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Spatial Distribution of *Microsporidia MB* Along Clinal Gradient and the Impact of Its Infection on Pyrethroid Resistance in *Anopheles gambiae s.l.* Mosquitoes from Nigeria and Niger Republic

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Abstract

Microsporidia MB (*MB*), a promising biological control agent, suppresses *Plasmodium falciparum* transmission in *Anopheles* mosquitoes. This study examined the spatial distribution of *MB* infection in natural populations of *An. gambiae s.l.* mosquitoes collected in Nigeria and Niger Republic, and its association with insecticide susceptibility in the mosquitoes. *Microsporidia MB* has wide geographic distribution across Nigeria and Niger Republic. The overall prevalence of *MB* in F_0 mosquitoes was 12.25% (95% CI: 7.76–16.75%); 25 mosquitoes out of 204 were positive. Geographic variation was observed, with a higher prevalence (5/15 mosquitoes) in Ebonyi State (33.33%, CI: 9.48–57.19%, Fisher's exact test, $p = 0.008$). Infection rates were higher in *An. coluzzii* mosquitoes (21/133 mosquitoes), estimated at 15.79% (CI: 9.59–21.99%) compared to *An. gambiae s.s.* mosquitoes (4/71), with approximately 5.63% (CI: 0.27–11.00%, $\chi^2 = 4.44$; df = 1, $p = 0.035$). Resistant mosquitoes had a significantly higher prevalence of *MB* infection than susceptible mosquitos at 28.57% (CI: 16.74–40.40%) with an odds ratio of 3.33 (CI: 1.23–9.03, $p = 0.017$). These findings suggests that *MB* can be exploited as an alternative for vector control in Nigeria and Niger, but its possible association with pyrethroid resistance suggests that it should be taken into account as a potential confounder when designing insecticide resistance management strategies.

Keywords: *Anopheles*; malaria; *Microsporidia MB*; control; insecticide resistance; spatial distribution

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1. Introduction

Malaria remains a significant global public health issue, particularly in Africa, where over 94% of infection/cases and 95% of malaria-related deaths occur [1]. Nigeria has the highest burden of malaria in the world, accounting for ~26% of malaria cases and ~30% of malaria-related deaths globally. The neighbouring country of Niger also faces significant malaria challenges, contributing 5.9% of malaria-related deaths globally, ranking third [1].

The major malaria vectors found in Nigeria and Niger Republic (hereafter Niger) belong to the *Anopheles gambiae* complex (principally *An. gambiae s.s.*, *An. coluzzii*, and *An. arabiensis*) and secondarily the *Anopheles funestus s.s.* [2–7]. These species exhibit considerable ecological diversity and wide distribution patterns across different habitats [8–10]. Studies conducted in Nigeria and Niger have highlighted the abundance and broad distribution of species within the *Anopheles gambiae* complex and *Anopheles funestus* group along various ecological zones [3,6,11].

Vector control is the cornerstone of the fight against malaria, with the major control tools being insecticide-treated bed nets and indoor residual spraying [12–15]. Unfortunately, the use of insecticides has led to the development of resistance to nearly all classes of public health insecticides, with pyrethroids, the key ingredients in insecticide-treated bed nets (ITNs), affected disproportionately [16]. In Niger, pyrethroid resistance towards alpha-cypermethrin, deltamethrin, and permethrin has been documented [11,12,17]. In Nigeria, widespread pyrethroid resistance has been reported in *Anopheles* mosquitoes. Studies have shown high resistance levels in southern and northern Nigeria, with significant operational implications for ITN effectiveness [3,18,19]. In both countries, high pyrethroid resistance in *Anopheles gambiae* is associated with increased activities of metabolic enzymes (e.g., P450s and GSTs) and the West African knockdown resistance (*kdr-w*) mutation [12,20–23].

The bulk of research on resistance mechanisms has focused on the role of metabolic genes and target site mutations [24–27], with alternative mechanisms such as the role of the gut microbiome in mediating resistance receiving little attention. However, Dada and colleagues have demonstrated that variations in the *Anopheles* gut microbiome can influence pyrethroid resistance, suggesting an additional layer of complexity to resistance management [28].

In the face of the resistance challenges associated with synthetic insecticides, efforts are being increasingly invested towards alternative, more eco-friendly control measures, such as *Wolbachia* [29–31] and *Bacillus thuringiensis* (*Bti*) [32,33]. The discovery of *Microsporidia MB*, an intracellular symbiont in *Anopheles* mosquitoes, has sparked interest due to its potential for malaria control. The presence of *MB* in *Anopheles* mosquitoes negatively impacts their ability to transmit *Plasmodium* [34]. *MB* accumulates in mosquito tissues, with sexual and vertical transmission ensuring its persistence and transmission [34–36]. The presence of *MB* has been confirmed in several *Anopheles* species across Africa, including Kenya, Ghana, Niger, and Benin [6,34,37,38]. Studies have shown that its prevalence varies by geographic region, habitat type, and climatic conditions. For instance, in Kenya, *MB* prevalence in *An. gambiae* complex mosquitoes was higher in low-altitude areas with warm temperatures and high rainfall compared to high-altitude areas with cooler temperatures and lower rainfall [34]. Similarly, in Ghana, *MB* prevalence in *An. gambiae s.l.* was higher in forest zones compared to savanna zones [38]. Recently, a variation in *MB* infection was reported in two geographical localities of Benin republic [39]. The above findings underscore the significant role of environmental conditions in shaping the geographic distribution of *MB*.

While *MB* shows promise as a biological control agent [40], its effectiveness may be influenced by environmental conditions and inherent variability in transmission rates, complicating its use. Moreover, its potential to influence mosquito phenotypes, particu-

larly insecticide resistance, remains largely unexplored. Understanding interspecies and intraspecies variations in *MB* infection between *Anopheles* populations and the potential role of *MB* resistance is essential for informing malaria control strategies. This study investigated the spatial distribution of *MB* and assessed its association with insecticide resistance phenotypes in *Anopheles gambiae s.l.* mosquitoes from Nigeria and Niger.

2. Materials and Methods

2.1. Collection of Anopheles Mosquitoes

Mosquitoes were collected across twelve sites, spanning contrasting ecological settings: one site in Niger and eleven in Nigeria (Figure 1). These sites cover mangrove swamp, tropical rainforest, Guinea savanna, Sudan savannah, and the Sahel (Table 1). Blood-fed female *Anopheles* mosquitoes (F_0) resting indoors were collected using Prokopack electric aspirators (John W. Hook, Gainesville, FL, USA) early in the morning, between 05:00 and 06:00 a.m. The mosquitoes were transferred to Bayero University Kano in paper cups (using cooling boxes) and maintained under standard insectarium conditions (25 °C and 75% relative humidity, with 12 h:12 h light/dark cycles), fed with 10% sugar. Collections were conducted in Nigeria between June and September and in Niger in September 2024. Gravid females were forced to lay eggs individually and, following oviposition, identified to species level using PCR, before eggs from identical species were pooled and reared to adulthood for insecticide bioassays.

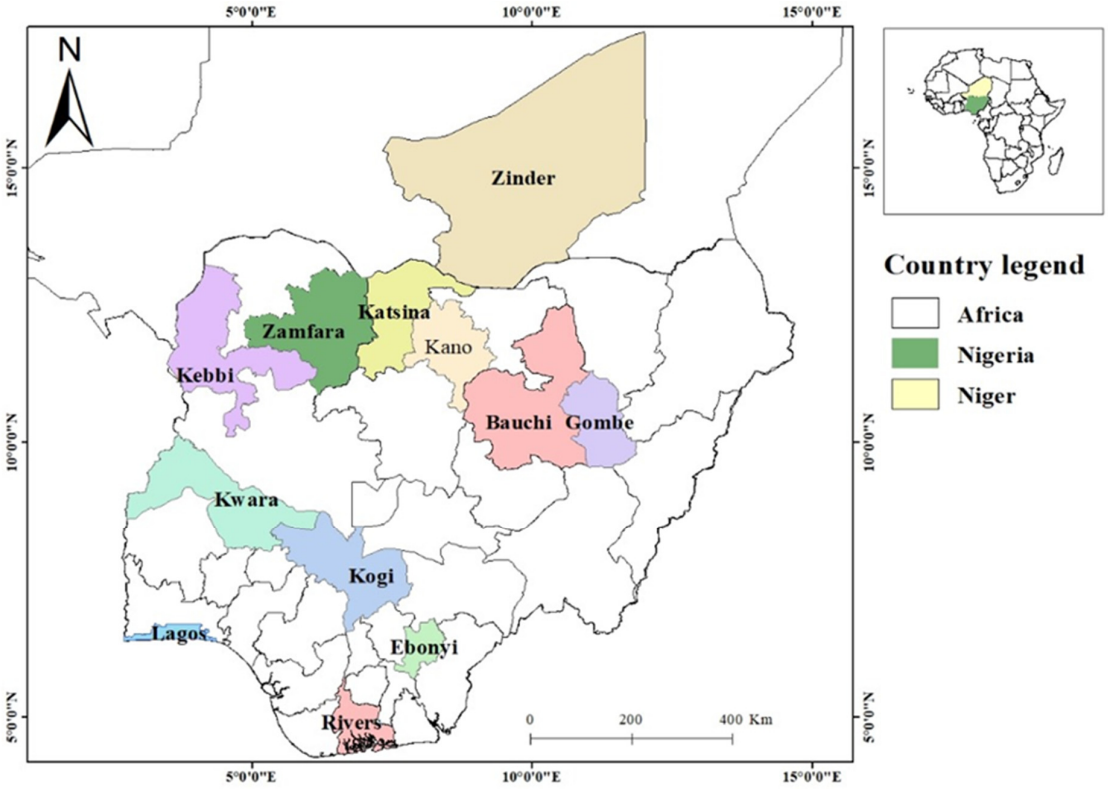


Figure 1. Map of sampling locations in Nigeria and Niger.

Table 1. Sampling design and localities for *Anopheles* mosquito collection and bioassays.

Locality	*n*	*Species*	Bioassay	Ecological Setting
F_0 parents caught indoor using Prokopack aspirators				
Gayi Niger, Magaria	32	*An. coluzzii*	-	Sahel
Gajerar Giwa, Katsina State	32	*An. coluzzii*		Sahel
Gaa-Bolorunduro, Kwara State	15	*An. gambiae s.s.*	-	Guinea Savanna
Zariagi, Lokoja, Kogi State	32	*An. gambiae s.s.*	-	Guinea Savanna
Gotomo, Kebbi State	15	*An. coluzzii*	-	Sudan Savanna
Tsunami, Gusau, Zamfara State	15	*An. coluzzii*	-	Sudan Savanna
Ugalaba, Ezza North, Ebonyi State	15	*An. coluzzii*	-	Tropical Rainforest
Obio/Akpor LGA, Rivers State	24	*An. coluzzii*	-	Mangrove Swamp
Badagry, Lagos State	24	*An. gambiae s.s.*	-	Mangrove Swamp
F_1 progenies used for bioassays				
Gajerar Giwa, Katsina State	16	*An. coluzzii*	Permethrin	Sahel
Dadin Kowa, Gombe State	16	*An. coluzzii*	Deltamethrin	Guinea Savanna
Zariagi, Lokoja, Kogi State	24	*An. gambiae s.s.*	Deltamethrin	Guinea Savana
Gamjin Bappa, Karaye, Kano State	16	*An. coluzzii*	Deltamethrin	Guinea Savana
Gadau, Bauchi State	16	*An. coluzzii*	Deltamethrin	Sudan Savanna
Obio/Akpor LGA, Rivers State	24	*An. coluzzii*	Permethrin	Mangrove Swamp

2.2. Morphological and Molecular Identification of Anopheles Mosquitoes to Species Level

Gravid F_0 female mosquitoes (4–5 d old after blood meal) were forced to lay eggs inside 1.5 mL Eppendorf tubes. They were morphologically identified to species complex level using keys described by Gillies and Coetzee [41] based on traits such as palpus length, wing spots pattern, and leg banding. Molecular identification was conducted using SINE200 PCR [42]. Genomic DNA (gDNA) was extracted using the LIVAK protocol [43]. Each gDNA premix of 14 µL comprised 1.5 µL of 10x Taq A buffer, 0.75 µL of $MgCl_2$, 0.12 µL of 10 mM dNTP mixes, 0.51 µL (0.34 µM) each of the SINE200 F and R primers, 0.12 µL of 5 U/µL KapaTaq Polymerase (Kapa Biosciences, Wilmington, MA, USA), and 10.49 µL of ddH_20, to which 1 µL of gDNA was added. The thermal cycling conditions were an initial denaturation at 95 °C for 5 min, followed by 35 cycles each of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 1 min. A final extension step was performed at 72 °C for 5 min. PCR products were separated on 1.5% agarose gel stained with pEqGREEN, and visualised using InGenius 3 GelDoc, (Syngene, Cambridge, UK) with expected band sizes of 479 bp, 245 bp, and 220 bp, respectively, for *An. coluzzii*, *An. gambiae s.s.*, and *An. arabiensis*. Genomic DNA samples from previously PCR-confirmed *An. coluzzii*, *An. gambiae s.s.*, and *arabiensis* were used for positive controls, and for a negative control, nuclease-free water was used.

2.3. Insecticide Susceptibility Bioassays

Tube bioassays were conducted using the World Health Organization (WHO) protocol [44,45]. Briefly, for each test, 4 replicates of 20 to 25 non-blood-fed adult female mosquitoes (3–4 d old) were exposed to papers impregnated with either 0.05% deltamethrin or 0.75% permethrin. The test papers were purchased from Universiti Sains Malaysia (Penang, Malaysia). The mosquitoes were exposed for 1 h, transferred to holding tubes,

and supplied with sugar. Mortalities were recorded at 24 h and mosquitoes alive at 24 h were separated from those which died.

2.4. Investigation of Spatial Distribution of MB Infection in Anopheles Mosquitoes

Subsets of gDNA extracted from the F_0 females mentioned above (Table 1) were used for the PCR identification of *MB* infection. Amplification of the 18S rRNA gene was performed using *MB18SF* and *MB18SR* primers according to a previously published protocol [28]. Briefly, 3 µL of 5x master mix (Solis Biodyne, Tartu, Estonia), comprising 7.5 mM of $MgCl_2$, 2 mM of each dNTP mix, and HOT FIREPol® DNA polymerase was mixed with 0.75 µL (0.5 µM) each of F and R primer, 3 µL of DNA template, and 7.5 µL of nuclease-free water. The thermal cycling conditions included an initial denaturation at 95 °C for 15 min, followed by 35 cycles each of denaturation at 95 °C for 30 s, annealing at 59 °C for 45 s, and extension at 72 °C for 45 s. The PCR products were separated on 1.5% agarose gel stained with pEqGREEN, and visualised using GelDoc for the expected band size of approximately 500 bp characteristic of *MB*. Genomic DNA from previously confirmed MB-positive *An. coluzzii* [6] was used as a positive control. Nuclease-free water was used as a negative control.

2.5. Investigation of the Association of Insecticide Resistance and MB Infection

The F_1 mosquitoes from six sites in Nigeria, which were phenotyped with pyrethroid insecticides, were selected to screen *MB* infection (Table 1). From each site, an equal number of mosquitoes from the contrasting phenotypes [alive (resistant, R) and dead (susceptible, S)] were randomly selected and screened for infection.

2.6. Data Analysis

Data were analysed using XLSTAT BASIC 2024.4.2 [46] to investigate the relationships between *MB* infection status, *Anopheles* species, locality, and insecticide resistance. To establish the relationships between variables, a Chi-square test or a Fisher's exact test of independence was applied to determine whether *MB* infection status is associated with species, locality, and insecticide susceptibility. The odds ratio (OR) was determined to measure the strength of association between variables. p-values < 0.05 were considered statistically significant. For each site, 95% confidence intervals (CIs) for the prevalence of *MB* infection were calculated using the exact binomial method, based on the number of infected mosquitoes out of the total number tested per site.

Multiple Correspondence Analysis (MCA) was conducted using XLSTAT's "Factor Analysis" package, a multivariate approach designed for categorical data. The MCA was applied to explore key relationships, including the association between *MB* infection status and *Anopheles* species, the relationship between infection status and mosquito insecticide susceptibility (resistant vs. susceptible), and the spatial distribution of *MB* across sampled localities. MCA was conducted using the following categorical variables: *Microsporidia MB* infection status (*MB*+ and *MB*−) for infected and non-infected mosquitoes, respectively; mosquito species (*An. coluzzii*, *An. gambiae s.s.*); and insecticide susceptibility phenotype (Resistant/Susceptible). A breakdown of the number of *Anopheles* species used for MCA is provided in Table S1 in Supplementary File S2.

3. Results

3.1. Microsporidia MB Infects Anopheles Mosquitoes from Contrasting Ecological Settings

A total of 204 adult female F_0 mosquitoes were analysed for *MB* infection, of which 25 were infected (gel pictures in File S1), with 12.25% prevalence (95% CI: 7.76–16.75%). *Microsporidia MB* was found to be widespread across sites with only 2 out of the 12 sites

being negative (Figure 2). The prevalence of infection varied, ranging from of 0% to 33% (Table S2, File S2). For F_0 females, the infection rate was highest in Ebonyi State, where 5 out of 15 mosquitoes were infected (33.33%, 95% CI: 9.48–57.19%), followed by Rivers State, where 7 out of 24 mosquitoes were positive for infection (29.17%, 95% CI: 10.98–47.35%). However, mosquitoes from Kwara and Gombe States were not infected. A Fisher's exact test confirmed a statistically significant association between *MB* infection status and locality ($p = 0.008$).

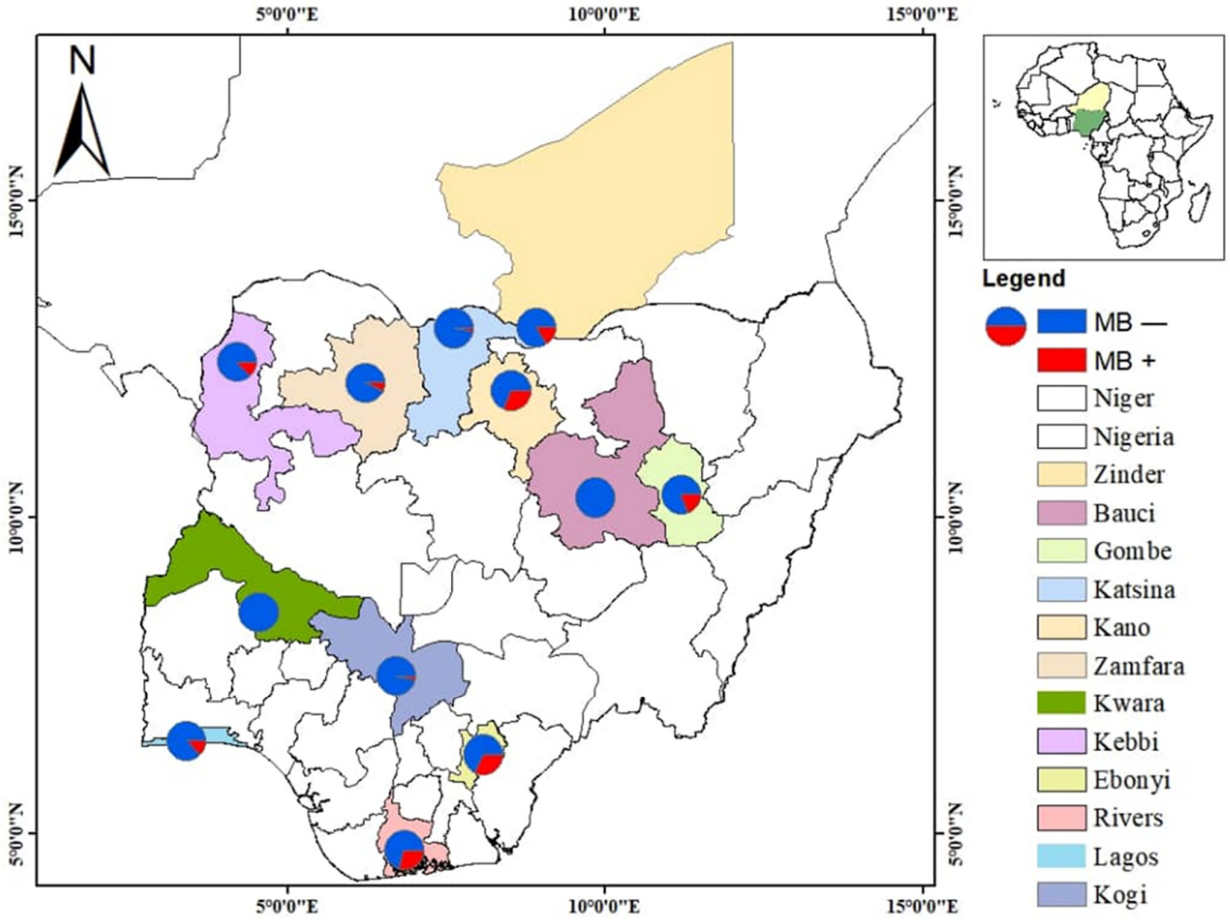


Figure 2. Spatial distribution of *MB* along clinal gradient of aridity spanning Nigeria and Niger.

Infection varied across *Anopheles* species with *An. coluzzii* exhibiting a higher infection rate. A total of 21 out of 133 *An. coluzzii* mosquitoes were infected, translating into prevalence of 15.79% (95% CI: 9.59–21.99%), compared to only 4 out of 71 for *An. gambiae s.s.* (prevalence = 5.63%, 95% CI: 0.27–11.00%) (Table S1, File S2). A statistically significant association was observed between *MB* infection status and mosquito species ($\chi^2 = 4.44$; $df = 1$, $p = 0.035$). Furthermore, when comparing the likelihood of infection between species, *An. coluzzii* had significantly higher odds of being infected with *MB* (odds ratio = 3.14, 95% CI: 1.03–9.54, $p = 0.035$), whereas *An. gambiae s.s.* was predicted to be likely not infected (odds ratio = 0.32, 95% CI: 0.11–0.91). The relationship between *MB* infection status and *Anopheles* species was also analysed using Multiple Correspondence Analysis (Figure 3).

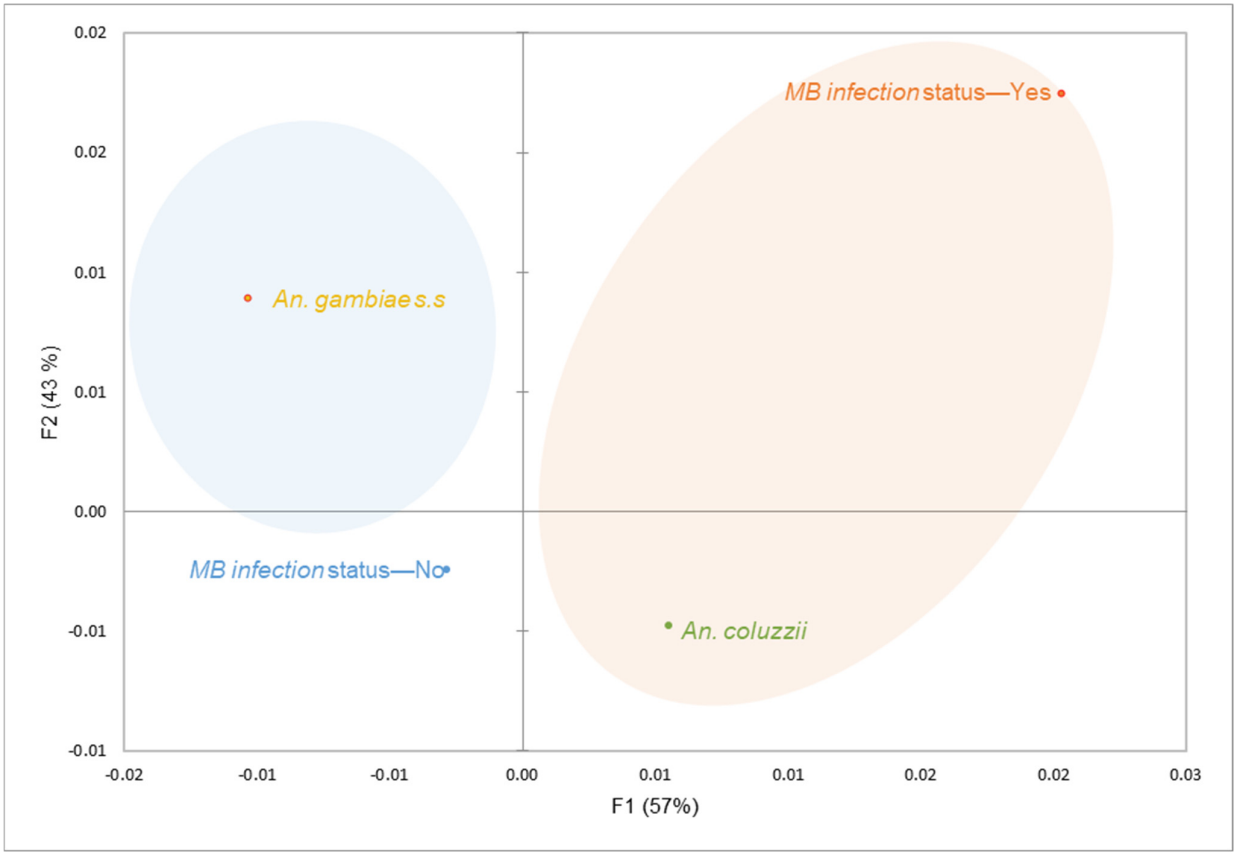


Figure 3. Spatial association between *MB* infection status and *Anopheles* species. *MB* infection status—No = *MB*-negative and *MB* infection status—Yes = *MB*-positive phenotypes.

The MCA plot illustrates the association between *MB* infection status and *Anopheles* species by clustering variable categories with similar profiles on the factor map. The two dimensions (F1 and F2) together explain 100% of the variability in the dataset, with F1 accounting for 57% and F2 explaining 43% of the variance. On the map, *An. coluzzii* and *MB*+ variables clustered together in the right-hand-side quadrant of dimension F1. This clustering indicates that these categories share similar profiles, consistent with the significantly higher prevalence of *MB* infection observed in *An. coluzzii*. Conversely, the *MB*-negative (MB infection status—No) variable and *An. gambiae s.s.* clustered in the left-hand-side quadrant of dimension F2. This separation possibly reflects the lower prevalence of infection in *An. gambiae s.s.* This clear spatial differentiation on the MCA plot corroborates the statistical findings, where *An. coluzzii* exhibited a significantly higher *MB* infection compared to *An. gambiae s.s.*

3.2. Microsporidia MB Infection Probably Correlates with Pyrethroid Resistance

Bioassays revealed resistance in all populations towards pyrethroid insecticides, with the lowest mortalities observed in the *An. coluzzii* populations from Gadau in Bauchi State (deltamethrin mortality = 18.75%), followed by Obio/Akpor in Bauchi State (permethrin mortality = 29.46%) (Figure 4). The lowest resistance was observed for the *An. gambiae* s.s. population from Zariagi, Kogi State (deltamethrin mortality = 85%).

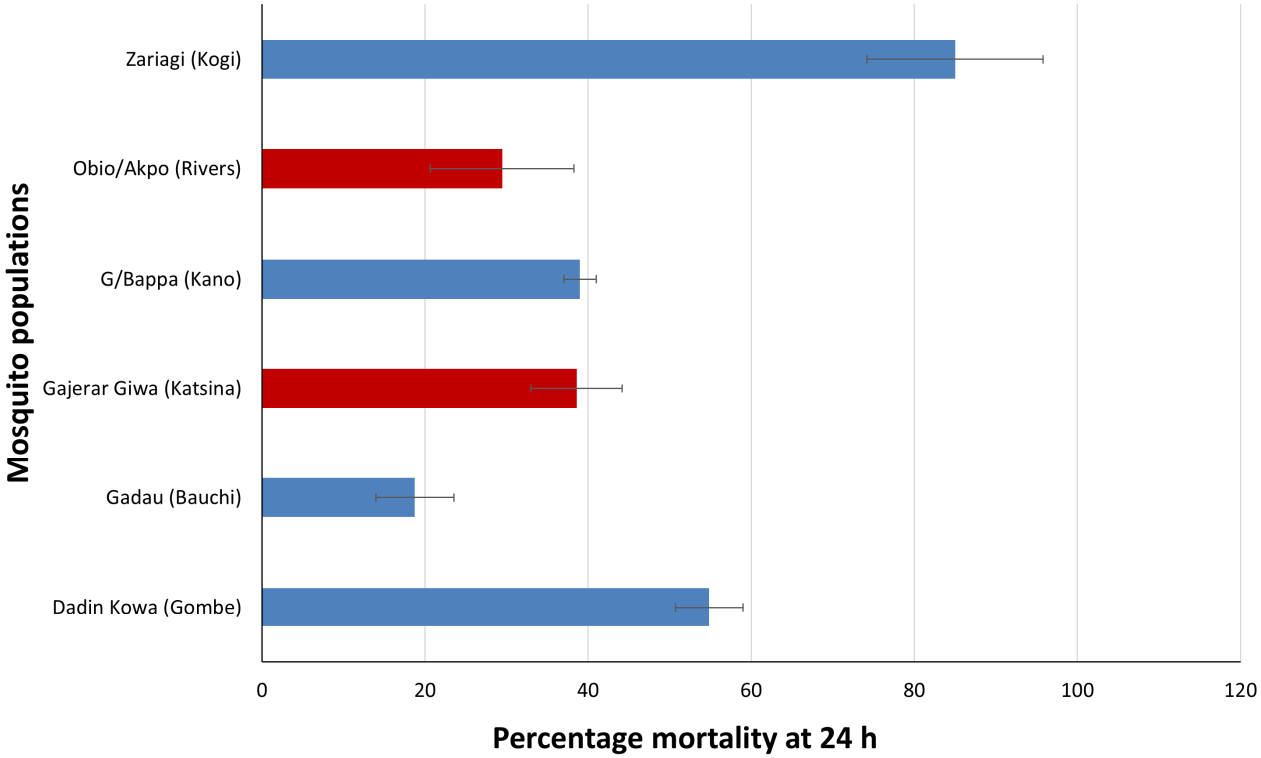


Figure 4. Pyrethroid resistance profiles of F_1 *Anopheles* mosquitoes from contrasting ecological settings. Results are the average of percentage mortalities from four or five replicates of 20–25 mosquitoes each ± standard error of mean (SEM). Blue bars are for deltamethrin and red bars for permethrin.

To test the hypothesis that *MB* infection may be associated with insecticide resistance, a subset of F_1 female mosquitoes previously exposed to pyrethroids were PCR-screened to detect *MB* infection. Of the 112 females tested with pyrethroid, an equal proportion of 50% susceptible (S) and 50% resistant (R) mosquitoes were utilised. Overall, 16 of 56 resistant (R) mosquitoes (28.57%, 95% CI: 16.74–40.40%) were infected with *MB*, compared to 6 of 56 susceptible (S) mosquitoes (10.71%, 95% CI: 2.61–18.82%). These differences in infection were statistically significant ($\chi^2 = 5.65$, $df = 1$, $p = 0.017$). The odds ratio of the likelihood of surviving pyrethroid exposure (R phenotype) when *MB*-infected was significant (OR = 3.33, 95% CI: 1.23–9.03, $p = 0.017$), compared with the non-significant odds ratio of 0.30 (95% CI: 0.11–0.81) observed in susceptible mosquitoes. The MCA plot in Figure 5 depicts the association between *MB* infection status and phenotypes (resistant vs. susceptible) by clustering variable categories with similar profiles. The two dimensions (F1 and F2) explain 100% of the variability, with F1 accounting for 61% and F2 explaining 39% of the variance.

The variables Resistant (R) and *MB*+ (positive infection status) cluster closely in the positive quadrant of F1. This indicates an association between the resistance phenotype and *MB* infection status. In contrast, the Susceptible (S) phenotype and *MB*− (negative infection status) cluster in the negative and positive quadrants of F2, respectively. This suggests an association between low *MB* infection and pyrethroid susceptibility.

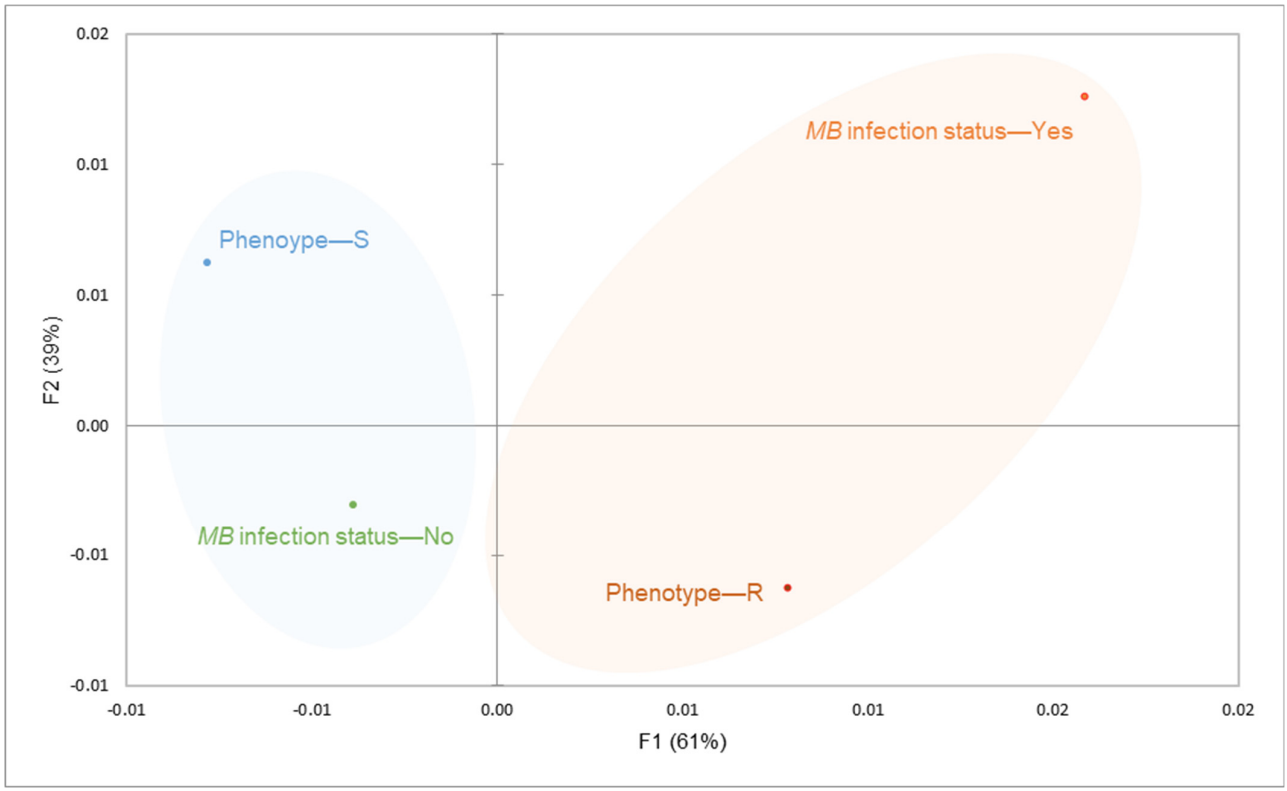


Figure 5. Spatial depiction of potential association between *MB* infection and pyrethroid resistance. *MB* infection status—No = *MB*-negative and *MB* infection status—Yes = *MB*-positive phenotypes.

4. Discussion

The threat of insecticide resistance spurs efforts for alternative control measures, including using biological agents such as *Wolbachia* and *B. thuringiensis*. The discovery of *MB* and the recent description of its ability to reduce *Plasmodium* infection in malaria mosquitoes [34] has catalysed new efforts to understand the bionomics of this promising fungus.

This primary study generates information about the distribution of *MB* in *Anopheles* mosquitoes from contrasting ecological settings and its association with insecticide resistance. *MB* is relatively well established in mosquito populations from Nigeria and Niger, with ten populations testing positive. However, it was not detected in two populations. This observation needs to be confirmed with a larger sample size. Indeed, previous studies have suggested that local and ecological variations influence the presence of *MB* [38,39,47]. Our results show that the differences in *MB* prevalence between mosquito species and geographic locations are possibly shaped by the underlying ecological origins of the mosquitoes. Indeed, humidity, temperature, breeding site characteristics, vegetation cover, and urbanisation can influence mosquito abundance, microbiota composition, and insecticide resistance phenotype [39,48–51]. For instance, the states of Ebonyi and Rivers, where *MB* prevalence was highest, are located in humid, vegetated zones that may support higher microbial diversity. Future studies should integrate detailed ecological and environmental data to disentangle these potential confounding effects.

Across Africa, *MB* appears to be well established in the main malaria vectors. The 12.25% *MB* infection prevalence observed in this study was double the 6.8% (17/251) prevalence reported in *An. coluzzii* in a study in which no infection was observed in *An. gambiae s.s.* from Zinder city in Niger [6]. However, it is lower than the 53.4% prevalence previously reported in Benin [37]. The *MB* prevalence observed in Ghana (1.8%) [38] and

Kenya (1.7%) [47] was much lower than our findings (though fewer mosquito samples were screened in our study).

This study demonstrated a significantly higher prevalence of *MB* in *An. coluzzii* compared to *An. gambiae s.s.* A similar pattern has been described in a previous study from Benin, with a prevalence of 41.0% in *An. gambiae s.s.* versus 57.0% in *An. coluzzii* [37]. Most studies in East Africa indicate that *MB* infects *An. arabiensis* [34,35,47], while in West Africa it primarily infects *An. coluzzii* [6,37,38].

The *MB* infection rates varied significantly across the study sites, reflecting a likely influence of geographic and ecological factors. The highest infection rates were observed in Ebonyi State (a tropical rainforest site) and Rivers State (mangrove swamp) in the wild-caught mosquitoes, possibly because these localities offer particularly favourable conditions for optimal *MB* growth, including high humidity, conducive temperatures, and suitable habitats for optimal development in mosquito vectors. These findings are consistent with a previous study that reported that *MB* prevalence is influenced by species-specific and ecological factors [34]. Similarly, other studies [39,47,52] have documented geographic variation in *MB* prevalence, with higher rates in humid environments.

The higher prevalence of *MB* in pyrethroid-resistant mosquitoes raises important questions about its potential role in insecticide resistance. However, variations in insecticide exposure history between the different populations used in this study could also shape resistance phenotypes independent of *MB* infection. It is also important to acknowledge that the mosquitoes utilised for the bioassays were disproportionately *An. coluzzii*, with a limited number of *An. gambiae s.s.* (only 24 mosquitoes from one population out of the 112 F_1 mosquitoes screened). Future studies should explore within-species variability in *MB* infection between resistant and susceptible mosquitoes, using a stratified approach to confirm the potential association between the infection and resistance phenotype. Several studies have linked the microbiome with insecticide resistance, showing that pyrethroid-resistant mosquitoes harbour distinct *microbiota* compared to the pyrethroid-susceptible strains [28,53]. For example, in the major malaria vector *An. coluzzii*, an abundance of *Asaia* and *Serratia* bacteria has been associated with deltamethrin resistance [54]. Resistant mosquitoes have been found to be enriched with bacteria and enzymes that can degrade insecticides, suggesting that the microbiota may play a role in resistance [28]. It has also been shown that *MB* infection can influence the phenotype of *Anopheles* such as adaptation to varying dietary conditions [55], faster larval development, and higher adult emergence rates [56], all which suggest an impact on various phenotypes. Although few samples were utilised, our finding of a possible association between pyrethroid resistance and *MB* infection highlights the need for further research into its roles in insecticide resistance, particularly towards key malaria control tools, such as long-lasting insecticidal bed nets and the ingredients used for indoor residual spraying. Understanding these may strengthen the management of resistance and improve malaria control efforts, and could lead to novel strategies that use insecticide resistance to facilitate the spread of *MB* through mosquito populations for sustainable malaria control.

5. Conclusions

Microsporidia MB is detected across multiple eco-geographic settings in *Anopheles* mosquitoes across Nigeria, and predominantly in *An. coluzzii* in both Nigeria and Niger. The detection of *MB* in multiple localities and its strong association with *An. coluzzii*, a major vector in West Africa, highlight its potential for biological control. However, more screening of mosquitoes should be conducted with larger sample sizes to confirm this pattern. The finding of a possible association between *MB* infection and the ability to survive insecticide exposure in *Anopheles* mosquitoes in this study is of interest to control

programmes. However, there is a need to conduct further studies, ideally longitudinally, with higher mosquito numbers to confirm this observation. Future research should also incorporate environmental and entomological variables, and explore the functional role of *MB* in insecticide resistance. Such investigations will be essential to assess the feasibility of harnessing *MB* as a biological tool for integrated malaria vector management programmes.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/parasitologia5030031/s1; File S1: Agarose gel of *MB* PCR identification; File S2: Prevalence of *MB* infection across geographical sites and in mosquito species.

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