

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

Jewelna Akorli (✉ jakorli@noguchi.ug.edu.gh)

Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana

Esinam Akorli

Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana

Seraphim Tetteh

Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana

Godwin Amlalo

Vestergaard-NMIMR Vector Labs, Noguchi Memorial Institute for Medical Research, University of Ghana

Rebecca Pwalia

Vestergaard-NMIMR Vector Labs, Noguchi Memorial Institute for Medical Research, University of Ghana

Michelle Adimazoya

Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana

Dorcas Atibilla

Entomology Unit, Kintampo Health Research Centre

Sellase Pi-Bansa

Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana

Joseph Chabi

Vestergaard-NMIMR Vector Labs, Noguchi Memorial Institute for Medical Research, University of Ghana

Samuel Dadzie

Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana

Research Article

Keywords: Microsporidia MB, *Anopheles Gambiae* s.s, *Anopheles Coluzzii*, Plasmodium development

Posted Date: May 21st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-540732/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

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

3
4 Jewelna Akorli^{1*}, Esinam Abla Akorli¹, Seraphim Naa Afoley Tetteh¹, Godwin Kwame
5 Amlalo², Rebecca Pwalia², Michelle Adimazoya¹, Dorcas Atibilla³, Sellase Pi-Bansa¹,
6 Joseph Chabi², Samuel K. Dadzie¹

7
8 1. Department of Parasitology, Noguchi Memorial Institute for Medical Research,
9 University of Ghana, P. O. Box LG 581, Legon, Accra, Ghana

10 2. Vestergaard-NMIMR Vector Labs, Noguchi Memorial Institute for Medical
11 Research, University of Ghana, P. O. Box LG 581, Legon, Accra, Ghana

12 3. Entomology Unit, Department of Clinical Laboratory, Kintampo Health Research
13 Centre, P.O. Box 200, Kintampo, Ghana

14
15 *Correspondence: jakorli@noguchi.ug.edu.gh; jewelna.akorli@gmail.com

16
17 **Abstract**

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

31
32
33 **Introduction**

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

40
41 It has recently been demonstrated that a vertically transmitted microsporidian
42 prevalent in *An. arabiensis* in Kenya disrupts *Plasmodium* development ². Contrary to
43 other mosquito-associated microsporidians, *Microsporidia MB* does not confer any
44 significant negative effect on fertility, fecundity, development, and longevity of its host

45 2-4. These characteristics make *Microsporidia MB* an appealing candidate for control
46 of parasite transmission in *Anopheles* mosquitoes.

47

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

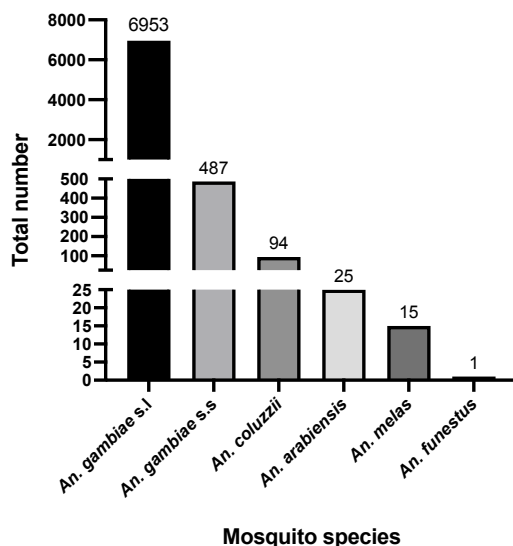
58

59 Results

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

61 *Microsporidia MB* was detected in 133 DNA samples. However, accounting for the
62 number of individual mosquitoes that went into pools for DNA extraction, a total of
63 7575 individual mosquitoes were involved in this analysis (Fig 1), giving an overall *MB*
64 prevalence of 1.8%. *Microsporidia MB* was identified only in DNA from *An. gambiae*
65 s.s and *An. coluzzii*; the former constituting 87.2% of *MB*-positive mosquitoes (Fig 2;
66 Supplementary Table S1). The distribution of *MB* infection among *An. gambiae* s.s
67 and *An. coluzzii* was 23.9% and 19.3%, respectively (Fig 3) and this rate did not differ
68 significantly between the two sibling species ($\chi^2=0.66$; $p=0.42$). *MB* was only detected
69 in mosquitoes from 3 southern regions of Ghana (Fig 4).

70

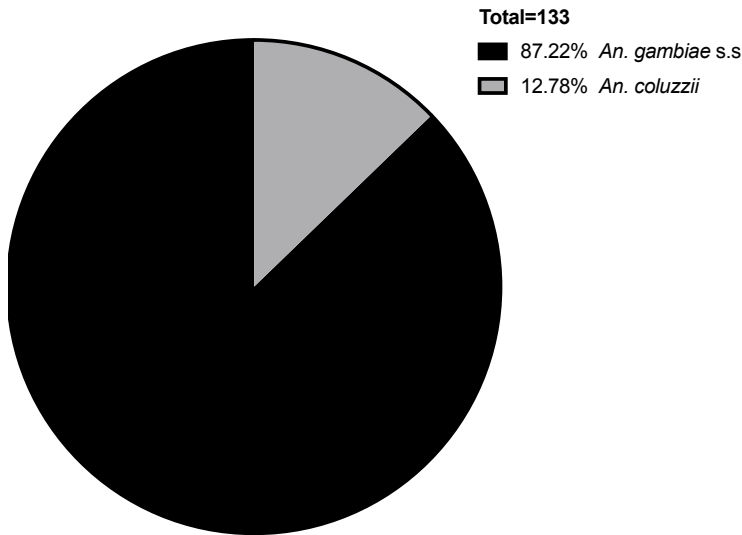


71

72

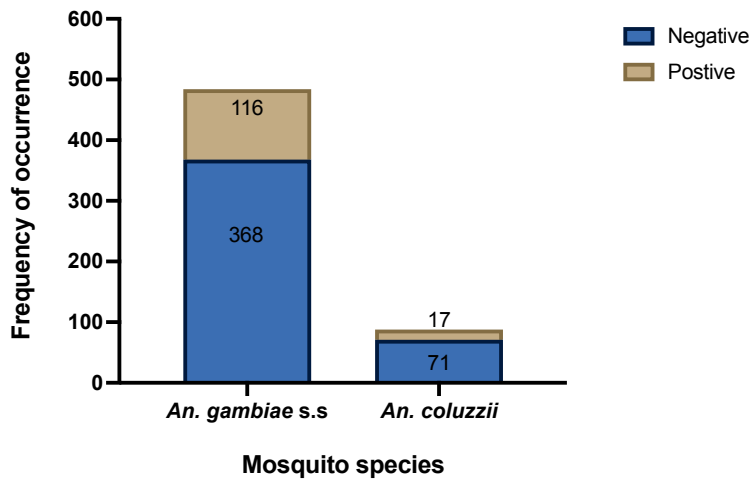
73 **Fig 1: The total number of each member of *Anopheles gambiae* complex**
74 **analysed.** The overall total number of mosquitoes involved in the analysis was
75 **N=7575.**

76



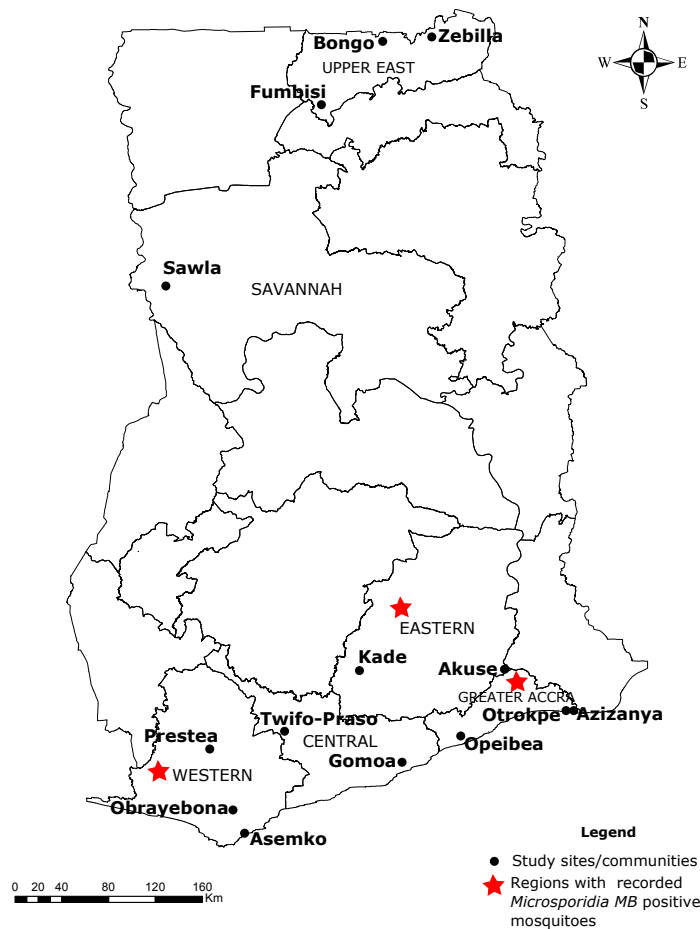
77
78
79
80
81
82

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.



83
84
85
86
87

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.



88
89

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

94

95 *Higher MB detection rate in field-caught larvae than adults*

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

104

105 **Discussion**

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

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

114

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

127

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

139

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

146

147 **Methods**

148 *Description of Anopheles samples*

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

154 samples were from single female mosquitoes while few were extracted from pools of
155 25 (Supplementary Table S1). The species information on the samples were retrieved
156 from the different projects. Where the mosquitoes were only identified as *An. gambiae*
157 s.l, these were further assessed for their sibling species identification using Restriction
158 Fragment Length Polymorphism (RFLP) ¹³ or SINE ¹⁴ methods when they were
159 positive for *Microsporidium MB* infection.

160

161 *Quality check of archived samples*

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

171

172 *Detection of Microsporidia MB*

173 A total of 1158 DNA samples were screened for *Microsporidia MB* using MB18SF/
174 MB18SR primers ² and with *Microsporidia MB* DNA obtained from Jeremy Herren's lab
175 as positive control in each set of reactions run. Diluted DNA samples were used in a
176 first round of PCR reactions. The PCR included in the final reaction 1X One-Taq
177 Master mix, 0.4μM of each primer and 1μL of DNA template. The cycling conditions
178 were as described in ². Products were loaded and run on a 2% agarose gel stained
179 with SYBR Safe DNA stain (Invitrogen) and viewed under a blue-light transilluminator.
180 The PCR reaction was repeated for samples that showed no band for *Microsporidia*
181 *MB* using an increased volume of the diluted DNA and/or using 1μL of the stock DNA
182 sample to confirm the initial results. The band size for *Microsporidia MB* detection was
183 ~500bp (Supplementary Fig S1).

184

185 *Statistical analyses*

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

191

192 **References**

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

- 196 2. Herren, J. K. *et al.* A microsporidian impairs Plasmodium falciparum
197 transmission in Anopheles arabiensis mosquitoes. *Nature Communications* **11**,
198 2187 (2020).
- 199 3. Agnew, P., Bedhomme, S., Haussy, C. & Michalakis, Y. Age and size at
200 maturity of the mosquito Culex pipiens infected by the microsporidian parasite
201 Vavraia culicis. *Proceedings of the Royal Society B: Biological Sciences* **266**,
202 947–952 (1999).
- 203 4. Undeen, A. H. & Alger, N. E. The effect of the microsporidan, Nosema algerae,
204 on Anopheles stephensi. *Journal of Invertebrate Pathology* **25**, 19–24 (1975).
- 205 5. Wiebe, A. *et al.* Geographical distributions of African malaria vector sibling
206 species and evidence for insecticide resistance. *Malaria Journal* **16**, 85 (2017).
- 207 6. Sinka, M. E. *et al.* A global map of dominant malaria vectors. *Parasites and*
208 *Vectors* **5**, 69 (2012).
- 209 7. de Souza, D., Kelly-Hope, L., Lawson, B., Wilson, M. & Boakye, D.
210 Environmental factors associated with the distribution of Anopheles gambiae
211 s.s in Ghana; an important vector of lymphatic filariasis and malaria. *PLoS One*
212 **5**, e9927 (2010).
- 213 8. Mattah, P. A. D. *et al.* Diversity in breeding sites and distribution of Anopheles
214 mosquitoes in selected urban areas of southern Ghana. *Parasites and Vectors*
215 **10**, 1–15 (2017).
- 216 9. Kudom, A. A. Larval ecology of Anopheles coluzzii in Cape Coast, Ghana:
217 water quality, nature of habitat and implication for larval control. *Malaria journal*
218 **14**, 447 (2015).
- 219 10. Akorli, J. *et al.* Seasonality and Locality Affect the Diversity of Anopheles
220 gambiae and Anopheles coluzzii Midgut Microbiota from Ghana. *PLoS ONE*
221 **11**, e0157529 (2016).
- 222 11. Hamid-Adiamoh, M. *et al.* Insecticide resistance in indoor and outdoor-resting
223 Anopheles gambiae in Northern Ghana. *Malaria Journal* **19**, 314 (2020).
- 224 12. ANDREADIS, T. G. & HALL, D. W. Development, Ultrastructure, and Mode of
225 Transmission of Amblyospora sp. (Microspora) in the Mosquito. *The Journal of*
226 *Protozoology* **26**, 444–452 (1979).
- 227 13. Fanello, C., Santolamazza, F. & della Torre, a. Simultaneous identification of
228 species and molecular forms of the Anopheles gambiae complex by PCR-
229 RFLP. *Medical and veterinary entomology* **16**, 461–4 (2002).
- 230 14. Santolamazza, F. *et al.* Insertion polymorphisms of SINE200 retrotransposons
231 within speciation islands of Anopheles gambiae molecular forms. *Malaria*
232 *Journal* **7**, (2008).
- 233
234

235 Acknowledgements

236 We thank Jeremy Herren, ICIPE, Kenya for sharing DNA sample of *Microsporida MB*
237 which was used as positive control.

238

239 Author contributions

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

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

245

246 **Competing interests**

247 The authors declare no competing interests.

248

249

Figures

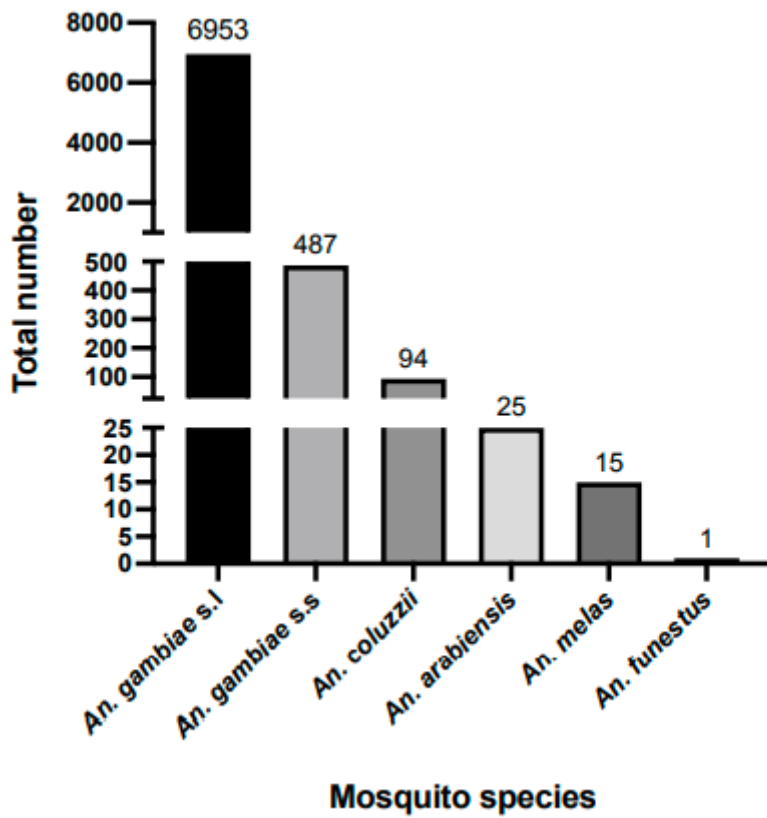


Figure 1

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

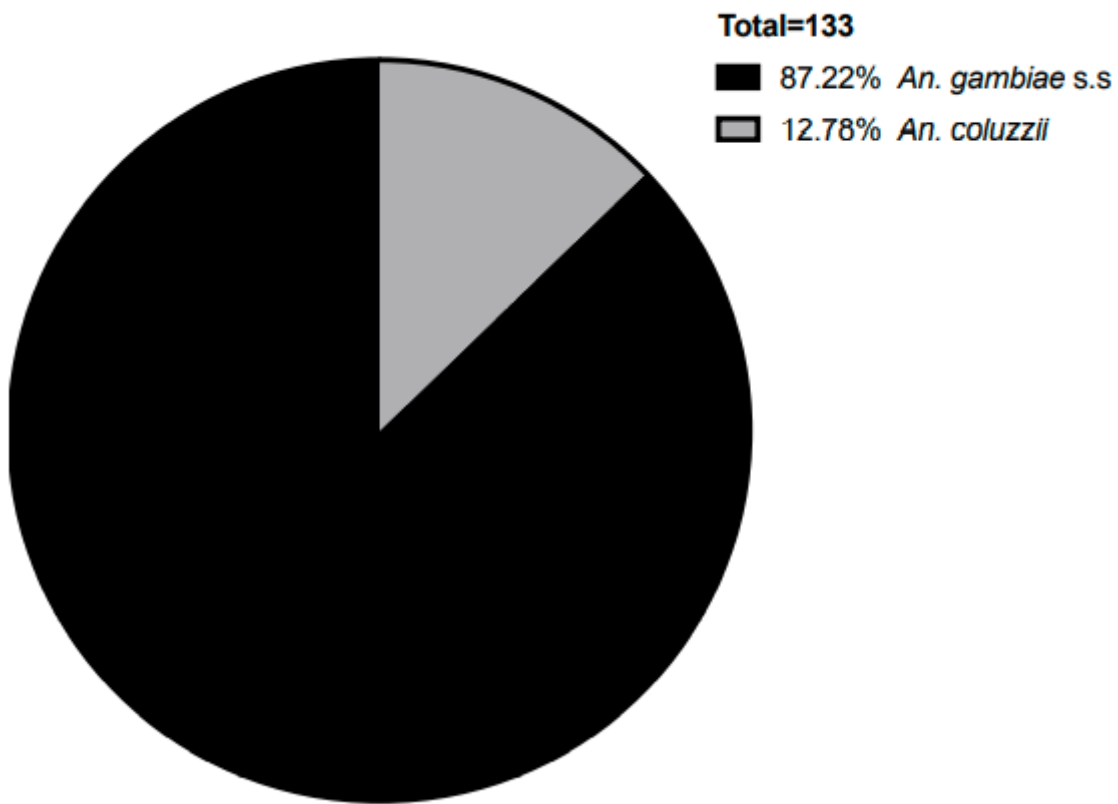


Figure 2

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.

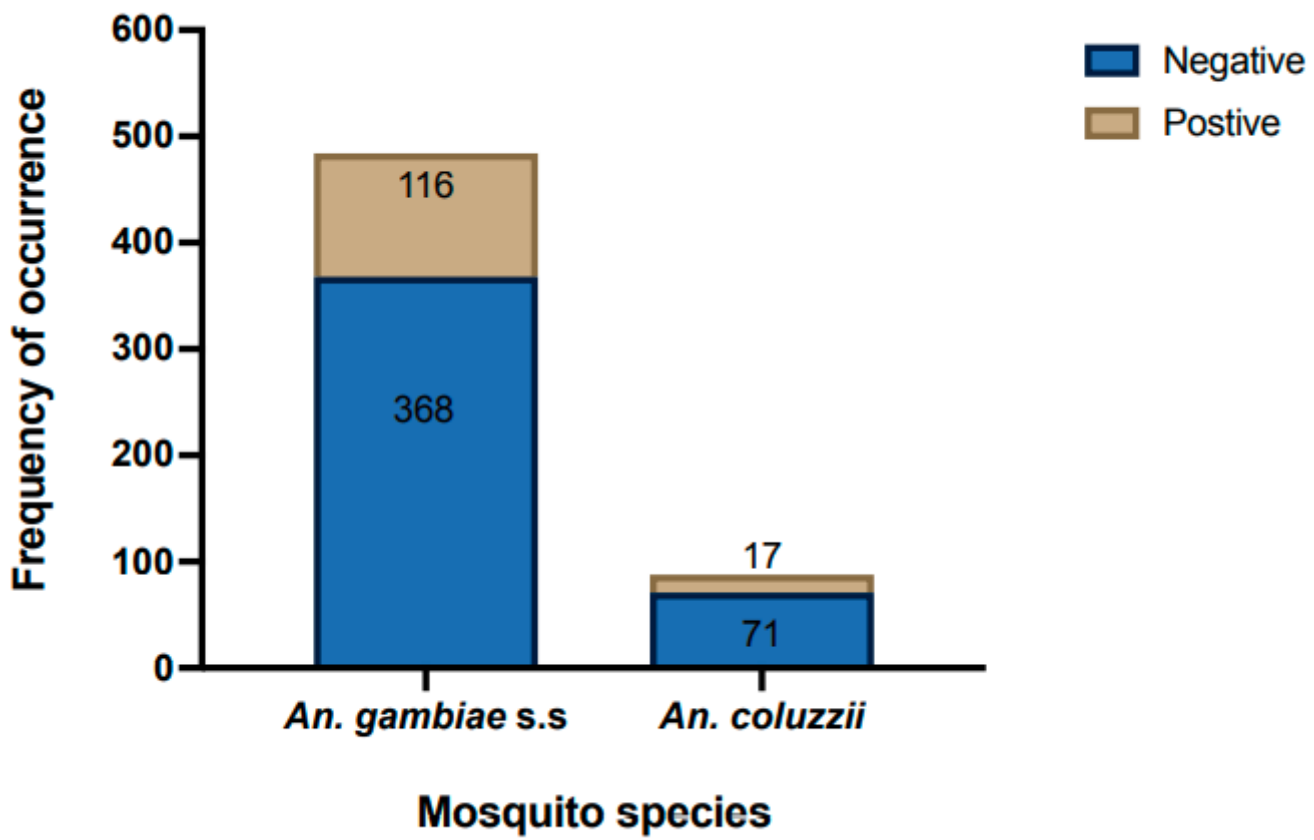


Figure 3

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.

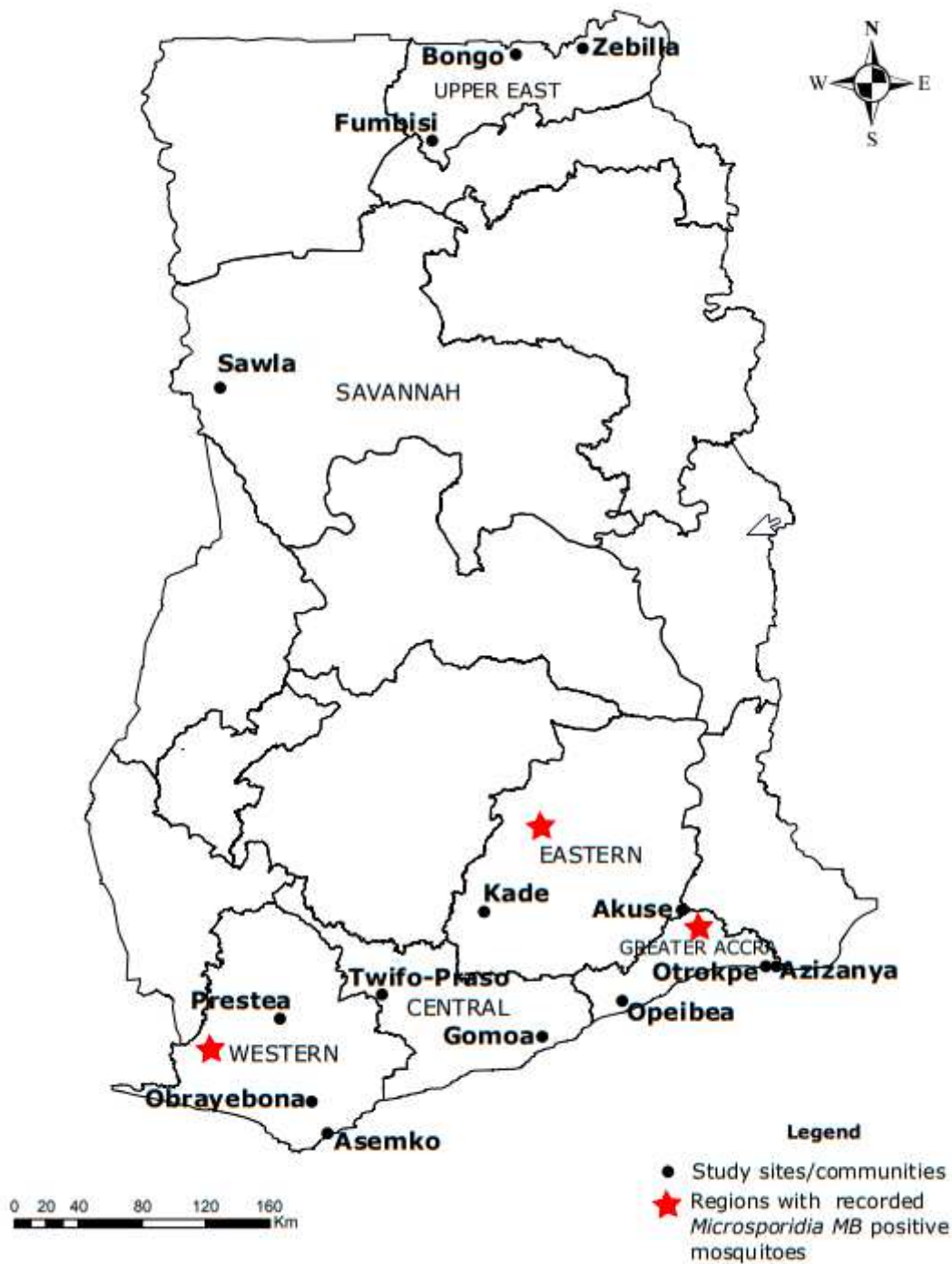


Figure 4

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

authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaryinformation.docx](#)