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Transgenerational and Sexual Auto-Dissemination within *Anopheles* Mosquitoes of the Malaria Parasite Transmission Blocking *Microsporidia Sp* MB in Burkina Faso

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Abstract

Introduction: Malaria control needs the development of complementary and/or alternative strategies such as biological controls. Despite, malaria's current control efforts, the spread and the emergence of insecticide resistance in vectors undermine the fight against vectors. Endonsymbiotic fungi can be a good candidate to include in the existence of Arsenal. However, we know little about endosymbiotic fungi transmission and its impact on malaria transmission. In this paper, the authors aimed to investigate *Microsporidia sp* MB transgenerational and sexual autodissemination and malaria parasites within Anopheline mosquitoes. **Methods:** An entomology survey was conducted in Burkina Faso for one year (June 2020 to June 2021) using the Residual Fauna Capture method. Infection rates in collected females, sexual autodissemination of *Microsporidia sp MB* in both *Microsporidia sp MB* negative females and *Microsporidia sp MB* negative males through mating, transgenerational prevalence from parents to offsprings and the correlation between *Microsporidia sp MB and Plasmodium falciparum* were investigated. **Results:** Results *show Microsporidia sp MB* infection in *An. gambaie* s.l in Burkina Faso. The prevalence was significantly higher (21.78%) in *An. coluzzii* than *An. gambiae s.s* (16.89%) (*p-value* = 0.03). Sexual auto-dissemination of *Microsporidia sp MB* in *Microsporidia sp MB* uninfected females was significantly 3-fold lower than

those in *Microsporidia sp MB* uninfected males (9.23% and 33.33%, *p-value* = 0.03) during mating for *An. coluzzii* lines. *Microsporidia sp MB* prevalence was significantly higher through mosquitos' generations in *An. gambaie* s.s than *An. coluzzii* (30.23% vs 26.41%, *p-value* < 0.001). A significant negative correlation was observed between *Microsporidia sp MB* and *Plasmodium* infection rate with 73% of *Microsporidia sp* MB positive mosquitoes which were negative at *Plasmodium* infection (p-value < 0.01). **Conclusions:** Our findings pave the road to developing new malaria control technologies by making *Microsporidia sp MB*-positive males sexually competitive with wild males to spread the fungus to wild female mosquitoes.

Keywords

Transgenerational, Sexual Autodissemination, Correlation, Prevalence, *Microsporidia Sp MB*, *Anopheles*, *Plasmodium Falciparum*, Malaria, Burkina Faso

1. Introduction

Malaria is one of the important vector-borne diseases in the world that decimates more than half of 1 million lives each year [1]. In 2022, 249 million cases and 608000 deaths were reported globally in 85 endemic countries with 94% of deaths attributable to WHO Africa region [2]. In Burkina Faso, malaria is a health concern because it is endemic throughout the country with a seasonal upsurge from June through October. Overall, Burkina Faso is among the ten countries with the highest number of malaria cases and deaths (3.3% of global malaria cases and 3.4% of global malaria deaths in 2021). Malaria is responsible for 43% of health provider consultations and 22% of deaths. The country accounted for an estimated 7% of total malaria cases in West Africa in 2020 [3]. Funding for malaria control increased from US$ 1.3 billion in 2017 to US$ 2.6 billion in 2019 [4], but globally malaria control progress has stalled in recent years, leading to the urgent need to develop and implement novel control strategies [2]. Malaria vectors are Anopheline mosquitoes which are the dominant species for transmission of *Plasmodium*. In sub-Saharan Africa, transmission is dominated by three widespread vectors: *Anopheles gambiae* s.s, *An. arabiensis*, and *An. funestus* [5]. Previous vector control methods showed that Long-Lasting Insecticide Nets (LLINs) and Indoor Residual Spraying (IRS), reduced malaria incidence to 68% and 10% respectively [6]. In Nigeria, studies showed that there is a positive linear correlation between LLIN use and a decline in malaria prevalence in pregnant women and caregivers who had at least a child under the age of five years from the 2015 malaria indicator surveys in six geographical regions [7]. Current malaria management methods are essentially based on preventive methods such as the administration of doses of the RTS, AS01 and R21/Matrix-M (R21) vaccines recommended respectively in 2021 and 2023 by the WHO. However, intermittent preventive treatments based on sulfadoxine-pyrimethamine by (IPTp) and

sulfadoxine-pyrimethamine-amodiaquine (IPTi) respectively also remain another preventive tool for vulnerable individuals such as pregnant women and children under 5 years of age [2] [8]. In addition to these preventive methods against the malaria parasite, case management based essentially on early diagnosis using rapid diagnostic tests (RDTs) and microscopy, as well as comprehensive treatment of the disease, is a key indicator that has a direct impact on preventing mortality and reducing morbidity [9]. There are also preventive methods of vector control, such as the use of long-acting insecticide-impregnated mosquito nets and indoor residual spraying. Recently, for better management of insecticide resistance in vectors, the WHO has recommended combining molecules such as pyrethroids with a synergist, piperonyl butoxide (PBO), or with a growth regulator, Chlorfenapyr, in areas of pyrethroid resistance [8]. In areas where pyrethroids are only used to control malaria, a combination of pyrethroids with pyriproxyfen has been recommended. Despite, the massive distribution of LLINs and mass IRS campaigns, the spread and emergence of insecticide resistance in vectors understand the fight for vector control [10]-[13]. In addition, agricultural use of insecticides is involved in the selection of resistance to these compounds in field populations of mosquitoes in Burkina Faso. *Anopheles gambiae* s.l. was resistant to permethrin and DDT in cotton growing and urban areas [14]**.** Novel strategies such as those that aimed at focusing transgenerational, sexual autodissemination of an endosymbiotic microsporidian, *Microsporidia sp* MB and malaria parasite within Anopheline mosquitoes could overcome insecticide resistance. Here, we screened *Microsporidia sp* MB in wild *Anopheles gambiae* complex which are resistant to pyrethroids, the common insecticide used in impregnated nets and are potential for transmission of *Plasmodium* to humans. Belong biological vector controls, there are some that now focus on the use of genetically modified microorganisms to block the development of the malaria parasite in the *Anopheles* vector [15] or to target the vector itself [16]. The use of entomopathogenic microorganisms against malaria vectors has been demonstrated in Burkina Faso by the use of two local strains of *Metarhizium pingshaense* (Met_S26 and Met_S10), that specifically target mosquitoes, which rapidly kill 80% insecticide-resistant mosquitoes, *Anopheles coluzzii* with low-dose of conidia on 7-day post-infection [17]. Several works found links between *Anopheles* and entomopathogenic microsporidians. Most of them revealed that entomopathogenic microsporidians like *Vavraia culicis* and *A. algerae* reduced the intensity of *P. yoellii* and *P. berghei* oocysts by 70% and 23%, respectively in *An. stephensi* and *An. gambiae* [18] [19]**.**

Endosymbiont microorganisms offer advantages in blocking malaria transmission. Many biological control strategies use endosymbiont microorganisms to block malaria. This is the case of *Wolbachia*, which has shown its effectiveness by significantly reducing the number of sporozoites in mosquitoes [20]. Current knowledge of this reduction involves the influence of this endosymbiont bacterium on the mosquito's immune system, nutritional competition, the mosquito's lifespan and its interaction with the mosquito's microbiome [21]-[24]. As a result, recent work has

shown that *Wolbachia* induces immunity in *An. stephensi* mosquitoes to plasmodial infection through the anti-*Plasmodium* genes, TEP1, LRIM1, the Toll Rel1 pathway gene, and the effector Defensin 1 [25]. The use of *Wolbachia-infected* female mosquitoes as a method of replacing the wild mosquito population in the intervention area has an advantage which is the reproduction of new mosquitoes [26] [27]. However, this technique does not respect ethical considerations, given that the females could transmit the parasite because of their "biting-sucking" proboscis. The release of *Wolbachia-infected* males could be a solution to the problem of using *Wolbachia-infected* females. However, there have been no reports of Wolbachia being used to block malaria transmission; in particular, large-scale trials have shown progress in blocking dengue fever [26] [28]. This transmission blocking strategy (TBS) using *Wolbachia* requires an understanding of the establishment of the symbiotic relationship between *Wolbachia* and the host mosquito in order to control its stability and infection, which can be sustained over the long term through vertical and horizontal transmission [29]-[31]. In addition, several endosymbiont microorganisms of the intestinal flora (*Serratia marescens* Y1, *Serratia marescens*, *Asaia*, *Enterobacter*) of mosquitoes have shown their anti-plasmodial activity by inducing immunity in the vector, making it less susceptible to plasmodial infection, which varied according to the vector species and the plasmodial species [32]-[34]. Recent studies identified an endosymbiont microsporidian, *Microsporidia sp MB* in the malaria vectors including *An. arabiensis*, *An. gambiae s.s*, *An funestus* and *An. coluzzii* in several countries in sub-Saharan Africa [35]-[40]. *Microsporidia sp MB* was found in higher infection intensity in male mosquitoes' reproductive organs than those of female mosquitoes and in midgut [35] [37]. This endosymbiont can impair *Plasmodium* transmission in *An. arabiensis* [35] and can be transmitted vertically and sexually through mating. Here, we carried out entomological survey in order to screen *Microsporidia sp MB* in *An. gambiae* complex collected in the field. Then, we established *Microsporidia sp* MB *An. coluzzii* lines. Furthermore, we investigated transgenerational transmission between F0 to F3 and evaluated sexual autodissemination of *Microsporidia sp* MB with infected males crossed by females uninfected and between males uninfected and females infected. Finally, we estimated the correlation in both *Micropsoridia sp* MB and *P. falciparum* in *An. coluzzii* collected in the field.

2. Methodology

Study site and Mosquito collection

Fed female *Anopheles gambiae* complex mosquitoes were collected inside dwelling houses and enclosures using the Residual Fauna Capture (RFC) method in Vallée du Kou and Soumousso, two rural areas near Bob-Dioulasso. In Burkina Faso, from 2013 to 2016, two malaria transmission periods were observed: low from January to July and high from August to December [41]. These two rural areas are located in the south-west of Burkina Faso in the province of Houet, an area where malaria transmission is permanent [42]. This area is characterized by two seasons:

a dry season from November to April and a rainy season from May to October. The average annual rainfall in this area is between 800 and 1100 mm, with an uneven distribution and average annual temperatures of 25˚C and 30˚C. Savannah vegetation accounts for around 31.19% of the total surface area of the Houet region. Studies have shown a positive correlation between vegetation density and malaria prevalence, while temperature, soil clay content, annual rainfall and distance to the nearest water source were negatively associated with the prevalence of the disease [43]. "Mosquitos collection" was carried out using a mouth aspirator. This site is a rice-growing area of 1200 hectares located 30 Km northwest of Bobo-Dioulasso the second largest town of Burkina Faso with a predominant vector of *An. coluzzii* has a high density but low malaria transmission. Moreover, Soumousso is a savannah area in which S molecular form is predominant with high malaria transmission despite a low density of malaria vectors, following with *An. arabiensis*, *An. funestus* and *An. nili*. Fed females caught using the Residual Fauna capture method inside houses or enclosures between 6 to 9:00 a.m during four months (June to September 2020) and were maintained in cages and brought back to Centre MURAZ insectary. There, mosquitoes were morphologically identified under binocular glass using morphological key determination [44] [45]. Only fed and gravid female' mosquitoes belonging to *An. gambiae* complex were taken into account in our study. Mosquitoes were placed in cages, maintained in insectary conditions, and fed daily with 5% glucose solution.

Mosquito rearing

Larvae and adult mosquitoes were reared under standard conditions, at 27˚C ± 2˚C and 75% ± 5% relative humidity and photoperiodicity 12h in light and 12h in dark. Larvae were fed with Tretamin baby fish® and adult mosquitoes with glucose solution 5%. F1 from adult fed females collected to vallée du Kou and Soumousso were obtained after putting them in individual oviposition which was monitored daily during 7 days. After laying eggs, each female mosquito belonging to *An. gambiae* complex was removed and stored in an Eppendorf tube with Ethanol solution 70% at 4°C. In addition, PCR technique was performed to molecularly detect *Microsporidia sp MB* and *P. falciparum* infections.

Establishment of *Microsporidia sp MB* positive *An. coluzzii and An. gambiae* s.s lines

We established *An. coluzzii* and *An. gambaie* lines with or without *Microsporidia sp MB* are in our insectary. *Microsporidia sp MB* prevalence was screened between generations from F0, F1, F2 and F3. Conventional PCR was performed to determine *Microsporidia sp MB* presence in each generation according to Herren's protocol (Herren *et al*., 2020). Adults Mosquitoes were provided with a glucose solution of 5% and larvae were fed with Tetramin baby fish®. Mosquitoes were maintained at insectary conditions, 27˚C ± 2˚C and 70% ± 5% relative humidity and under at 12h light: 12h dark photoperiod. About 3,350 adult female mosquitoes from each generation F0, F1, F2 and F3 and both gambiae strains, respectively were analysed using *Microsporidia sp MB*.

Sexual auto-dissemination assays of *Microsporidia MB with An. coluzzii lines*

F1 progeny from *An. coluzzii* lines established in our laboratory with or without *Microsporidia sp MB*. First-generation adult mosquitoes that had emerged from pupae were immediately sexed to avoid any mating. Virgin males and females of *An. coluzzii* lines with or without *Microsporidia sp MB* were maintained in different 30 × 30 × 30 cm cages. Moreover, only 2 - 5-day-old infected male mosquitoes to *Microsporidia sp MB* (IM) were crossed with 2 - 5-day-old uninfected females (UF). In addition, infected female mosquitoes (IF) were allowed to mate with uninfected males (UM). These two bioassays were performed using three sex-ratio *i.e.*, 1:2,1:3 and 1:5 during 2 days. After 2 days of mating, uninfected females and males were removed and stored in Ethanol solution 70% (Table 1).

Table 1. Mating between males and females based on *Microsporidia sp MB* infection.

	Infected Females (IF)	Uninfected Females (UF)
Infected Males (IM)	IMxUF	
Uninfected Males (UM)	UMxIF	

IM Infected Males, IF Infected Females, UM uninfected Males, UF Uninfected Females.

DNA extraction from mosquitoes

Individually oviposited and bi-sex (males and females) uninfected mated mosquitoes were preserved in ethanol at −20˚C in 1.5 mL Eppendorf tubes. In addition, DNA of F0, F1, F2 and F3 mosquitoes from *An. coluzzii* and *An. gambiae* were analysed. Finally, Desoxyribonucleic Acid (DNA) from these mosquitoes was extracted using Cetyl Trimethyl Ammonium Bromide 2% (CTAB 2%) according to Myriam and Cecile's protocol (Myriam and Cecile, 2003). Mosquito's DNA obtained from RFC method, individual oviposition (n = 1260), mating (n = 71), and between generation (n = 3350) was used in further molecular analysis.

Molecular assays

Molecular identification of *An. gambiae s.l* strains

To detect molecular strains of *An. gambiae* s.l, PCR was performed using the Santolamazza *et al.* protocol amplification [46]. Specific primers were used for identification strains (S200X 6.1. F: 5'-TCGCCTTAGACCTTGCGTTA-3'; S200X 6.1. R: 5'-CGCTTCAAGAATTCGAGATAC-3'). Migration bands were expected at 479 bp for *An. coluzzii*, 249 bp for *An. gambiae s.s* and 223 bp for *An. arabiensis*. Finally, prevalence was calculated by dividing the number of positive mosquito specimens by the total number of mosquitoes tested by PCR for each treatment.

Monitoring for *Microsporidia sp MB* infection in *An. gambiae* strains

Molecular detection of the presence of *Microsporidia MB* was also carried out using mosquito's DNA extracts using *Microsporidia sp MB* PCR according to the protocol of Herren *et al.* [35]. *Microsporidia sp MB* specific primers (MB18SF: 5'-CGCCGGCCGTGAAAAATTTA-3' and MB18SR: 5'-CCTTGGACGTGGGAGCT ATC-3') were designed to target the *Microsporidia sp MB* 18S rRNA gene region.

Migration bands are expected at 450 bp for sporozoite-positive samples. In addition, prevalence is calculated by dividing the number of positive mosquito specimens by the total number of mosquitoes tested by PCR for each treatment. Moreover, only mosquito's DNA from RFC method, individual oviposition, each generation and mating were screened for this molecular analysis.

Molecular detection of sporozoite rate (SR) of *P. falciparum* in *Microsporidia sp MB* positive *An. coluzzii* from field

A total of 37 DNA's mosquito from *An. coluzzii* caught in the field using RFC method was tested to identify the presence of circumsporozoite protein membrane which is specific for *P. falciparum*, infectious stage to humans called sporozoite.

Ethics approval and consent to participate

The study had the approval of the Institutional Ethics Committee for Health Sciences Research: CEIRES (No. A-40/2020-CEIRES granted on November 3rd 2020). Informed written consent was obtained from trained volunteers who participated in mosquito collections. Heads of families were requested through individual discussions and group meetings, prior to the selection of their house for mosquito collection in the study. Permission was requested and obtained from inhabitants to conduct mosquito collections both indoors and outdoors.

Data analysis

All data were entered in Microsoft (MS) Excel version 2016. All statistical analyses were performed with R software version 4.4.1 for data manipulation and visualization. Logistic regression using a Generalized Linear Model (GLM) with binomial distribution was used in order to estimate the effects of *An. gambiae* complex strains (two modalities: *An. coluzzii* and *An. gambiae* s.s) and sex-ratio (three modalities: 1:2, 1:3 and 1:5) on *Microsporidia sp MB* prevalence (GLM, binomial errors). In addition, the effects of generations (4 modalities: F0, F1, F2 and F3) on *Microsporidia sp MB* prevalence were assessed using GLM approach with binomial distribution. Moreover, a correlation between *P. falciparum* and *Microsporidia sp MB* was analysed using GLM with a family binomial distribution. A suitable minimum model was selected by progressively eliminating non-significant terms using a Likelihood Ratio Test (LRT). The terms retained in the minimal model were those that significantly reduced the explanatory power of the model ($P < 0.05$) (Crawley, 2007). Multiple comparison tests (chisq. test) were used to compare proportions and means respectively between "gambiae" "generations *i.e.*, F0, F1, F2 and F3" and "*Microsporidia sp MB* mosquitoes with or without *P. falciparum*" variables when the latter were significant. Raw data and supplementary R codes of the analysis are available on the GitHub repository at:
https://github.com/EtienneBilgo/Millogo-et-al-Advances-in-Bioscience-and-Biotechnology

3. Results

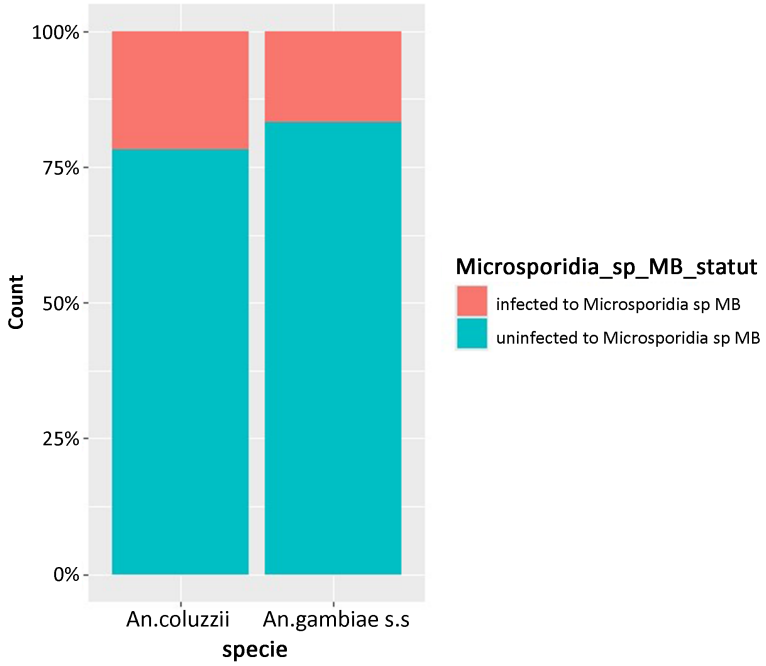
Mosquito species composition

A total of 1260 adult female *An. gambiae* complex mosquitoes collected by RFC

method were analysed of which 58.65% were *An. coluzzii* and the remainder *An. gambiae* s.s.

Infection prevalence with *Microsporidia sp MB* in *An. gambiae* complex

The highest *Microsporidia sp* MB infection rate was obtained in *An. coluzzii* and the lowest in *An. gambiae* s.s (Figure 1). No infection was observed in *An. arabiensis* during our study. Infection prevalence with *Microsporidia sp* MB was between 16.89% (95%CI 0.19 - 0.25) and 21.78% (95%CI 0.14 - 0.20) with the significant highest prevalence in *An. coluzzii* than *An. gambiae* s.s ($X^2 = 4.31$, df = 1, *p-value* = 0.03).



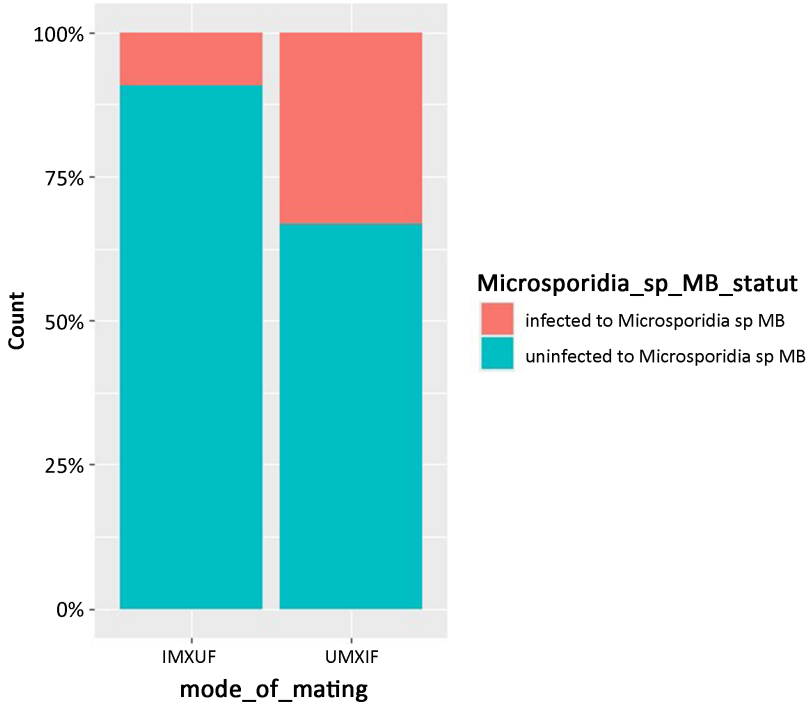
RFC method: Residual Fauna Capture method.

Figure 1. Prevalence of *Microsporidia sp* MB in *An. gambiae* complex strains collected by RFC method in Bama and Soumousso.

Sexual auto-dissemination of *Microsporidia sp MB* transmission with Infected males (IM) crossed uninfected females (UF) and vice versa (UMxIF) in *An. coluzzii*

The diversity of infection prevalence with *Microsporidia sp* MB in Uninfected Males (UM) and Uninfected Females (UF) has been shown in Figure 2. Overall; 71 *An. coluzzii* positive lines to *Microsporidia sp* MB were tested to screen its sexual auto-dissemination during mating. When we crossed Infected males (IM) with Uninfected females (UF), infection prevalence with *Microsporidia sp* MB in UF was significant 3-fold lower (9.23% (95%CI 0.04 - 0.18)) than those Uninfected Males (UM) (33.33% (95%CI 0.09 - 0.70)), When we mated Uninfected Males with Infected Females (IF) ($X^2 = 4.4185$, df = 1, *p-value* = 0.03). However, there was no significant effect of sex-ratio in *Microsporidia sp* MB prevalence in UM and UF ($X^2 = 2.52$, df = 2 *p-value* = 0.28), when we used three different sex ratio

(1:2, 1:3 and 1:5).



IM: Infected Males; UF: Uninfected Females; UM: Uninfected Males; IF: Infected Females

Figure 2. Infection prevalence with *Microsporidia sp* MB during sexual autodissemination (STD) with two mating modes, Infected males (IM) with Uninfected Females (UF) and vice versa (UMxIF) in *An. coluzzii* lines.

Transgenerational transmission of *Microsporidia sp* MB between F0, F1, F2 and F3 generations of *An. coluzzii* and *An. gambiae* s.s

Figure 3 indicates transgenerational transmission of *Microsporidia sp* MB in *An. coluzzii* and *An. gambiae* s.s between F0, F1, F2 and F3 through *An. coluzzii* and *An. gambiae* s.s positive lines to *Microsporidia sp* MB established. Among a total of 3350 adult female mosquitoes, 1614 belong to *An. coluzzii* and 1736 are *An. gambiae* s.s. In these four generations (F0, F1, F2 and F3), the mean prevalence was 22.19% for *An. Coluzzii*. In addition, the prevalence was 21.25% (95%CI 0.17 - 0.26), 21.94% (95%CI 0.18 - 0.25), 22.69% (95%CI 0.19 - 0.26) and 22.88% (0.17 - 0.29) respectively for F0, F1, F2 and F3 in this mosquito's specie. However, the mean infection rate with *Microsporidia sp* MB was 30,23% in *An. gambiae* s.s which was significantly higher than those in *An. coluzzii*. In addition, the prevalence was estimated at 26.41% (95%CI 0.2 - 0.31), 27.31% (95%CI 0.27 - 0.35), 31.63% (95%CI 0.27 - 0.35) and 35.57% (95%CI 0.29 - 0.41) for F0, F1, F2 and F3, respectively for *An. gambiae* s.s. Then, the infection rate was significantly different between *An. coluzzii* and *An. gambiae* s.s ($X^2 = 33.70$, df = 1, *p-value* < 0.001). However, there was no significant different in *Microsporidia sp* MB infection rates between generation F0, F1, F2 and F3 for each *An. gambiae* sibling species ($X^2 = 6.41$, df = 3, p = 0.09).

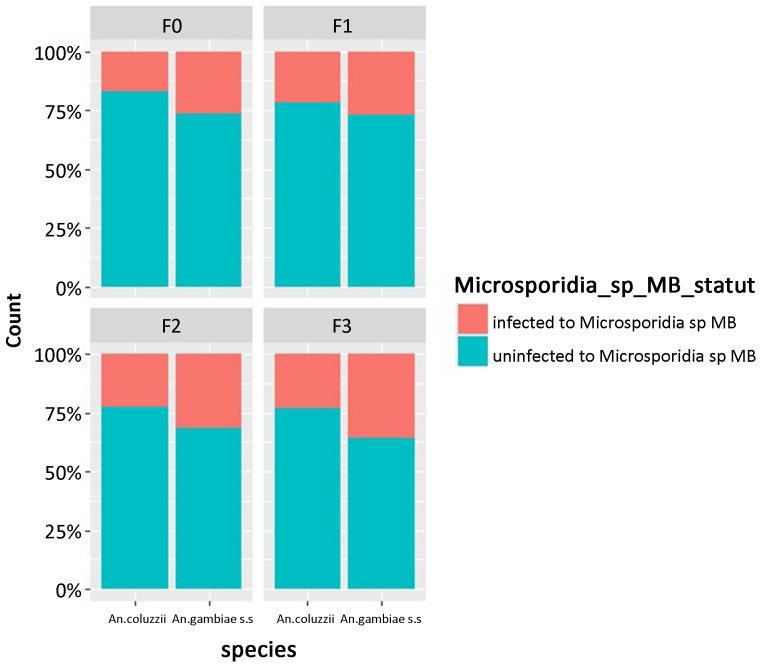


Figure 3. Prevalence of *Microsporidia sp* MB in *An. coluzzii* and *An. gambiae* s.s between F0, F1, F2 and F3 generations.

Correlation between *Microsporidia sp MB* and *P. falciparum* in infected wild mosquitoes

Figure 4 shows the correlation between *Microsporidia* sp *MB* and *P. falciparum* in infected wild mosquitoes.

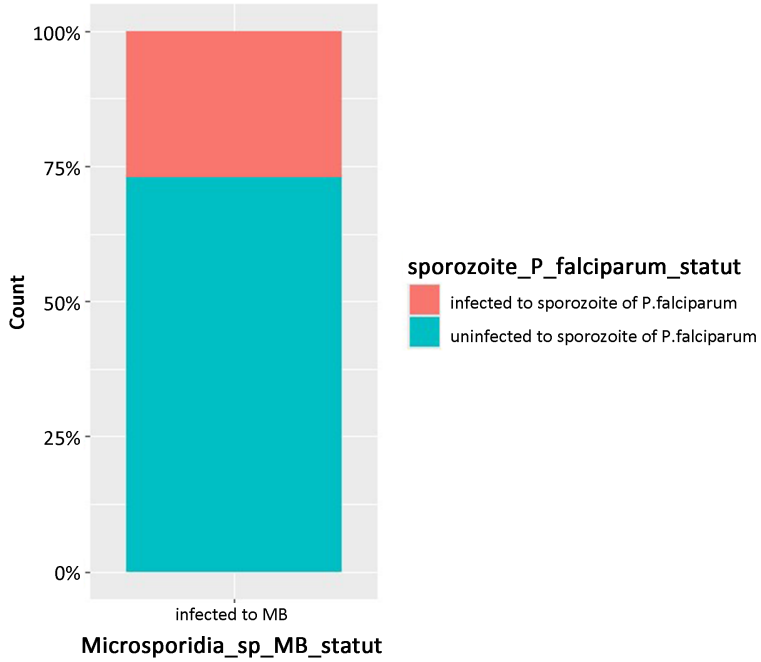


Figure 4. Sporozoite infection rate on *An. coluzzii* positive to *Microsporidia sp* MB collected in the field using RFC method.

We tested 37 positives *Microsporidia sp MB An. coluzzii* caught in the field by RFC method. A molecular assay using PCR to detect circumsporozoite protein which is specific to sporozoite, an infectious stage of *P. falciparum* in mosquito's salivary glands. Around 72.97% (95%CI (0.57-0.84) (27/37) mosquitoes were positive for *P. falciparum* and 27.03% were negative for *P. falciparum* (10/37). The was a higher negative correlation between *Microsporidia sp* MB and *P. falciparum* in positive mosquitoes to *Microsporidia sp MB* (X^2 =7.81, df =1, *p-value* <0.01).

4. Discussion

The prevalence of *Microsporidia sp* MB infection was between 16.39% and 21.78% respectively for *An. gambiae* s.s and *An. coluzzii* during our study. These frequencies were higher than those reported in previous studies in countries such as Kenya, Ghana and Niger, which recorded frequencies of (0% - 9%) [35], (0.198% - 1.71%) [37], (1.8%) [36], (1.98%) [38] and 6.8% [39]. These high frequencies of infection could be explained by a high density of natural infection with *Microsporidia sp* MB in *An. gambiae* s.l. In addition, our high frequencies may reflect the sensitivity of the total mosquito DNA extraction methods used in these earlier studies, which may be less suitable (Trizol RNA/DNA and columns). However, our frequencies were lower than those reported by recent studies in Benin which found frequencies of 41% and 57% for *An. gambiae* s.s and *An. coluzzii* respectively [40]. Our low frequencies could explain a difference in the sensitivity of our extraction method, which is less optimal (CTAB2%) compared with that of the previous study, which used commercial kits such as the Qiagen kit, whose sensitivity is very high. During our study, no *Microsporidia sp* MB infections within the *An. arabiensis* species were recorded. These results could indicate the predominance of this mosquito in urban areas, as reported by recent studies which have shown that this species is the main malaria vector in urban and peri-urban areas [47], while its sibling species, *An. gambiae* s.s and *An. coluzzii*, remain the main vectors in rural areas [48] in the western part of Burkina Faso.

Entomopathogenic microsporidia have been associated with the infection of *Anopheles* and the transmission of malaria. It is only recently that an endosymbiont microsporidian called *Microsporidia sp* MB has been discovered and is thought to be involved in blocking *Plasmodium falciparum transmission*. In order to facilitate the dissemination of this fungus in vectors using the sterile insect technique (SIT) and the population replacement method for genetic control, laboratory studies on its sexual and vertical transmission and its impact on the life history traits of mosquitoes are being carried out in many countries. Our results revealed the possibility of transmission of *Microsporidia sp* MB by males and females carrying *An. coluzzii* during mating, with frequencies of infection of 9.23% and 33.33% respectively. Our results corroborate those found by Nattoh *et al.* studies have shown the effectiveness of sexual transmission or "STD" in *An. arabiensis* and *An. gambiae* s.s in Kenya [37] [38] with the reported prevalence of infection of 3.45% - 12.5% by *An. gambiae* s.s positive males. However, a high

prevalence of transmission of *Microsporidia sp* MB from positive females to uninfected males was recorded in our study compared to that of Nattoh *et al.* who reported a prevalence of 6.25%. This difference in prevalence in our case could be explained by the sensitivity of the amplification technique, *i.e.* conventional PCR, compared with that used by Nattoh *et al.* which is the most sensitive, *i.e.* qPCR, and can detect the fungus even with a low DNA density. In addition, our results could also explain the susceptibility of *An. coluzzii* to infection by *Microsporidia sp* MB compared with its sibling species *An. gambiae* s.s. This hypothesis confirms our results on the prevalence of *Microsporidia sp* MB and those of Ahouandjinou *et al.* [40] who found a higher prevalence among *An. coluzzii* in Benin and Burkina Faso than among *An. gambiae* s.s. However, our results are at odds with those found by Nattoh *et al.* [37] which showed a high prevalence of *Microsporidia sp* MB in negative females (56%) compared with negative males (33%).

Our findings show the lower transmission rate of *Microsporidia sp* MB during matin within negative females and males in *An. coluzzii* mosquitoes suggesting that DNA amplification techniques such as conventional PCR could have an impact on infection rate. In addition, our small sample size (n = 71) could increase or decrease the infection rate. In addition, the technique-dependent horizontal transmission rate of *Microsporidia sp* MB could also be linked to mosquito species, as the prevalence of *Microsporidia sp* MB transmission decreases according to the following species: *An. arabiensis* (33% and 56%), *An. coluzzii* (33.33% and 9.23%) and *An. gambiae s.s* (3.28% - 12.5% and 6.25%) respectively in negative males and females. This suggests a greater susceptibility of *An. arabiensis* to *Microsporidia sp* MB than the other two species. However, this difference in transmission rates between species could be explained by the quality and composition of collection sites, which vary from one species to another. Indeed, sexual transmission of bacterial microorganisms of the *Serratia* AS1 genus and *Wolbachia* has been demonstrated in previous studies [49] [50]. Female mosquitos or insects with the potential to disperse are of great importance to the many types of symbiont transfer. However, due to the endophagous/exophagous nature of mosquitos, they face ethical challenges. Indeed, investigations on symbiotic paternal transfer in aphids have been described [51]. As a result, maternal horizontal and vertical transmission of the *Microsporidia sp* MB symbiont must coexist with paternal transmission, as demonstrated by the collaboration of *An. stephensi* and *Asaia. sp* for paratransgenic control using releases of non-biting transgenic males, a potential malaria control solution [52]. Other insects' results have revealed that parasites can influence host reproduction. This is the case, for example, with trematodes, which cause the snails they infect to mate more frequently [53]. Male midges are similarly affected when infected with mites that have a high mating rate [54] [55]. In mammals, host manipulation in *Toxoplasma gondii*-infected rats has been reported, which on the one hand does not induce rapid, innate, and stereotyped defensive actions, but on the other hand boosts the sexual desire of infected males [56]. The parasite can also cause an exponential secretion of volatile chemical

molecules known as pheromones in its host, which causes greater reproduction. Female moths (*Helicoverpa zea*) that devastate maize harvests in the USA, for example, release 3 to 7 times more pheromones when infected with the virus.

Transovarial transmission is the most important vertical route amongst parasites of invertebrates and is used by a number of intracellular parasites including viruses, bacteria and protists [57]. For example, transovarial transmission has been reported for the Ross River virus infecting the mosquito *Aedes vigilax* [58], for a diverse range of bacteria infecting insects and crustaceans [59] and for a number of microsporidia infecting insects and crustaceans [57] [60]. Recent studies show that *Microsporidia sp* MB is vertically transmitted in *An. arabiensis* and *An. gambiae* s.s in Ken [35] [38]. Our results showed that *Microsporidia sp* MB is clearly transmitted in *An. coluzzii* from maternal to offsprings with *Microsporidia sp* MB prevalence between 23% to 29%. Those results are in the same line with those found in Kenya which described vertical transmission of *Microsporidia MB* by *Microporidia sp* MB positive males in uninfected females in two subspecies of *An. gambiae* s.l in Kenya. In the same way as the release of sterile [59], it is necessary to develop a malaria control strategy aimed at mass release of *Microsporidia MB*-positive males. However, the sexual competitiveness of these *Microsporidia sp* MB-positive males needs to be assessed against that of wild males in order for this strategy to be successful. These findings could be the basis for a dissemination strategy that involves the targeted release of *Microsporidia sp* MB infected male *Anopheles* mosquitoes, potentially avoiding the need to release biting females, which would be advantageous in terms of community engagement and acceptance of the intervention [38] These endosymbiotic strategies in malaria vectors will be disseminated through several generations and reduce malaria transmission in female mosquitoes.

We observed 73% *Microsporidia sp* MB positive adults female *An. coluzzii* that were negative to csp-protein specific to an infectious stage of *Plasmodium falciparum* to humans named sporozoite, the most dangerous malaria parasite among others because it is responsible for 90% of deaths in Sub-Saharan Africa. We found a negative correlation between *Microsporidia sp* MB and *Plasmodium falciparum* in field mosquitoes, suggesting that Microsporidia sp MB can reduce malaria transmission among primary malaria vectors in the field. However, our results are controversial with those found by Herren *et al.* [35] which show an absence of *Plasmodium falciparum* in positive mosquitoes to *Microsporidia sp* MB (head-thorax) and an absence of *Microsporidia sp* MB in positive *Plasmodium* mosquitoes head-thorax in lab conditions. 27% positive *An. coluzzii* to *Microsporidia sp* MB caught in the field mosquitoes could explain mosquitoes the diversity between malaria parasite strains and Microsporidia strains to grow up within its insect-host.

5. Conclusion

In summary, this study shows that *Microsporidia sp* MB is present in both species

An. coluzzii and *An. gambiae*. s.s. For *Microsporidia sp* MB transgenerational transmission between three generations, *Microsporidia sp* MB transmission rate was on average to %. Then, we found *Microsporidia sp* MB auto-dissemination in both negative females and negative males by crossing them with *Microsporidia sp MB* positive lines established in the Lab. However, these results are based on a controlled environment such as Lab conditions. Our findings are paving the road to developing new malaria control technologies by making *Microsporidia sp MB*-positive males sexually competitive with wild males to spread this malaria parasite transmission blocking *Microsporidia sp* MB to wild female mosquitoes and studying more about tripartite interaction between *Anopheles Microsporidia sp* MB *and Plasmodium falciparum.*

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Authors' Contributions

SAM, EB and AD conceived and designed the study. SAM, SI and EB performed the field and laboratory experiments, analysed both field and laboratory data and drafted the manuscript. MK and SAM involved in molecular assays. EB, SI, LDL, MK, RB, KRD, EJG, GAMB and AD critically reviewed the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interest in the publication of this article

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