

Malaria vector surveillance report update, South Africa, January – December 2023

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Summary

Malaria transmission in South Africa is seasonal and primarily occurs in the Limpopo, Mpumalanga, and KwaZulu-Natal provinces. Control of malaria vectors is by indoor spraying of residual insecticides and limited larval source management. As the country moves towards malaria elimination, enhanced vector surveillance is necessary in order to obtain comprehensive information on the distribution and relative density of all the vectors responsible for ongoing residual malaria transmission. This report summarises the findings of malaria vector surveillance from the three endemic provinces that was conducted in 2023 by the provincial malaria control entomology teams in collaboration with research institutes. The specimens analysed were collected from the KwaZulu-Natal (27%, n=2 525), Mpumalanga (6%, n=509), and Limpopo (67%, n=6 172) provinces. The surveillance revealed the presence of several mosquito species previously incriminated as vectors in South Africa – *Anopheles arabiensis* (n=1 504, 16%), *An. vaneedeni* (n=237, 3%), *An. parensis* (n=227, 2%), and *An. funestus* (n=1). These contribute in varying degrees to ongoing residual malaria transmission. *Anopheles* species implicated as vectors in other African localities, but not in South Africa, as well as several closely related non-vector species, were also collected. The surveillance information confirms malaria receptivity in all three endemic provinces and supports the ongoing implementation of indoor residual insecticide spraying for vector control. We recommend ongoing vector surveillance and the collection of insecticide susceptibility data for vector populations in endemic districts. Vigilance for signs of increasing urban malaria is also necessary, especially given the range expansion of the Asian malaria vector *An. stephensi* into parts of sub-Saharan Africa – although not detected in southern Africa to date.

Introduction

South Africa's malaria-affected areas include the low-altitude border regions of the Limpopo, Mpumalanga, and KwaZulu-Natal (KZN) provinces. These regions typically experience active malaria transmission, especially during the peak malaria season that spans the months of November to May. Malaria incidence in South Africa is generally low (the number of total cases for the period 2019 to 2023 ranged from 5 889 to 13 711).¹ The Department of Health, Republic of South Africa, issued a media statement on 25 April 2024 stating that in 2023, there were 9 795 malaria cases and 106 deaths.²

South Africa's malaria-endemic provinces – Limpopo, Mpumalanga, and KwaZulu-Natal – have developed and implemented well-co-ordinated malaria control operations, including routine vector control, which is primarily based on the application of indoor residual insecticide spraying (IRS) and larval source management.³ Although IRS has proven efficacy spanning many decades, residual malaria transmission continues and is likely caused by outdoor-feeding and outdoor-resting *Anopheles* vector mosquitoes that are less exposed to indoor applications of insecticides.^{4,5,6} In addition, populations of the major malaria vector species *Anopheles funestus* and *An. arabiensis* have developed resistance to insecticides, especially in northern KwaZulu-Natal.^{3,7} Pyrethroid resistance in *An. arabiensis* in this region is currently of low intensity, i.e., a mild expression of the phenotype based on the World Health Organization bioassay method for assessing resistance intensity⁸, and is therefore not considered to be operationally significant as yet. This is in contrast to the pyrethroid-carbamate resistance profile in *An. funestus* that is of high intensity, is highly significant epidemiologically, and was at least partly causative of the malaria epidemic experienced in South Africa during the period of 1996 to 2000.⁹



Residual malaria transmission and insecticide resistance in vector populations within South Africa's borders necessitate ongoing and enhanced vector surveillance to inform best practices for control and elimination. This is especially pertinent in terms of South Africa's malaria elimination agenda,¹⁰ because the presence of vectors indicates malaria receptivity and therefore a risk of malaria reintroduction in areas cleared of local transmission. Currently, surveillance is routinely conducted by the entomology teams of the Mpumalanga, KwaZulu-Natal, and Limpopo provinces with support from partner institutions including the National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Service (NHLS); the Wits Research Institute for Malaria (WRIM) of the University of the Witwatersrand; the UP Institute for Sustainable Malaria Control (UP ISMC) of the University of Pretoria; and the South African Medical Research Council (SAMRC).

This report summarises malaria vector surveillance in South Africa in 2023 based on specimens referred to the Vector Control Reference Laboratory (VCRL) of the Centre for Emerging Zoonotic and Parasitic Diseases (CEZPD), NICD, as well as specimens collected and analysed by personnel from the UP ISMC.

Methods

Anopheles mosquitoes and larvae were collected at selected sentinel sites in KwaZulu-Natal, Mpumalanga, and Limpopo from January to December 2023 (Figure 1), either on a weekly or monthly basis. These specimens were either collected by VCRL and UP ISMC personnel or were referred to the VCRL by partner institutions and provincial malaria control programme entomology teams.

Adult *Anopheles* mosquitoes were collected via CO₂-baited tent traps, cattle-baited tent traps, human landing catches, CDC and Encephalitis Vector Survey (EVS) traps, cattle-kraal collections, pit traps, pyrethrum spray collections, outdoor-placed clay pots, modified plastic buckets and discarded tyres. Other specimens were collected as larvae using dippers/scoops and were reared to adults before analysis. *Anopheles* specimens were collected from several sentinel sites (Figure 1). Preservation of adult specimens was by desiccation on silica gel in 1.5ml microcentrifuge tubes. Initial morphological identification of each specimen using dichotomous keys^{11,12} was conducted by VCRL, partner institutions and/or provincial malaria control programme personnel. Specimens identified as members of the *An. gambiae* complex or *An. funestus* group were subsequently identified to species using standard polymerase chain reaction (PCR) assays^{13,14,15} by VCRL and UP ISMC personnel.

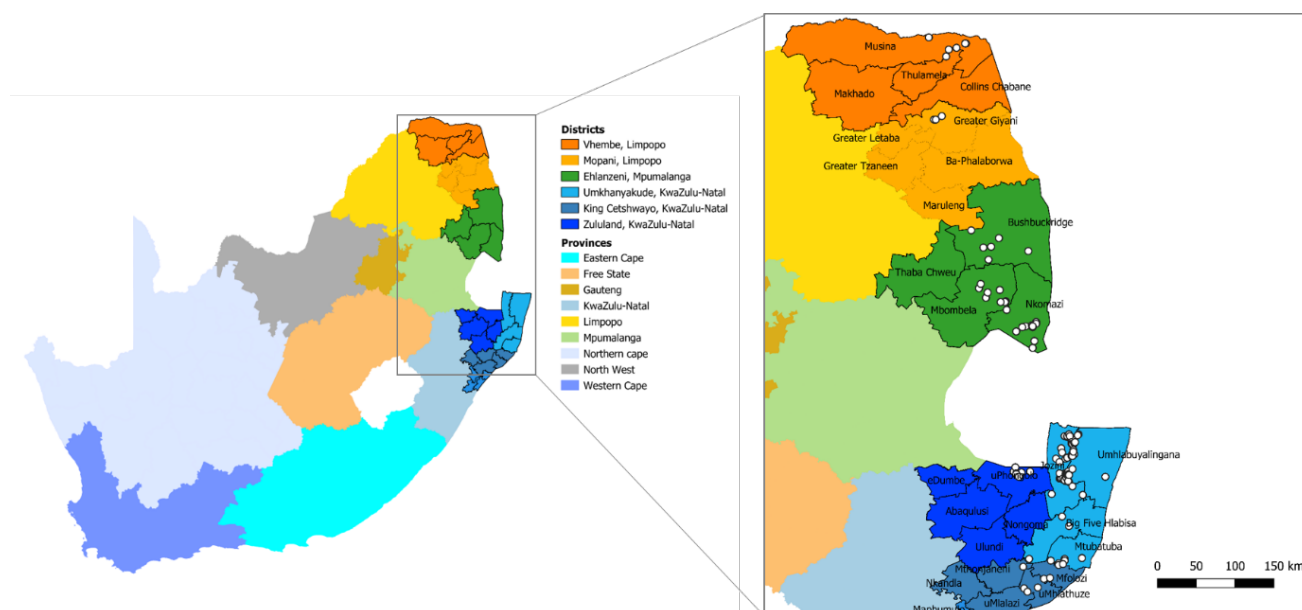


Figure 1. Sentinel sites (white dots) in the KwaZulu-Natal, Mpumalanga, and Limpopo provinces from where *Anopheles* specimens were collected, South Africa, 2023. All maps were created using the QGIS Geographic Information System (Open Source Geospatial Foundation Project, <http://qgis.org>).

Results

In total, 9 206 *Anopheles* mosquitoes (larvae and adults) were collected from the uMkhanyakude, King Cetshwayo, and Zululand districts of KwaZulu-Natal, the Ehlanzeni District of Mpumalanga, and the Vhembe and Mopani districts of Limpopo. Most of the specimens were collected from Limpopo (67%, $n=6\,172$), followed by KwaZulu-Natal (27%, $n=2\,525$) and Mpumalanga (6%, $n=509$) (Table 1). These were subsequently clustered as either *An. gambiae* complex (37%, $n=3\,408$), *An. funestus* group (12%, $n=1\,137$), or other (miscellaneous) *Anopheles* species (51%, $n=4\,661$). *Anopheles pretoriensis* predominated the collections (25%, $n=2\,263$), especially in Limpopo, while *An. arabiensis* (16%, $n=1\,504$) and *An. merus* (8%, $n=732$) predominated the collections from KwaZulu-Natal and Mpumalanga, respectively (Table 1).



Table 1. Numbers of collected *Anopheles* specimens by species and province, South Africa, 2023.

| <i>Anopheles</i> species complex, group, or other | Species | KwaZulu-Natal | Mpumalanga | Limpopo | Total |
|---|-------------------------------|---------------|------------|---------|-------|
| An. gambiae complex | <i>An. arabiensis</i> | 1 298 | 87 | 119 | 1 504 |
| | <i>An. merus</i> | 368 | 328 | 36 | 732 |
| | <i>An. quadriannulatus</i> | 91 | 52 | 1 029 | 1 172 |
| An. funestus group | <i>An. funestus</i> | 0 | 0 | 1 | 1 |
| | <i>An. lesoni</i> | 23 | 0 | 58 | 81 |
| | <i>An. parensis</i> | 227 | 0 | 0 | 227 |
| | <i>An. rivulorum</i> | 162 | 3 | 266 | 431 |
| | <i>An. rivulorum-like</i> | 0 | 0 | 160 | 160 |
| | <i>An. vaneedeni</i> | 151 | 2 | 84 | 237 |
| Other (miscellaneous) <i>Anopheles</i> species | <i>An. coustani</i> | 0 | 12 | 103 | 115 |
| | <i>An. demeilloni</i> | 2 | 0 | 3 | 5 |
| | <i>An. flavicosta</i> | 0 | 0 | 4 | 4 |
| | <i>An. gabonesis</i> | 0 | 0 | 1 | 1 |
| | <i>An. gibbinsi</i> | 0 | 0 | 29 | 29 |
| | <i>An. listeri</i> | 0 | 0 | 945 | 945 |
| | <i>An. maculipalpis</i> | 55 | 5 | 6 | 66 |
| | <i>An. marshallii complex</i> | 90 | 0 | 0 | 90 |
| | <i>An. nili</i> | 0 | 0 | 101 | 101 |
| | <i>An. ovengensis</i> | 0 | 0 | 1 | 1 |
| | <i>An. pharoensis</i> | 3 | 0 | 0 | 3 |
| | <i>An. pretoriensis</i> | 0 | 5 | 2 258 | 2 263 |
| | <i>An. rhodesiensis</i> | 0 | 0 | 236 | 236 |
| | <i>An. rufipes</i> | 3 | 14 | 632 | 649 |
| | <i>An. squamosus</i> | 1 | 0 | 13 | 14 |
| | <i>An. tenebrosus</i> | 51 | 1 | 82 | 134 |
| | <i>An. theileri</i> | 0 | 0 | 5 | 5 |
| Total | | 2 525 | 509 | 6 172 | 9 206 |

Various vector species from the *Anopheles gambiae* complex (*An. arabiensis* and/or *An. merus*)^{5,16} and the *An. funestus* group (*An. funestus sensu stricto.*, *An. lesoni*, *An. Rivulorum*, and/or *An. vaneedeni*)^{4,17,18} were collected from sentinel sites in the three endemic provinces (Table 2, Figure 2). These species were primarily collected from the Jozini municipality of the uMkhanyakude District, KwaZulu-Natal; the Nkomazi Local Municipality of the Ehlanzeni District, Mpumalanga; and the Musina and Greater Giyani municipalities of the Vhembe and Mopani districts, Limpopo, respectively (Table 2, Figure 2).

Anopheles coustani, *An. demeilloni*, *An. gibbinsi*, *An. marshallii complex*, *An. nili*, *An. ovengensis*, *An. pharoensis*, *An. pretoriensis*, *An. rufipes*, *An. squamosus*, *An. Tenebrosus*, and *An. theileri* are directly implicated in malaria transmission in various African regions^{11,18-26} but not in South Africa to date. Miscellaneous *Anopheles* specimens were collected from the Jozini and uMhlabyalingana municipalities of the uMkhanyakude District, KwaZulu-Natal; the Nkomazi Local Municipality of the Ehlanzeni District, Mpumalanga; and the Musina and Thulamela municipalities of the Vhembe District, Limpopo (Table 2, Figure 2).



Table 2. Numbers of collected *Anopheles* specimens belonging to taxa implicated in malaria transmission in South Africa and/or other African regions. These are shown by province, district, and municipality, South Africa, 2023.

| Province | District | Municipality | <i>An. gambiae</i> complex | <i>An. funestus</i> group | Miscellaneous <i>Anopheles</i> |
|---------------|----------------|------------------|----------------------------|---------------------------|--------------------------------|
| KwaZulu-Natal | uMkhanyakude | uMhlabuyalingana | 29 | 71 | 9 |
| | | Jozini | 1 431 | 348 | 196 |
| | | Big Five Hlabisa | 8 | 1 | |
| | | Mtubatuba | 149 | 1 | |
| | Zululand | uPhongolo | 24 | 77 | |
| | King Cetshwayo | Mfolozi | 6 | 3 | |
| | | uMhlathuze | 17 | 34 | |
| | | Mthonjaneni | | 21 | |
| | | uMlalazi | 2 | | |
| | Mpumalanga | Ehlanzeni | Bushbuckridge | 14 | |
| Nkomazi | | | 404 | 5 | 32 |
| Mbombela | | | 1 | | |
| Limpopo | Vhembe | Musina | 75 | 322 | 2 513 |
| | | Thulamela | | 26 | 714 |
| | Mopani | Greater Giyani | 80 | 61 | |

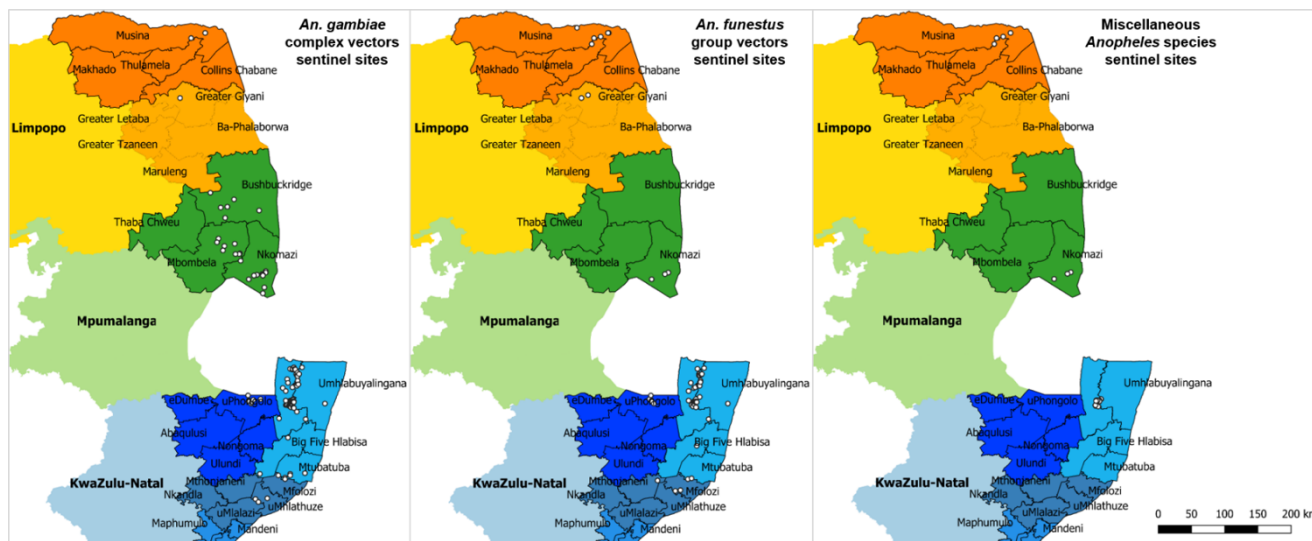


Figure 2. Sentinel sites (white dots) in the KwaZulu-Natal, Mpumalanga, and Limpopo provinces that yielded *Anopheles* specimens belonging to taxa implicated in malaria transmission in South Africa and/or other African regions, 2023. These include vector species from the *An. gambiae* complex (*An. arabiensis* and *An. merus*); *An. funestus* group (*An. funestus* s.s., *An. leesoni*, *An. parensis*, *An. rivulorum*, and *An. vaneedeni*); and miscellaneous *Anopheles* species (*An. coustani*, *An. demeilloni*, *An. gibbinsi*, *An. marshallii* complex, *An. nili*, *An. ovengensis*, *An. pharoensis*, *An. pretoriensis*, *An. rufipes*, *An. squamosus*, *An. tenebrosus*, and *An. theileri*).



The number of anophelines collected by species during specific seasons was highly variable across the three endemic provinces. *Anopheles arabiensis* was particularly prevalent during summer (01 January to 28 February and 01 to 31 December), autumn (01 March to 31 May), and winter (01 June to 31 August) in KwaZulu-Natal, while *An. merus* was prevalent throughout the year in Mpumalanga (Figure 3). *Anopheles quadriannulatus* predominated the *An. gambiae* complex collections from Limpopo during autumn, spring (01 September to 30 November), and early summer (01 to 31 December). Of the *An. funestus* group, *An. vaneedeni* was most common during summer and winter in KwaZulu-Natal. *Anopheles rivulorum* and *An. vaneedeni* were collected during autumn and were the only *An. funestus* group specimens from Mpumalanga. *Anopheles rivulorum*, *An. Leesonii*, and *An. vaneedeni* dominated the collections in Limpopo during autumn, spring, and early summer (December), respectively (Figure 4). Miscellaneous *Anopheles* species collections in KwaZulu-Natal showed that *An. marshallii* complex species predominated the autumn and winter collections, while *An. rufipes* and *An. coustani* predominated the autumn collections from Mpumalanga (Figure 5). *Anopheles pretoriensis* was predominant in winter and spring, while in early summer, *An. listeri* predominated the collections of miscellaneous specimens in Limpopo.

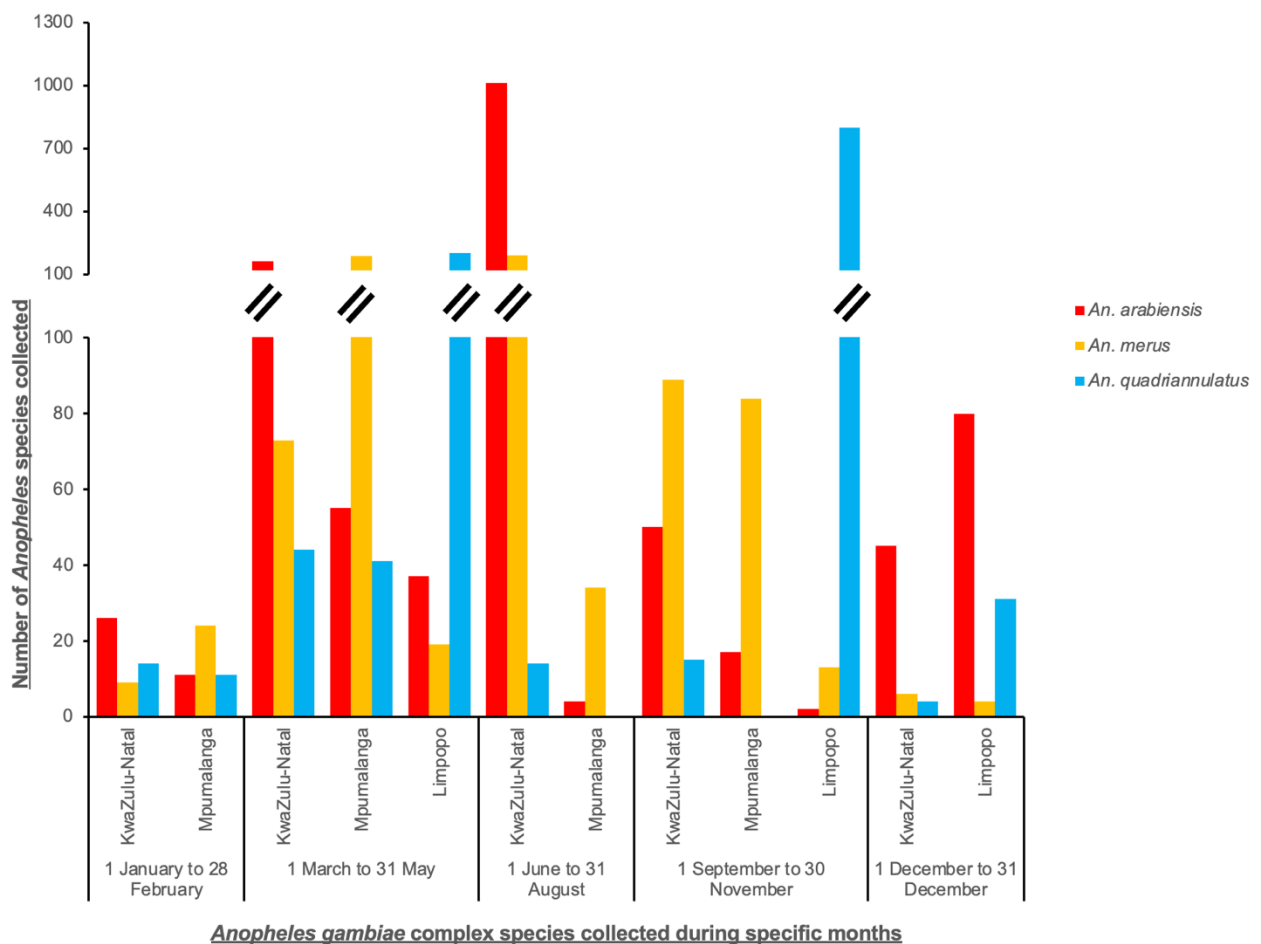


Figure 3. Distribution (in absolute numbers) of *Anopheles gambiae* complex specimens by species, province, and season, South Africa, 2023. Summer = 01 January to 28 February and December; Autumn = 01 March to 31 May; Winter = 01 June to 31 August; Spring = 01 September to 30 November.

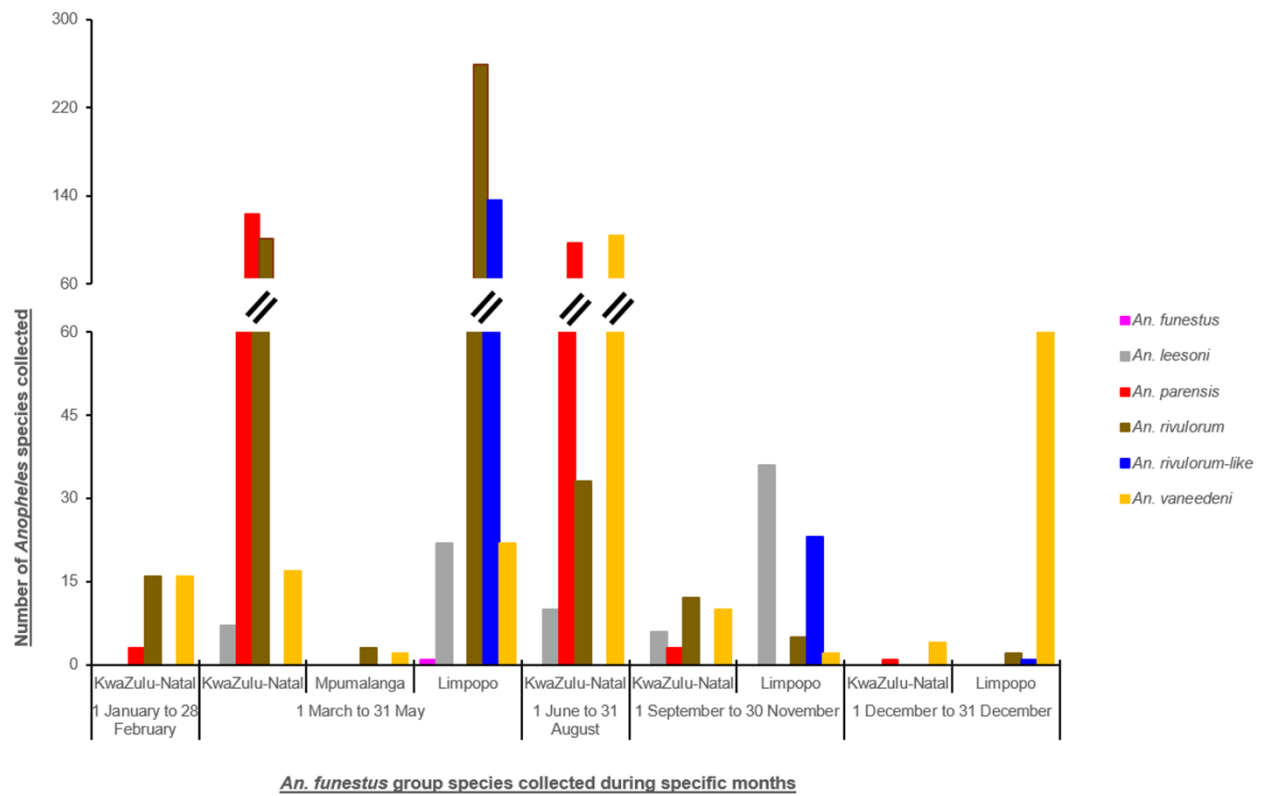


Figure 4. Distribution (in absolute numbers) of *Anopheles funestus* group specimens by species, province, and season, South Africa, 2023. Summer = 01 January to 28 February and December; Autumn = 01 March to 31 May; Winter = 01 June to 31 August; Spring = 01 September to 30 November.

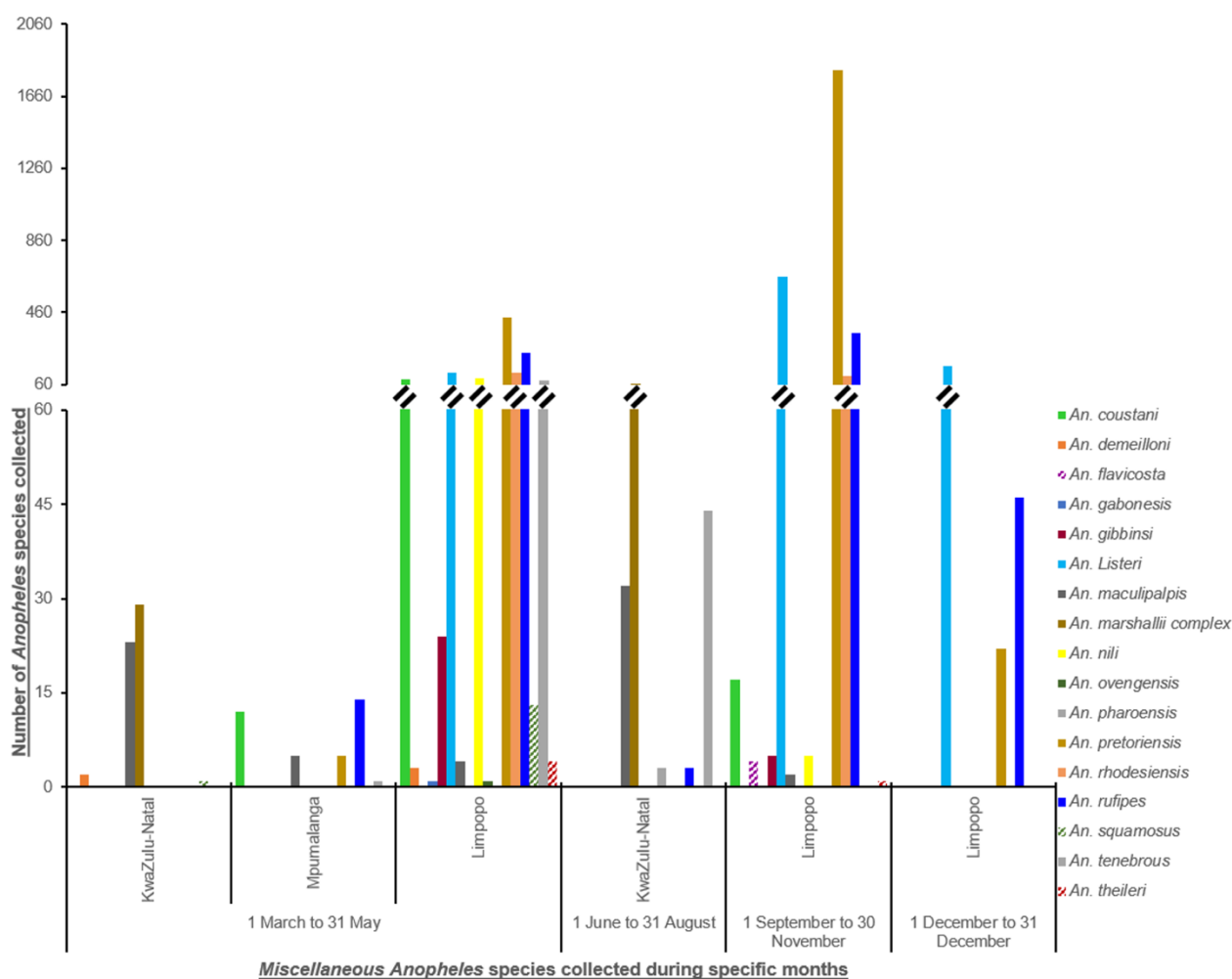


Figure 5. Distribution (in absolute numbers) of miscellaneous *Anopheles* specimens by species, province, and season, South Africa, 2023. Summer = 01 January to 28 February and December; Autumn = 01 March to 31 May; Winter = 01 June to 31 August; Spring = 01 September to 30 November.

The *Anopheles* specimens were sampled as either larvae or adults. In KwaZulu-Natal, 85% of specimens were collected as adults, while in Limpopo and Mpumalanga, 28% and 17% of *Anopheles* specimens were collected as adults, respectively. In all three provinces, CO₂-baited tent traps were used to collect adult mosquitoes. CO₂-baited tent traps were especially effective in Limpopo, where 73% (n=1 248) of the adult mosquitoes collected were sampled using this method. In Mpumalanga, 41% (n=35) of the specimens were collected using this sampling method. Clay pots were especially effective in KwaZulu-Natal (64%, n=1 372), followed by cattle-kraal catches (23%, n=487). Table 3 shows the numbers of specimens by species collected in each province across all sampling methods

Table 3. Numbers of collected adult *Anopheles* specimens by province, species, and sampling method, South Africa 2023.

| Taxon | Species | Clay pot | | CO ₂ tent trap | | Cattle-baited tent traps | Human landing catches | | | Aspiration | CDC-light trap | EVS traps | Cattle-kraal catch | | | Drum | Modified bucket | Tyre | | Block cement | Pyrethrum Spray collection | Pit trap | |
|--|-------------------------------|----------|----|---------------------------|----|--------------------------|-----------------------|-----|----|------------|----------------|-----------|--------------------|----|----|------|-----------------|------|----|--------------|----------------------------|----------|----|
| | | KZN | MP | KZN | MP | LP | LP | KZN | MP | LP | LP | LP | KZN | MP | LP | KZN | KZN | KZN | MP | KZN | KZN | MP | LP |
| <i>An. gambiae</i> complex | <i>An. arabiensis</i> | 823 | 5 | 6 | 4 | 2 | | 13 | 3 | | | | 107 | | | 14 | 7 | 145 | 4 | 7 | | | |
| | <i>An. merus</i> | 96 | 2 | | 15 | 6 | 3 | | 5 | | | | 88 | 1 | | 6 | | 25 | | | 1 | 16 | |
| | <i>An. quadriannulatus</i> | 9 | | | 4 | 275 | 99 | | | | 1 | 1 | 54 | | | | | 3 | 1 | | | | |
| <i>An. funestus</i> group | <i>An. funestus</i> | | | | | | | | | | | 1 | | | | | | | | | | | |
| | <i>An. leesoni</i> | 6 | | | | 39 | 10 | | | | 4 | 3 | 16 | | | | | | | | | | |
| | <i>An. parensis</i> | 184 | | | | | | | | | | | 22 | | | | | 20 | | | | | |
| | <i>An. rivulorum</i> | 21 | | 2 | 2 | 160 | 6 | | 1 | | 71 | 22 | 134 | | 1 | 1 | | 1 | | | 2 | | |
| | <i>An. rivulorum-like</i> | | | | | 132 | 4 | | | | 1 | 10 | | | | | | | | | | | |
| | <i>An. vaneedeni</i> | 50 | | | | 15 | | | 2 | 4 | | | 66 | | | | | 2 | | | | | 56 |
| Miscellaneous <i>Anopheles</i> species | <i>An. coustani</i> | | | | 7 | 65 | 15 | | 3 | | 1 | 7 | | | 2 | | | | | | | | |
| | <i>An. demeilloni</i> | 2 | | | | 2 | | | | | | | | | | | | | | | | | |
| | <i>An. flavicosta</i> | | | | | 4 | | | | | | | | | | | | | | | | | |
| | <i>An. gabonesis</i> | | | | | 1 | | | | | | | | | | | | | | | | | |
| | <i>An. gibbinsi</i> | | | | | 26 | | | | | | | | | | | | | | | | | |
| | <i>An. listeri</i> | | | | | 36 | 17 | | | | 3 | | | | | | | | | | | | |
| | <i>An. maculipalpis</i> | 47 | | | | 2 | 1 | | | | | | 1 | | | | 2 | 6 | | | | | |
| | <i>An. marshallii</i> complex | 83 | | | | | | | | | | | | | | | | 7 | | | | | |
| | <i>An. nili</i> | | | | | 55 | 19 | | | | 5 | | | | | | | | | | | | |
| | <i>An. ovengensis</i> | | | | | 1 | | | | | | | | | | | | | | | | | |
| | <i>An. pharoensis</i> | 3 | | | | | | | | | | | | | | | | | | | | | |
| | <i>An. pretoriensis</i> | | | | | 256 | 24 | | 1 | | 2 | 4 | | | | | | | 1 | | | | |
| | <i>An. rhodesiensis</i> | | | | | 21 | | | | | 2 | 2 | | | | | | | | | | | |
| | <i>An. rufipes</i> | 2 | | | 3 | 93 | 14 | | | | 1 | 1 | 1 | | | | | 1 | 1 | | | | |
| | <i>An. squamosus</i> | 1 | | | | 10 | 3 | | | | | | | | | | | | | | | | |
| | <i>An. tenebrosus</i> | 45 | | | | 43 | | | 1 | | 25 | 13 | | | | | | 6 | | | | | |
| | <i>An. theileri</i> | | | | | 4 | 1 | | | | | | | | | | | | | | | | |

KZN = KwaZulu-Natal; MP = Mpumalanga; LP = Limpopo



Discussion

Malaria vector surveillance in 2023 in the KwaZulu-Natal, Mpumalanga, and Limpopo provinces of South Africa revealed the presence of 19 *Anopheles* species of interest in malaria transmission. The collections included species previously incriminated as vectors in South Africa – *An. funestus* s.s., *An. arabiensis*, *An. Parensis*, and *An. vaneedeni*^{4,5,9,17} – and species incriminated as vectors in other African localities – *An. merus*, *An. lesoni*, *An. rivulorum*, *An. coustani*, *An. demeilloni*, *An. gibbinsi*, *An. marshallii* complex, *An. nili*, *An. ovengensis*, *An. pharoensis*, *An. pretoriensis*, *An. rufipes*, *An. squamosus*, *An. Tenebrosus*, and *An. theileri*.¹⁸⁻²⁶

The major malaria vector *An. arabiensis* was present in all three endemic provinces but was most prevalent in KwaZulu-Natal. This species is currently the major vector of malaria in South Africa following the near eradication of *An. funestus* by intensive IRS campaigns over the last three decades.^{3,27} Since *An. arabiensis* females are at least partially inclined to feed and rest outdoors, they are less susceptible to control by IRS.^{5,6} This species is therefore the primary, but not the only, vector of residual malaria in South Africa.⁵

Anopheles merus specimens were only collected from KwaZulu-Natal and Mpumalanga during the surveillance period, but it likely also occurs in Limpopo. Although *An. merus* has not been directly implicated in malaria transmission in South Africa, its confirmed vector status in countries along the east coast of Africa, including nearby southern Mozambique – the sporozoite rate for *An. merus* in the Boane District recorded at 4.2%¹⁶ – suggests that it is most likely an important secondary malaria vector in South Africa. This species is primarily a coastal saltwater breeder, although it has also been collected from freshwater larval habitats in southern Africa, including sites in South Africa.²⁸

Anopheles parensis and *An. vaneedeni* have been implicated as secondary malaria vectors in South Africa,^{4,17} while other members of the *An. funestus* group – *An. rivulorum* and *An. lesoni* – have been implicated as secondary vectors in East Africa.¹⁸ Collections of *An. vaneedeni* and *An. rivulorum* were from all three of South Africa's endemic provinces. *Anopheles lesoni* was detected in KwaZulu-Natal and Limpopo. *Anopheles parensis* was only collected from KwaZulu-Natal in 2023. *Anopheles vaneedeni* likely contributes to residual malaria transmission in South Africa given its tendency to rest outdoors and to feed on humans amongst other vertebrate hosts.⁴ *Anopheles parensis* is primarily zoophilic and rests indoors and outdoors. This species will also occasionally feed on humans²⁹ and potentially contributes to residual malaria transmission in South Africa, as is the case for *An. rivulorum* and *An. Leesonii*, although neither of these two species have been directly implicated in malaria transmission in South Africa to date.

A single specimen of *An. funestus* s.s., a primary vector of malaria in neighbouring Mozambique and Zimbabwe, was detected in the Limpopo in 2023. The scarcity of this species in South Africa can be attributed to year-on-year effective IRS programmes in the malaria-endemic provinces. Ongoing vigilance for the presence of this species is, however, important. *Anopheles funestus* is an especially efficient malaria vector that can cause outbreaks and epidemics in comparatively short time frames.

Other species that were collected in South Africa in 2023 and incriminated as malaria vectors in various African localities included *An. coustani*, *An. demeilloni*, *An. gibbinsi*, *An. marshallii* complex, *An. nili*, *An. ovengensis*, *An. pharoensis*, *An. pretoriensis*, *An. rufipes*, *An. squamosus*, *An. Tenebrosus*, and *An. theilei*.¹⁸⁻²⁶



These species were particularly prevalent in Limpopo, although some of these were also detected in Mpumalanga and KwaZulu-Natal. It is possible that one or more of these species plays a role in residual malaria transmission in South Africa, especially in the Limpopo region, where primary vectors are scarce. The presence of species from the *An. nili* group (*An. nili* and *An. ovengensis*) needs to be monitored, as these species are important vectors in several regions of Africa^{25,26} and may contribute to malaria transmission in Limpopo.

Anopheles population densities tend to fluctuate between seasons, as indicated in the collection data for 2023. They are generally highest during the spring and summer months, congruent with increased rainfall,⁵ and translating into higher malaria transmission rates during the malaria season (September to May). It is for this reason that South Africa's provincial IRS campaigns begin in spring, shortly before the onset of the rains.¹⁰ Winter larviciding of perennial *Anopheles* breeding sites is also indicated as a precursor to IRS each year.

Collections of the adult *Anopheles* species incriminated as vectors in South Africa, *An. arabiensis* and *An. parensis*, were predominantly from clay pots, while *An. vaneedeni* was predominantly collected from cattle-kraal posts and *An. funestus* s.s. was collected by EVS traps. Collections of other potential secondary vectors were predominantly from CO₂- and cattle-baited tent traps, clay pots, cattle-kraal posts, discarded tyres, and CDC-light and EVS traps. These data show that collection methods targeting adult mosquitoes yield critical surveillance information, especially in terms of vector species assemblage (risk and receptivity) and vector incrimination. As each collection method on its own is not likely to yield all *Anopheles* species of interest, the use of several collection methods at each sentinel site is necessary.

The urban malaria vector *An. stephensi* has not been detected in southern Africa to date, but is nevertheless increasing its range in Africa.³⁰ This species is endemic to South-East Asia and parts of the Arabian Peninsula and has recently been detected in the horn of Africa, Sudan, and most recently in East and West Africa. *Anopheles stephensi* generally breeds in clean, potable water, and adult females take blood from humans and livestock. Its mode of spread into Africa is evidently by shipping, and based on an analysis of global shipping networks, South Africa is at risk of importing this species.³¹ Vigilance for *An. stephensi* in east coast seaports and urban and peri-urban areas of malaria-endemic districts is therefore indicated.

The occurrence of primary and secondary vector species in all three of South Africa's malaria-endemic provinces shows that the affected districts/municipalities remain highly receptive to malaria despite ongoing IRS operations each year. During 2023, Limpopo recorded the highest number of local malaria cases, primarily in the Mopani and Vhembe districts,³² where the primary vectors *An. arabiensis* and *An. funestus*, although scarce, were detected. It is also likely that secondary vector species play an especially important role in ongoing malaria transmission in Limpopo, which may also be true for the other endemic provinces as well, but to a lesser extent.

Prior to the collection of a single *An. funestus* s.s. specimen in the Vhembe District in 2023, this species had rarely been detected in South Africa since the early 2000s, with only a single specimen collected in the area in 2018.²⁷ Its recent occurrence in Limpopo is therefore of concern and may be linked to sub-optimal IRS operations in that province, although this has not been definitively determined. This species is common in the bordering regions of southern Mozambique and southern and eastern Zimbabwe, and is highly resistant to pyrethroid insecticides.^{3,9} Ongoing vigilance for the presence of this species is especially important in terms of maintaining the efficacy of



control operations and reducing the incidence of locally acquired malaria in South Africa's endemic regions, especially along the border regions of the Mopani and Vhembe districts in Limpopo.

Conclusion

Several malaria vector species occur in the low-altitude, north-eastern border regions of South Africa, with their relative abundances remaining comparatively high through the dry winter months in some instances. These data indicate a high receptivity for malaria and therefore a high risk of resurgence in endemic areas currently cleared of malaria or at low incidence. Despite co-ordinated provincial IRS programmes that usually achieve high spray-coverage rates (80% or more of targeted structures in endemic areas), populations of vector species persist, and at least four of them – *An. funestus* s.s., *An. arabiensis*, *An. Vaneedeni*, and *An. parensis* – have previously been implicated in ongoing residual transmission in South Africa (*An. merus* is also a highly likely contributor). The reasons for this certainly include outdoor-biting and outdoor-resting behaviours in some of these species. The vector surveillance information by province and district/municipality from 2023 supports the ongoing implementation of a stratified IRS-based vector control strategy supported by larval source management for the control of residual malaria.

Recommendations

- Maintenance of malaria vector surveillance in South Africa's endemic provinces on a weekly to monthly basis, especially during summer and autumn, by provincial entomology teams with the support of partner institutions (NICD, UP ISMC, SAMRC, and WRIM);
- Prioritisation of insecticide susceptibility data, especially for populations of major vector species. Collection of susceptibility data should be annual and conducted in collaboration with partner institutions. Priority insecticides include deltamethrin, pirimiphos methyl, and clothianidin, if possible;
- Emphasis on the collection of adult *Anopheles* mosquitoes using an array of proven methods, especially in terms of surveillance for *An. funestus sensu stricto*. This necessarily involves night-time collections by surveillance teams and personnel of partner institutions. Larval collections, conducted during the day, are also important, primarily for the detection and geolocation of breeding sites;
- Biannual vector surveillance (by provincial entomology team personnel) in those districts or municipalities in endemic provinces that are currently malaria-free. This provides important information on malaria receptivity and the risk of re-introduction;
- Annual sampling – by provincial entomology team personnel and partner institutions – of aquatic-stage mosquitoes from potential *An. stephensi* breeding sites, especially in east coast seaports and urban and peri-urban areas in malaria-endemic districts; and
- Use of the provincial DHIS2 systems for the collation of vector surveillance data. Senior entomology team members with the support of information officers can do this. Partner institutions are strongly encouraged to share their surveillance data with the national and provincial control programmes by uploading pertinent data onto the DHIS2 databases.



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Conflict of interest

The authors declare no conflict of interest.



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