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Inventory and faunistic analysis of Calliphoridae and Mesembrinellidae at Três Picos State Park, Brazil

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The Atlantic Forest encompasses forests, sandbanks, mangroves and high-altitude grasslands. This biome has high biodiversity, including more than 15,000 species of endemic vascular plants. This study aimed to inventory and analyze the Calliphoridae and Mesembrinellidae fauna at Três Picos State Park, in Cachoeiras de Macacu, Rio de Janeiro, Brazil. Two traps containing preserved beef liver bait were installed at each site in a gradient from 0 to 1.000 meters from the edge. Samples of 15 species were collected quarterly, between June 2021 and May 2023. The specimens were euthanized with an ethyl alcohol and ethyl acetate solution, then transferred to the laboratory for identification based on morphological characteristics observed under a stereoscope microscope and consultation of descriptions and taxonomic keys. The species were classified according to abundance and frequency, and diversity indices were calculated. A total of 5,476 dipterans of 15 species were collected, of which five were Calliphoridae (77.1%), with *Lucilia eximia* (Wiedemann, 1819) (59.3%) and *Hemilucilia segmentaria* (Fabricius, 1805) (12.9%) being the most abundant species. Ten were Mesembrinellidae (22.9%), with *Mesembrinella bellardiana* (Aldrich, 1922) (16.3%) and *Laneella nigripes* (Guimarães, 1977) (4.5%) being the most abundant species. Six species were common and one was rare in terms of abundance, and five were constant and seven accidentals in frequency. Rényi's diversity profile varied throughout the sampling period, with higher richness and diversity in winter and lower diversity in autumn. We highlight the occurrence of *Mesembrinella currani* (Guimarães, 1977), a species previously known to be restricted to the Amazon Forest.

KEYWORDS

asynanthopes, biodiversity, blowflies, neotropical, rainforest

Introduction

The Atlantic Rainforest comprises different types of vegetation, including forests, sandbanks, mangroves and high-altitude fields. Covering only 7% of its original territory, this biome is considered one of the world's 36 hotspots due to its extraordinary diversity of more than 15,000 endemic vascular plant species (1, 2). In addition to its flora, the Atlantic Rainforest is home to one of the world's greatest concentrations of endemic animal species. This biome extends across 17 Brazilian states but has suffered constant anthropogenic pressure due to resource extraction and intense urbanization (3–5). Biodiversity loss due to human action is one of the primary reasons for establishing environmental protection areas, and has been the subject of extensive debate (6–8). Consequently, the number of environmental protection areas in Atlantic Rainforest fragments increased in recent years (5). However, due to its high biodiversity and intricate biotic interactions, it merits even greater concern (6, 9).

The class Insecta is the most diverse group of animals, containing three times more species than all other groups combined. Insects are ecologically important, as pollinators, carriers of pathogen-causing agents, parasites, economic pests and decomposers (10, 11). Diptera is one of the most diverse insect orders, and is present in almost all environments and niches (12, 13). The Calliphoridae family, commonly known as blowflies (14), is widely distributed around the world, with over 1,000 species and around 150 recognized genera (15). The immature stages of this family can be biontophagous (feeding on healthy tissue from a living host), scavengers (feeding on decomposing organic matter, such as garbage, feces and carcasses), or necrobiontophagous (feeding on necrotic tissue in living hosts). These maggots cause obligate and facultative myiasis, making these dipterans extremely important to animal (16) and human health (17). In forensic entomology, data from their biology has been used to solve criminal cases by identifying suspects through molecular analyses, evidencing the transposition of corpses, detecting ingested chemical substances, and estimating the postmortem interval (PMI) (18–21).

The Mesembrinellidae family, previously considered a subfamily of the Calliphoridae family (22), exhibits similar scavenging habits. This small group of exclusively Neotropical flies is highly related to forest areas, where they are abundant and diverse. They are considered potential biological indicators of preserved forest areas and respond to different types of environmental impacts (6, 19, 20, 23).

However, the forest species of Calliphoridae and Mesembrinellidae are little known. This is largely due to the difficulty of rearing or keeping these insects out of their habitat for extended periods, which makes observing their bionomy difficult (4, 6, 23). Furthermore, the taxonomic classification of these dipterans is still the subject of extensive discussion. Mesembrinellidae has been elevated to family status, ceasing to be a subfamily of Calliphoridae (22, 24). A recent study unified *Albuquerquea* (Mello, 1967), *Eumesebrinella* (Towsend, 1931), *Henriquella* Bonatto in Bonatto and Marinoni, 2005, *Huascaromusca* (Towsend, 1918), *Giovanella* Bonatto in Bonatto and Marinoni, 2005 and *Thompsoniella* (Guimarães, 1977) with *Mesebrinella* (Giglio-Toss, 1893) (25).

Understanding the Calliphoridae and Mesembrinellidae dipteran fauna in this Atlantic Rainforest unit will help us to better understand species diversity in these families. Therefore, this study aimed to inventory the Calliphoridae and Mesembrinellidae fauna in the Três Picos State Park (TPSP), by carrying out a faunal analysis of these insect communities over two years using diversity indices (Rényi, Richness, Shannon diversity, Simpson's Dominance and Pielou's evenness) and constancy and frequency analyses of the collected species.

Materials and methods

Ethics statement

All research was conducted in accordance with Scientific License Number 019/2020 (Extension Number 068/2022), which was provided by the State Environmental Institute (INEA). Yellow fever vaccine was administered to all sampling personnel and the risks in the study areas were acknowledged.

Study site

Samples were collected near the headquarters of the Três Picos State Park (TPSP) in Cachoeiras de Macacu (Figure 1). The TPSP was established in 2002 to preserve remnants of the Atlantic Forest, restore degraded areas of Serra do Mar, and promote environmental education. In 2009, its jurisdiction expanded, and it now covers around 58,790 hectares encompassing the following municipalities: Cachoeiras de Macacu, Nova Friburgo, Teresópolis, Guapimirim and Silva Jardim. The TPSP is currently the largest fully protected area in the state and makes up Rio de Janeiro's central Atlantic Forest ecological corridor. The park's great diversity of habitats and altitude gradient, ranging from 100 to 2,310 meters, are reflected in its diverse fauna and flora. Experts consider it to be one of the priority areas for Atlantic Forest conservation in Brazil (2).

The TPSP serves as both an environmental protection area and an ecological corridor between various conservation units. These include the Serra dos Órgãos National Park and the Environmental Protection Areas of Petrópolis, Macaé de Cima and Rio São João. However, in addition to the areas open to visitors, the park faces anthropogenic pressure from private properties for expropriation, irregular occupation, hunting, extraction of plants and forest fires (2).

Sampling methods

Samplings were carried out quarterly between June 2021 and May 2023, with the aim of capturing in the middle of each season. Five sites were selected along an edge gradient at distances of 0, 200, 400, 700, and 1,000 meters from the park entrance (Figure 1; Table 1) to bypass edge-effect biases. Two traps containing preserved bovine liver were exposed for 48 hours at each site. The traps were placed on trees at least

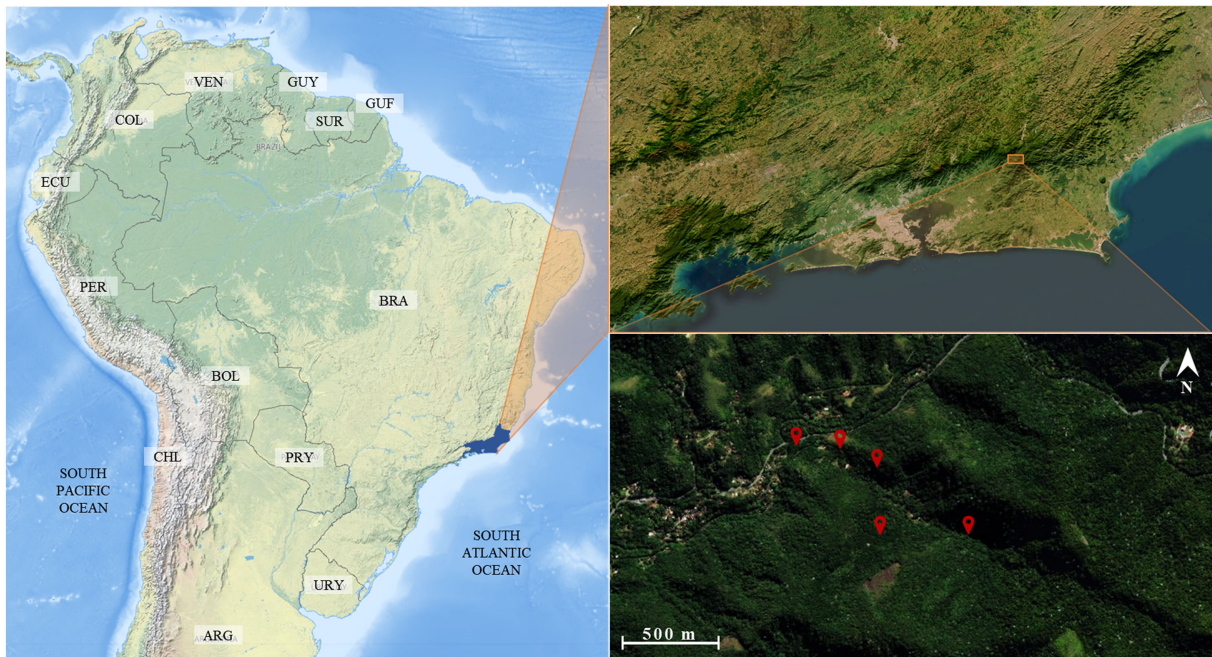


FIGURE 1
Location of the sampling sites on a gradient of distance (0, 200, 400, 700 and 1,000 meters) from the entrance of the Jequitibá Nucleus of Três Picos State Park, municipality of Cachoeiras de Macacu, State of Rio de Janeiro, Brazil.

five meters apart, at a height of 1,5 meters from the soil. Thus, there was a total of ten traps per sampling and 80 traps in total. The traps followed the description by Mello et al. (26), and consisted of a PVC tube base with lateral holes for insects to enter. The odor-attracting bait was placed inside the base, which was covered by a transparent polyethylene container containing a funnel that retained insects through positive phototropism (Figure 2). After sampling, the specimens were transferred to sequentially numbered polyethylene containers and recorded on field cards with a description of the sampling point and the sampling date. The collected individuals were then sacrificed using cotton wool soaked in a solution of ethyl alcohol and ethyl acetate. The containers with the specimens were transported to the Laboratory for Dipteran Studies at the Federal University of the State of Rio de Janeiro (LED-UNIRIO), where the specimens were kept in a freezer at -5°C until they were screened and identified at the species level.

Screening consisted of sorting the insects based on morphological characteristics to separate the Calliphoridae and Mesembrinellidae from other possible species collected in the traps. For taxonomic identification, the insects were dried under incident light on absorbent paper. The insects were pinned, and the species were identified based on direct observation of morphological characteristics visible under a stereoscopic microscope, as well as consultation of respective species descriptions and diagnoses using dichotomous keys developed by Mello (27) and Kosmann et al. (28), with updates by Whitworth and Yussef-Venegas (25). Some of the samples (n = 4 per sampling point, or fewer if there were fewer individuals) were pinned to be sent to the entomological samplings of the LED and the National Museum of the Federal University of Rio de Janeiro (UFRJ). The rest of the material was stored in entomological envelopes with labels containing the sampling and identification information, and kept in the LED collection.

TABLE 1 Gerorreference of the study sites for samplings of Calliphoridae and Mesembrinellidae at distances of 0, 200, 400, 700 and 1,000 meters from the Jequitibá Nucleus entrance of Três Picos State Park, municipality of Cachoeiras de Macacu, State of Rio de Janeiro, between August 2021 and May 2023.

Site	Distance (m)	Latitude	Longitude	Altitude (m)
A	0	-22.413700°	-42.614583°	284 m
B	200	-22.413675°	-42.612788°	334 m
C	400	-22.414380°	-42.610400°	387 m
D	700	-22.417783°	-42.609783°	422 m
E	1000	-22.417800°	-42.604883°	436 m



FIGURE 2

Trap following Mello et al. (26) description, used for Diptera (Calliphoridae and Mesembrinellidae) sampling at Três Picos State Park, municipality of Cachoeiras de Macacu, State of Rio de Janeiro, between June 2021 and May 2023.

Data on temperature, rainfall, and relative humidity from the sampling days were obtained to characterize the sampling periods. These data were obtained from the National Institute of Meteorology's Meteorological Database (BDMEP: <http://www.inmet.gov.br/>) for the Salinas meteorological station, Nova Friburgo (A624). The mean values for each sampling period were computed.

Statistical analysis

Species abundances were tabulated in Excel and imported into RStudio 2023.12.0 + 369 for statistical analyses. A Coleman accumulation curve was produced to assess the sufficiency of the sampling effort. Following the methodology proposed by (29),

species were classified according to their frequency as follows: constant (occurring in more than 50% of the samples), accessory (occurring between 25 and 50% of the samples), or accidental (occurring in less than 25% of the samples). According to their abundance, species were classified as common (52 or more individuals), intermediate (three to 51 individuals) and rare (one or two specimens) following the methodology of (29). To compare the abundance of the most abundant species across the studied seasons, we applied the Kruskal-Wallis test, followed by the Conover *post hoc* test. The Rényi diversity profile was plotted using the “renyi” and “plot” functions from the “vegan” package. To the plots were used to characterize species diversity, highlighting the richness (S), diversity (Shannon-Wiener index, H'), dominance (inverted Simpson index, $1-D$), and evenness (Pielou index, J') indices.

Results

A total of 5,476 dipterans representing 15 species from the Calliphoridae and Mesembrinellidae families were collected at the TPSP during the two-year study period (Table 2). Of those, 77.1% were representatives of five Calliphoridae species, with *Lucilia eximia* (Wiedemann, 1819) (Diptera: Calliphoridae) as the most

abundant (59.3% of the total collected). The Mesembrinellidae family accounted for 22.9% of the dipterofauna, contributing ten species, with *Mesembrinella bellardiana* (Aldrich, 1922) (Diptera: Mesembrinellidae) as the most abundant species (16.3% of the total). The sampling effort was sufficient to characterize the dipteran community, as indicated by the asymptotic trend in Coleman's species accumulation curve. (Figure 3).

TABLE 2 Absolute and relative abundance of species of the families Calliphoridae and Mesembrinellidae captured in different seasons at the Jequitibá Nucleus of Três Picos State Park, municipality of Cachoeiras de Macacu, State of Rio de Janeiro, between August 2021 and May 2023.

Taxa	Autumn		Winter		Spring		Summer		Total	
	n	%	n	%	n	%	n	%	n	%
Calliphoridae									4,217	77.0
Luciliinae										
<i>Lucilia eximia</i> (Wiedemann, 1819)	541	52,2	193	40,5	739	70,9	1,774	60,8	3,247 ^c	59.3
Chrysomyinae										
<i>Hemilucilia segmentaria</i> (Fabricius, 1805)	210	20,3	27	5,7	55	5,3	414	14,2	706 ^c	12.9
<i>Hemilucilia semidiaphana</i> (Rondani, 1850)	16	1,5	3	0,6	10	1,0	171	5,9	200 ^c	3.7
<i>Hemilucilia benoisti</i> (Séguy, 1925)	4	0,4	8	1,7	7	0,7	17	0,6	36 ⁱ	0.7
<i>Paralucilia nigrofacialis</i> (Mello, 1969)	–	–	–	–	–	–	28	1	28 ⁱ	0.5
Mesembrinellidae									1,259	23.0
Mesembrinellinae										
<i>Mesembrinella bellardiana</i> (Aldrich, 1922)	177	17,1	162	34,0	164	15,7	388	13,3	891 ^c	16.3
<i>Mesembrinella peregrina</i> (Aldrich, 1922)	2	0,2	5	1,0	–	–	–	–	7 ⁱ	0.1
<i>Mesembrinella semihyalina</i> (Mello, 1967)	24	2,3	27	5,7	5	0,5	21	0,7	77 ^c	1.4
<i>Mesembrinella currani</i> (Guimarães, 1977)	3	0,3	2	0,4	4	0,4	3	0,1	12 ⁱ	0.2
<i>Mesembrinella quadrilineata</i> (Fabricius, 1805)	–	–	–	–	1	<0.1	3	0,1	4 ⁱ	0.1
<i>Mesembrinella cyaneicynta</i> (Surcouf, 1919)	–	–	3	0,6	4	0,4	–	–	7 ⁱ	0.1
<i>Mesembrinella randa</i> (Walker, 1849)	–	–	–	–	1	<0.1	–	–	1 ⁱ	<0.1
<i>Mesembrinella aeneiventris</i> (Wiedemann, 1830)	–	–	4	0,8	1	<0.1	2	<0,1	7 ⁱ	0.1
<i>Mesembrinella purpurata</i> (Aldrich, 1922)	–	–	2	0,4	–	–	5	0,2	7 ⁱ	0.1
Laneellinae										
<i>Laneella nigripes</i> (Guimarães, 1977)	59	5,7	41	8,6	52	5,0	94	3,2	246 ^c	4.5
Total	1,036	100	477	100	1,043	100	2,920	100	5,476	100

Classification of species according to abundance: Common (n^c); Intermediate (nⁱ); Rare (n^r).

Classification of species according to frequency: Constant (n_c); Accessory (n_a); Accidental (n_m).

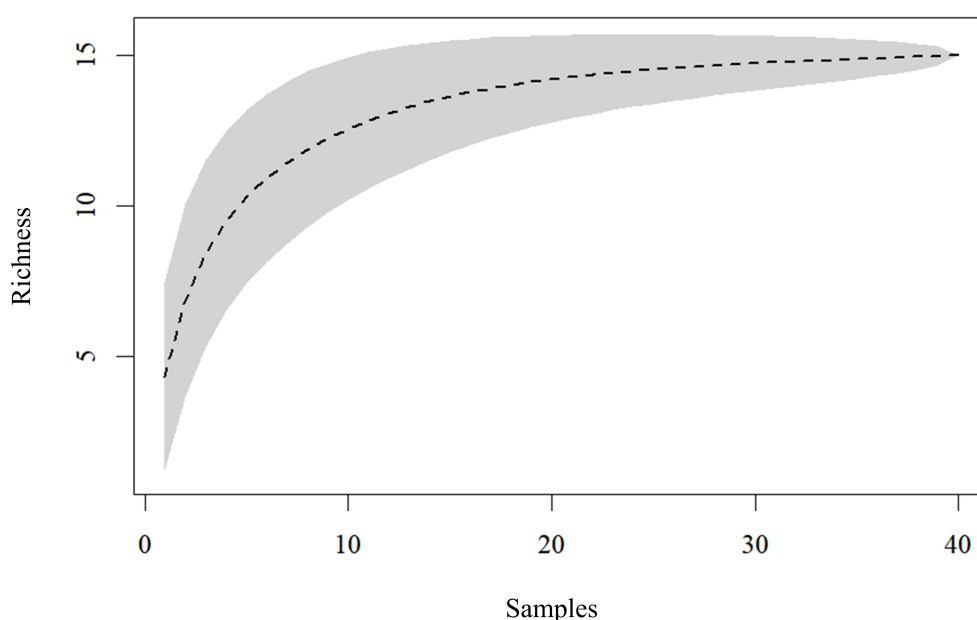


FIGURE 3

Coleman collector's curve illustrating the sampling effort of Calliphoridae and Mesembrinellidae collected at Três Picos State Park, municipality of Cachoeiras de Macacu, State of Rio de Janeiro, between June 2021 and May 2023.

Analyzing the fauna in terms of species abundance (Table 2), the following species were classified as common, with abundances greater than 51 specimens: *Hemilucilia segmentaria* (Fabricius, 1805) (Diptera: Calliphoridae), *Hemilucilia semidiaphana* (Rondani, 1850) (Diptera: Calliphoridae), *L. eximia*, *Laneella nigripes* (Guimarães, 1977) (Diptera: Mesembrinellidae), *M. bellardiana* and *Mesembrinella semihyalina* (Mello, 1967) (Diptera: Mesembrinellidae). Eight species were considered intermediate, with abundances between three and 51 individuals: *Hemilucilia benoisti* (Séguy, 1925), (Diptera: Calliphoridae), *Paralucilia nigrofacialis* (Mello, 1969) (Diptera: Calliphoridae), *Mesembrinella peregrina* (Aldrich, 1922) (Diptera: Mesembrinellidae), *Mesembrinella currani* (Guimarães, 1977) (Diptera: Mesembrinellidae), *Mesembrinella quadrilineata* (Fabricius, 1805) (Diptera: Mesembrinellidae), *Mesembrinella cyaneicynta* (Surcouf, 1919) (Diptera: Mesembrinellidae), *Mesembrinella aeneiventris* (Wiedemann, 1830) (Diptera: Mesembrinellidae) and *Mesembrinella purpurata* (Aldrich, 1922) (Diptera: Calliphoridae). *Mesembrinella randa* (Walker, 1849) (Diptera: Mesembrinellidae) was the only rare species, with only one specimen occurring in the spring.

It is numerically possible to observe the preference of some species for certain seasons. The abiotic variables of temperature, relative humidity, and rainfall are available in Table 3. *Paralucilia nigrofacialis* occurred exclusively in the summer, while other species, such as *Lucilia eximia* and *H. segmentaria*, were more abundant in this season, especially in the summer of 2022. *Hemilucilia semidiaphana* was abundant in both summers (Figure 4). *Mesembrinella bellardiana* showed the highest peak in summer 2023 and a lower peak in autumn 2022. *Laneella nigripes* was most abundant in summer 2022 and did not change during the other

seasons. Kruskal-Wallis tests show significant differences in the abundances of the evaluated species across the seasons: *L. eximia* ($p = 0.0046$), *H. segmentaria* ($p = 0.0262$), *H. semidiaphana* ($p = 0.0109$), *M. bellardiana* ($p = 0.0015$), and *L. nigripes* ($p = 0.0117$). The Conover post-tests highlight the differences across seasons. *Lucilia eximia* exhibited higher abundance in the summer of 2022 than in winter ($p = 0.0002$) and autumn ($p = 0.0130$) of 2021, and winter of 2022 ($p = 0.0136$). Winter of 2021 also showed lower abundance than the spring of that year ($p = 0.0223$). *Hemilucilia segmentaria* also had higher abundance during the summer of 2022 compared to the winter of 2021 ($p = 0.032$) and spring of 2022 ($p = 0.036$). Similar patterns were observed for *H. semidiaphana*, which was more abundant in the summer of 2022 than in the winter of 2021 ($p = 0.019$) and spring of 2022 ($p = 0.019$). *Mesembrinella bellardiana* had lower abundance in the winter of 2021 than in the winter ($p = 0.0283$) and autumn ($p = 0.0092$) of 2022, and summer of 2023 ($p = 0.0001$). The autumn of 2021 exhibited lower abundance than the autumn of 2022 ($p = 0.0175$) and the summer of 2023 ($p = 0.0003$). This species also exhibited lower abundance in the spring of 2021 compared to the summer of 2023 ($p = 0.0167$). Comparing the abundance of *L. nigripes* across seasons reveals that it was lower in the winter of 2021 compared to the winter ($p = 0.0338$), autumn ($p = 0.0159$), and summer ($p = 0.0032$) of 2022.

In terms of frequency, *H. segmentaria*, *L. eximia*, *La. nigripes*, *M. bellardiana*, and *M. semihyalina* were considered constant species, occurring in more than 50% of the samples. *H. semidiaphana*, *H. benoisti*, and *M. currani* were considered accessory species, occurring in 25–50% of samples. *Paralucilia nigrofacialis*, *M. peregrina*, *M. quadrilineata*, *M. cyaneicynta*, *M. randa*, *M. aeneiventris* and *M. purpurata* were considered accidental, occurring in less than 25% of samples.

TABLE 3 Temperature (°C), Relative Humidity (%) and Precipitation (mm) measurements during the sampling periods of Calliphoridae and Mesembrinellidae in the Jequitibá Nucleus of Três Picos State Park, municipality of Cachoeiras de Macacu, State of Rio de Janeiro, between August 2021 and May 2023.

Year	Season	Maximum Temperature (°C)	Mean Temperature (°C)	Minimum Temperature (°C)	Relative Humidity (%)	Mean Rainfall (mm)
2021	Autumn	21.7	15.6	10.3	78.0	0
	Winter	18.8	13.5	8.0	77.0	0.1
	Spring	16.9	13.0	9.6	82.8	7.3
2022	Summer	23.3	18.7	16.2	85.3	36.9
	Autumn	19.0	14.2	10.7	76.2	4.5
	Winter	18.0	12.0	7.9	80.3	5.3
	Spring	16.4	12.5	8.8	81.5	4.1
	Mean	20.2	15.1	11.0	79.7	7.6
2023	Summer	27.6	21.3	16.5	76.5	2.9

National Institute of Meteorology (BDMEP-INMET: <http://www.inmet.gov.br/>), referring to the Salinas meteorological station, Nova Friburgo (A624).

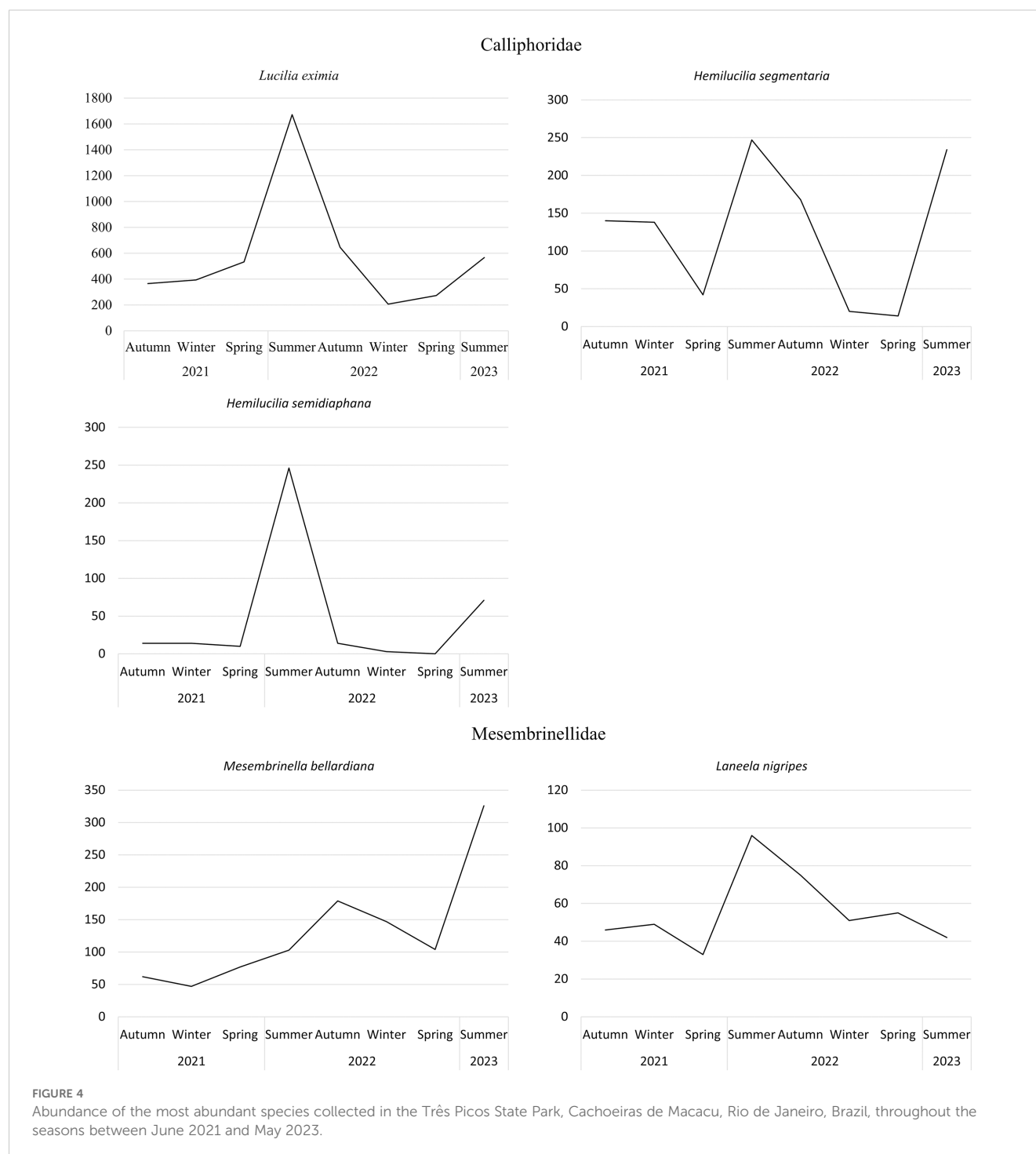
Analysis of Rényi's diversity profile by sampling period (Figure 5, Table 4) revealed the highest richness ($\alpha = 0$) during winter ($S = 15$), while the lowest number of species ($S = 11$) was observed in autumn. Winter also had the highest diversity ($\alpha = 1$, $H = 1.451$) and evenness ($\alpha = \text{inf}$, $J = 0.528$). The lowest diversity was observed during the spring ($H = 1.066$), which also showed the highest dominance ($\alpha = 2$, $1-D = 0.507$) and consequently the lowest evenness ($\alpha = \text{inf}$, $J = 0.416$). Overall, the park exhibited a diversity ($\alpha = 1$) of $H = 1.304$. Dominance ($\alpha = 2$) was $1-D = 0.602$, and evenness ($\alpha = \text{inf}$) was 0.482 .

Discussion

Scavenging dipterans play a fundamental ecological role in nutrient cycling. The immature stages of some saprophagous species can develop on decaying plant and animal matter, as well as on excrement and other organic waste. Maggots can become contaminated by direct contact with microorganisms living in these substrates. Adults, in turn, frequent these environments to obviate and feed and can carry pathogenic agents when they land on food, our body and surfaces we use, potentially transferring those pathogens and causing diseases and infections. Studies by Förster et al. (30, 31) in rural Germany identified various potentially harmful fungi and bacteria in synanthropic flies, including *Enterobacter aerogenes*, *Enterococcus faecium*, *Staphylococcus aureus*, *Mucor* sp., and different strains of *Escherichia coli*. In Thailand, Chaiwong et al. (32) found that the presence of *C. megacephala* flies contaminated with potentially harmful bacteria was correlated with the frequency of diarrhea cases. A review study by Khamesipour et al. (33) also observed the occurrence of viruses in *Musca domestica* Lineaus. *Taenia* spp. eggs have been found on the bodies of Calliphoridae in various studies, as reviewed by Benelli et al. (34). Thus, these insects can carry pathogens and are of great economic and health importance.

In the medical field, these dipterans are also relevant because they cause myiasis, an infestation of living vertebrates that can debilitate or even kill the host (35, 36). Conversely, scavenger species can be used to promote debridement, disinfection, and healing of chronic wounds in a treatment known as biotherapy or larval therapy (37). These insect's scavenging habits also allow them to be used in forensic entomology, the study of insects and other arthropods to help solve criminal or civil cases (38). *Lucilia eximia* has been reported to cause human myiasis (39) and exhibits synanthropic tolerance, as observed with *H. segmentaria* and *H. semidiaphana* (40). Thus, these species are potentially medically relevant as they can carry pathogens and infest humans and animals. Additionally, they have been used several times as estimators of postmortem intervals, helping to elucidate forensic investigations (20, 41, 42).

On the other hand, these organisms act as pollinators (43), and serve as food for spiders, beetles, amphibians, birds, and mites in the trophic chain (44). Finally, these dipterans can be used as bioindicators. Santos Jr. et al. (45) observed that different species of *Chrysomya* Robineau-Desvoidy, 1830 were able to detect and bioaccumulate heavy metals present in the soil during larval development. They can also indicate environmental quality because some species have very specific synanthropic preferences (i.e., a preference to human-altered environments) (40). Mesembrinellidae are exclusively forest-dwelling and Neotropical. *Mesembrinella bellardiana* and *La. nigripes* are often reported as abundant in well-preserved forest areas. Despite the marked presence of Calliphoridae, the presence of *M. bellardiana* in forest remnants is common in the literature (46–48), showing that this species has great plasticity. Of particular note, *M. curranii* known distribution is expanded by this study since previous records were limited to the Northern and Northeastern regions of Brazil and other countries farther North (40). The occurrence of this species suggests that there is efficient habitat connectivity between the Atlantic and the Amazon forests, or that there was in the past. The distribution may also been underestimated due to a lack of studies. Another possibility is that



this species is expanding or rearranging its biogeographical distribution for some reason (e.g., climatic changes, habitat loss). However, further studies are necessary to test these hypotheses.

Calliphoridae species, on the other hand, thrive in warmer environments, finding favorable conditions for reproduction, which may explain their high abundance during the summer. Carmo et al. (49), when studying an Atlantic Forest fragment in Recife, Brazil, found only two species from the Mesembrinellidae family, *M. bellardiana* and *Mesembrinella bicolor* (Fabricius, 1805), in low abundance. Meanwhile, species from the Calliphoridae family occurred in high abundance.

Another noteworthy finding of this study was the replacement of Calliphoridae species. The genus *Hemilucilia* (Brauer, 1895), as well as the species *Lucilia eximia*, are commonly abundant in forest environments but become less abundant as the environment becomes drier and more open. In these environments, species of the genera *Cochliomyia* (Coquerel, 1858), and *Chrysomya* begin to dominate.

When studying a 2,000-meter edge gradient of the Tinguá Biological Reserve, in Rio de Janeiro, using sardines as bait, Ferraz et al. (50) and Gadelha et al. (4) found *H. semidiaphana*, *La. Nigripes*, and *M. bellardiana* in high abundance, as well as *L.*

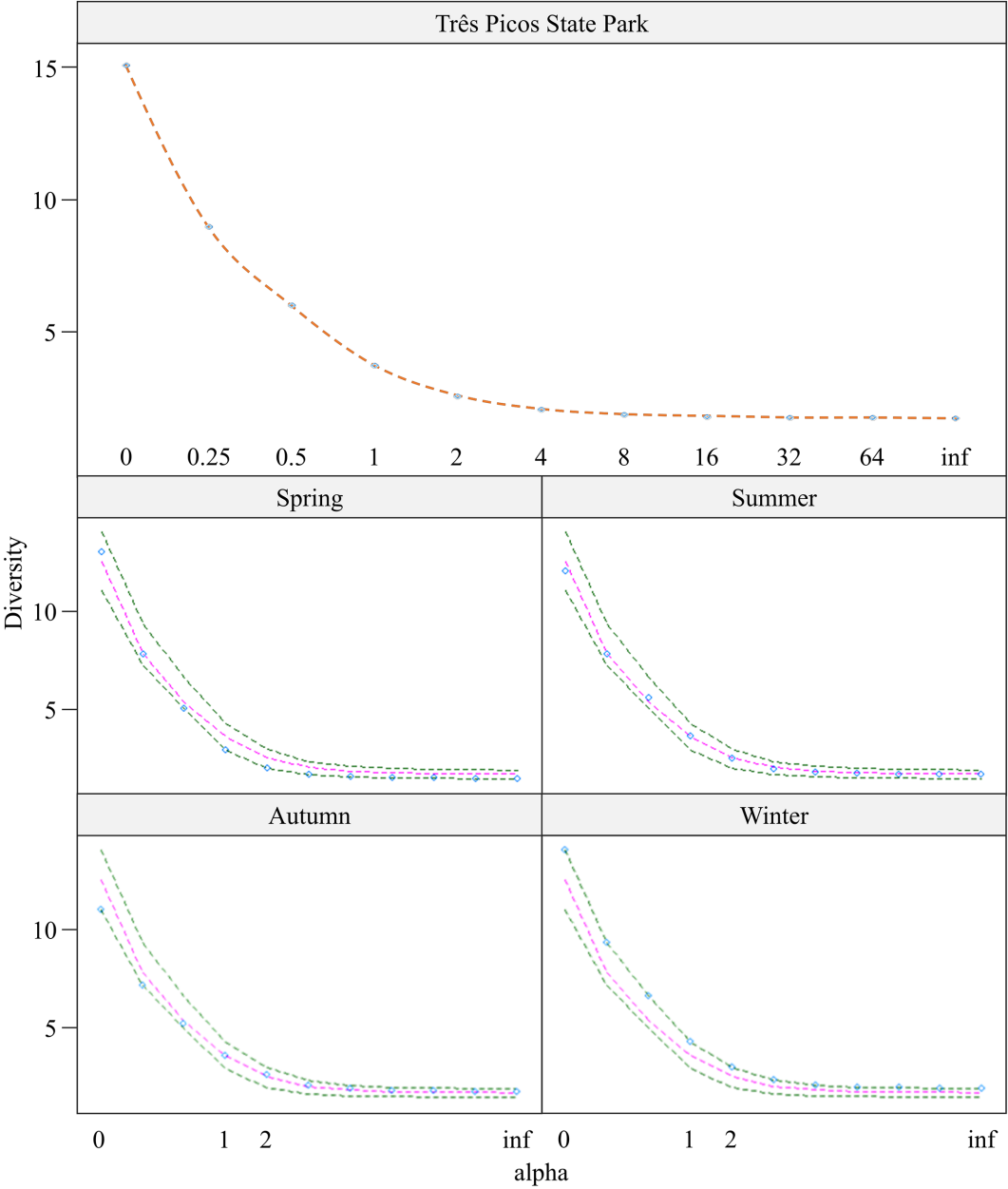


FIGURE 5
Rényi diversity profile, by climatic season, of the Calliphoridae and Mesembrinellidae samplings captured at different points in the Jequitibá Nucleus of the Três Picos State Park, municipality of Cachoeiras de Macacu, State of Rio de Janeiro, between June 2021 and May 2023.

TABLE 4 Rényi diversity profile, by climatic season, of the Calliphoridae and Mesembrinellidae samplings captured at different sites in the Jequitibá Nucleus of Três Picos State Park (TPSP), municipality of Cachoeiras de Macacu, State of Rio de Janeiro, between August 2021 and May 2023.

Season	S (0)	H (1)	1-D (2)	J (Inf)
TPSP	15	1.304	0.602	0.482
Autumn	11	1.267	0.612	0.528
Winter	14	1.451	0.662	0.550
Spring	13	1.066	0.493	0.416
Summer	12	1.287	0.594	0.518

S, Richness; H, Shannon Diversity; 1-D, Inverted Simpson Dominance; J, Pielou Evenness).

eximia, *H. segmentaria*, *M. semihyalina*, *M. aeneiventris*, *M. quadrilineata*, *P. nigrofascialis*, *M. randa* and *M. purpurata* in lower abundance. However, these authors did not record *H. benoisti*, *M. currani* or *M. cyaneicincta*. They also recorded 12 additional species of Calliphoridae and three species of Mesembrinellidae that were not found in the present study, likely due to the difference in bait used.

When studying the fauna of scavenging dipterans using sardines in the Tijuca National Park, Gadelha et al. (23) recorded the occurrence of *L. eximia*, *M. bellardiana*, *H. segmentaria* and *La. nigripes*, as common and constant species, as well as *H. semidiaphana* as the fourth most abundant species. They also recorded *M. aeneiventris* and *M. peregrina* in low abundance, as well as eight additional species of Calliphoridae and two of Mesembrinellidae that were not recorded in the TPSP. However, they did not record two species of Calliphoridae and five species of Mesembrinellidae that were observed in this study.

Carvalho et al. (19) and Azevedo et al. (20), in studies also carried out in the Tijuca National Park using rat carcasses as bait, found three Mesembrinellidae species that were also present in the Três Picos State Park, *L. eximia*, *H. segmentaria* and *H. semidiaphana*. The differences in richness may have been due to the baits used. Bovine liver has been used successfully to attract necrophagous and biontophagous flies (40, 51, 52). Bovine liver is sometimes used as bait to collect fertile females of the screwworm *Cochliomyia hominivorax*, which are used in eradication programs (53). Due to its strong odor, sardine bait proved to be very effective in capturing scavenging dipterans, allowing for a higher richness to be captured. Unlike the liver used in this study, rat carcass bait has a milder odor. Additionally, studies show that using a whole organism can be more attractive, depending on each species' preferences of each species due to the release of volatile organic compounds from different organs (48, 49, 54). Other factors that may explain the difference in dipterofauna observed between these Atlantic Forest environments include the degree of preservation, variations in climate and microhabitats, and the geographical location of the study sites. Altitude can be a barrier for some species, and other geographical barriers may occur between latitudes and longitudes.

Species constancy likely reflects their biological adaptability to environmental conditions (55), since they persist in environments suffering minimal impact from biotic and abiotic variations. Rare species are most susceptible to extinction, which defines patterns of diversity. These species play important roles in biological communities and perform essential ecological functions and processes, such as nutrient cycling, decomposition, trophic interactions. They influence how species respond to human activities. Organizations such as the IUCN use them as a criterion for identifying key biodiversity areas, covering species that are most appealing to interventions (56, 57). A species' frequency seems to reflect its population size and its fluctuations. Constant species have stable populations and are sampled throughout most of the study. Accidental species, on the other hand, may undergo fluctuations due environmental variations, experiencing reduced populations in

certain periods and consequently decreasing their likelihood of being sampled. Accidental species may also occur due to ineffective traps for a particular taxon.

Although the literature indicates that rare species are the majority in terms of richness, our study revealed only *Mesembrinella randa* to be rare and accidental. As expected, a few more common and dominant species accounted for nearly all of the observed abundance, demonstrating their high degree of adaptation to this environment. These species have been recorded by other authors in Atlantic Forest environments, as previously discussed.

Thus, the diversity profiles reflect what has already been discussed. Mesembrinellidae species, which are typically forest-dwelling, are adapted to milder climates and more humid environments, while Calliphoridae species benefit from higher temperatures. For this reason, the highest levels of richness, diversity, and evenness were observed during the winter, when the greater abundance of Mesembrinellidae reduces the dominance of Calliphoridae. During the summer and autumn, when Calliphoridae species find more suitable conditions and multiply significantly, becoming dominant and decreasing diversity and evenness. The seasonal variation was not significant for the Mesembrinellidae species, with only a slight increase in abundance during the summer for *M. bellardiana* and *La. nigripes*. This suggests that these organisms are well adapted to variations in this environment. A more in-depth analysis of the biotic and abiotic parameters influencing this community during the sampling periods will be presented in another article. Other potential causes of this variation, such as resource availability, could not be measured during this study.

Conclusion

The dipterofauna of the Três Picos State Park consists of five Calliphoridae species and 11 Mesembrinellidae species. Seven of these species are common (occurring in more than 51 individuals), is rare (occurring in one individual), five are constant (occurring in more than 50% of samples) and seven are accidental (occurring in less than 25% of samples). The most abundant Calliphoridae species are *Lucilia eximia* and *Hemilucilia segmentaria*. The most abundant Mesembrinellidae species are *Mesembrinella bellardiana* and *Laneella nigripes*. Diversity indices vary according to the seasonal climate period of sampling. The greatest richness, diversity, and evenness are recorded during the winter climate period.

Finally, this study reports a unique record of *Mesembrinella currani* occurring in this biome, updating its current distribution from the Amazon Forest to include the Atlantic Forest. This observation may fill a data gap, since Mesembrinellidae are little studied. However, it may also suggest more serious scenarios, such as habitat loss and climate changes.

Besides providing useful data for identifying species of medical and forensic relevance, knowledge of the diversity of dipterans in different forest remnants, along with knowledge of the biology and ecology of the species, helps us understand the dynamics of these environments and identify the need for protective actions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

WA: Writing – original draft, Writing – review & editing, Formal Analysis, Methodology, Conceptualization. MN: Methodology, Writing – original draft, Writing – review & editing. VMLA: Writing – review & editing, Formal Analysis, Methodology. CL: Conceptualization, Methodology, Writing – review & editing. JA: Conceptualization, Formal Analysis, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. VMA: Conceptualization, Formal Analysis, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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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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