Spatial distribution of *Microsporidia MB* infection in *Anopheles gambiae* s.l. in three different ecoclimatic areas in Burkina Faso

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Abstract

Introduction. In sub-saharan Africa, malaria control faces significant challenges due to the increasing vector resistance, highlighting the need for innovative approaches. Symbiotic microorganisms associated with mosquitoes are being explored as potential tools to reduce vector competence. Among these microorganisms, *Microsporidia MB* has emerged as a promising candidate. This study aims to investigate the natural occurrence of *Microsporidia MB* infection in *Anopheles (An.) gambiae sensu lato* (s.l.)

Methodology. We screened *An. gambiae* s.l. collected in the field in 2021 across 14 sites covering different ecoclimatic areas in Burkina Faso. Molecular identification of species within the *Anopheles gambiae* complex, as well as detection of *Microsporidia MB* infection, was performed using PCR.

Results. A total of 1,176 DNA samples of *An. gambiae* s.l. were screened, including 337, 424, and 415 samples from the Sahelian, Sudano-Sahelian, and Sudanian ecoclimatic zones, respectively. Three species of the *Anopheles gambiae* complex were identified: *An. coluzzii*, *An. gambiae* and *An. arabiensis*. In the Sahelian and Sudano-Sahelian zone, *An. coluzzii* was the predominant species, with 97.62% and 83.37% of specimens, respectively. In contrast, *An. gambiae* was more frequently identified in the Sudanian ecoclimatic zone (58.25%). The highest *Microsporidia MB*

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infection rate was observed in *Anopheles* mosquitoes from the Sudano-Sahelian zone (9.8%) followed by the Sahelian (7.12%) and Sudanian (3.77%) zones. The prevalence of *Microsporidia MB* infection varied among species, with rates of 7.8% in *An. coluzzii*, 5% in *An. gambiae* and 0% in *An. arabiensis*.
Conclusion. This study revealed a widespread distribution of *Microsporidia MB* among *Anopheles gambiae* s.l. species in Burkina Faso. The absence of *Microsporidia MB* infection in *An. arabiensis* observed in our study may be attributable to the relatively small number (29) of *An. arabiensis* specimens included in our sample.

Keyword: *Anopheles gambiae* s.l., *Microsporidia MB*, Infection, ecoclimatic, Burkina Faso

Distribution spatiale de l'infection à *Microsporidia MB* chez *Anopheles gambiae* s.l. dans les trois zones écoclimatiques du Burkina Faso

Résumé

Introduction. Les microorganismes symbiotiques associés aux moustiques sont actuellement étudiés comme outils potentiels pour réduire la compétence vectorielle des vecteurs du *Plasmodium*. Parmi ces microorganismes, *Microsporidia MB* apparaît comme un candidat prometteur. Notre étude vise à investiguer l'infection à *Microsporidia MB* au sein de la faune sauvage d'*Anopheles gambiae sensu lato* (s.l.) afin de comprendre sa distribution spatiale dans les trois zones écoclimatiques du Burkina Faso.
Méthodes. Les moustiques *An. gambiae* s.l. ont été collectés sur le terrain en 2021 dans 14 sites repartis au sein des zones écoclimatiques du Burkina Faso. L'identification moléculaire des espèces du complexe *Anopheles gambiae* ainsi que la détection de l'infection à *Microsporidia MB* ont été réalisées par PCR sur les échantillons collectés.
Résultats. Au total, 1 176 *An. gambiae* s.l. ont été analysés, dont 337, 424 et 415 provenaient respectivement de la zone sahélienne, soudano-sahélienne et soudanienne. *An. coluzzii* était l'espèce prédominante dans la zone sahélienne (97,62 %) et soudano-sahélienne (83,37 %). En revanche, *An. gambiae* était fréquemment identifiée dans la zone soudanienne (58,25%). La prévalence de l'infection à *Microsporidia MB* variait selon les espèces, avec un taux de 7,8 % chez *An. coluzzii*, 5 % chez *An. gambiae* et 0 % chez *An. arabiensis*.
Conclusion. Cette étude a révélé une large distribution de *Microsporidia MB* chez les espèces du complexe *Anopheles gambiae* au Burkina Faso. L'absence d'infection à *Microsporidia MB* chez *An. arabiensis* pourrait être attribuer au nombre relativement faible (n = 29) de cette espèce dans notre étude.

Mots clés: *Anopheles gambiae* s.l*., Microsporidia MB,* infection, Burkina Faso

Background

Malaria remains the vector borne disease that records the highest number of cases and deaths each year in sub-saharan Africa, despite the

implementation of several prevention and control strategies targeting the vectors (1): i) insecticide-treated nets (ITNs), ii) indoor residual spraying (IRS) and the *Plasmodium* parasites by iii) intermittent preventive treatment (IPT) for pregnant women, iv) Seasonal Malaria Chemoprevention (SMC) for children from 0 to 5 years old. The success of these strategies has been limited by insecticide resistance in *Anopheles* mosquito species (2,3) and by drug resistance in treatment of malaria due to *Plasmodium falciparum* (4), both of which are widespread across Africa. This situation highlights the limitations of current control measures and underscores the need for new strategies targeting *Anopheles* mosquito populations or their capacity to transmit *Plasmodium* parasites to achieve substantial reductions in malaria incidence. One of these novel strategies involves the use of symbiotic microorganisms through paratransgenic, transgenic, or natural approaches such as *Microsporidia, Wolbachia, Serratia, Asaia, Enterobacter, Chromobacterium* and *Metarhizium* in *Anopheles* mosquitoes to reduce their vectorial competence for transmitting *Plasmodium* parasites (5–7). A recent study conducted in Kenya demonstrated that *Microsporidia MB*, a natural symbiont in the *An. gambiae* complex has the ability to impair *Plasmodium* transmission (6). The presence of *Microsporidia MB* in *An. gambiae* mosquitoes offer advantage to block *Plasmodium* development within the mosquito. Therefore reducing vector competence and potentially decreasing the capacity of these mosquitoes to transmit malaria. This finding was reported in *An. arabiensis* sampling in different geographical area in Kenya, with the highest infection prevalence occurring after the peak rainfall period. Since then, studies have been conducted in several African countries, firstly to identify natural *Microsporidia MB* infection in major malaria vectors such as the *An. gambiae* complex. Subsequently, investigations were carried out by researchers from Ghana (8), Niger (9) and Benin (10) to understand *Microsporidia MB* infection within their vector populations. The results of these studies show that *An. gambiae* and *An. coluzzii* are the two species of *An*. *gambiae* complex found to be infected by *Microsporidia MB*. The prevalence of *Microsporidia MB* in these countries was 14 % for *An. coluzzii* in sub equatorial climate zone (Benin), 6.8% for *An. coluzzii* in the Sahelian zone (Niger) and 23.9% for *An. gambiae* and 19.3% for *An. coluzzii* in southern zone (Ghana). In Burkina Faso Millogo and al. (11), reported substantially higher infection prevalences, reaching 21.78% in *An. coluzzii* and 16.89% in *An. gambiae*. However, mosquitoes in that study were collected in Vallée

du kou and Soumousso, both located in Sudanian ecoclimatic zone of Burkina Faso. These findings suggest that *Microsporidia MB* prevalence may vary according to climatic conditions. Indeed, Burkina Faso comprises three major ecoclimatic zones (Sahelian, Sudano-Sahelian, and Sudanian) each characterized by distinct environmental and climatic conditions that may influence the distribution and prevalence of *Microsporidia MB*. Therefore, determining the infection status of *Microsporidia MB* in the *Anopheles gambiae* complex across the three major ecoclimatic zones of Burkina Faso is essential? To address this question, we investigated *Microsporidia MB* infection in the *An. gambiae* complex and examined its spatial distribution among species across three distinct ecoclimatic zones in Burkina Faso.

Methodology

Sampling sites

Mosquitoes were collected in 14 sites across from the 3 different ecoclimatic zone of Burkina Faso (12) (**Figure 1**). These three ecoclimatic zones have three distinct climatic characteristics. The Sahelian zone is in northern part of the country and includes four (4) sampling sites (Kalsaka, Kongoussi, Seguenega and Yilou) (**Figure 1**). This Sahelian zone is the driest of the three ecoclimatic zones with a short raining season occurring from July to September. Annual rainfall ranges from 300 to 600 mm, and temperatures generally range from 25 to 41°C. The Sudano-Sahelian zone is in central part of the country, with four (4) sampling sites: Bobo-biron, Mollé, Nouna and Solenzo (**Figure 1**). The rainy season occurs from June to September, with annual rainfall ranging from 600 to 900 mm, and temperatures generally ranging from 20 to 40°C. The Sudanian zone is the most humid ecoclimatic zone and is in the Southern part of the country. Six (6) sampling sites were selected in this zone (Banlo, Gaoua, Kampti, Karangasso-vigue, Loglona and Soumousso) (**Figure 1**). The rainy season is longer, occurring from June to October, and temperatures range from 18 to 35°C (12).

Mosquito sampling

Archived *Anopheles gambiae* sensu lato (s.l.) specimens, originally collected as part of routine entomological surveillance programs to monitor malaria vector species composition and distribution, were used in this study. The mosquitoes were preserved in silica gel and stored at

the *Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest* (IRSS/DRO), Bobo-Dioulasso, Burkina Faso, until molecular analyses.

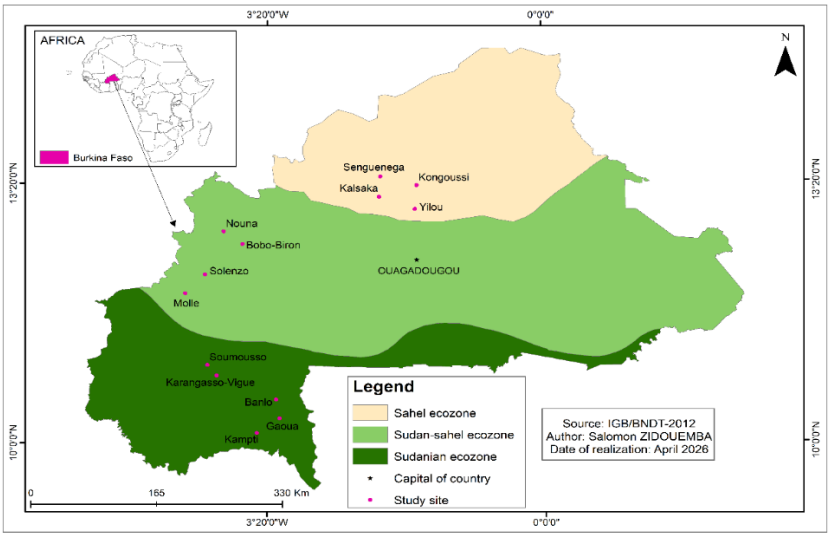

Figure 1: Mosquito sampling sites across each ecoclimatic zone.

Adults' mosquitoes were collected by indoor Pyrethroid Spray Catches (PSC) method during the rainy season period, June to September 2021. PSC was conducted once per month in 20 randomly selected houses at each study site to collect indoor-resting mosquitoes. Early each morning, the rooms were prepared by covering the floors with white sheets and sealing all openings. A non-residual pyrethroid was then sprayed, and the rooms were kept closed for 15 min. Knocked-down mosquitoes were collected using forceps and transferred to Petri dishes for subsequent identification**.** Blood-fed *An. gambiae* s.l. was the most frequently collected among the other female's status. All blood-fed females were used for molecular analysis. (**Table I**).

Molecular analysis

DNA extraction

Mosquito Desoxyribonucleic Acid (DNA) was extracted from the abdomens of blood-fed females using 2% cetyltrimethylammonium bromide (CTAB) following the protocol described by Myriam and Cécile (13).

Molecular identification of *Anopheles gambiae* s.l.

Molecular identification of *Anopheles gambiae* s.l. species was carried out on 1176 DNA samples (337, 415 and 424 respectively from Sahelian, Sudano-sahelian and Sudanian zone) (**Table I**) following Santolamazza protocol (14). The primers pairs (S200X 6.1. F: 5'-TCGCCTTAGACCTTGCGTTA-3'and S200X 6.1. R: 5'-CGCTTCAAGAATTCGAGATAC-3') were used for identification. The PCR product was migrated and visualized on 2% agarose gels.

Table I: Blood-fed *Anopheles gambiae* s.l. specimens selected for molecular analyses

Sampling period	Ecoclimatic zone	Sampling sites	Total number by sites	Total number by ecoclimatic zone
June to september 2021	Sahelian	Kalsaka	45	337
		Kongoussi	154	
		Seguenega	69	
		Yilou	69	
	Sudano-sahelian	Mollé	66	415
		Nouna	141	
		Solenzo	87	
		Bobo-biron	121	
	Sudanian	Gaoua	134	424
		Kampti	4	
		Banlo	129	
		Loglona	21	
		Karangasso-vigué	60	
		Soumousso	76	

Migration bands were expected at 479 bp for *An. coluzzii,* 249 bp for *An. gambiae* and 223 bp for *An. arabiensis* (14).

Molecular screening for *Microsporidia MB*

The screening of *Microsporidium MB* infection was carried out on the same samples used for molecular identification of *Anopheles gambiae* s.l. species (1176 DNA samples). We screened *Microsporidia MB* using the primers pair (MB18SF: 5' CGCCGGCCGTGAAAAATTTA-3' and MB18SR: 5'-CCTTGGACGTGGGAGCT ATC-3') that were designed

to target the *Microsporidia MB* 18S rRNA gene region (6). PCR reaction volume was 20 µl containing 13 µl of H2O, 4 µl of FIREPol Master Mix Ready to Load ,0.5 µl of each primer and 2 µl of template DNA. The PCR cycling conditions were as follows: initial denaturation at 95 °C for 15 min, further denaturation at 95 °C for 1 min, followed by annealing at 62 °C for 90 s and extension at 72 °C for a further 60 s, all done for 35 cycles. Final elongation was done at 72 °C for 5 min (6). The PCR product was migrated and visualized on 2% agarose gels. The positive samples band size was expected at ~ 500 pb for *Microsporidia MB* (**Figure 3**).

Statistical analyses

Statistical analyses were performed using R software (version 4.4.2) to compare the proportions of *Anopheles gambiae* s.l. species and their *Microsporidia MB* infection rates across ecoclimatic zones. Pearson's chi-squared test was used to assess differences between groups, and statistical significance was defined as $p < 0.05$.

Results

Anopheles gambiae sl species composition

In the sahelian ecoclimatic zone, only two species of the *An. gambiae* complex species (*An. gambiae* and *An. coluzzii*) were identified across all 4 sampling sites, with *An. coluzzii* being the predominant species (97.62%) (**Figure 2**). Within the Sudano-sahelian zone, *An. coluzzii* and *An. gambiae* were predominant species, accounting for 83.37% and 16.14% of specimens, respectively. *An. arabiensis* was detected at a low frequency (0.48%) and was restricted to a single collection site (Bobo-biron), (**Figure 2**). The Sudanian zone harboured all three species, with *An. gambiae* being the most abundant (58.25%), followed by *An. coluzzii* (35.37%) and *An. arabiensis* (6.36%) (**Figure 2**).

Anopheles gambiae s.l. species infection status by *Microsporidia MB*

Overall, *Microsporidia MB* prevalence was 6.88% (81/1176) (95% CI 5.53 – 8.52) in the total samples analyzed. *Microsporidia MB* was detected exclusively in *An. gambiae* and *An. coluzzii* and no significant difference in infection rates was observed between these two species ($X^2 = 5.25$, $df = 2$; $p=0.07$). The infection rate was 5% (16/306) (95% CI 3.11 – 8.52) and 7.8% (65/760) (95% CI 6.70 – 10.82) respectively in *An. gambiae* and *An. coluzzii* (**Figure 4**).

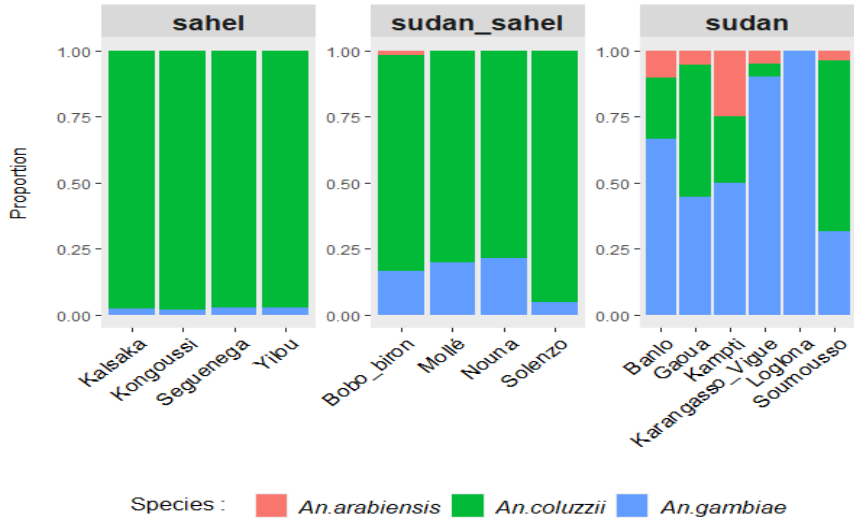

Figure 2: *Anopheles gambiae* complex species composition by collection sites and ecoclimatic zone.

No *Microsporidia MB* infection was detected in *An. arabiensis* (0/29) (**Figure 4**). The prevalence of *Microsporidia MB* infection in mosquitoes differed significantly across the three ecoclimatic zones ($X^2 = 12.23$, $df= 2$; $p=0.002$). The highest infection rate was observed in *Anopheles* mosquitoes from the Sudano-Sahelian zone 9.8% (95% CI 7.26 – 13.26), followed by the Sahelian 7.12% (95% CI 4.71 – 10.54) and Sudanian 3.77% (95% CI 2.24 – 6.18) zones. (**Figure 4**). Kalsaka, located in the Sahelian zone and Nouna in the Sudano-Sahelian zone, were the mosquito sampling sites with higher infection rates compared to the other study sites (**Figure 5**).

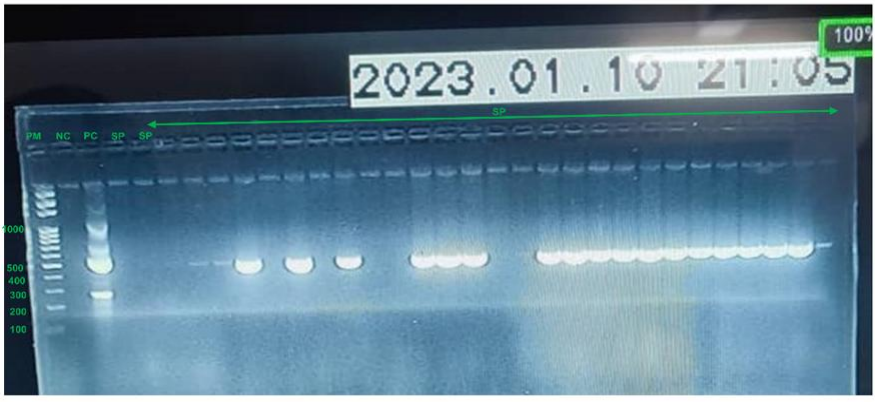

Figure 3: Representative agarose gel electrophoresis of replicate positive samples. Well PM: 100 bp DNA ladder; well NC: negative control; well PC: positive

control; well SP: samples. The smear in well PC resulted from the high concentration of the positive control DNA. Positives samples band size was ~ 500pb.

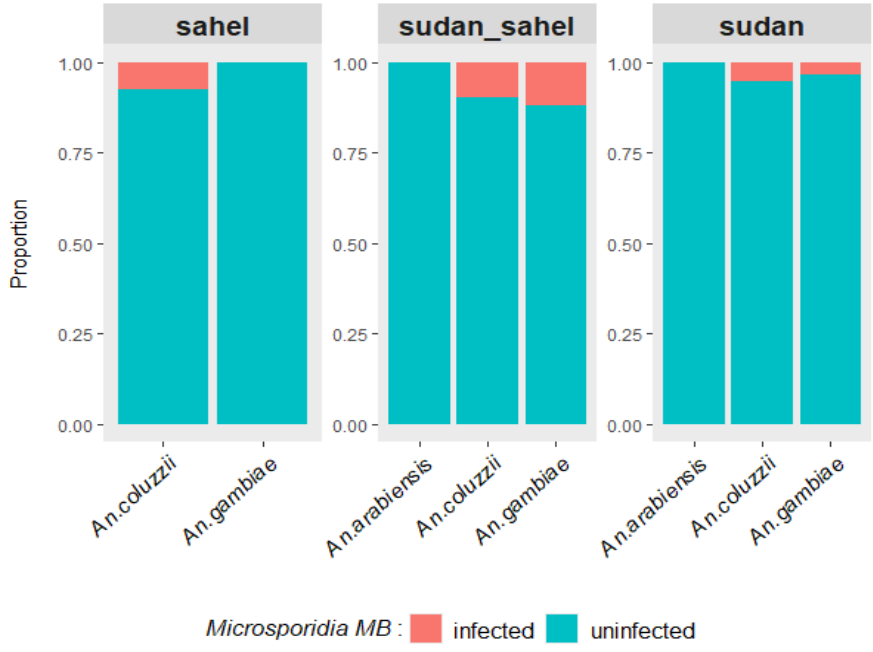

Figure 4: *Microsporidia MB* infection rate in *Anopheles gambiae* s.l. species by ecoclimatic zone.

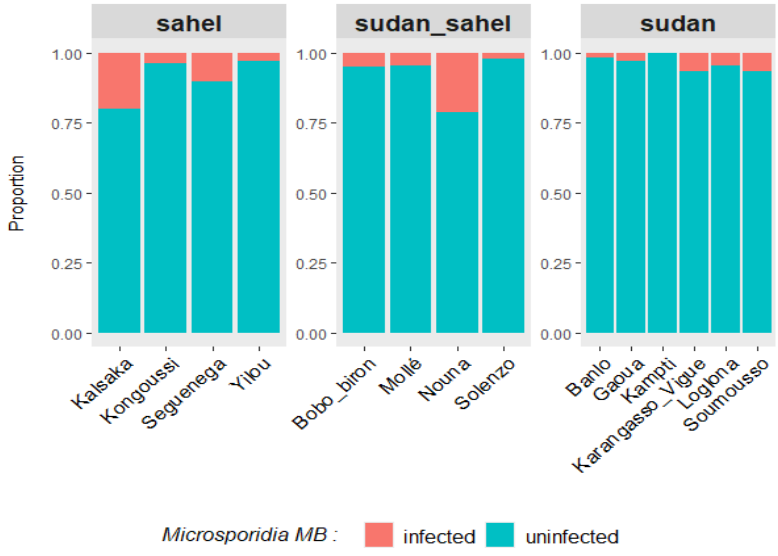

Figure 5: *Microsporidia MB* infection rate by sites and ecoclimatic zone.

Discussion

This study was conducted to provide baseline information on the ecological distribution of *Microsporidia MB* associated with the *Anopheles gambiae* complex across the ecoclimatic gradient of Burkina Faso, ranging from the arid Sahelian zone in the north to the humid Sudanian zone in the south. Molecular analyses on *An. gambiae* complex to determine the specific *Anopheles'* composition in each site and ecoclimatic area revealed the *An. coluzzii* was dominant in north (sahel) and Central (Sudan-sahel) zones. In Burkina Faso, *An. coluzzii* is known to be the predominant *Anopheles* species in arid and semi-arid zone of the country, consistent with findings from previous studies (15,16). *An. gambiae* was predominant in humid (Sudanian) area, this result align with previous studies (17,18). The humid ecoclimatic area have favorable breeding sites for *An. gambiae* such as unpolluted stagnant water without submerged vegetation (19). *An. arabiensis* was detected at low frequency and was restricted to the Sudanian zone. This suggests that *An. arabiensis* prefers humid breeding sites and may be more abundant in urban areas with polluted and stagnant water (17,20). Screening of *Anopheles gambiae* s.l. for *Microsporidia MB* revealed that only *An. gambiae* and *An. coluzzii* were infected, with infection prevalences of 5.0% and 7.8%, respectively. These findings are consistent with those reported from other West African countries. In Niger (9), the prevalence of *Microsporidia MB* in *An. coluzzii* collected during the rainy season was 6.8%. In Benin (10), the prevalence reached 14% in *An. coluzzii* sampled during the dry season but decreased markedly to 0.7% in mosquitoes collected from the same area during the rainy season (10).

Taken together, these observations may suggest that *Microsporidia MB* is more frequently associated with specific mosquito species, particularly *An. coluzzii*, and that environmental conditions, including seasonality, may influence its prevalence. However, this pattern is not consistent across West Africa. Indeed, studies conducted in Ghana (8) and Burkina Faso (11), reported substantially higher infection rates (>20%) in both *An. gambiae* and *An. coluzzii*, despite mosquitoes being collected during the rainy season in the Sudanian ecoclimatic zone. These contrasting findings indicate that the distribution and prevalence of *Microsporidia MB* are likely influenced by multiple interacting factors, including local ecological conditions, and seasonal variation. No *Microsporidia MB* infection was detected in *An. arabiensis*. Conversely, in Kenya, *Microsporidia MB* infection has been reported in

An. arabiensis in a previous study (6). The absence of *Microsporidia MB* infection in *An. arabiensis* could be explain in our study both the few numbers of *An. arabiensis* reported in molecular identification and then their low distribution in three different ecoclimatic area. *Microsporidia MB* infection was reported in 13 out of 14 mosquito sampling sites. This result suggests *Microsporidia MB* is widespread in large area in Burkina Faso and then the three distinct areas provide favorable climatic conditions for *Microsporidia MB* to infect mosquitoes. However, it is important to note that mosquito sampling was conducted during the rainy season (June to September), a period characterized by optimal environmental conditions such as high humidity and suitable temperatures, which may be influence *Microsporidia MB* infection.

The natural occurrence of *Microsporidia MB* in local *Anopheles* populations suggests that it may represent a promising candidate for approaches aimed at exploiting or enhancing symbiotic mechanisms to reduce malaria transmission.

Further studies are therefore needed to better understand the ecological determinants governing *Microsporidia MB* infection in malaria vectors across different West African settings. Also, the characterization of the interactions between *Microsporidia MB*, its *Anopheles* host, and *Plasmodium* could help elucidate the mechanisms underlying parasite development inhibition.

Conclusion

This study revealed the presence of *Microsporidia MB* in *An. gambiae* and *An. coluzzii* across three distinct ecoclimatic zones in Burkina Faso. The infection rate between these species was homogeneous across the ecoclimatic zones. This finding opens the way for further investigations to better characterize the dynamics of *Microsporidia MB* in local *Anopheles* populations and to assess its potential as a complementary tool for malaria vector control.

The next step of our study will involve phylogenetic analyses of positive samples to better characterize the strains circulating across the different ecoclimatic zones and to investigate their genetic diversity.

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Authors contribution

RKD was the principal scientific investigator of the study. DPAK, SPS and KRD designed the study. DPAK, KLN, DDS, DJJT, RB, SM, DOK, NDCD and ASH coordinated the lab and field activities. SZ realized the map. DPAK analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

Competing interest

The authors declare no competing interest.

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The mosquitoes analyzed in this study were collected during US President Malaria Initiative (PMI) VectorLink project conducted by IRSS/DRO. The funders had no role in the design of the study such as *Microsporidia MB* analysis, interpretation of data and in writing the manuscript

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