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Natural *Wolbachia* Infections in *Anopheles funestus* Complex Mosquitoes in Burkina Faso

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Abstract

Wolbachia is a maternally transmitted endosymbiotic bacterium with demonstrated potential for modulating vector competence and suppressing *Plasmodium falciparum* development in *Anopheles* mosquitoes. While naturally occurring *Wolbachia* infections have been reported in *Anopheles gambiae* sensu lato in Burkina Faso, the extent of its distribution among other key malaria vectors, particularly within the *Anopheles funestus* complex remains unclear. This study aimed to assess the presence and prevalence of natural *Wolbachia* infections in wild *An. funestus* complex populations from western Burkina Faso. Adult females were collected from Soumousso and screened for *Wolbachia* using a quantitative PCR assay targeting the 16S rRNA gene (W16S-qPCR). Molecular species identification was conducted on a subsample to resolve intra-group composition. Of 329 individuals identified, *An. funestus* sensu stricto accounted for 50.2% of the sample, with *An. rivulorum-like* (23.4%) and *An. leesoni* (5.8%) also present. A notable proportion of specimens exhibited mixed molecular profiles, indicating possible limitations in marker resolution or interspecific hybridization. Out of 510 specimens tested by qPCR, *Wolbachia* DNA was detected in 178 individuals, yielding an overall infection prevalence of 34.9%. Stratified by species, *An. funestus sensu stricto* exhibited the highest prevalence (36.4%), followed by *An. leesoni* and *An. rivulorum*-like. These findings provide the first molecular evidence of natural *Wolbachia* infections in *An. funestus* complex mosquitoes in Burkina Faso. The observed infection rates, particularly in *An. funestus* s.s., a major malaria vector, underscore the potential epidemiological relevance of this symbiosis. Further genomic and functional characterization of the detected *Wolbachia* strains is essential to

elucidate their phylogenetic origins, tissue tropism, and potential influence on Plasmodium development. Such insights will be critical for evaluating the suitability of *Wolbachia*-based biocontrol strategies in African vector populations where *An. funestus* plays a dominant role in residual malaria transmission.

Keywords

Wolbachia, Natural Infection, *Anopheles funestus*, Malaria, Burkina Faso

1. Introduction

Wolbachia is a maternally inherited intracellular bacterium that infects a wide range of arthropods and some nematodes [1]. In mosquitoes, *Wolbachia* has shown great promise as a symbiont capable of reducing vector competence for *Plasmodium falciparum*, the parasite responsible for the most lethal form of malaria [2]. While transinfected *Wolbachia* strains have been used in pilot malaria control programs, naturally occurring infections in African malaria vectors remain poorly characterized [3]. Previous evidence has demonstrated natural *Wolbachia* infections in *An. gambiae* (s.l.) in Burkina Faso [4], raising the question of whether similar infections occur in other primary vectors such as *An. funestus*, which contributes substantially to residual malaria transmission. This study investigates the prevalence and species-specific distribution of natural *Wolbachia* infections in *An. funestus* complex mosquitoes collected in western Burkina Faso.

2. Materials and Methods

Mosquitoes from the *Anopheles funestus* group were collected in Soumousso (Houet Province, western Burkina Faso) using CDC light traps between June and November 2022. Adult females were morphologically identified and further processed for species-level confirmation using ITS2-PCR molecular assays according to the protocol described by [5], which includes species-specific primers. This approach allows the differentiation of the six members of the *An. funestus* group, including *An. rivulorum-like*, a distinct species within the complex that was first described in Burkina Faso in 2000 and later reported in Cameroon and Zambia.

Total DNA was extracted from individual mosquitoes using the CTAB protocol. The presence of *Wolbachia* was assessed using a qPCR assay targeting the *Wolbachia* 16S rRNA gene (W16S-qPCR), using the primer pair W16S-qPCR_F (CATACCTATTCGAAGGGATAG) and W16S-qPCR_R (TTGCGGGACTTAACCCAACA). qPCR reactions were performed using the 5x HOT FIREPol EvaGreen qPCR Mix Plus (ROX) kit (Solis BioDyne, Tartu, Estonie). The cycling conditions consisted of an initial denaturation at 95˚C for 12 minutes, followed by 40 cycles of 15 s denaturation at 95˚C, 20 s annealing at 58˚C, and 20 s

extension at 72˚C. All reactions were run on QuantStudio 5 (Thermo Fisher Scientific, USA) qPCR platform. Each qPCR plate contained extraction blanks, and a *Wolbachia*-infected mosquito DNA control to monitor contamination and assay performance. Samples with Ct values <35 were considered positif. A subset of samples underwent molecular species identification to determine intra-group composition and assess infection prevalence by species. Statistical analysis was performed to calculate prevalence estimates and 95% confidence intervals.

3. Results

3.1. Specie Composition of the *Anopheles funestus* Complex

The species distribution within the *Anopheles funestus* group revealed a predominance of *An. funestus sensu stricto* (50.15%), followed by *An. rivulorum-like* (23.4%) out of a total of 329 identified mosquitoes. *An. leesoni* was detected at lower proportions (5.8%). Mixed molecular profiles (*An. leesoni/An. funestus*, *An. leesoni/An. rivulorum-like*, *An. rivulorum-like/An. funestus*) were also observed, likely indicating limitations in the molecular identification method (Figure 1).

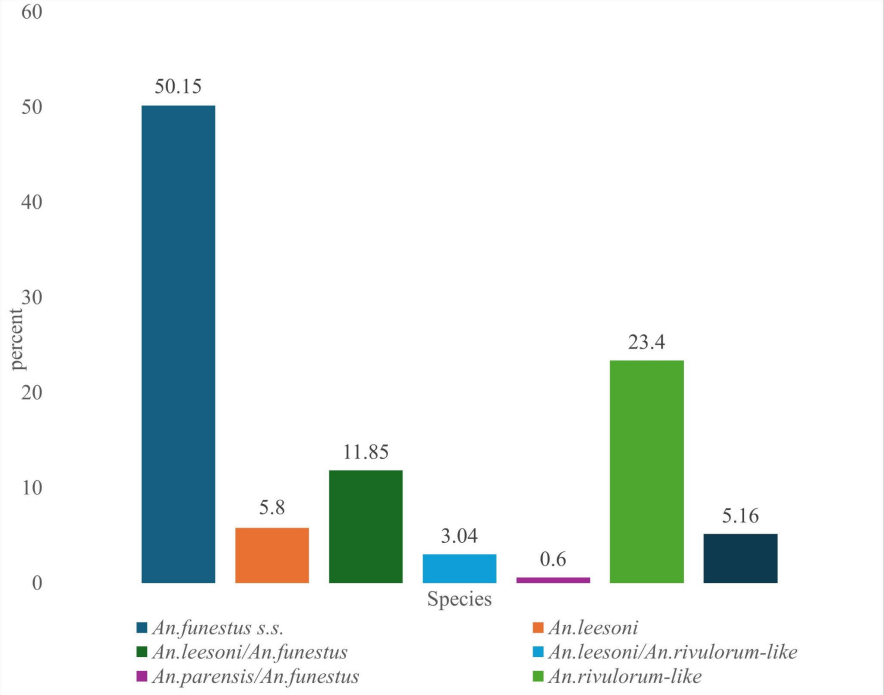

Figure 1. Percentage composition of species *Within the An. funestus Complex*.

3.2. *Wolbachia* Infections in Wild *Anopheles funestus* Populations

Among 510 *An. funestus* group mosquitoes tested using real-time PCR, *Wolbachia* DNA was detected in 178 individuals, corresponding to an infection frequency of 34.9%. Species-specific prevalence rates were 36.36% in *An. funestus s.s.* and 25.97% in *An. rivulorum-like* (Table 1).

Table 1. Prevalence of *Wolbachia* Infection by Species within the *Anopheles funestus* complex.

Mosquito species	Sample size (n)	Prevalence (%) [95% IC]
An. funestus s.s.	165	36.36 (29.03 - 44.2)
An. leesoni	19	26.32 (9.15 - 51.2)
An. rivulorum-like	77	25.97 (16.64 - 37.23)

4. Discussion

This study provides molecular evidence of natural *Wolbachia* infections in *An. funestus* complex mosquitoes in Burkina Faso. The relatively high prevalence observed, particularly in *An. funestus s.s.*, suggests that *Wolbachia* may be more widely distributed among African malaria vectors than previously assumed. This observation is consistent with emerging reports from other regions in sub-Saharan Africa [6]-[8]. This high natural prevalence of *Wolbachia* may reflect a stable ecological association, even though evidence from [9] suggests that it is unlikely to result from long-term coevolution.

Mixed ITS2-PCR profiles were observed in some specimens. These patterns may arise from several methodological limitations inherent to the ITS2 marker. First, cross-amplification can occur among closely related species that share highly similar ITS2 sequences [10] [11]. Second, intragenomic variation within the ribosomal DNA array documented in several *Anopheles* taxa can generate heterogeneous ITS2 copies within a single individual, leading to mixed amplification products [12]. Finally, although rarely reported, hybridization events between sibling species within the *An. funestus* complex cannot be fully excluded and may also contribute to atypical PCR profiles [11]. This underscores the need to refine molecular diagnostic tools for species delineation within the funestus complex.

The higher prevalence of *Wolbachia* observed in *An. funestus sensu stricto* is particularly noteworthy. It is well established that *An. funestus s.s.* is the primary vector species within the *An. funestus* group [13]. Although the mechanisms underlying *Wolbachia-Plasmodium* interactions in *Anopheles* remain poorly understood [14], several studies have shown that certain *Wolbachia* strains can modulate vector competence by affecting mosquito survival, fecundity, or parasite development [15] [16]. In field settings, natural infections have also been associated with reduced sporozoite prevalence or decreased mosquito longevity [2] [17] [18]. Therefore, in a highly anthropophilic and endophilic vector such as *An. funestus s.s.*, even moderate *Wolbachia* effects could have more direct consequences on *Plasmodium* transmission dynamics.

Although this study did not determine the *Wolbachia* strain types or their effects on *Plasmodium* development, the findings support further investigation into the functional role of naturally occurring *Wolbachia* in malaria transmission dynamics. Genomic characterization, tissue tropism analysis, and parasite interaction studies will be essential to evaluate whether these native infections offer

transmission-blocking potential. Such evidence would be critical for informing *Wolbachia*-based strategies as part of integrated malaria control programs

5. Conclusion

We report the presence of natural *Wolbachia* infections in *Anopheles funestus* complex mosquitoes from western Burkina Faso, with a substantial infection rate in *An. funestus sensu stricto*. These findings expand the known host range of *Wolbachia* among African malaria vectors and provide a foundation for future work to assess their utility in malaria control. Continued research is needed to characterize the strains and understand their biological significance.

Ethics Approval and Consent to Participate

Not applicable (mosquito sampling only).

Consent for Publication

All authors' consent to publication.

Availability of Data and Materials

Available upon reasonable request.

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Authors' contributions

MRdS, AD, MKG and EB design the study. MRdS EJG performed the assays and MRdS and EB wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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