Microsporidia MB is Found Predominantly Associated with Anopheles Gambiae s.s and Anopheles Coluzzii in Ghana

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Microsporidia MB is found predominantly associated with *Anopheles gambiae* s.s and *Anopheles coluzzii* in Ghana

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Abstract

A vertically transmitted microsporidian, *Microsporidia MB*, with the ability to disrupt *Plasmodium* development was reported in *Anopheles arabiensis* from Kenya, East Africa. To demonstrate its range of incidence, archived DNA samples from 7575 *Anopheles* mosquitoes collected from Ghana were screened. *MB* prevalence was observed at 1.8%. *An. gambiae* s.s constituted 87% of positive mosquitoes while the remaining were from *An. coluzzii*. Both sibling species had similar positivity rates (24% and 19%; p= 0.42) despite the significantly higher number of *An. gambiae* s.s analysed (*An. gambiae* s.s= 487; *An. coluzzii*= 94; p= 0.0005). The microsporidian was also more prevalent in field-collected larvae than adults (p< 0.0001) suggestive of an efficient vertical transmission and/or horizontal transfer among larvae. This is the first report of *Microsporidia MB* in *Anopheles* mosquitoes in West Africa. It indicates possible widespread among malaria vector species and warrants investigations into the symbiont's diversity across the sub-Saharan Africa.

Introduction

Mosquitoes remain very important vectors of human disease, and several efforts are being made to reduce the burden they pose to human and animal health. As the progress of chemical-based vector control interventions is continually threatened, there is increased focus on various other strategies including biotechnological ones for disease control [1]. Therefore, the search for natural mosquito-associated symbionts with the ability to reduce vector competence has been a growing interest.

It has recently been demonstrated that a vertically transmitted microsporidian prevalent in *An. arabiensis* in Kenya disrupts *Plasmodium* development [2]. Contrary to other mosquito-associated microsporidians, *Microsporidia MB* does not confer any significant negative effect on fertility, fecundity, development, and longevity of its host

[2–4]. These characteristics make *Microsporidia MB* an appealing candidate for control of parasite transmission in *Anopheles* mosquitoes.

The study by Herren and colleagues revealed the prevalence of *Microsporidia MB* only in *An. arabiensis* [2]. However, malaria is transmitted by a wide range of Anopheline species with different ecological ranges and varied efficiencies in transmitting *Plasmodium* [5,6]. In this study, we sought to extend the geographical range and identification of *Microsporidia MB* to other *Anopheles* species. We screened archived *Anopheles* mosquito DNA from field collections of several unrelated projects to detect the existence of *Microsporidia MB* among *Anopheles* populations in Ghana. To the best of our knowledge, this is the first study since the discovery of this microsporidian in *An. arabiensis* in Kenya that has investigated its incidence in different *Anopheles* mosquito species and from another geographically distant African population.

Results

Microsporidia MB detected in *An. gambiae* s.s and *An. coluzzii*

Microsporidia MB was detected in 133 DNA samples. However, accounting for the number of individual mosquitoes that went into pools for DNA extraction, a total of 7575 individual mosquitoes were involved in this analysis (Fig 1), giving an overall *MB* prevalence of 1.8%. *Microsporidia MB* was identified only in DNA from *An. gambiae* s.s and *An. coluzzii*; the former constituting 87.2% of *MB*-positive mosquitoes (Fig 2; Supplementary Table S1). The distribution of *MB* infection among *An. gambiae* s.s and *An. coluzzii* was 23.9% and 19.3%, respectively (Fig 3) and this rate did not differ significantly between the two sibling species (χ^2=0.66; p=0.42). *MB* was only detected in mosquitoes from 3 southern regions of Ghana (Fig 4).

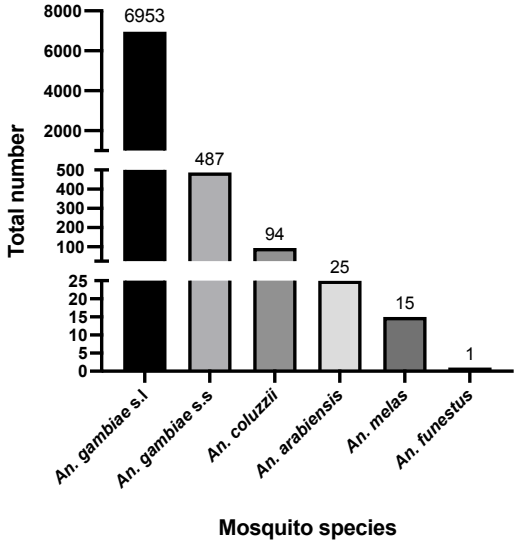

Fig 1: The total number of each member of *Anopheles gambiae* complex analysed. The overall total number of mosquitoes involved in the analysis was N=7575.

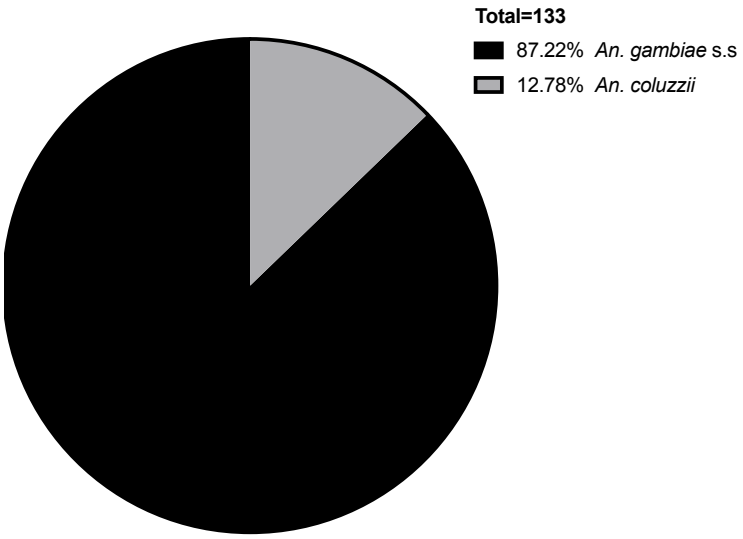

Fig 2: Pie chart showing the contribution of *An. gambiae* s.s and *An. coluzzii* to overall *MB*-positivity. The number of positive *An. gambiae* s.s and *An. coluzzii* was 116 and 17, respectively.

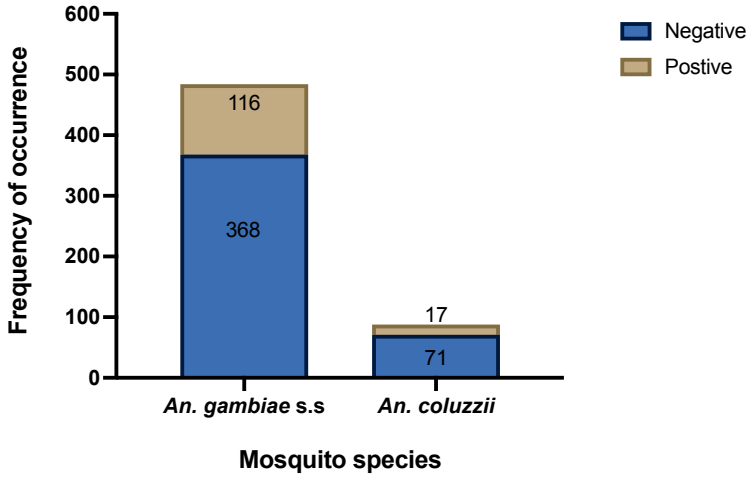

Fig 3: Microsporidia positivity rate in *An. gambiae* s.s and *An. coluzzii*. Numbers within the bar plot show the total number of mosquitoes observed for each status.

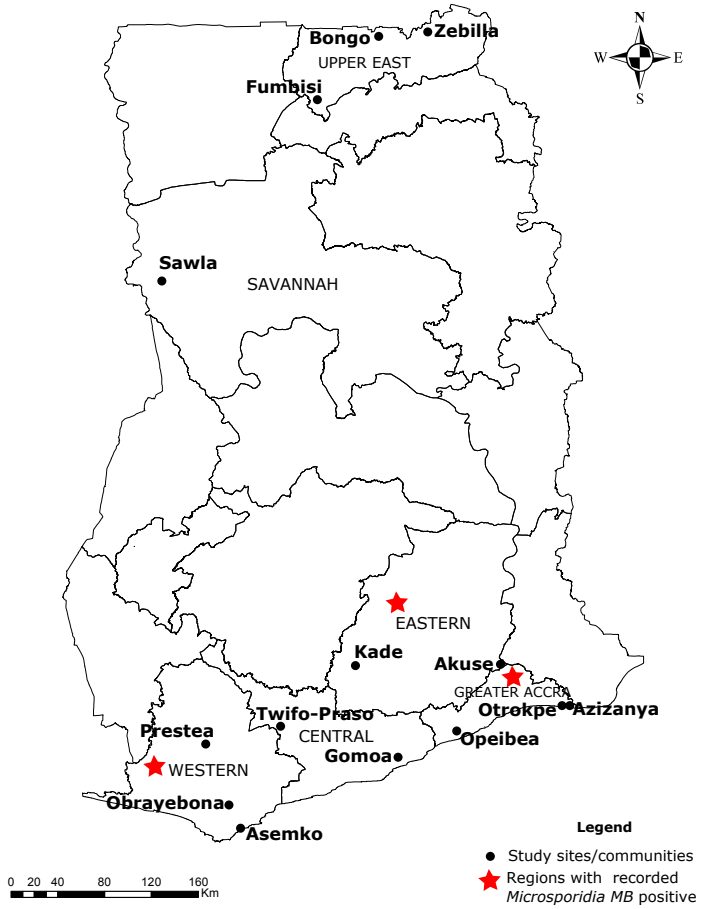

Fig 4: Map of Ghana showing study sites where mosquitoes were collected. Only the regions (6 out of 16) where the study sites are located are named in upper cases. Study sites are named in bold. Red stars depict regions where MB positive mosquitoes were recorded.

Higher MB detection rate in field-caught larvae than adults

The DNA samples used in this study comprised those extracted from adults that emerged from field-collected larvae and those that were collected from the field as adults. We were interested in establishing whether infections were more likely to be identified in field-caught larvae or adults. For this analysis, *An. arabiensis*, *An. melas* and *An. funestus* were excluded since they did not show positive infection. Of the remaining 7534 *An. gambiae* s.l mosquitoes, ~90% were field-caught adults. However, prevalence of *MB* was significantly higher in larvae (χ^2= 1092; p<0.0001) which showed a positivity rate of 17.3% versus 0.13% of field-caught adults.

Discussion

We make the first report of *Microsporidia MB* in *An. gambiae* s.s and *An. coluzzii* following identification of the symbiont in *An. arabiensis*. This does not only demonstrate the existence of the microsporidian in another predominant malaria vector species in Africa but also extends its incidence from East to West Africa. The

prevalence of *MB*-positive mosquitoes was estimated to be 1.8%, which is within the rate of <1-9% reported for *An. arabiensis* [2]. The present study took advantage of archived mosquito DNA samples which were either collected from the field as larvae or adults from different study sites over 5 years.

Anopheles gambiae s.s and *An. coluzzii* are the predominant malaria vectors in Ghana [7–9]. They are often found in sympatry where one is usually more abundant [9,10]. Contrary to the study by Herren and colleagues [2], a handful of *An. arabiensis* was analysed in the present study. *Anopheles arabiensis* is more commonly found in the arid north of Ghana where rainfall is observed within a few months in a year. In studies conducted in Ghana that are focused on *Anopheles* distribution, between 2-3% are *An. arabiensis* despite collection of large numbers of mosquitoes [7,11]. However, we acknowledge that there were more collections from the south of the country, especially Greater Accra, which contributed 40% of the DNA samples used in this study and 89% of *MB*-positive mosquitoes. Further studies to investigate variations in mosquito species density and seasonal prevalence of *Microsporidia MB* will shed more light on on the field dynamics of the symbiont in these mosquito populations.

DNA samples from mosquitoes initially collected as larvae from the field showed significantly higher *MB*-positivity than those collected as adults. Microsporidians can be transmitted both vertically and horizontally [12]. The efficiency with which *MB* is transmitted vertically depends on the intensity in the ovaries of the female parent. It also appears that horizontal transfer is also more likely to occur in the larval habitat [2] thereby increasing the probability of finding larvae that are infected. Given that collecting large numbers of adult mosquitoes in the field may prove more challenging than larval sampling, the latter has also shown to have the advantage of increasing chances of finding *MB* infections in a population. It remains yet to be demonstrated why prevalence is low among adult mosquitoes while in larvae it could be as high [2] as observed in this study.

While the data presented here shows basic information about the ecological spread of *Microsporidia MB*, it has nonetheless demonstrated a potential widespread occurrence of *Microsporidia MB* among *Anopheles* mosquitoes across sub-Saharan Africa. It warrants further investigation of the diversity, environmental dynamics, and interactions with other mosquito symbionts for a clearer understanding of their possible use in malaria control.

Methods

Description of Anopheles samples

We retrieved *Anopheles* mosquito DNA samples from various studies that have been conducted at the Noguchi Memorial Institute for Medical Research, University of Ghana between 2014 - 2019. Mosquitoes had been collected from different sites across the country either as larvae or adults. When collected as larvae, they were reared to adults for experimental assays before DNA was extracted. Most DNA

samples were from single female mosquitoes while few were extracted from pools of 25 (Supplementary Table S1). The species information on the samples were retrieved from the different projects. Where the mosquitoes were only identified as *An. gambiae* s.l, these were further assessed for their sibling species identification using Restriction Fragment Length Polymorphism (RFLP) [13] or SINE [14] methods when they were positive for *Microsporidium MB* infection.

Quality check of archived samples

Different extraction methods, including CTAB, Trizol RNA/DNA and columns had been used by the various projects to obtain DNA. It was therefore expected that the DNA integrity would differ among samples. The samples would have also gone through some freeze-thaw cycles which would compromise the quality of the DNA and result in false negatives. To address this concern, we randomly selected samples for DNA quantification with Qubit Fluorometer (Thermofisher, UK) and purity using the BioDrop (Biochrom, UK). Average DNA concentration was 34.8ng/μL (1.02 - 204ng/μ) and average purity was 1.7 (1.14 – 2.17). To reduce potential contaminants that would limit PCR, DNA samples were diluted 1 in 10.

Detection of Microsporidia MB

A total of 1158 DNA samples were screened for *Microsporidia MB* using MB18SF/ MB18SR primers [2]and with *Microsporidia MB* DNA obtained from Jeremy Herren's lab as positive control in each set of reactions run. Diluted DNA samples were used in a first round of PCR reactions. The PCR included in the final reaction 1X One-Taq Master mix, 0.4μM of each primer and 1μL of DNA template. The cycling conditions were as described in [2]. Products were loaded and run on a 2% agarose gel stained with SYBR Safe DNA stain (Invitrogen) and viewed under a blue-light transilluminator. The PCR reaction was repeated for samples that showed no band for *Microsporidia MB* using an increased volume of the diluted DNA and/or using 1μL of the stock DNA sample to confirm the initial results. The band size for *Microsporidia MB* detection was ~500bp (Supplementary Fig S1).

Statistical analyses

Contingency analyses to compare independence observed positivity between mosquito species was performed using a two-sided Chi-squared test with Yate's correction. The distribution of *An. gambiae* s.s and *An. coluzzii* from the study sites was tested with a two-tailed unpaired Mann-Whitney non-parametric test. All test significance was accepted a $p<0.05$.

References

1. Jones, R. T., Ant, T. H., Cameron, M. M. & Logan, J. G. Novel control strategies for mosquito-borne diseases. *Philosophical Transactions of the Royal Society B: Biological Sciences* **376**, 20190802 (2021).

2. Herren, J. K. *et al.* A microsporidian impairs Plasmodium falciparum transmission in Anopheles arabiensis mosquitoes. *Nature Communications* **11**, 2187 (2020).
3. Agnew, P., Bedhomme, S., Haussy, C. & Michalakis, Y. Age and size at maturity of the mosquito Culex pipiens infected by the microsporidian parasite Vavraia culicis. *Proceedings of the Royal Society B: Biological Sciences* **266**, 947–952 (1999).
4. Undeen, A. H. & Alger, N. E. The effect of the microsporidan, Nosema algerae, on Anopheles stephensi. *Journal of Invertebrate Pathology* **25**, 19–24 (1975).
5. Wiebe, A. *et al.* Geographical distributions of African malaria vector sibling species and evidence for insecticide resistance. *Malaria Journal* **16**, 85 (2017).
6. Sinka, M. E. *et al.* A global map of dominant malaria vectors. *Parasites and Vectors* **5**, 69 (2012).
7. de Souza, D., Kelly-Hope, L., Lawson, B., Wilson, M. & Boakye, D. Environmental factors associated with the distribution of Anopheles gambiae s.s in Ghana; an important vector of lymphatic filariasis and malaria. *PLoS One* **5**, e9927 (2010).
8. Mattah, P. A. D. *et al.* Diversity in breeding sites and distribution of Anopheles mosquitoes in selected urban areas of southern Ghana. *Parasites and Vectors* **10**, 1–15 (2017).
9. Kudom, A. A. Larval ecology of Anopheles coluzzii in Cape Coast, Ghana: water quality, nature of habitat and implication for larval control. *Malaria journal* **14**, 447 (2015).
10. Akorli, J. *et al.* Seasonality and Locality Affect the Diversity of Anopheles gambiae and Anopheles coluzzii Midgut Microbiota from Ghana. *PLoS ONE* **11**, e0157529 (2016).
11. Hamid-Adiamoh, M. *et al.* Insecticide resistance in indoor and outdoor-resting Anopheles gambiae in Northern Ghana. *Malaria Journal* **19**, 314 (2020).
12. ANDREADIS, T. G. & HALL, D. W. Development, Ultrastructure, and Mode of Transmission of Amblyospora sp. (Microspora) in the Mosquito. *The Journal of Protozoology* **26**, 444–452 (1979).
13. Fanello, C., Santolamazza, F. & della Torre, a. Simultaneous identification of species and molecular forms of the Anopheles gambiae complex by PCR-RFLP. *Medical and veterinary entomology* **16**, 461–4 (2002).
14. Santolamazza, F. *et al.* Insertion polymorphisms of SINE200 retrotransposons within speciation islands of Anopheles gambiae molecular forms. *Malaria Journal* **7**, (2008).

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Author contributions

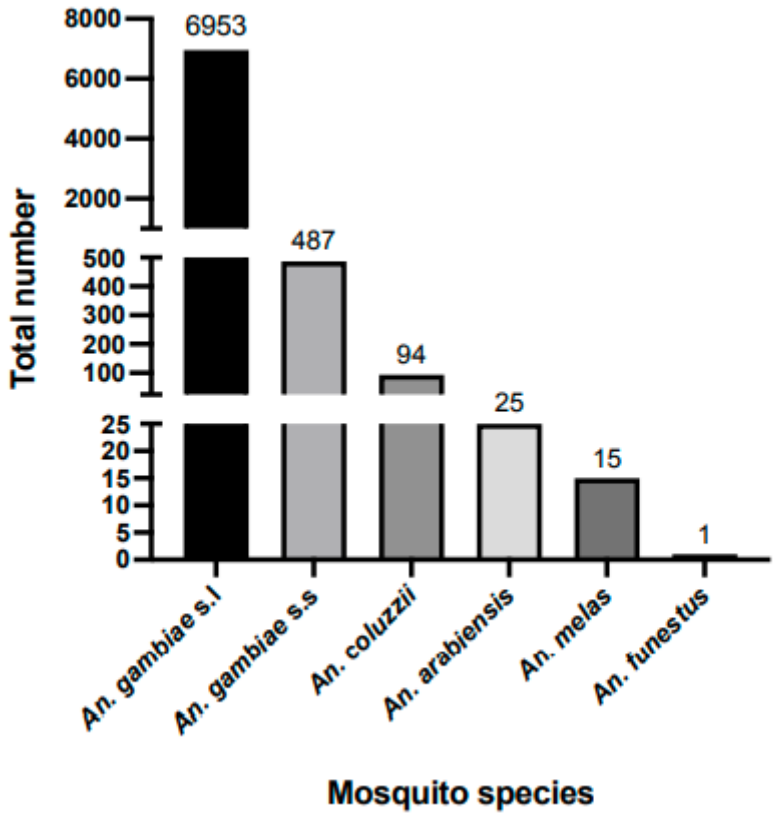
JA conceived and co-ordinated the study. EAA, ST screened all samples for microsporidian infection. GA, RP, MA, DA, SP-B, JC, SKD carried out or co-ordinated field collection and archiving of the mosquitoes used in the study. JA analysed the

data and wrote the manuscript. All authors reviewed, edited and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Figures



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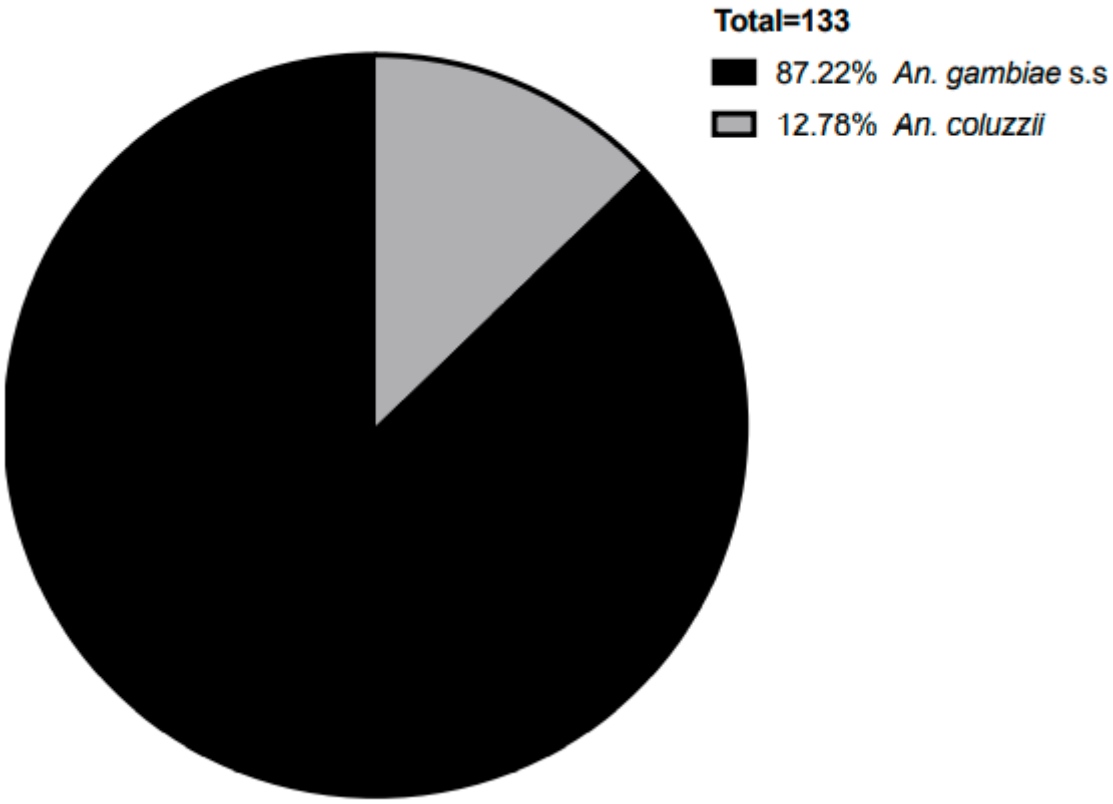

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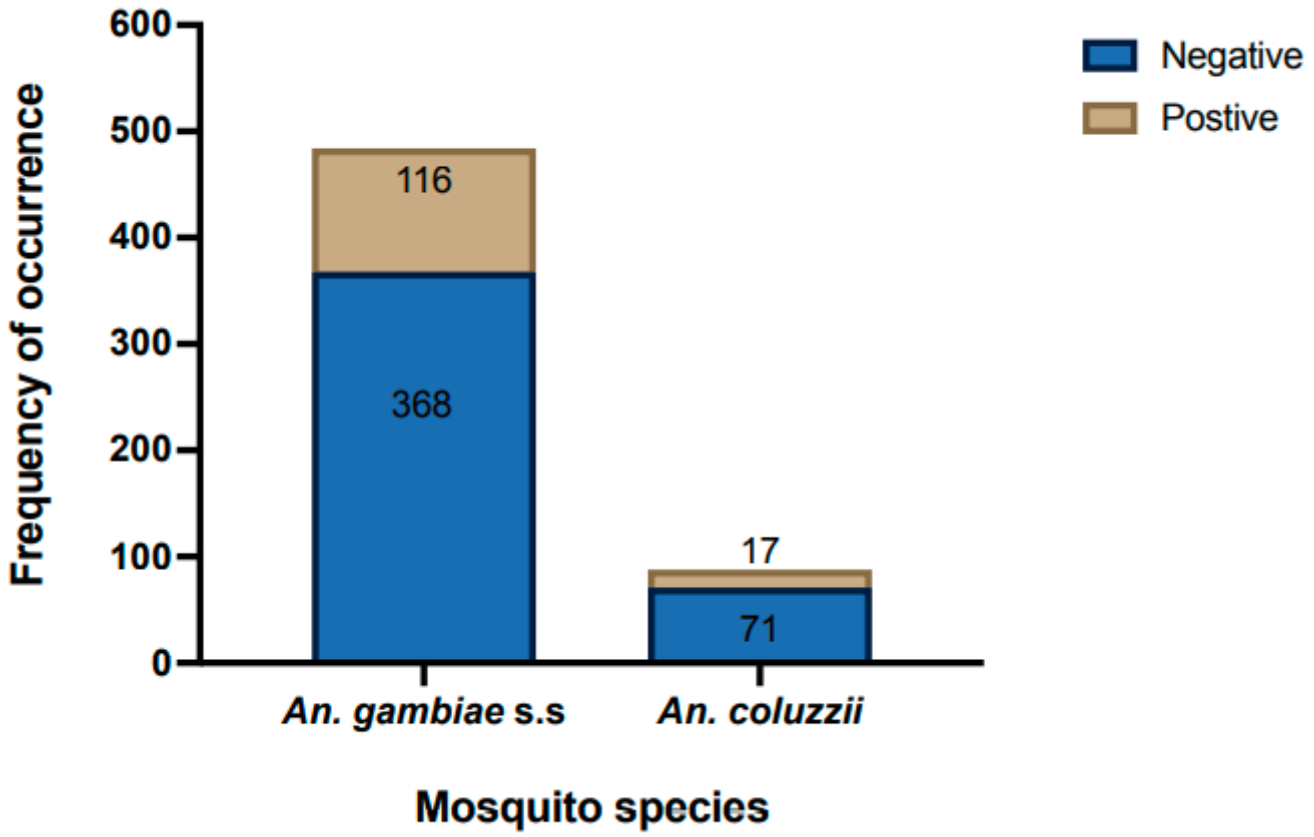

Figure 3

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Figure 4

Map of Ghana showing study sites where mosquitoes were collected. Only the regions (6 out of 16) where the study sites are located are named in upper cases. Study sites are named in bold. Red stars depict regions where MB positive mosquitoes were recorded. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its

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