medical sciences forum

MDPI

Proceeding Paper

Species Composition, Diversity and Insecticide Susceptibility of *Anopheles* Mosquitoes in Bushbuckridge, an Endemic Malaria Transmission Setting in South Africa †

Thabo Mashatola [1,2,*], Basil D. Brooke [1,2], Zuziwe Mthiyane [2,3], Shüné V. Oliver [1,2], Jaishree Raman [1,2,4] and Givemore Munhenga [1,2]

[1] Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases, Division of the National Health Laboratory Service, Johannesburg 2131, South Africa; basilb@nicd.ac.za (B.D.B.); shuneo@nicd.ac.za (S.V.O.); jaishreer@nicd.ac.za (J.R.); givemorem@nicd.ac.za (G.M.)
[2] Wits Research Institute for Malaria, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2193, South Africa; nyawozuziwe@gmail.com
[3] Malaria Control Programme, Mpumalanga Department of Health, Mbombela 1200, South Africa
[4] UP Institute for Sustainable Malaria Control, Faculty of Health Sciences, University of Pretoria, Pretoria 0007, South Africa
* Correspondence: rhinethabo@gmail.com
† Presented at the 2026 International Online Conference on Tropical Medicine and Infectious Disease (CTMID 2026), 1–2 July 2026; Available online: https://sciforum.net/event/CTMID2026.

Abstract

Bushbuckridge, South Africa, remains a challenging setting for malaria elimination due to residual transmission and diverse *Anopheles* populations. This study assessed *Anopheles* species diversity and insecticide susceptibility across 29 localities between 2022 and 2024. Morphological and PCR-based identification revealed 18 taxa, primarily *Anopheles pretoriensis*, *An. quadriannulatus*, and *An. rufipes*, and the primary vector *An. arabiensis* accounted for 7.1% of collections. Two putative *An. arabiensis*/*An. quadriannulatus* hybrids were detected, warranting further genomic investigation. While vector populations were largely susceptible to DDT, deltamethrin, and pirimiphos-methyl, reduced DDT susceptibility was observed in the *An. funestus* group; however, due to small sample sizes, this result remains inconclusive. These findings underscore the necessity of sustained, integrated vector surveillance to inform strategic control in complex transmission landscapes.

Keywords: *Anopheles arabiensis*; *Anopheles* mosquitoes; insecticide susceptibility; malaria vectors; residual transmission; species diversity; South Africa; vector surveillance

1. Introduction

Malaria remains a primary global public health challenge, with approximately 94% of cases and 95% of deaths occurring in sub-Saharan Africa [1]. In South Africa, transmission is primarily driven by *Anopheles arabiensis* Patton, 1905, with secondary contributions from species such as *An. merus* Dönitz, 1902, *An. vaneedeni* Gillies & Coetzee, 1987, *An. parensis* Gillies & de Meillon, 1968, and potentially *An. funestus* Giles, 1900 [2]. These vectors are largely restricted to the low-altitude regions of the Limpopo, Mpumalanga and KwaZulu-Natal provinces [3].

Despite long-standing vector control interventions, focal residual transmission persists in several areas, including the Bushbuckridge Local Municipality in Mpumalanga. This

Check for updates

Academic Editor: Constantinos Tsioutis

Published: 21 September 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

region presents a complex transmission landscape, characterized by ecological heterogeneity, diverse *Anopheles* populations, and proximity to higher-burden districts in Limpopo and neighbouring Mozambique [2]. The municipality encompasses a mosaic of rural settlements, agricultural zones and conservation areas, which provide a wide array of breeding sites capable of supporting both primary and secondary vector species. Identifying the full spectrum of vectors present, particularly lesser-known species that may sustain residual transmission, is essential for optimizing current control strategies.

Furthermore, the emergence and spread of insecticide resistance remain significant threats to the efficacy of malaria interventions. While widespread resistance to pyrethroids and organochlorines has been documented among malaria vectors across sub-Saharan Africa [4], the resistance profile in South Africa is more nuanced. Previous reports have documented resistance in *An. funestus sensu stricto* [5] and low-level resistance in *An. arabiensis* [6], though these levels have historically been considered operationally insignificant. Given the potential for shifting resistance patterns, continuous entomological surveillance is critical to inform insecticide selection and maintain control effectiveness.

This study aimed to comprehensively assess the species composition, diversity, and insecticide susceptibility profiles of *Anopheles* mosquitoes in Bushbuckridge. By generating this baseline data, we seek to support more precise entomological surveillance and evidence-informed vector control planning in this endemic setting.

2. Materials and Methods

2.1. Study Area

The study was conducted in Bushbuckridge Local Municipality, Mpumalanga, South Africa (Figure 1a,b). The municipality shares borders with Limpopo province, Eswatini and Mozambique, covering approximately 1 million hectares of diverse landscapes, including rural settlements, agricultural zones, and conservation areas adjacent to the Kruger National Park.

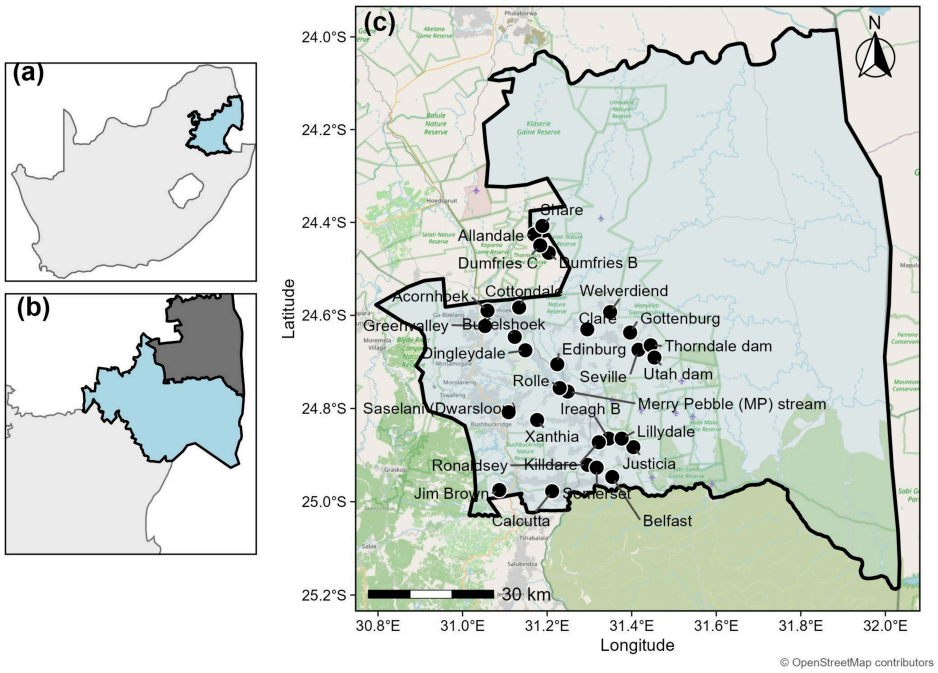


Figure 1. Mosquito collection sites in Bushbuckridge Local Municipality, Mpumalanga, South Africa. (**a**) Mpumalanga province within South Africa; (**b**) Ehlanzeni District and Bushbuckridge Local Municipality within Mpumalanga; and (**c**) distribution of the 29 mosquito collection sites within Bushbuckridge Local Municipality, shown over an OpenStreetMap base layer. Black circles indicate mosquito collection localities. © OpenStreetMap contributors.

While study sites were initially identified through a random selection framework to ensure broad spatial coverage, actual sampling visits were conducted opportunistically between June 2022 and April 2024, contingent on site accessibility and the presence of standing water. Consequently, the sampling effort and collection frequency varied across the 29 localities. Diversity indices reported here provide a snapshot of the local *Anopheles* assemblage rather than a strictly standardized quantitative assessment of temporal trends.

2.2. Larval Collection and Rearing

Larvae were collected from aquatic habitats (springs, streams, marshes, roadside puddles, irrigation canals) (Figures 1c and A1) using standard dipping techniques [7,8]. Collected larvae were transported to the Botha DeMeillon Insectary at the National Institute for Communicable Diseases in Johannesburg, and reared under standard insectary conditions (25 ± 2 °C room temperature, 80 ± 5% relative humidity, 12 h photoperiod). Larvae were fed a standardized diet consisting of three parts, by weight, finely crushed peanut-butter-flavoured dog biscuits (West's Beeno Traditional Crunchy Biscuit Treats; Martin and Martin, Kempton Park, South Africa) and one part brewer's yeast (Vital Health Foods, Cape Town, South Africa), with the quantity of food adjusted according to larval developmental stage, following established rearing protocols [9,10]. Upon pupation, pupae were collected and transferred into BugDorm cages (30 × 30 × 30 cm; MegaView Science Co., Ltd., Taichung, Taiwan) for adult emergence. All adult mosquitoes used in susceptibility assays were F0 generation specimens, 2–5 days post-emergence, provided with 10% sugar solution and maintained for 2–5 days prior to insecticide susceptibility bioassays.

2.3. Insecticide Susceptibility Testing

Standard World Health Organisation (WHO) tube bioassays were performed using insecticide-impregnated papers (dichlorodiphenyltrichloroethane (DDT) 4%, deltamethrin 0.05%, pirimiphos-methyl 0.25%; sourced from Universiti Sains Malaysia) [11]. The insecticide papers were validated for insecticidal toxicity using a susceptible *An. arabiensis* laboratory strain (100% mortality).

Bioassays utilized batches of 20–25 non-blood-fed adult mosquitoes per tube, with a minimum of four replicates per insecticide and control group. Both male and female mosquitoes were included to maximize data acquisition from the limited F0 progeny obtained from field-collected larvae. Following the 60 min exposure, mosquitoes were transferred to holding tubes, and mortality was recorded after 24 h.

2.4. Species Identification

All adults were identified morphologically using dichotomous keys [12]. Specimens identified as *An. gambiae* complex or *An. funestus* group were further analyzed using species-specific Polymerase Chain Reaction (PCR) assays [13,14]. Samples that failed to amplify were re-tested twice.

2.5. Data Analysis

Species composition and relative abundance were determined for each locality. Species richness, Shannon (H′), Simpson (D), and Pielou's evenness (J) indices were calculated per locality. Insecticide susceptibility was analyzed by grouping specimens into three categories: *An. gambiae* complex, *An. funestus* group, and "other *Anopheles*" species. Mortality percentages were calculated for each *Anopheles* species group and insecticide combination and classified as susceptible (98–100%), possible resistance (90–97%), or resistance (<90%), according to WHO criteria [11]. Results for the *An. funestus* group ($n < 100$) are reported as "inconclusive" to avoid misinterpretation of potential resistance. Controls consistently

showed <5% mortality; therefore, correction using Abbott's formula was not required. Statistical analyses were performed in R 4.3.1, with significance set at $p < 0.05$.

3. Results

3.1. Species Composition and Diversity

A total of 4454 adult *Anopheles* emerged, representing 18 distinct taxa (Table 1, Figure A2). *Anopheles pretoriensis* Theobald, 1903 was most abundant (33.4%, *n* = 1487), followed by *An. quadriannulatus* Theobald, 1911, (20.7%, *n* = 921) and *An. rufipes* Gough, 1910, (20.0%, *n* = 890). The primary vector, *An. arabiensis* accounted for 7.1% (*n* = 316) of total collections. Other secondary vectors, including *An. merus* Dönitz, 1902, *An. vaneedeni* Gillies & Coetzee, 1987, and *An. parensis* Gillies & de Meillon, 1968) were present at lower frequencies. Notably, two specimens were identified as putative *An. arabiensis*/*An. quadriannulatus* hybrids (0.004%). PCR amplification was unsuccessful in 3% of *An. gambiae* complex and 16% of *An. funestus* group specimens.

Table 1. *Anopheles* species composition and relative abundance from the Bushbuckridge Local Municipality, Mpumalanga, South Africa, stratified by species and sex. Relative abundance is expressed as a percentage of the total collected over the study period (*n*).

Species	*n*	Relative Abundance (%)
An. pretoriensis	1487	33.4
An. quadriannulatus	921	20.7
An. rufipes	890	20
An. maculipalpis	320	7.2
An. arabiensis	316	7.1
An. coustani	185	4.2
An. squamosus	116	2.6
An. vaneedeni	98	2.2
An. gambiae complex [1]	36	0.8
An. demeilloni	31	0.7
An. funestus group [1]	21	0.5
An. marshalli	13	0.3
An. rivulorum	7	0.2
An. merus	5	0.1
An. leesoni	3	0.1
An. parensis	2	0.04
An. arabiensis/*An. quadriannulatus hybrid*	2	0.04
An. tenebrosus	1	0.02
Total	4454	100
Sex distribution		
Males	2011	45.1
Females	2443	54.9
Total	4454	100

[1] Did not amplify following molecular testing.

Diversity indices showed marked spatial variation in species across the 29 localities (Table 2). Species richness ranged from 2 (Cottondale, Dumfries B) to 11 (Ronaldsey). The

Shannon index was highest at Saselani (Dwarsloop) (H′ = 1.894) and was lowest at Killdare Bridge (H′ = 0.256).

Table 2. Diversity indices (species richness (R), Shannon Index (H′), Simpson Index (D), Pielou's evenness index (J)) of *Anopheles* mosquitoes collected from the Bushbuckridge Local Municipality, Mpumalanga, South Africa from June 2022–April 2024.

Locality	Species Richness (R)	Shannon Index (H′)	Simpson Index (D)	Pielou's Evenness Index (J)
Acornhoek	6	0.907	0.428	0.506
Allandale	10	0.869	0.363	0.377
Belfast	6	1.081	0.516	0.603
Buffelshoek	5	1.222	0.632	0.759
Calcutta	8	1.396	0.686	0.672
Clare	3	0.746	0.504	0.679
Cottondale	2	0.429	0.26	0.619
Dingleydale	5	0.499	0.221	0.31
Dumfries B	2	0.367	0.211	0.529
Dumfries C	10	1.214	0.576	0.527
Edinburg	3	0.736	0.406	0.67
Gottenburg	7	0.93	0.42	0.478
Greenvalley	5	0.389	0.163	0.242
Ireagh B	4	0.84	0.452	0.606
Jim Brown	5	1.421	0.725	0.883
Justicia	7	0.896	0.428	0.46
Killdare bridge	3	0.256	0.125	0.233
Lillydale dam	4	0.852	0.535	0.614
Merry Pebble (MP) stream	4	0.929	0.513	0.67
Rolle	6	0.979	0.511	0.546
Ronaldsey	11	1.538	0.702	0.641
Saselani (Dwarsloop)	9	1.894	0.822	0.862
Seville	10	1.41	0.698	0.612
Share	9	1.495	0.728	0.68
Somerset	6	0.818	0.375	0.457
Thorndale dam	5	0.864	0.471	0.537
Utah dam	3	0.777	0.461	0.708
Welverdiend	8	1.197	0.591	0.576
Xanthia	9	0.959	0.476	0.437

3.2. Insecticide Susceptibility

Susceptibility results are summarized in Table 3 and Figure A3. *Anopheles gambiae* complex demonstrated high susceptibility to all three insecticides: 99.8% mortality to DDT, 99.7% to deltamethrin, and 100% to pirimiphos-methyl. Similarly, the "Other *Anopheles*" group showed high susceptibility: 99% to DDT, 98.8% to deltamethrin, and 98.8% to pirimiphos-methyl. These mortality rates all exceeded the WHO threshold (≥98%).

Table 3. WHO insecticide susceptibility of adult Anopheles mosquitoes by species group, insecticide, and sex.

Taxonomic Group	Insecticide	Sex	*n*	n_{rep}	Mortality Rate (%)	SD	95% CI (Lower)	95% CI (Upper)	Status *
An. funestus group †	DDT	Female	55	3	98.2	13.5	94.6	100	Inconclusive ($n < 100$)
An. funestus group †	DDT	Male	37	2	94.6	22.9	87.2	100	Inconclusive ($n < 100$)
An. funestus group †	Deltamethrin	Female	13	1	100	0	100	100	Inconclusive ($n < 100$)
An. funestus group †	Deltamethrin	Male	10	1	100	0	100	100	Inconclusive ($n < 100$)
An. funestus group †	Pirimiphos-methyl	Female	15	1	100	0	100	100	Inconclusive ($n < 100$)
An. funestus group †	Pirimiphos-methyl	Male	1	1	100	NA	NA	NA	Inconclusive ($n < 100$)
An. gambiae complex	DDT	Female	320	13	99.7	5.6	99.1	100	Susceptible
An. gambiae complex	DDT	Male	308	13	100	0	100	100	Susceptible
An. gambiae complex	Deltamethrin	Female	208	9	100	0	100	100	Susceptible
An. gambiae complex	Deltamethrin	Male	180	8	99.4	7.5	98.4	100	Susceptible
An. gambiae complex	Pirimiphos-methyl	Female	144	6	100	0	100	100	Susceptible
An. gambiae complex	Pirimiphos-methyl	Male	120	5	100	0	100	100	Susceptible
Other *Anopheles*	DDT	Female	959	40	98.7	11.1	98	99.5	Susceptible
Other *Anopheles*	DDT	Male	755	31	99.2	8.9	98.6	99.8	Susceptible
Other *Anopheles*	Deltamethrin	Female	432	18	99.5	6.8	98.9	100	Susceptible
Other *Anopheles*	Deltamethrin	Male	462	19	98.1	13.8	96.8	99.3	Susceptible
Other *Anopheles*	Pirimiphos-methyl	Female	297	12	98.3	12.9	96.9	99.8	Susceptible
Other *Anopheles*	Pirimiphos-methyl	Male	138	6	99.3	8.5	97.9	100	Susceptible

n_{rep} = number of replicates. CI = Confidence Interval. WHO Susceptibility Criteria (per 24 h observation): ≥98% mortality = Susceptible; 90–97% mortality = Possible Resistance; <90% mortality = Confirmed Resistance. * Status based on WHO criteria and sample size considerations. † Inconclusive: Sample size < 100 per WHO guidelines, despite high overall mortality. NA = not applicable (single specimen).

Specimens in the *An. funestus* group showed 100% mortality to both deltamethrin and pirimiphos-methyl. However, mortality to DDT was 96.4%. Following WHO guidelines, this result is classified as "Inconclusive" due to the limited sample size ($n < 100$), and thus cannot be definitively interpreted as resistance.

4. Discussion

This study provides a comprehensive assessment of the *Anopheles* species assemblage in Bushbuckridge, a setting characterized by persistent residual malaria transmission. Our findings reveal a diverse community, including the primary vectors *An. arabiensis*, several secondary vectors (*An. merus*, *An. vaneedeni*, *An. parensis*), and species not currently recognized as malaria vectors [15–17]. The dominance of *An. pretoriensis* and *An. quadriannulatus*, species often considered secondary or non-vectors, raises questions regarding their potential role in local transmission dynamics, particularly in scenarios where primary vector populations are suppressed by intervention.

The detection of two putative *An. arabiensis*/*An. quadriannulatus* hybrids (0.004%) is a noteworthy sentinel event. Hybridization within the *An. gambiae* complex can potentially alter phenotypic traits, such as host preferences or vector competence, which may complicate control efforts [18,19]. These findings are preliminary; future high-resolution genomic confirmation is required to determine the identity of these specimens and to assess the prevalence of hybridization in the region.

Regarding insecticide susceptibility assays confirmed susceptibility, our results confirm that *An. gambiae* complex and other *Anopheles* species remain highly susceptible to DDT, deltamethrin, and pirimiphos-methyl across the species groups, consistent with recent studies conducted in other regions of South Africa [6,20]. This supports the continued utility of these insecticides for indoor residual spraying in Bushbuckridge. The 96.4% mortality observed for the *An. funestus* group when exposed to DDT is noteworthy; however, because the sample size was below the WHO-recommended threshold ($n < 100$), this result is classified as inconclusive. Consequently, we emphasize the need for continued, targeted surveillance of the *An. funestus* group to monitor for potential resistance development.

The inclusion of male mosquitoes in our bioassays provided an exploratory assessment of mortality trends. While we acknowledge that physiological and behavioral differences between sexes could influence outcomes, we observed minimal sexual dimorphism in mortality across species groups. This aligns with recent reports of comparable insecticide susceptibility between sexes in *An. coluzzii* Coetzee & Wilkerson, 2013 [21]. Integrating male mosquitoes into resistance monitoring is increasingly relevant due to emerging male-targeted genetic control strategies, such as the sterile insect technique and gene drives [22,23]. Although our findings support the potential for monitoring male susceptibility, these data remain preliminary. Further research is required to validate whether male mosquitoes can serve as reliable, statistically robust proxies for females in operational programs, ensuring that any deviation from established WHO protocols is grounded in rigorous validation.

Finally, several limitations of this study should be acknowledged. Although our sampling strategy was geographically broad, it was opportunistic and contingent on site accessibility, which may have resulted in uneven spatial and seasonal representation. Molecular identification of some specimens within the *An. gambiae* complex and the *An. funestus* group remained unresolved, thereby limiting taxonomic resolution [24,25]. In addition, environmental characteristics of breeding sites were not systematically recorded, and the restriction of sampling to Bushbuckridge limits the generalisability of the findings to other regions of South Africa. Furthermore, the aggregation of species into groups for susceptibility testing, while a pragmatic necessity for baseline surveillance, may mask

species-specific resistance profiles. Future research should prioritize systematic, multi-seasonal environmental monitoring to better elucidate the drivers of species diversity and integrate advanced molecular tools to enhance the taxonomic resolution of the *Anopheles* community in South Africa.

5. Conclusions

This assessment of *Anopheles* diversity and insecticide susceptibility in Bushbuckridge provides critical baseline information for malaria vector surveillance in a setting with residual focal transmission. The detection of a diverse vector community, including secondary and non-traditional vectors, coupled with high baseline susceptibility to key insecticides, underscores the importance of integrated, strategic vector management. The detection of rare hybridisation events add value for emerging genetic vector control initiatives. Future research should integrate advanced molecular tools, environmental characterisation, and multi-species surveillance to enhance understanding of malaria transmission dynamics and guide evidence-informed control strategies.

Author Contributions: Conceptualization, T.M., B.D.B. and G.M.; methodology, T.M., Z.M. and G.M.; formal analysis, T.M. and G.M.; investigation, T.M.; resources, B.D.B., S.V.O. and J.R.; data curation, T.M.; writing—original draft preparation, T.M.; writing—review and editing, B.D.B., Z.M., S.V.O., J.R. and G.M.; visualization, T.M.; supervision, B.D.B. and G.M.; funding acquisition, B.D.B. and J.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Health Laboratory Service (NHLS) Research Trust Award to Prof. Basil Brooke as well as partially by the International Atomic Energy Agency (IAEA) under their Technical Cooperation Programme (SAF 5014/5017, Research Contract No. 19099), and by the South African National Research Foundation (NRF) research grants (grant numbers 119765 and 107428), the Bill and Melinda Gates Foundation (grant no. OPP1210314) and the South African Medical Research Council's Grants Innovation and Product Development funds received from the Department of Science and Innovation awarded to Dr. Givemore Munhenga, National Research Foundation of South Africa (Grant numbers CSRP210405592233 and SRUG2204133394), Female Academic Leadership Fellowship (OLIS022), and the South African Medical Research Council Self-Initiated Research Grant (OLVR023) awarded to Dr Shune V. Oliver and the Global Fund (sub-award QPA-M-E8S from the Elimination Eight) awarded to Dr Jaishree Raman. Field entomologists were compensated for overtime by the Mpumalanga Department of Health. The content and findings reported are the sole deduction, view, and responsibility of the authors and do not reflect the official position and sentiments of the funders.

Institutional Review Board Statement: The study was approved by the Animal Research Ethics Committee (AREC) of the University of the Witwatersrand, Johannesburg, South Africa (Ref: CR 20200218-10/AREC-101210-002, dated 18 February 2020).

Informed Consent Statement: Permissions and verbal consent for larval collections were obtained from local authorities and community leaders. Not applicable for studies not involving humans.

Data Availability Statement: The original contributions presented in this study are included in the article. All collected mosquito specimens are preserved at the Vector Control Reference Laboratory (VCRL), National Institute for Communicable Diseases, Johannesburg, and are available upon request.

Acknowledgments: We thank the Mpumalanga Department of Health Malaria Control Programme for field support, tribal chiefs and community leaders for their cooperation, Dumisani Dlamini for morphological identifications, and VCRL staff (Avhatakali Matamba, Malibongwe Zulu) for technical assistance.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

CI	Confidence Interval
D	Simpson's diversity index
DDT	Dichlorodiphenyltrichloroethane
H′	Shannon diversity index
IRS	Indoor Residual Spraying
J	Pielou's Evenness index
n_{rep}	number of replicates
PCR	Polymerase Chain Reaction
SD	Standard Deviation
WHO	World Health Organization

Appendix A



Figure A1. Field collection of *Anopheles* larvae from diverse breeding habitats in Bushbuckridge, Mpumalanga Province, South Africa. Larval sampling sites encompassed diverse *Anopheles* breeding habitats representative of ecological heterogeneity in the region. Representative habitats included (**a**) concrete water storage structures and anthropogenic pools; (**b**) spring-fed seepage areas with emergent vegetation; (**c**) field personnel conducting larval surveys in semi-aquatic habitats; (**d**) water-logged meadows with dense aquatic vegetation; (**e**) muddy stream beds and swamps; and (**f**) irrigation/stream channels with flowing water.

Appendix B

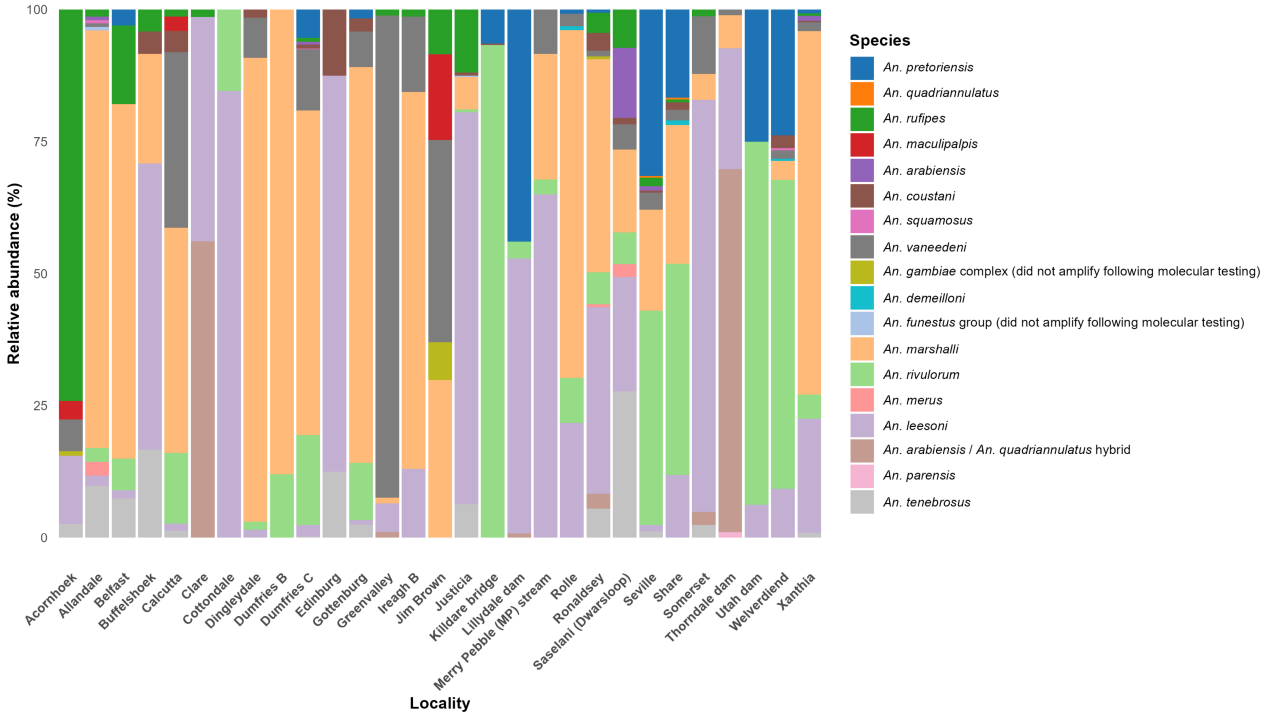


Figure A2. The relative abundance of *Anopheles* species collected across 29 localities of the Bushbuckridge Local Municipality, Mpumalanga, South Africa, June 2022–April 2024.

Appendix C

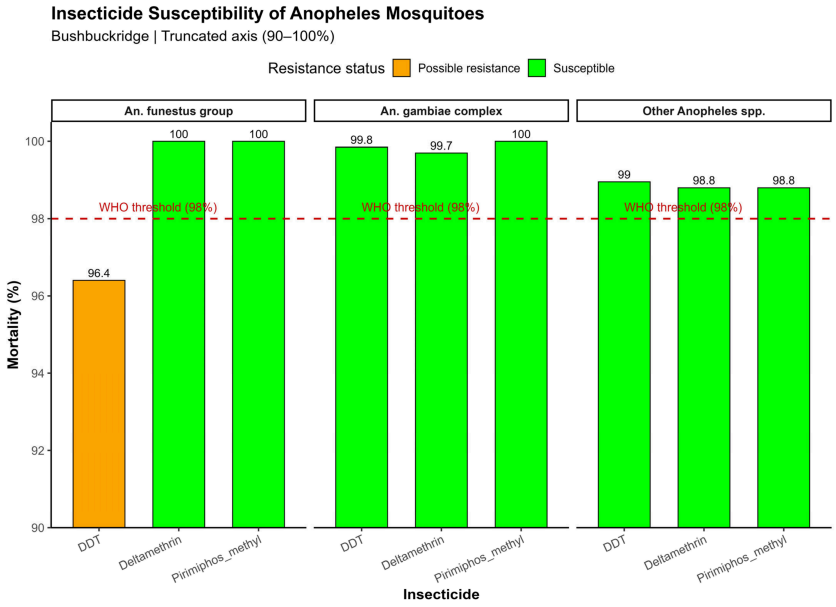


Figure A3. Insecticide susceptibilities of *Anopheles* mosquitoes collected from the Bushbuckridge Local Municipality, Mpumalanga, South Africa, June 2022–April 2024. Results are shown by species group and insecticide class. Bars represent mean mortalities (%) 24 h post-exposure. Green indicates susceptible populations; orange indicates possible resistance (90–97% mortality). *Anopheles gambiae* complex and other *Anopheles* species demonstrated high susceptibility (≥98.8% mortality) to all three insecticides. *Anopheles funestus* group showed 100% mortality to deltamethrin and pirimiphos-methyl but 96.4% mortality to DDT, indicating possible resistance.

References

1. World Health Organization. *World Malaria Report 2025*; World Health Organization: Geneva, Switzerland, 2025. Available online: https://www.who.int/publications/i/item/9789240117822 (accessed on 12 January 2026).
2. Dahan-Moss, Y.; Munhenga, G.; Kaiser, M.L.; Riddin, M.; Matamba, A.; Shanahan, M.; Kachali, K.; Makhubela, M.; Marubini, E.; Sekgele, W.; et al. Malaria vector surveillance report update, South Africa, January–December 2023. *Public Health Bull. S. Afr.* **2025**, *3*, 40–56.
3. Balmith, M.; Basson, C.; Brand, S.J. The malaria burden: A South African perspective. *J. Trop. Med.* **2024**, *2024*, 6619010. [CrossRef] [PubMed]
4. Ranson, H.; Lissenden, N. Insecticide resistance in African *Anopheles* mosquitoes: A worsening situation that needs urgent action to maintain malaria control. *Trends Parasitol.* **2016**, *32*, 187–196. [CrossRef] [PubMed]
5. Hargreaves, K.; Koekemoer, L.L.; Brooke, B.D.; Hunt, R.H.; Mthembu, J.; Coetzee, M. *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Med. Vet. Entomol.* **2000**, *14*, 181–189. [CrossRef] [PubMed]
6. Munhenga, G.; Oliver, S.V.; Lobb, L.N.; Mazarire, T.T.; Sekgele, W.; Mashatola, T.; Mabaso, N.; Dlamini, D.M.; Zulu, M.; Moletsane, F.; et al. Malaria risk and receptivity: Continuing development of insecticide resistance in the major malaria vector *Anopheles arabiensis* in northern KwaZulu-Natal, South Africa. *S. Afr. J. Sci.* **2022**, *118*, 11755. [CrossRef] [PubMed]
7. Silver, J.B. *Mosquito Ecology: Field Sampling Methods*; Springer: Dordrecht, The Netherlands, 2008; ISBN 978-1-4020-6665-8. [CrossRef]
8. Russell, T.L.; Staunton, K.; Burkot, T.R. Standard Operating Procedure for Sampling *Anopheles* Larvae. protocols.io: 2022. Available online: https://pacmossi.org/carbon/assets/0007e8/00000a/PacMOSSI-SOP_Larval-sampling-Anopheles.pdf (accessed on 2 April 2026).
9. Hunt, R.H.; Brooke, B.D.; Pillay, C.; Koekemoer, L.L.; Coetzee, M. Laboratory selection for and characteristics of pyrethroid resistance in the malaria vector *Anopheles funestus*. *Med. Vet. Entomol.* **2005**, *19*, 271–275. [CrossRef] [PubMed]
10. Niain'ny Felamboahangy, L.; Kaiser, M.L.; Zengenene, M.P.; Okumu, F.; Munhenga, G.; Koekemoer, L.L. Optimisation of laboratory-rearing parameters for *Anopheles funestus* larvae and adults. *Acta Trop.* **2023**, *238*, 106785. [CrossRef] [PubMed]
11. World Health Organization. *Standard Operating Procedure for Testing Insecticide Susceptibility of Adult Mosquitoes in WHO Tube Tests*, 1st ed.; World Health Organization: Geneva, Switzerland, 2022; ISBN 978-92-4-004383-1.
12. Coetzee, M. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). *Malar. J.* **2020**, *19*, 70. [CrossRef] [PubMed]
13. Scott, J.A.; Brogdon, W.G.; Collins, F.H. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am. J. Trop. Med. Hyg.* **1993**, *49*, 520–529. [CrossRef] [PubMed]
14. Koekemoer, L.L.; Kamau, L.; Hunt, R.H.; Coetzee, M. A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *Am. J. Trop. Med. Hyg.* **2002**, *66*, 804–811. [CrossRef] [PubMed]
15. Afrane, Y.A.; Bonizzoni, M.; Yan, G. Secondary malaria vectors of sub-Saharan Africa: Threat to malaria elimination on the continent? In *Current Topics in Malaria*; Rodriguez-Morales, A.J., Ed.; IntechOpen: London, UK, 2016. [CrossRef] [PubMed]
16. Burke, A.; Dandalo, L.; Munhenga, G.; Dahan-Moss, Y.; Mbokazi, F.; Ngxongo, S.; Coetzee, M.; Koekemoer, L.; Brooke, B. A new malaria vector mosquito in South Africa. *Sci. Rep.* **2017**, *7*, 43779. [CrossRef] [PubMed]
17. Mbokazi, F.; Coetzee, M.; Brooke, B.; Govere, J.; Reid, A.; Owiti, P.; Kosgei, R.; Zhou, S.; Magagula, R.; Kok, G.; et al. Changing distribution and abundance of the malaria vector *Anopheles merus* in Mpumalanga Province, South Africa. *Public Health Action* **2018**, *8*, S39–S43. [CrossRef] [PubMed]
18. Temu, E.A.; Hunt, R.H.; Coetzee, M.; Minjas, J.N.; Shiff, C.J. Detection of hybrids in natural populations of the *Anopheles gambiae* complex by the rDNA-based PCR method. *Ann. Trop. Med. Parasitol.* **1997**, *91*, 963–965. [CrossRef]
19. Fontaine, M.C.; Pease, J.B.; Steele, A.; Waterhouse, R.M.; Neafsey, D.E.; Sharakhov, I.V.; Jiang, X.; Hall, A.B.; Catteruccia, F.; Kakani, E.; et al. Extensive introgression in a malaria vector species complex revealed by phylogenomics. *Science* **2015**, *347*, 1258524. [CrossRef] [PubMed]
20. Maharaj, R.; Seocharan, I.; Lakan, V.; Nyawo, Z.; Mkhabela, M.; Balakrishna, Y. Field evaluation of the residual efficacy of new generation insecticides for potential use in indoor residual spray programmes in South Africa. *Malar. J.* **2024**, *23*, 127. [CrossRef] [PubMed]
21. Maiga, H.; Millogo, A.S.; Bayili, K.; Bilgo, E.; Toe, I.; Dabiré, R.K.; Bouyer, J.; Diabaté, A. Screening the resistance of male *Aedes aegypti* and *Anopheles coluzzii* to insecticides in the context of using genetic control tools in Burkina Faso. *Insects* **2025**, *16*, 315. [CrossRef] [PubMed]
22. Lees, R.S.; Carvalho, D.O.; Bouyer, J. Potential impact of integrating the sterile insect technique into the fight against disease-transmitting mosquitoes. In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, 2nd ed.; Dyck, V.A., Hendrichs, J., Robinson, A.S., Eds.; CRC Press: Boca Raton, FL, USA, 2021; pp. 1081–1118. [CrossRef]
23. Naidoo, K.; Oliver, S.V. Gene drives: An alternative approach to malaria control? *Gene Ther.* **2025**, *32*, 25–37. [CrossRef] [PubMed]

24. Erlank, E.; Koekemoer, L.L.; Coetzee, M. The importance of morphological identification of African anopheline mosquitoes (Diptera: Culicidae) for malaria control programmes. *Malar. J.* **2018**, *17*, 43. [CrossRef] [PubMed]
25. Dahan-Moss, Y.; Hendershot, A.; Dhoogra, M.; Julius, H.; Zawada, J.; Kaiser, M.; Lobo, N.F.; Brooke, B.D.; Koekemoer, L.L. Member species of the *Anopheles gambiae* complex can be misidentified as *Anopheles leesoni*. *Malar. J.* **2020**, *19*, 89. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.