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Preliminary Inventory of *Stomoxys* spp., Potential Vectors of Pathogens in the Minkébé Forest Massif in the North-East of Gabon

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aims to characterize *Stomoxys* spp., potential vectors of pathogens in the north-east of Gabon.
Place and Duration of Study: This research was conducted in the Minkébé forest massif, Woleu-Ntem Province, Gabon from April to May 2023 (rainy season).
Methodology: The daily captures were conducted at Mikouka, Massoko and Minkébé using

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Vavoua and Nzi traps. All captured specimens were morphologically identified to species using binocular microscope (Leica Microsystems ©). The identification was aided by "specific" taxonomic keys. The collected data were organized from Microsoft Office Excel 2013. This database was used to calculate numbers and proportions of specimens observed by species. Several parameters including Apparent Density per Trap per day (ADT), Shannon, Simpson, Pielou and Bray-Curtis indices were evaluated.

Results: A total of 1552 *Stomoxys* were caught, including 610 at Massoko, 533 at Mikouka and 409 at Minkébé. These specimens belonged to two species: *Stomoxys niger niger* and *Stomoxys omega*. *Stomoxys niger niger* was the most abundant with 1176 specimens caught and an ADT of 4 *Stomoxys*/trap/day (s/p/d). On the other hand, *Stomoxys omega* was poorly captured (n= 376; ADT= 1.28). As for the biotopes surveyed, Massoko (n= 619; ADT= 6.42) and Mikouka (n= 533; ADT= 5.44) were the most infested with *Stomoxys* spp. However, the lowest catches were recorded at Minkébé (n= 409; ADT= 4.17). The Shannon, Pielou and Simpson index values indicate relatively low levels of diversity between sites. The Shannon index reached higher values at Minkébé than at the other study sites. The Simpson and Pielou indices also had higher values at Minkébé. As for the Bray-Curtis index, its values were low between Minkébé and Mikouka (0.592), between Mikouka and Massoko (0.302) and between Minkébé and Massoko (0.384).

Conclusion: Our findings show the coexistence of *Stomoxys niger niger* and *Stomoxys omega* in Minkébé. Their presence constitutes a health risk, because these vectors could play a role in the circulation of certain pathogens in the Minkébé forest massif. So, a longitudinal study of these vectors and a screening of their infectious agents are urgently needed.

Keywords: Stomoxys niger niger; Stomoxys omega; insect vectors; minkébé; Gabon.

1. INTRODUCTION

The vector-borne diseases are among the major causes of morbidity and mortality in many developing countries of tropical Africa [1, 2]. According to the World Health Organization (WHO), they account for 17% of infectious diseases worldwide and are responsible for more than one million deaths by year [2]. Among these diseases, trypanosomiasis and filariasis represent one of the main obstacles to the development of livestock farming, food security and human health [3-5]. In the livestock sector, the economic losses due to these diseases have been estimated at around 400 million dollars [6, 7]. These two vector-borne diseases are transmitted by numerous groups of insects, including *Stomoxys* [5].

The *Stomoxys* spp. are hematophagous insects [8] that play a vectorial role in the transmission of several pathogens [4, 5, 9, 10]. These flies occupy an important place in the epidemiological system and therefore constitute a major health problem for domestic and/or wild fauna, as well as for humans [11-16]. According to Raymond [17], *Stomoxys* are involved in the transmission of *Trypanosoma evansi* (Balbiani, 1888) in Africa, America and Asia.

In Gabon, the populations of Stomoxys are relatively well known in certain regions [16, 18-22]. However, in many other localities, they are still poorly known. This is the case of the Minkébé forest massif in North-East of Gabon [23] and where the meteorological and environmental conditions are favourable to the development and maintaining of *Stomoxys* spp., potential vectors of trypanosomes and filariae. To fill this gap, an entomological study was carried out at three sites in the Minkébé region in order to characterize the populations of *Stomoxys* present in the Minkébé forest massif.

2. MATERIAL AND METHODS

2.1 Study Area

This study was conducted in the Minkébé forest massif located in the Woleu-Ntem province in North-East of Gabon (Fig. 1). This region has an equatorial climate with two rainy seasons and two dry seasons [23]. The rainy seasons extend from October to December (short rainy season) and from March to May (long rainy season). On the other hand, the dry seasons extend from January to February (short dry season) and from June to August (long dry season). Average annual rainfall varies between 1,600 and 1,800 mm [23]. In general, average temperatures are between 23°C and 24°C, while humidity levels usually exceed 80%.

The vegetation of this study area is composed of Guinean-Congolian dense forests [23]. These

forests cover a large part of the region and form an almost continuous vegetation carpet marked by a mosaic of evergreen and semi-deciduous plant formations [24]. Waterbodies are often bordered by vast areas of floodplain and swamp forests [23]. Regarding savannahs, they are found only at Mvadi and in the south-west of the Minkébé National Park [24].

Moreover, the Minkébé forest massif has undergone several human disturbances, especially in the Minkébé area, due to the increase of the gold-washing between the 1950s and the 2000s [24]. This activity has had important repercussions on this forest massif through the establishment of gold-washing camps and the significant decline of wildlife in areas of human activities [24].

2.2 Capture of *Stomoxys* at the Study Surveyed

The specimens of *Stomoxys* were collected using 7 Vavoua and 7 Nzi traps [25, 26]. These traps have been used many times in the studies of hematophagous dipterans carried out in Gabon [19, 20, 22, 27]. They were installed at three different forest sites, namely Mikouka, Massoko and Minkébé, according to the following sequence: Mikouka-Massoko-Minkébé.

These traps were placed both in the forest environments and along the waterbodies, in order to maximize the quality and quantity of the collections. In this fact, each site surveyed was equipped with seven Vavoua traps and seven Nzi traps.

These traps were labelled with a corresponding number and were installed at given collection points. They were equidistant at least 500 m in order to better distribute the traps across the study sites. They were set up at 07:00 a.m. on the first day of trapping and removed at 06:00 p.m. on the last day. During the present study, the traps were continually in use and were checked at 6:00 p.m. daily. When these traps were visited, the captured insects were brought back to the camp and packaged for later identification in the laboratory in Libreville. All captures of *Stomoxys* were carried out between 28th April and 18th May 2023 (rainy season), with 7 days of capture for each study site.

2.3 Morphological identification of *Stomoxys*

All the captured insects were identified morphologically using binocular microscope at the Laboratoire d'Ecologie Vectorielle of Institut de Recherche en Ecologie Tropicale (LEV-IRET). To this end, an initial separation was made between *Stomoxys* and other insects. The specimens of *Stomoxys* were then carefully identified at the species level using the keys developed by Zumpt [12] and Garros et al. [28] in order to distinguish more easily *S. calcitrans* (Linnaeus, 1758) and *S. niger niger* (Macquart, 1851).

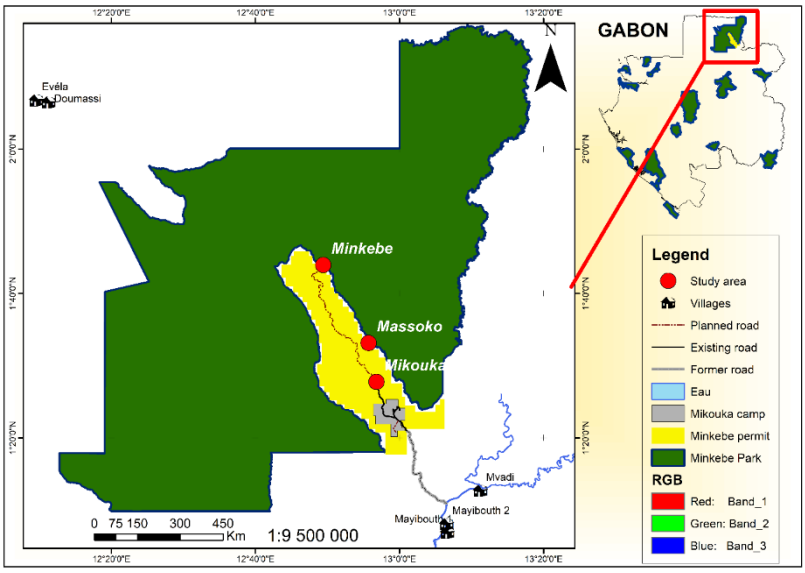

Fig. 1. Map of study area showing the three sites surveyed in Woleu-Ntem province

2.4 Data Analysis

The collected data were meticulously entered and organized from Microsoft Office Excel 2013. This spread sheet was used to calculate numbers and proportions of specimens observed by species. The data were then presented to make it easier to visualize the different results. Moreover, several parameters including Apparent Density per Trap per day, Shannon's index, Simpson's index, Pielou's index and Bray-Curtis's index, were also estimated in order to better understand the biodiversity of *Stomoxys* colonizing the study sites.

The Apparent Density per Trap per day (ADT) corresponds to the relative abundance of *Stomoxys* spp. in each surveyed site. It was calculated using the following formula:

$$ADT = \frac{Number\ of\ captured\ Stomoxys}{Number\ of\ traps\ x\ number\ of\ trapping\ days}$$

The Shannon's Diversity Index (H') was also calculated in order to assess the specific diversity of *Stomoxys* at the surveyed sites. This index is expressed according to the following formula: H'= -Σ (Ni/N) x log (Ni/N); where Ni: represents the number of individuals of a given species and N: the total number of individuals.

Regarding the Simpson's Dominance index (D), it was estimated in order to determine the dominance of particular *Stomoxys* spp. in the community according to the following formula: D = ∑ Ni (Ni-1)/N(N-1); where D: represents Simpson's index, Ni: the number of individuals of the species and N: the total number of individuals.

In addition, Pielou's index (J) and Bray-Curtis's index were evaluated to provide further information on the study area. Indeed, the Pielou index enables to measure the equitability of the distribution of individuals between the different species of *Stomoxys* present at each study site. Nevertheless, the Bray-Curtis's index is used to determine the similarity or dissimilarity between the species of *Stomoxys* captured in the different study sites.

Spatial analyses were performed using Quantum GIS version 3.10.7 (https://www.qgis.org/, accessed on 10 September 2023).

3. RESULTS AND DISCUSSION

3.1 Species Composition of *Stomoxys*

A total of 1552 *Stomoxys* were sampled in the study area with an apparent density per trap per day (ADT) of 5.28 *Stomoxys*/trap/day (s/t/d). Of these *Stomoxys*, 619 (39.30%) specimens were captured at Massoko with an ADT of 6.42 s/t/d and 533 (34.30%) at Mikouka with an ADT of 5.44 s/t/d. The lowest number of individuals was caught at Minkébé with 409 (26.40%) *Stomoxys* with an ADT of 4.17 s/t/d (Fig. 2).

In total, 2 species of *Stomoxys*, including *Stomoxys niger niger* and *Stomoxys omega* (Figs. 3 and 4), were identified during this study, with variable ADT. Indeed, *Stomoxys niger niger* was the most abundant species with 1176 individuals caught and an ADT of 4 s/t/d. Nevertheless, *Stomoxys omega* was weakly collected (n= 376; ADT= 1.28).

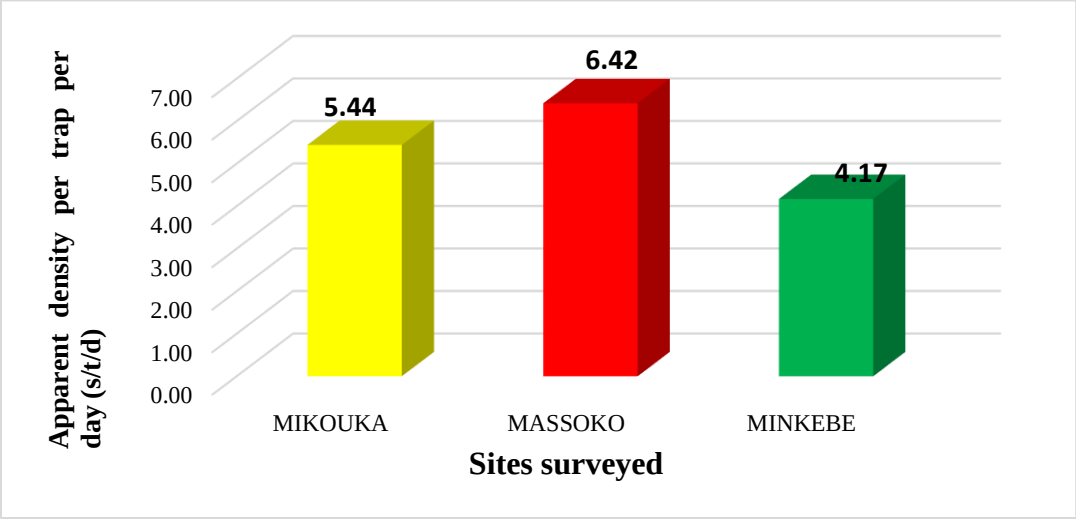

Fig. 2. Apparent density of *Stomoxys* per trap according to the sites surveyed

Fig. 3. *Stomoxys niger niger* captured

Fig. 4. *Stomoxys omega* collected

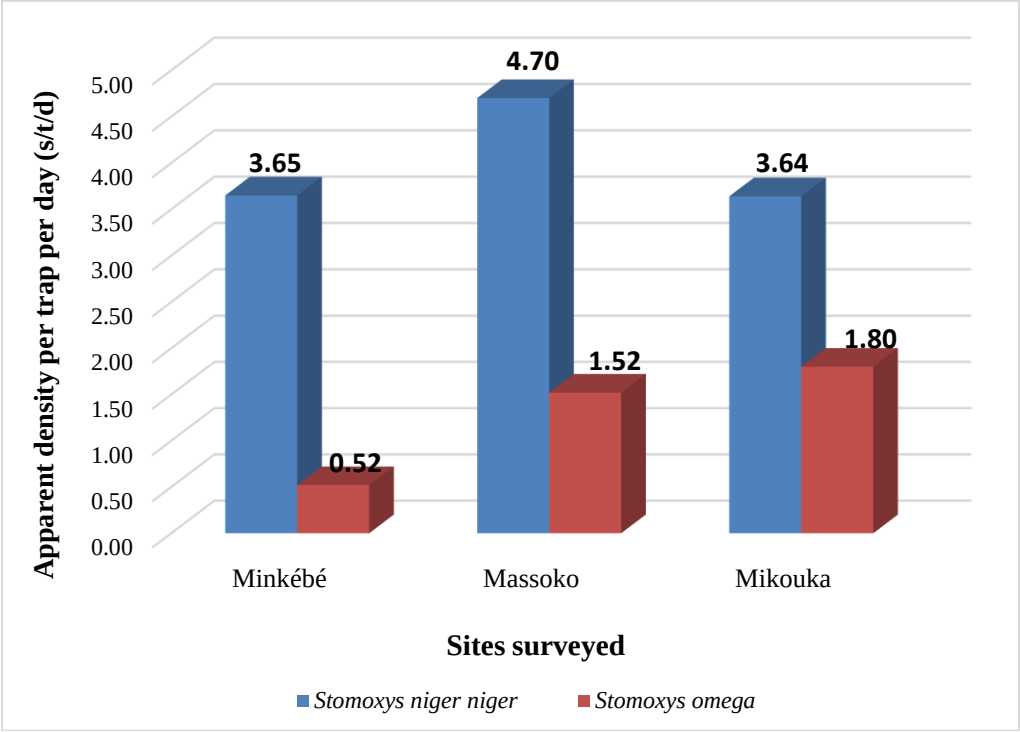

Fig. 5. Distribution of species of *Stomoxys* according to the sites surveyed

Table 1. Diversity and distribution of *Stomoxys* spp. according to the sites surveyed

Stomoxys	Variables	Massoko	Mikouka	Minkébé
S. niger niger	Percentage (%)	461 (39.20)	357 (30.36)	358 (30.44)
	ADT (s/t/j)	4.7	3.64	3.65
S. omega	Percentage (%)	149 (39.63)	176 (46.81)	51 (13.56)
	ADT (s/t/j)	1.52	1.80	0.52
	Total	533	610	409

Table 2. Indices of diversity of *Stomoxys* in the sites surveyed

Index	Massoko	Mikouka	Minkébé	Mean
Shannon (H)	0.737	0.736	0.829	0.767
Pielou (J)	0.555	0.554	0.625	0.578
Simpson (D)	0.678	0.646	0.759	0.694

Moreover, the ADTs of these two species vary according to the sites surveyed (Fig. 5). Indeed, *Stomoxys niger niger* was caught in abundance (461 individuals) at Massoko with an ADT of 4.70 s/t/d. In the other two environments, this species presented almost similar abundances with ADTs of 3.65 s/t/d and 3.64 s/t/d respectively.

Regarding *Stomoxys omega*, it was abundant at Mikouka and Massoko, with ADT of 1.80 s/t/d and 1.52 s/t/d respectively. On the other hand, at Minkébé, *Stomoxys omega* was collected very little, with 51 individuals and an ADT of 0.52 s/t/d (Table 1).

The Shannon's index, Simpson's index and Pielou's index showed higher values at Minkébé (H= 0.829; D= 0.759; J= 0.625) than at the other study sites (Table 2). However, the Bray-Curtis's index recorded the highest values between Minkébé and Mikouka (0.592), while between Mikouka and Massoko (0.302), then Minkébé and Massoko (0.384), the values of this index were low.

3.2 Discussion

This study revealed the concomitant presence of *Stomoxys niger niger* and *Stomoxys omega* with variable abundances. The coexistence of these two Stomoxys could represent a danger for animals and humans, because these insects are known to be mechanical and/or biological vectors of bacteria, viruses, helminths or trypanosomes [5, 29, 30]. Globally, the collections of *Stomoxys* were not abundant in the study area. This low abundance of catches could be explained by the reduced number of trapping days in this study compared to the trapping duration in previous studies [27]. However, the Nzi and Vavoua traps have confirmed their effectiveness in the capture of *Stomoxys* in Gabon as reported by other authors [8, 19, 20, 27, 31]. However, the combination of these attractants would certainly have made it possible to increase the size of collections and improve species diversity [32].

In addition, the low number of collections of *Stomoxys* could be attributed to the frequency of heavy rains and thunderstorms during the study period. These unfavourable weather conditions probably reduced the attractiveness of the traps used, causing thus fewer captures of these insects. From Raymond [33], the heavy rains have a negative impact on the development and the survival of hematophagous flies, as well as on the effectiveness of the traps. Besides, previous studies conducted by Mavoungou et al. [34] and Doumba Ndalembouly et al. [31] also reported a decrease in the abundance of hematophagous insects in response to rainfall in Makokou and Ndendé in Gabon.

Among species of Stomoxys identified, *Stomoxys niger niger* was more abundant than *Stomoxys omega*. This observation could be linked, on the one hand, to the ubiquity of *Stomoxys omega* and on the other hand, to the dependence of *Stomoxys niger niger* on forest environments [12, 27]. Various authors have also observed this predominance of *Stomoxys niger niger* compared with *Stomoxys omega* in Gabon and Cameroon [16, 35, 36]. Moreover, these two species of *Stomoxys* showed a heterogeneous distribution, as attested by the values of the ecological indices. This observation could be attributed to ecological and environmental factors, as well as to the specific characteristics of the sites surveyed. Indeed, in Mikouka and Massoko sites, characterized by a relatively intact forest ecosystem, we noted a higher abundance of *Stomoxys omega*. On the other hand, in the Minkébé site, which is heavily impacted by gold-washing and therefore submitted to environmental disturbances, this species was rather low in abundance. These results are similar to those reported by Mavoungou [27] and Desquesnes et al. [37, 38], who showed that there was a correlation between the abundance of some species of hematophagous insects, the presence of forest and human disturbances on their abundance. Furthermore, according to Mavoungou [27], at Makokou (north-east of Gabon), variation of the abundance of *Stomoxys omega* was strongly correlated with the presence of primary forest, whereas *Stomoxys niger niger* was closely linked to the presence of secondary forest and disturbed environments [39]. Consequently, it would be important that future studies incorporate an in-depth analysis of environmental factors to obtain a more complete understanding of the abundance and distribution of species of *Stomoxys* in the Minkébé forest massif.

In the global context of vector-borne infectious diseases, the identification of environmental factors determining the distribution of species of *Stomoxys* has a great importance. The analysis of the ecological indices revealed low levels of diversity between the sites surveyed. Although the minimum number of individuals was obtained at Minkébé, this environment seems to have the greatest diversity, which is reflected in the higher

values of these indices at this site. The Bray-Curtis's index revealed similarities between the communities of *Stomoxys* in the sites surveyed. This observation is coherent because the three study sites are forest environments with variable levels of anthropization.

So, for the future studies, we should detect or isolate pathogenic microorganisms in the species of *Stomoxys* studied in Minkébé region.

4. CONCLUSION

This study provides the first data on the populations of *Stomoxys* present in the Minkébé forest massif. We identified two species of *Stomoxys* including *Stomoxys niger niger* and *Stomoxys omega*, having a heterogeneous distribution. These hematophagous insects are already known to be biological and/or mechanical vectors of trypanosomes in humans and animals.

Therefore, a particular attention must be paid to monitoring these insects in order to better understand biology and ecology of these arthropods. This is essential to develop the strategies of vector control which are specific to this region. The results of this study constitute a useful information base for implementing of vector control measures in order to mitigate the associated risks and to prevent the spread of the diseases they transmit to their hosts. They also contribute to a better understanding of the abundance and distribution of *Stomoxys* in the Minkébé forest massif in Gabon, where hematophagous flies have never been recorded.

In order to better control these vectors, bioecological and microbiological studies are needed. This will make it possible to assess the epidemiological importance of these insects in the Minkébé forest massif and to identify the environmental and anthropogenic factors determining the dynamics of their population. It would also be interesting to examine the genetic diversity of *Stomoxys* spp. and their pathogens, in order to have a better understanding of the mechanisms involved in the vector transmission.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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