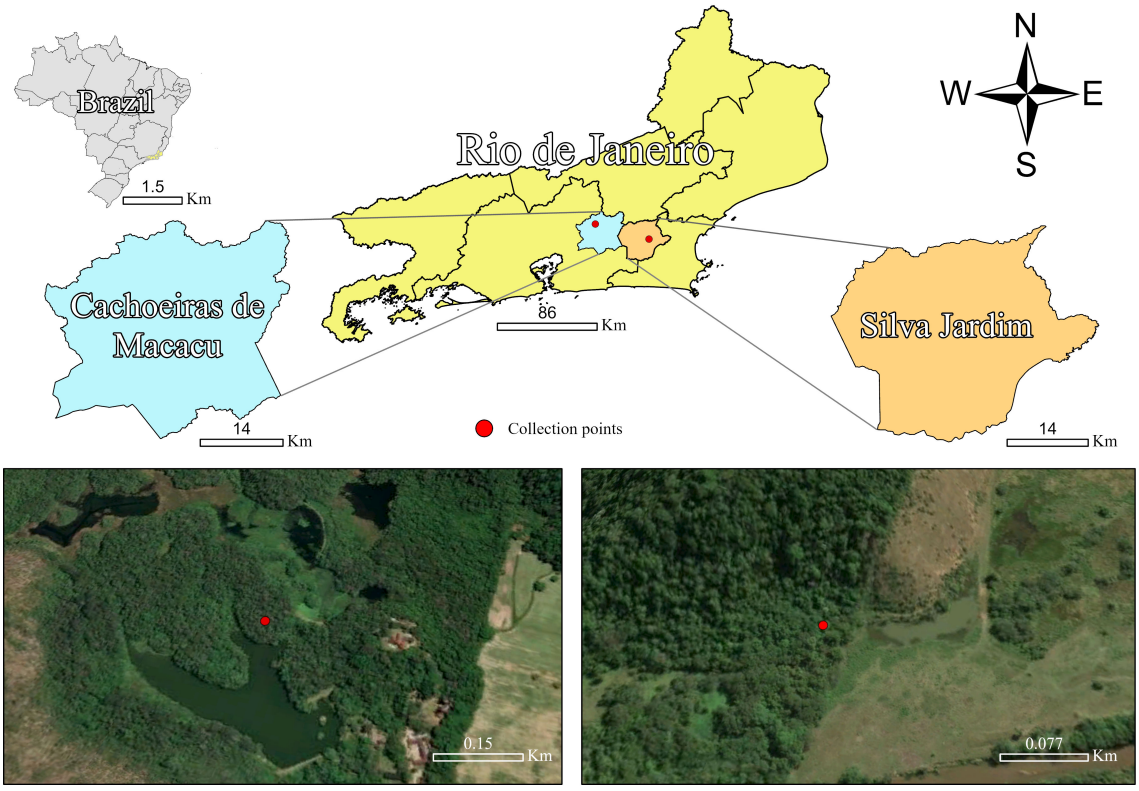

FIGURE 1
Map of the mosquito collection sites for this study. Sampling points: Cachoeiras de Macacu and Silva Jardim, located in the state of Rio de Janeiro, Brazil. Maps were prepared in QGIS version 3.34.9 (URL: https://qgis.org/. Acessed: 8 August 2024).

elevation and surrounds the entire lake, with vegetation that includes a mix of reintroduced native species; Point 2 (green trail), located at a higher elevation in the submontane zone, features dense and diverse forest with restored secondary forest terrain. Although these sampling points differ significantly in terms of elevation and possibly environmental complexity, they are both part of ecological restoration efforts and the promotion of local biodiversity.

The sampling point at Sítio Recanto Preservar is represented by an environment with a dense shrub layer, where tall trees are positioned very close to each other. This vegetation type suggests a dense forest ecosystem with a complex vertical structure that provides a range of unique conditions for local biodiversity.

The captured specimens were placed in polyethylene cages, labeled according to their origin, stored in thermal boxes, and transported alive to the field support laboratory. Subsequently, they were anesthetized by exposure to chloroform vapor and kept in a freezer (4 °C) to halt the digestive process.

Male and female specimens were separated and identified on a refrigerated table to preserve vertebrate DNA using dichotomous keys developed by Lane (1953a, b), Consoli & Lourenço-de-Oliveira (1994), Forattini (2002), and Marcondes & Alencar (2010). After identification, the specimens were stored in an ultra-freezer at -80°C for subsequent molecular analysis.

Molecular analysis

DNA extraction was performed using the TransGen Biotech kit: EasyPure Viral DNA/RNA (code#ER201–02 TransGen Biotech, Beijing, China), following the manufacturer's protocol from the macerated head and abdomen of the mosquito, previously separated with a scalpel. Then, the material was quantified and tested for quality using a Denovix DS-11 FX spectrophotometer (Denovix Inc., Wilmington, US); only good-quality samples proceeded to the next stage.

Food sources were identified using two pairs of primers that amplify a mitochondrial DNA (mtDNA) fragment of the cytochrome B (CytB) gene (Table 1). These primers are considered universal for vertebrates and do not amplify mosquito DNA; therefore, they are useful in amplifying only the blood meal they consumed (Malmqvist et al., 2004).

The protocol used for the L14841/H15149 primers was adapted from (Dias et al., 2011). The PCR reaction was performed in a final volume of 25µL, containing 2mM of MgCl2, 2mM of dNTP, 1.0µM of each primer, 2.5x of 10x buffer, 0.4µM of Taq DNA polymerase, 5µL of DNA, and ultrapure water to complete the final solution. Amplification involved an initial step at 95°C for 5 minutes, followed by 35 cycles at 95°C for 1 minute, 50°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

TABLE 1 Sequences of the two pairs of primers used and the size of their amplicons used in the present study.

Primer	Sequence (5′-3′)	Region	Amplicon size (pb)	References
L14841 Forward	AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA	14841	305	(Kocher et al., 1989)
H15149 Reverse	AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA	15149	305	(Kocher et al., 1989)
VF Forward	GAGGMCAAATATCATTCTGAGG	15150	457	(Townzen et al., 2008)
VR Reverse	TAGGGCVAGGACTCCTCCTAGT	15607	457	(Townzen et al., 2008)

VF, vertebrate forward; VR, vertebrate reverse.

The protocol used for the VF/VR primers was adapted from (Gyawali et al., 2019). The PCR reaction was also performed in a final volume of 25µL, containing 0.5µM of each primer, 1.5mM of MgCl2, 2.5 mM of dNTPs, 2.5x of 10x buffer, 0.4µM of Taq DNA polymerase, 5µL of sample DNA, and ultrapure water to complete the final volume. Amplification involved an initial step at 95°C for 1 minute, followed by 35 cycles at 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 40 seconds, and a final extension at 72°C for 10 minutes.

After PCR, 5µL of the amplified product was subjected to 1.5% agarose gel elec-trophoresis. The gels were placed in a container with 0.05% ethidium bromide solution for 5 to 10 minutes to visualize the bands under a UV transilluminator at 260nm.

The PCR product was purified for all materials that amplified the CytB mtDNA fragment using the protocol established in the PCR Purification Kit (Cellco Biotec do Brasil Ltda., São Carlos, Brazil—cat#DPK-106L). Part of the purified material was used for sequencing, and the remainder was preserved in an ultra-freezer at -80°C as a reserve for future confirmation if necessary. After purification, the amplified samples were sent for Sanger sequencing at the RPT-01A Sequencing Platform of the Oswaldo Cruz Institute–RJ (FIOCRUZ), Rio de Janeiro, Brazil.

Sequence analysis

The sequences obtained from sequencing were processed using Geneious R11 v.11.1.5 (Biomatters Ltd., Auckland, New Zealand). The food source was identified by aligning the sequencing data with sequences deposited in the GenBank database (NCBI: National Center for Biotechnology Information —https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the BLAST tool and the MUSCLE algorithm. A vertebrate was considered a food source when the sequences showed a similarity of > 95%.

Results

Sampling at the Guapiaçu Ecological Reserve (REGUA) and the Recanto Preservar site yielded a total of 1,714 mosquitoes. At Recanto Preservar, 653 specimens representing 21 species were collected, while 1,061 mosquitoes, corresponding to 31 species, were collected at REGUA (Table 2). Of the total captured mosquitoes, only 145 females (8.46%) were engorged. Subsequent molecular analysis showed that the DNA of only 55 of these engorged samples (37.93%) was successfully amplified. Blood meals could not be detected in the remaining 90 engorged females, as the tested primers did not yield any amplification.

First, PCR was performed on the blood samples using the L14841/H15149 primers, for which only 24 samples were amplified for Cytb. Subsequently, the VF/VR primers were tested, and all 55 samples were amplified for this gene.

TABLE 2 Total specimens captured at the Guapiaçu Ecological Reserve (REGUA), municipality of Cachoeiras de Macacu, and at the Recanto Preservar Farmstead (SRP), municipality of Silva Jardim, state of Rio de Janeiro, Brazil.

Species	REGUA	SRP	Total
Aedeomyia (Ady.) squamipennis (Lynch Arribálzaga, 1878)	4	–	4
Aedes (Och.) scapularis (Rondani, 1848)	7	184	191
Ae. (Och.) serratus (s.l.) (Theobald, 1901)	3	12	15
Ae. (Pro.) argyrothorax (Bonne-Wepster & Bonne, 1920)	9	–	9
Ae. (Stg.) albopictus (Skuse, 1895)	2	6	8
Anopheles (Nys.) albitarsis Lynch-Arribálzaga, 1878	51	1	52
Anopheles (Ker.) cruzii Dyar & Knab, 1908	2	–	2
An. (Nys.) deaneorum Rosa-Freitas, 1989	2	–	2
An. (Ano.) evandroi Costa Lima, 1937	1	–	1
An. (Nys.) evansae (Brèthes, 1926)	42	12	54
An. (Ano.) maculipes (Theobald, 1903)	4	–	4
Anopheles spp.	3	2	5
Chagasia fajardi (Lutz, 1904)	1	–	1
Coquillettidia (Rhy.) albicosta (Chagas, 1908)	21	–	21
Coquillettidia (Coq.) chrysosoma (Edwards, 1915)	–	1	1
Cq. (Rhy.) chrysonotum (Peryassú,1922)	3	6	9
Cq. (Rhy.) fasciolata (Lynch-Arribálzaga, 1891)	139	49	188
Cq. (Rhy.) juxtamansonia (Chagas, 1907)	12	–	12
Cq. (Rhy.) shannoni (Lane & Antunes, 1937)	–	6	6
Cq. (Rhy.) venezuelensis (Theobald, 1912)	25	–	25
Culex spp.	183	–	183

(Continued)

TABLE 2 Continued

Species	REGUA	SRP	Total
Cx. (Mel.) clarki Evans, 1924	21	–	21
Cx. (*Cux.*) spp.	126	19	145
Cx. (Mcx.) pleuristriatus Theobald, 1903	1	–	1
Cx. (Mcx.) sp.	11	–	11
Cx. (Mel.) erraticus (Dyar & Knab, 1906)	2	–	2
Cx. (Mel.) pereyrai Duret, 1967	2	–	2
Cx. (Mel.) spp.	235	22	257
Haemagogus (Hag.) leucocelaenus (Dyar & Shannon, 1924)	1	4	5
Limatus durhamii Theobald, 1901	1	1	2
Mansonia (Man.) humeralis Dyar & Knab, 1916	–	1	1
Mansonia (Man.) indubitans Dyar & Shannon, 1925	1	–	1
Ma. (Man.) titillans (Walker, 1848)	73	29	102
Psorophora (Jan.) albipes (Theobald, 1907)	–	121	121
Psorophora (Jan.) ferox (Humboldt, 1819)	5	169	174
Runchomyia (Run.) reversa (Lane & Cerqueira, 1942)	2	–	2
Sabethes (Sab.) chloropterus (Humboldt, 1819)	1	–	1
Sabethes (Pey.) identicus Dyar & Knab, 1907	–	2	2
Sabethes sp.	–	2	2
Trichoprosopon compressum Lutz, 1905	3	–	3
Tri. digitatum (Rondani, 1848)	1	–	1
Tr. pallidiventer (Lutz, 1905)	6	–	6
Trichoprosopon sp.	1	–	1
Uranotaenia davisi Lane, 1943	3	–	3
Ur. (Ura.) calosomata Dyar & Knab, 1907	2	–	2
Ur. (Ura.) geometrica Theobald, 1901	4	–	4
Ur. (Ura.) pulcherrima Lynch-Arribálzaga, 1891	8	4	12
Wyeomyia (Pho.) edwardsi (Lane & Cerqueira, 1942)	8	–	8
Wyeomyia spp.	29	–	29
Total	1061	653	1714

In the samples collected at Sítio Recanto Preservar, 653 mosquitoes were captured, of which 21 females (3.21%) were engorged. However, only eight samples (9.5%) were successfully amplified, and it was possible to identify the blood meal of four of them. The remaining four samples could not be identified.

The four samples with identified blood meals corresponded to three females of *Psorophora ferox* (Humboldt, 1819) and one of *Aedes scapularis* (Rondani, 1848) (Table 3).

At REGUA, 124 engorged females (11.69%) were captured out of a total of 1061 mosquitoes; however, only 47 samples (37.90%) were successfully amplified. At the green trail sampling point, 80 specimens were captured, of which only seven were engorged, comprising four species. Of the 955 specimens captured on the yellow trail, 117 females were engorged, comprising 16 species. No food sources were detected for the species on the green trail, so all 47 amplified samples were from the yellow trail.

Similar to observations at Sítio Recanto Preservar, the food source could not be detected for 27 specimens, while the blood meal of 20 specimens was successfully identified. The specimens corresponded to two females of *Uranotaenia geometrica* Theobald, 1901, ten *Coquillettidia fasciolata* (Lynch-Arribálzaga, 1891), two *Cq. venezuelensis* (Theobald, 1912), one *Aedes scapularis* (Rondani, 1848), one *Ae. serratus* (s.l.) (Theobald, 1901), one *Ae. albopictus* (Skuse, 1895), one *Anopheles evansae* (Brèthes, 1926), one *An. evandroi* Costa Lima, 1937 and one *Culex (Melanoconion)* sp. (Table 4).

The detected food sources of the 24 specimens included 18 humans, one amphibian, six birds, one canid, and one mouse. However, even with a similarity of >95% in all sequences, only the sequences of humans could be published. Two samples showed multiple meals, corresponding to *Cq. venezuelensis* with two sources, an amphibian and a human, and *Cq. fasciolata* with four sources, one specimen for mouse and bird and another for human and bird. Two identified samples corresponded only to a bird genus (Table 5).

TABLE 3 Engorged species captured at the Sítio Recanto Preservar, Silva Jardim, Rio de Janeiro, Brazil.

Species	Engorged females	Food sources
Aedes (Ochlerotatus) scapularis (Rondani, 1848)	5	1
Ae. (Ochlerotatus) serratus (s.l.) (Theobald, 1901)	1	–
Anopheles (Nyssorhynchus) albitarsis (Lynch-h-Arribálzaga, 1878)	1	–
An. (Nyssorhynchus) evansae (Brèthes, 1926)	1	–
Coquillettidia (Rhynchotaenia) fasciolata (Lynch-Arribálzaga, 1891)	1	–
Mansonia (Mansonia) humeralis Dyar & Knab, 1916	1	–
Ma. (Mansonia) titillans (Walker, 1848)	6	–
Psorophora (Janthinosoma) ferox (Humboldt, 1819)	5	3
Total	**21**	**4**

Total values are highlighted in bold at the end of the table.

TABLE 4 Engorged species captured at sampling points in the Guapiaçu Ecological Reserve, Cachoeiras de Macacu, Rio de Janeiro, Brazil.

Species	Engorged females	Yellow trail	Green trail	Food sources
Aedes (Stegomyia) albopictus (Skuse, 1895)	1	1	–	1
Ae. (Protomacleaya) argyrothorax (Bonne-Wepster & Bonne, 1919)	4	–	4	–
Aedes (Ochlerotatus) scapularis (Rondani, 1848)	5	5	–	1
Aedes (Ochlerotatus) serratus (Theobald, 1901)	1	1	–	1
Anopheles (Anopheles) evandroi Costa Lima, 1937	1	1	–	1
An. (Nyssorhynchus) albitarsis Lynch-Arribálzaga, 1878	4	4	–	–
Anopheles (Nyssorhynchus) evansae (Brèthes, 1926)	1	1	–	1
Coquillettidia (Rhynchotaenia) albicosta (Peryassú, 1908)	4	4	–	–
Cq. (Rhynchotaenia) chrysonotum (Peryassú,1908)	1	1	–	–
Cq. (Rhynchotaenia) fasciolata (Lynch-Arribálzaga, 1891)	38	38	–	10
Cq. (Rhynchotaenia) juxtamansonia (Chagas, 1907)	1	1	–	–
Cq. (Rhynchotaenia) venezuelensis (Theobald, 1912)	7	7	–	2
Culex (Culex) sp.	2	2	–	–
Culex (Melanoconion) spp.	18	18	–	1
Mansonia (Mansonia) titillans (Walker, 1848)	30	30	–	–
Psorophora (Janthinosoma) ferox (Humboldt, 1819)	1	–	1	–
Runchomyia (Runchomyia) reversa Lane & Cerqueira, 1942	1	–	1	–
Trichoprosopon digitatum (Rondani, 1848)	1	–	1	–
Uranotaenia (Uranotaenia) geometrica Theobald, 1901	2	2	–	2
Ur. (Uranotaenia) pulcherrima Lynch-Arribálzaga, 1891	1	1	–	–
Total	**124**	**117**	**7**	**20**

Total values are highlighted in bold at the end of the table.

TABLE 5 Mosquito species captured at Cachoeiras de Macacu and Silva Jardim, Rio de Janeiro, Brazil, and their respective food sources.

Mosquito species	Locality	Quantity	Food sources	Class
Aedes scapularis (Rondani, 1848)	Sitio Recanto Preservar	1	*Anas acuta*	
Coquilletidia. fasciolata (Lynch-Arribálzaga, 1891)	REGUA	1	*Gallus gallus*	Bird
		1	*Harpia harpyja*	
		1	*Meleagris gallopavo*	
		1	*Larus* sp	
		1	*Chroicocephalus* sp.	
Cq. venezuelensis (Theobald, 1912)	REGUA	1	*Pithecopus rohdei*	Amphibia
Anopheles evansae (Brèthes, 1926)	REGUA	1	*Canis lupus familiaris*	Mammalia
Coquilletidia. fasciolata (Lynch-Arribálzaga, 1891)	REGUA	1	*Mus musculus*	
Ae. albopictus (Skuse, 1895)	REGUA	1	*Homo sapiens*	
Ae. scapularis (Rondani, 1848)		1		
Ae. serratus (Theobald, 1901)		1		
Anopheles evandroi Lima, 1937		1		
Cq. fasciolata (Lynch-Arribálzaga, 1891)		6		

(Continued)

TABLE 5 Continued

Mosquito species	Locality	Quantity	Food sources	Class
Cq. venezuelensis (Theobald, 1912)		2		
Culex (Melanoconion) sp.		1		
Psorophora ferox (Humboldt, 1819)	Sitio Recanto Preservar	3		
Uranotaenia geometrica Theobald, 1901	REGUA	2		

Discussion

The present findings highlight the importance of considering not only human presence but also the behavior and feeding preferences of mosquitoes when planning vector control strategies and preventing pathogens transmitted by these insects. It is essential to emphasize the need for more research to clarify better the feeding patterns of mosquitoes and their implications for public health.

The biodiversity of the Atlantic Forest is considered of extreme biological importance; however, this richness of fauna and flora is threatened by deforestation. The greater the increase in deforested areas, the faster the rate and acceleration of biodiversity loss (Branco et al., 2021). The loss of native vegetation is associated with an increase in the transmission of etiological agents of arboviruses (dengue, Zika, Chikungunya, and yellow fever). Consequently, the natural habitats of vectors and their life cycles are altered, affecting their population density. It should be added that, with the degradation of forest areas and the increasing human occupation, biological vector insects approach homes and peridomestic areas, causing transmissions (Moreno, 2021).

Besides the small sample size, we have detected in the two specimens of *Ae. scapularis* at different sampling points, one belonging to human and one bird which is consistent with the previous studies indicating that this species exhibits a generalist behavior, feeding on birds, canids, cattle, horses, and humans (Forattini et al., 1989; Mucci et al., 2015; Santos et al., 2019), which corroborates our results.

Coquillettidia fasciolata was found to feed on humans, mouse and birds. It is worth noting that some bird species can disperse over long distances, frequenting terrestrial and aquatic systems (Martín-Vélez et al., 2020). Gulls, for example, are aquatic birds that can travel hundreds of kilometers between freshwater, marine, and terrestrial habitats (Viana D. S. et al., 2016). Two bird genera found in this study correspond to two gulls. However, there is no information on the occurrence of these bird genera at REGUA (Barbieri, 2008; Santos et al., 2021; Chupil et al., 2024). Therefore, two possibilities arise: these birds may have migrated to the region, or there was an error in sequencing editing and analysis.

In our research, the sequences corresponding to these two genera showed >95% similarity, indicating a high level of reliability. Based on this principle, one might assume that these animals were indeed present in the study area. However (Townzen et al., 2008), emphasize that verifying the distribution of vertebrates is as important as checking the similarity of sequences, as this set of variables influences the accurate identification of the blood meal taken by the mosquitoes. Therefore, although the sequences' similarity were within the expected range, the presence of gulls around REGUA is questionable due to the type of habitat that comprises the area and the lack of records of these animals in the location.

It is undeniable that with the advent of sequencing and the range of genomes present in current databases, studies on the blood meals of hematophagous arthropods have seen unprecedented advancement (Hopken et al., 2021; Muturi et al., 2021; Balasubramanian et al., 2022). However, these results highlight the importance of improving these tools and continuing research on the subject.

The food source of a single specimen of *An. evansae* was identified as a canid; however, we could not determine its blood-feeding patterns due to the low sample size. *Coquillettidia venezuelensis* was found to fed on two food sources: an amphibian and two humans. According to (De Carvalho et al., 2014) and (Alencar et al., 2015), the feeding habit of *Cq. venezuelensis* involves mammals and birds. However, in the present study, one specimen was found to have fed on an amphibian, which had not been previously recorded.

Light traps with CO_2 baits have been reported to attract females searching for hosts, so they are either not fed or are partially fed with blood (Thiemann and Reisen, 2012). In our study, few engorged mosquitoes were observed, possibly due to the capture method used, as CDC traps were employed. Female mosquitoes that have just taken a blood meal tend to rest (Duvall, 2019). Therefore, resting traps should be employed, such as entomological nets (Brugman et al., 2017; Melgarejo-Colmenares et al., 2022) and battery-powered aspirators (Vazquez-Prokopec et al., 2009) to achieve better results in future studies.

Mosquitoes can spread pathogens to various vertebrate hosts, causing human dis-eases due to their high vector capacity (Segura et al., 2021). In the present study, human blood meals were detected in nine species, notably *Ae. albopictus*, a vector of several important viruses, including dengue, yellow fever, Zika, and chikungunya (Ryan et al., 2019), and *Aedes serratus* (s.l.), *Ae. scapularis*, and *Ps. ferox*, vectors of the yellow fever virus (Cardoso et al., 2010; Moreno et al., 2011). These findings suggest a tendency toward human hosts among the mosquito species captured.

This dominance is likely due to the frequent presence of residents, tourists, and researchers in these forest fragments. Additionally, it is important to consider the possibility that the recorded blood meals were not taken from team members during the capture. Mosquito species are known to travel greater distances than expected, even under unfavorable conditions, to find hosts.

Mosquitoes are known for their behavioral plasticity and adaptability to environmental conditions, mainly due to their

ability to adapt to different food sources, including vertebrate blood and resources available in vegetation. However, in some situations, especially when there is a reduction in the number of vertebrate hosts, mosquito species may resort to alternative blood sources, such as humans (Alencar et al., 2015).

Physical and chemical factors like vision, smell, heat, and humidity, as well as behavior are directly involved in the search and orientation of a vertebrate host (Cardé, 2015; Coutinho-Abreu et al., 2022). *Anopheles gambie* and *Ae. aegypti*, for example, when coming into contact with air laden with CO_2 odors, fly upwind towards the vertebrate trail (Dekker and Cardé, 2011; Hinze et al., 2021). Were observed a tendency for human blood meals in *Cq. fasciolata* and *Ps. ferox*, which, according to the literature, can fly an average of 2500 meters, which is in line with previous studies (Verdonschot and Besse-Lototskaya, 2014).

The successful identification of blood meals using PCR-based methods can present some limitations, such as the low quality and quantity of the host DNA in the mosquito's abdomen (Gómez-Díaz and Figuerola, 2010); preservation, transportation, and storage after collection (Mukabana et al., 2002); and DNA degradation during the conservation period before extraction (King et al., 2008). The effectiveness of food source analyses varies widely between studies (Thiemann et al., 2011; Hernández-Triana et al., 2017; Santos et al., 2019). In our work, 37.93% of the samples successfully amplified host DNA.

We must consider that the Sanger sequencing system used in this research has a methodological limitation, as it can only detect the target with the highest concentration. If more than one source is present, the amplified product may show multiple peaks in the chromatogram, even with highly specific primers are used, hampering their accurate identification (Avanesyan et al., 2021; Nagaki et al., 2021; Trivellone et al., 2022; Alonso et al., 2023).

Another possibility highlighted in a study by (Trivellone et al., 2022) is that short primers (<25 bp) used for Sanger sequencing, can lead to conflicting results and low specificity compared to other methods like next-generation sequencing (NGS) (Martínez-Porchas et al., 2016; Trivellone et al., 2022). In this regard the sequences from the amphibian, birds, canid, and mouse were of low quality, with less than 200 bp; therefore. they were not accepted for inclusion in the NCBI database.

Our study indicates that while molecular biology techniques hold promise, various factors can influence their outcomes. This suggests that, like collection methods, these techniques still require refinement and adjustment to enhance their sensitivity.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The research was conducted in accordance with scientific license number 44333 provided by the Ministry of the Environment (MMA), Chico Mendes Institute for Bio-diversity Conservation (ICMBio), and the Biodiversity Information and Authorization System (SISBIO). Mosquitoes were collected with the consent and cooperation of landowners, residents, and local authorities. All collection team members were vaccinated against yellow fever and were aware of the potential risks in the study areas.

Author contributions

DA: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. SM: Writing – review & editing, Methodology, Writing – original draft, Conceptualization. JS: Methodology, Writing – original draft. NA: Methodology, Writing – original draft. RD: Methodology, Writing – original draft. SS: Writing – original draft, Writing – review & editing. JA: Conceptualization, Writing – review & editing, Supervision, Funding acquisition, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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