

# Diversity and Insecticide Susceptibility of *Anopheles* Mosquitoes in Bushbuckridge, a Residual Malaria Transmission Setting in South Africa

Thabo Mashatola<sup>1,2</sup>, Zuziwe Mthiyane<sup>3</sup>, Shüné Oliver<sup>1,2</sup>, Jaishree Raman<sup>1,2</sup>, Basil Brooke<sup>1, 2</sup>, and Givemore Munhenga<sup>1,2</sup>



<sup>1</sup>Centre for Emerging Zoonotic & Parasitic Diseases, National Institute for Communicable Diseases, Johannesburg  
<sup>2</sup>Wits Research Institute for Malaria, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg  
<sup>3</sup>Mpumalanga Department of Health, Malaria Control Programme, Mbombela, South Africa

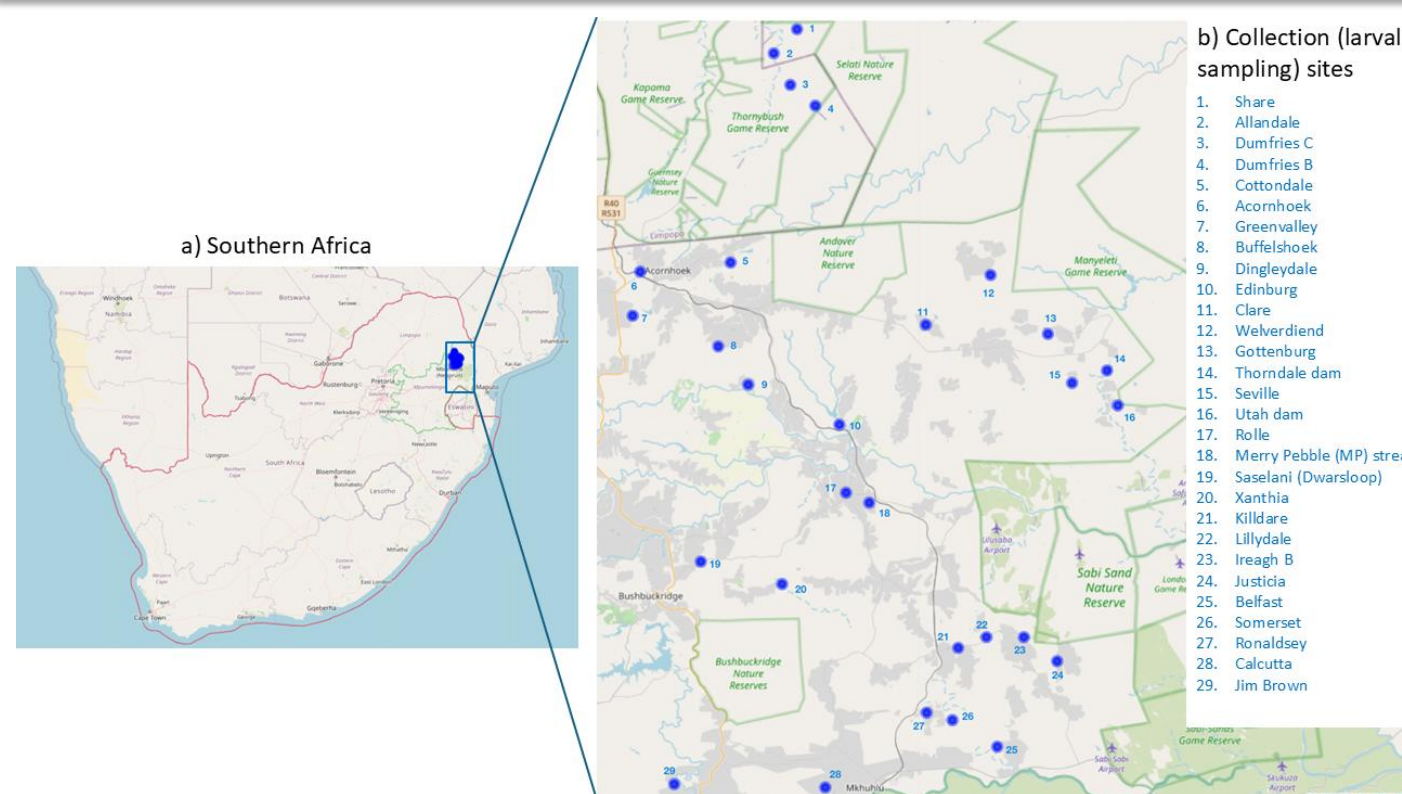


## INTRODUCTION & AIM

- Bushbuckridge lies within South Africa's lowveld malaria belt and experiences persistent residual malaria transmission due to its proximity to Mozambique and Eswatini.
- Understanding local vector diversity and insecticide susceptibility is critical for:
  - Detecting changes in vector populations
  - Monitoring emerging resistance
  - Informing evidence-based malaria control strategies
- Study Objective
  - To assess *Anopheles* species diversity and insecticide susceptibility across Bushbuckridge between 2022 and 2024

## METHOD

### Study area



### Larval collection

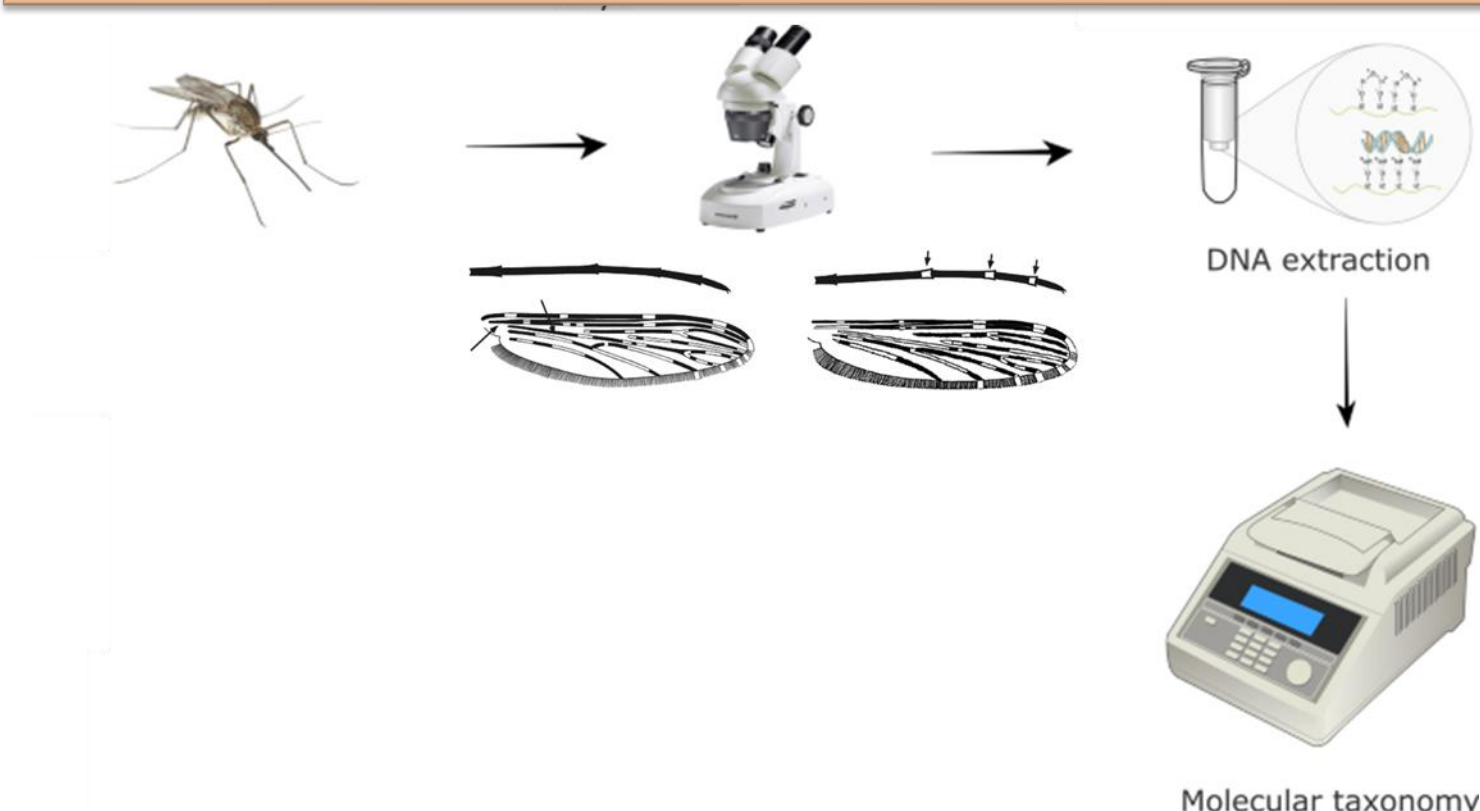


### Adult exposure to insecticides

World Health Organization  
Standard operating procedure for testing insecticide susceptibility of adult mosquitoes in WHO tube tests

| Insecticide       | Concentration |
|-------------------|---------------|
| Deltamethrin      | 0.05%         |
| DDT               | 4%            |
| Pirimiphos-methyl | 0.25%         |

### Adult morphological and molecular ID



### Species diversity

18 taxa identified, n=4,453.

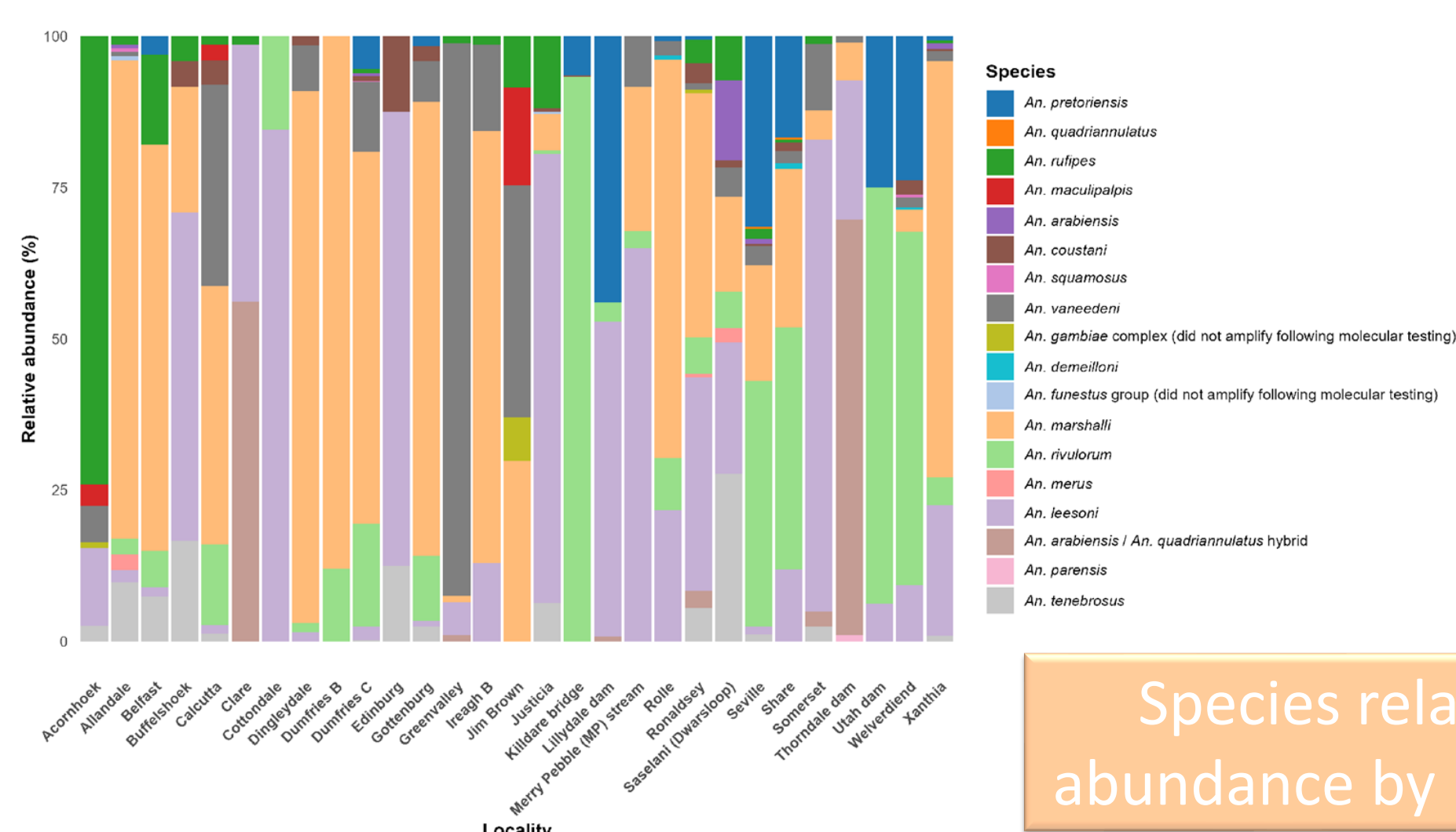
| Species  | n            | %          |
|--|--------------|------------|
| <i>An. pretoriensis</i>                                  | 1,487        | 33.4       |
| <i>An. quadriannulatus</i>                               | 921          | 20.7       |
| <i>An. rufipes</i>                                       | 890          | 20.0       |
| <i>An. maculipalpis</i>                                  | 320          | 7.2        |
| <i>An. arabiensis</i> ★                                  | 316          | 7.1        |
| <i>An. coustani</i>                                      | 185          | 4.2        |
| <i>An. squamosus</i>                                     | 116          | 2.6        |
| <i>An. vaneedeni</i> †                                   | 98           | 2.2        |
| <i>An. gambiae</i> complex (PCR fail)                    | 36           | 0.8        |
| <i>An. demeilloni</i>                                    | 31           | 0.7        |
| <i>An. funestus</i> group (PCR fail)                     | 21           | 0.5        |
| Others ( <i>An. marshalli</i> , <i>An. merus</i> , etc.) | 16           | 0.4        |
| <i>An. arabiensis/quadriannulatus</i> hybrid ◆           | 2            | 0.04       |
| <b>TOTAL</b>   | <b>4,454</b> | <b>100</b> |

★ Primary vector † Secondary vector ◆ Putative hybrid (n=2; PCR-confirmed)

### Insecticide susceptibility

- High susceptibility observed across most populations
- Mortality generally exceeded WHO susceptibility thresholds
- Reduced mortality (94.6%) observed in DDT-exposed *An. funestus* group
- Indicates possible emerging resistance requiring continued monitoring

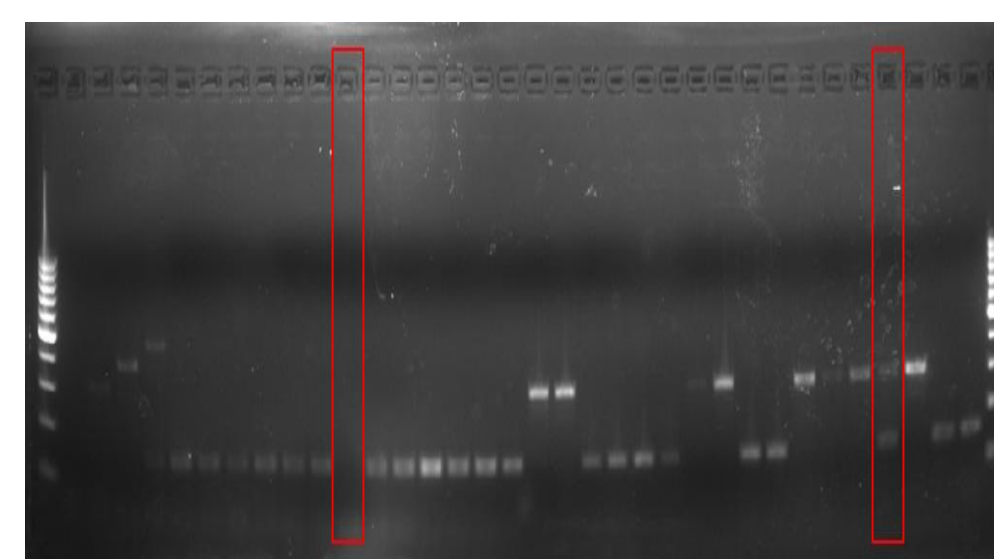
## RESULTS & DISCUSSION



Species relative abundance by locality

**Hybridisation finding:** Two putative *An. arabiensis* / *An. quadriannulatus* hybrids detected by PCR — a rare occurrence (<0.1% frequency in natural populations). May produce altered host preference, vector competence or insecticide susceptibility. Genomic sequencing recommended for confirmation.

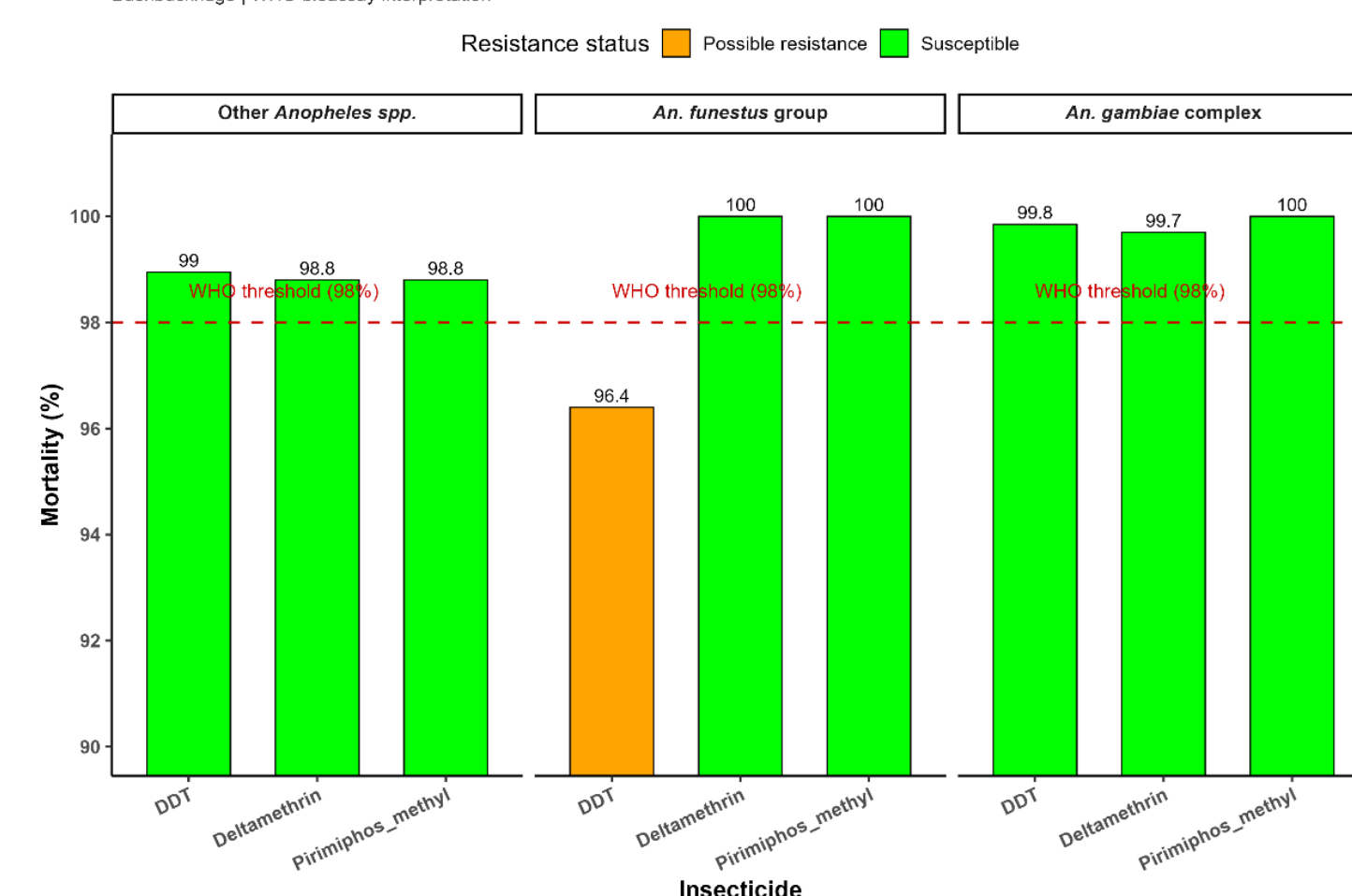
**PCR failures:** 3% of *An. gambiae* complex and 16% of *An. funestus* group specimens could not be resolved — attributed to DNA degradation, primer mismatches or possibly cryptic/novel taxa.



PCR multiplex gel: *An. gambiae* complex. Red boxes = PCR fail or putative hybrid banding patterns.

### Insecticide Susceptibility of *Anopheles* Mosquitoes

Bushbuckridge | WHO bioassay interpretation



## CONCLUSION / FUTURE WORK

- Bushbuckridge supports a diverse *Anopheles* community in a residual malaria transmission setting.
- Most populations remain susceptible to key insecticides, although reduced DDT susceptibility was detected.
- Potential hybridisation and emerging resistance patterns highlight the need for continued surveillance to support adaptive malaria vector control.