SPECIAL PUBLIC HEALTH SURVEILLANCE BULLETIN

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

VOLUME 19. SUPPLEMENTARY ISSUE 3 | 23 NOVEMBER 2021

MALARIA VECTOR SURVEILLANCE REPORT, SOUTH AFRICA, JANUARY – DECEMBER 2020

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SUMMARY

Malaria in South Africa is seasonal and primarily occurs in the Limpopo, Mpumalanga and KwaZulu-Natal provinces. The control of malaria vectors is based on indoor spraying of residual insecticides (IRS) and limited larval source management. There were 8126 malaria cases resulting in 38 confirmed deaths in South Africa in 2020. Vector surveillance in collaboration with the National Institute for Communicable Diseases (NICD) during 2020 revealed the presence of four malaria vector species - *Anopheles arabiensis* (n=9,325, 77%), *An. merus* (n=530, 4%), *An. parensis* (n=88, 0.7%) and *An. vaneedeni* (n=93, 0.8%). These have previously been implicated in ongoing residual malaria transmission in South Africa. Several closely related non-vector *Anopheles* species were also collected. The specimens analysed were collected from KwaZulu-Natal (84%, n=10,085), Mpumalanga (5%, n=583) and Limpopo (11%, n=1,380) provinces. Selected adult female *An. arabiensis* (n=530) and *An. merus* (n=8) specimens, collected from KwaZulu-Natal Province, all tested negative for the presence of *P. falciparum* circumsporozoites. The surveillance information by province and municipality shows that IRS based vector control needs to be maintained at a high rate of coverage in areas of active transmission, and that spraying should ideally be completed before the onset of each malaria season. Consideration can be given to a more targeted or reactive approach in areas where no local cases have been recorded for three or more years. Given that all sporozoite positive (and therefore malaria infective) adult *Anopheles* females collected in the recent years were found resting outdoors, and given that there are no large-scale vector control tools targeting outdoor-resting mosquitoes, larviciding, including the treatment of winter breeding sites, should continue to be used as a complimentary method to enhance the effect of IRS in high incidence

areas. It is also recommended that entomological surveillance be enhanced in the endemic provinces to monitor the bionomics of vectors responsible for residual transmission. In the context of the current COVID-19 pandemic, it is recommended that all malaria vector control activities be conducted especially timeously and efficiently. This will reduce the risk of co-infection in affected communities and reduce malaria-related hospitalisations.

INTRODUCTION

South Africa's malaria affected areas include the low altitude border regions of Limpopo, Mpumalanga and KwaZulu-Natal (KZN) provinces. These regions typically experience active malaria transmission, especially during the peak malaria season that spans the summer months of November to April. Malaria incidence in 2020 (8,126 cases) was substantially lower than that recorded in 2019 (13,833 cases), but still higher than the number of cases recorded in 2016 (5,842 cases)[1].

Each of South Africa's malaria endemic provinces have developed well-coordinated malaria control operations including routine vector control which is primarily based on the application of indoor residual insecticide spraying (IRS) and, to a lesser extent, larval source management [2]. Although IRS has proven efficacy spanning many decades, residual malaria transmission continues and is likely caused by outdoor feeding and resting *Anopheles* vector mosquitoes that are unaffected by indoor applications of insecticide [3,4,5]. In addition, populations of the major malaria vector species, *Anopheles funestus* and *An. arabiensis*, have developed resistance to insecticides, especially in northern KwaZulu-Natal [2,6]. The pyrethroid resistance phenotype in *An. arabiensis* in this region is however of low intensity currently and is not considered to be operationally significant yet. This is in contrast to the pyrethroid-carbamate resistance profile in *An. funestus* which is of high intensity, highly significant epidemiologically and was at least partly causative of the malaria epidemic experienced in South Africa during the period 1996 to 2000 [7].

Residual malaria transmission, comparatively high incidence and burgeoning insecticide resistance in malaria vector populations within South Africa's borders necessitate ongoing and enhanced vector surveillance to inform best practices for control. This is especially pertinent in terms of South Africa's malaria elimination agenda[8] and the current COVID-19 pandemic, during which it is especially important to reduce disease burden as much as possible[9]. Currently, surveillance is routinely conducted by the entomology teams of Mpumalanga, KwaZulu-Natal and Limpopo provinces with support from partner institutions including the National Institute for Communicable Diseases (NICD), the Wits Research Institute for Malaria (WRIM) of the University of the Witwatersrand, the UP Institute for Sustainable Malaria Control of the University of Pretoria, and the South African Medical Research Council.

This report summarises malaria vector surveillance in South Africa in 2020 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 University of Pretoria.

METHODS

Anopheles mosquitoes were collected from sentinel sites in KwaZulu-Natal, Mpumalanga and Limpopo provinces (Figure 1). These specimens were either collected by VCRL and University of Pretoria personnel, or referred to the VCRL by partner institutions and provincial malaria control programme entomology teams from January to December 2020.

Adult *Anopheles* mosquitoes were collected by human-baited net traps, human landing catches, CDC-light traps, BG-sentinel traps, CO_2 net traps, and outdoor placed clay pots, modified buckets and tyres. Other specimens were collected as larvae and were reared to adults for subsequent analysis. One or more of these collection techniques were deployed at each sentinel site (Figure 1). Adult specimens were preserved on silica gel in 1.5ml microcentrifuge tubes and were identified as far as possible using external morphological characters by VCRL, partner institution 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[10,11,12]. A standardised ELISA[13, 14] assay was used to detect the presence of *Plasmodium falciparum* circumsporozoites in selected adult female specimens. The VCRL is a SANAS accredited laboratory and quality assurance based on the ISO 17025:2017 standard was used to ensure the quality of results obtained for all specimens received and analysed.

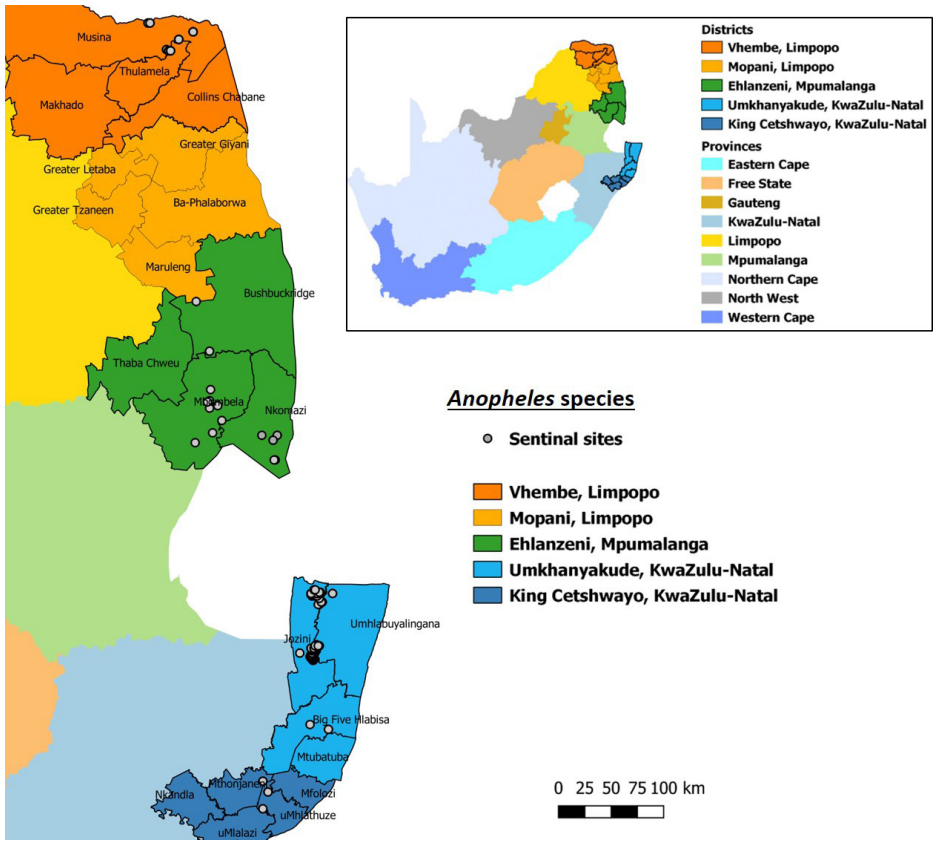

Figure 1. Sentinel sites in KwaZulu-Natal, Mpumalanga and Limpopo provinces from which *Anopheles* specimens were collected, South Africa, 2020.

RESULTS

A total of 12,048 *Anopheles* mosquitoes was collected from sentinel sites in the Umkhanyakude and King Cetshwayo districts of KwaZulu-Natal Province, the Ehlanzeni district of Mpumalanga Province and the Vhembe District of Limpopo Province. Most of the specimens were collected from KwaZulu-Natal (84%, n=10,085) followed by Limpopo (11%, n=1,380) and Mpumalanga (5%, n=583) provinces (Table 1). These were subsequently clustered as either *An. gambiae* complex (87%, n=10,451), *An. funestus* group (6%, n=682) or other *Anopheles* species (8%, n=915). *Anopheles arabiensis* predominated the collections (77%, n=9,325), especially in KwaZulu-Natal, although substantial numbers of *An. quadriannulatus*, *An. merus*, *An. rufipes*, *An. pretoriensis* and *An. rivulorum* were also obtained. *Anopheles merus* and *An. quadriannulatus* predominated in Mpumalanga and Limpopo provinces, respectively (Table 1). Adult female *An. arabiensis* (n=530) and *An. merus* (n=8) specimens, collected from KwaZulu-Natal Province, all tested negative for the presence of *P. falciparum* circumsporozoites.

Table 1. Numbers of *Anopheles* specimens collected by species and province, South Africa, 2020.

***Anopheles* species complex, group or other**	**species**	**KwaZulu-Natal**	**Mpumalanga**	**Limpopo**	**Total**
An. gambiae complex	*An. arabiensis*	9,275	49	1	9,276
	An. merus	220	308	2	478
	An. quadriannulatus	7	20	569	593
An. funestus group	*An. leesoni*	31	6	166	198
	An. parensis	88	0	0	85
	An. rivulorum	30	60	172	216
	An. rivulorum-like	0	0	36	36
	An. vaneedeni	59	20	14	86
Other *Anopheles* species	*An. coustani*	41	4	15	60
	An. demeilloni	11	1	23	35
	An. maculipalpis	10	17	0	27
	An. marshallii complex	38	0	0	38
	An. pharoensis	11	0	0	11
	An. pretoriensis	35	61	198	294
	An. rhodesiensis	0	0	12	12
	An. rufipes	210	37	130	377
	An. squamosus	18	0	0	18
	An. tenebrous	0	0	41	41
	An. ziemanni	1	0	1	2
Total		**10,085**	**583**	**1,380**	**12,048**

The malaria vectors *An. arabiensis* and *An. merus* (members of the *An. gambiae* species complex) were collected from sentinel sites in all the endemic provinces (Figure 2). In KwaZulu-Natal Province, populations of these species were found in all the municipalities of the Umkhanyakude District and the Mthonjaneni, uMhlahuse and uMlalazi municipalities of the King Cetshwayo District. In Mpumalanga, populations of these species were found in the Nkomazi, Bushbuckridge and Mbombela municipalities of the Ehlanzeni District. In Limpopo Province, these species were found in the Musina municipality of the Vhembe district.

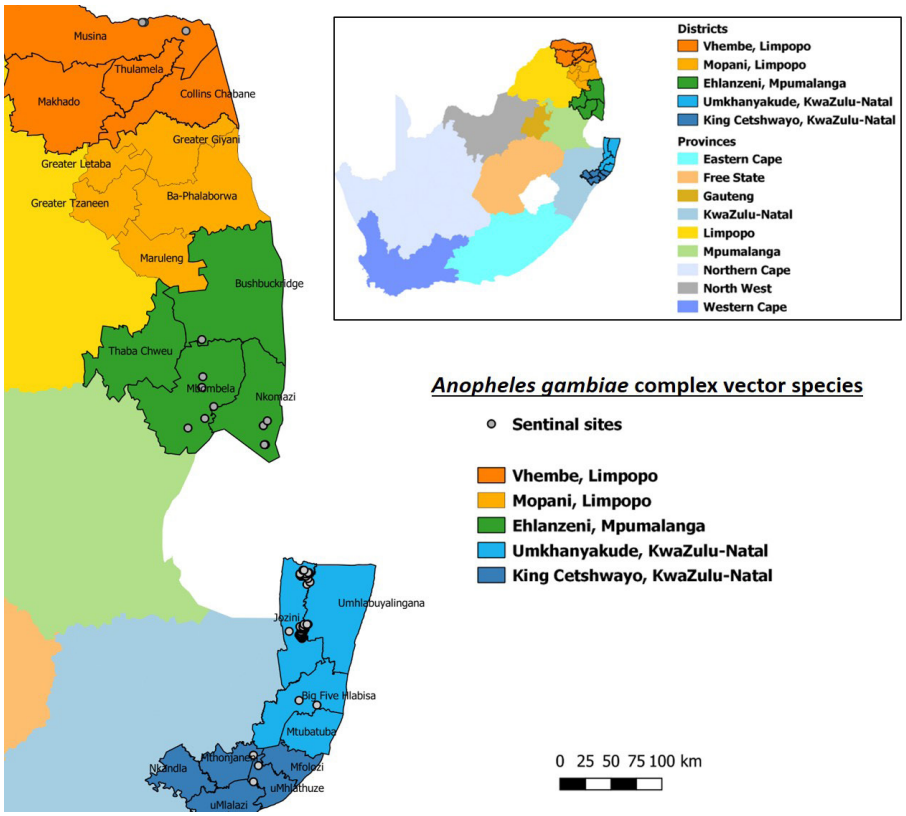

Figure 2. Sentinel sites in KwaZulu-Natal, Mpumalanga and Limpopo provinces from which samples of *Anopheles arabiensis* and *An. merus* (*Anopheles gambiae* complex) were collected, South Africa, 2020.

The potential secondary malaria vector species *An. vaneedeni* [3] was collected from sentinel sites in all three endemic provinces while *An. parensis*, also a potential secondary vector [15], was only collected in KwaZulu-Natal Province (Table 1). Other potential malaria vector species within the *An. funestus* group that were collected from sentinel sites in these three provinces included *An. leesoni* and *An. rivulorum* (Table 1). Collection sites for all known and suspected vector species within the *An. funestus* group are shown in Figure 3. Specimens of these species were collected in the Jozini and Umhlabuyalingana municipalities of the Umkhanyakude District, northern KwaZulu-Natal Province, in Nkomazi and Bushbuckridge of the Ehlanzeni District of Mpumalanga Province and in the Musina and Thulamela municipalities of the Vhembe district of Limpopo.

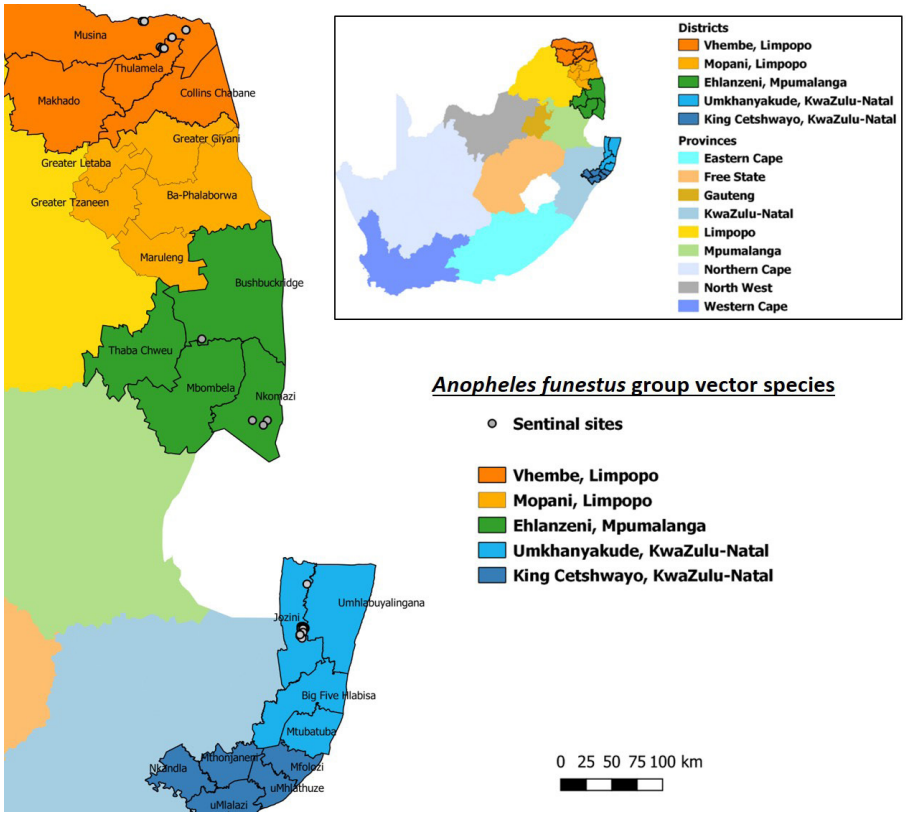

Figure 3. Sentinel sites in KwaZulu-Natal, Mpumalanga and Limpopo provinces from which samples of the known and potential secondary malaria vectors *Anopheles vaneedeni, An. parensis, An. rivulorum* and *An. leesoni* (*An. funestus* group) were collected, South Africa, 2020.

Anopheles coustani, An. demeilloni, An. marshallii complex, *An. pharoensis, An. pretoriensis, An. rufipes, An. squamosus* and *An. ziemanni* have been incriminated as malaria vectors in other regions of Africa[16,17,18,19, 20] but not in South Africa. The distribution of these potential vector species is shown in Figure 4. Specimens of these species were collected in the Jozini and Umhlabuyalingana municipalities in the Umkhanyakude District of KwaZulu-Natal Province, in Bushbuckridge of the Ehlanzeni District of Mpumalanga Province and in the Musina and Thulamela municipalities of the Vhembe district of Limpopo Province.

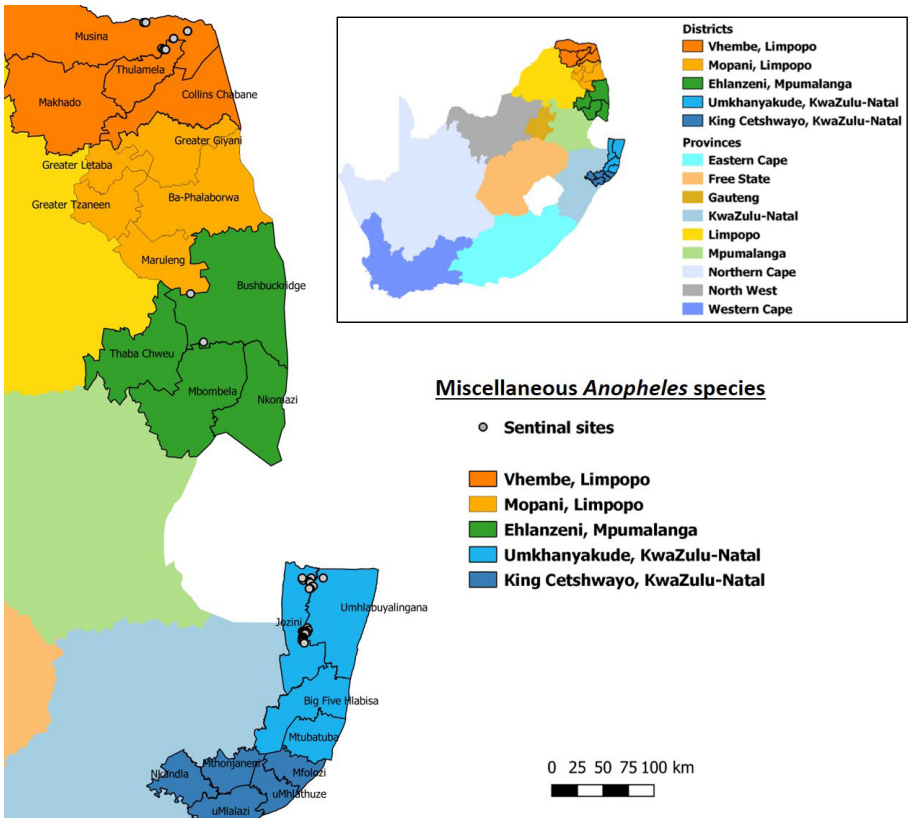

Figure 4. Sentinel sites in KwaZulu-Natal, Mpumalanga and Limpopo provinces from which samples of miscellaneous *Anopheles* species (species not belonging to the *An. gambiae* complex or *An. funestus* group) were collected. These sites included the collection of potential secondary malaria vectors *Anopheles* coustani, *An. demeilloni, An. marshallii* complex, *An. pharoensis, An. pretoriensis, An. rufipes, An. squamosus*, and *An. ziemanni*, South Africa, 2020.

The number of anophelines collected by species at the specific seasons was highly variable across the three endemic provinces. For example, *An. arabiensis* was prevalent throughout the year in KwaZulu-Natal Province while *An. merus* was particularly prevalent during winter and spring in Mpumalanga Province (Figure 5). *Anopheles quadriannulatus* predominated the collections in Limpopo Province during spring. *Anopheles parensis* were most common during spring and early summer in KwaZulu-Natal Province. *Anopheles rivulorum* predominated in late summer, autumn and spring in Mpumalanga Province (Figure 6). *Anopheles rufipes* was the most collected miscellaneous *Anopheles* species throughout most of the year in KwaZulu-Natal Province (Figure 7). *Anopheles pretoriensis* predominated the collections of miscellaneous species during the middle to late summer (January to February) and winter months in Mpumalanga Province. *Anopheles pretoriensis* followed by *An. rufipes* were the most collected miscellaneous species in spring in Limpopo Province.

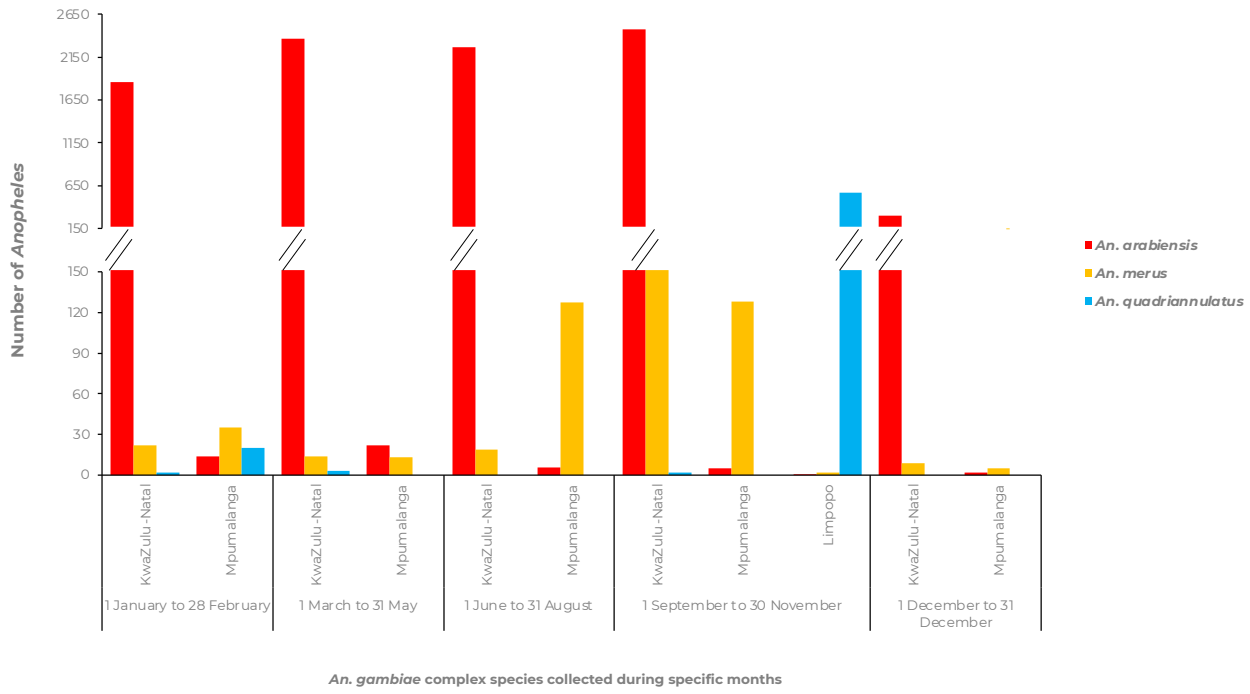

Figure 5. Distribution (in absolute numbers) of *Anopheles gambiae* complex specimens collected by species, province and season, South Africa, 2020.

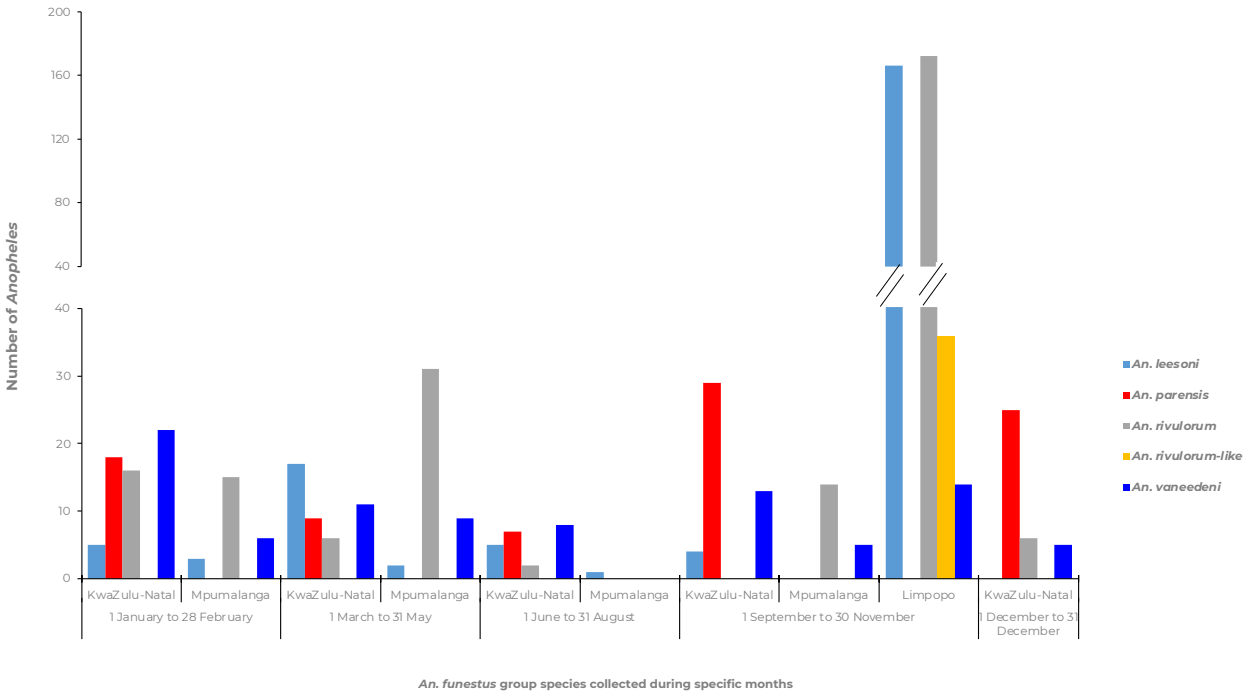

Figure 6. Distribution (in absolute numbers) of *Anopheles funestus* group specimens collected by species, province and season, South Africa, 2020.

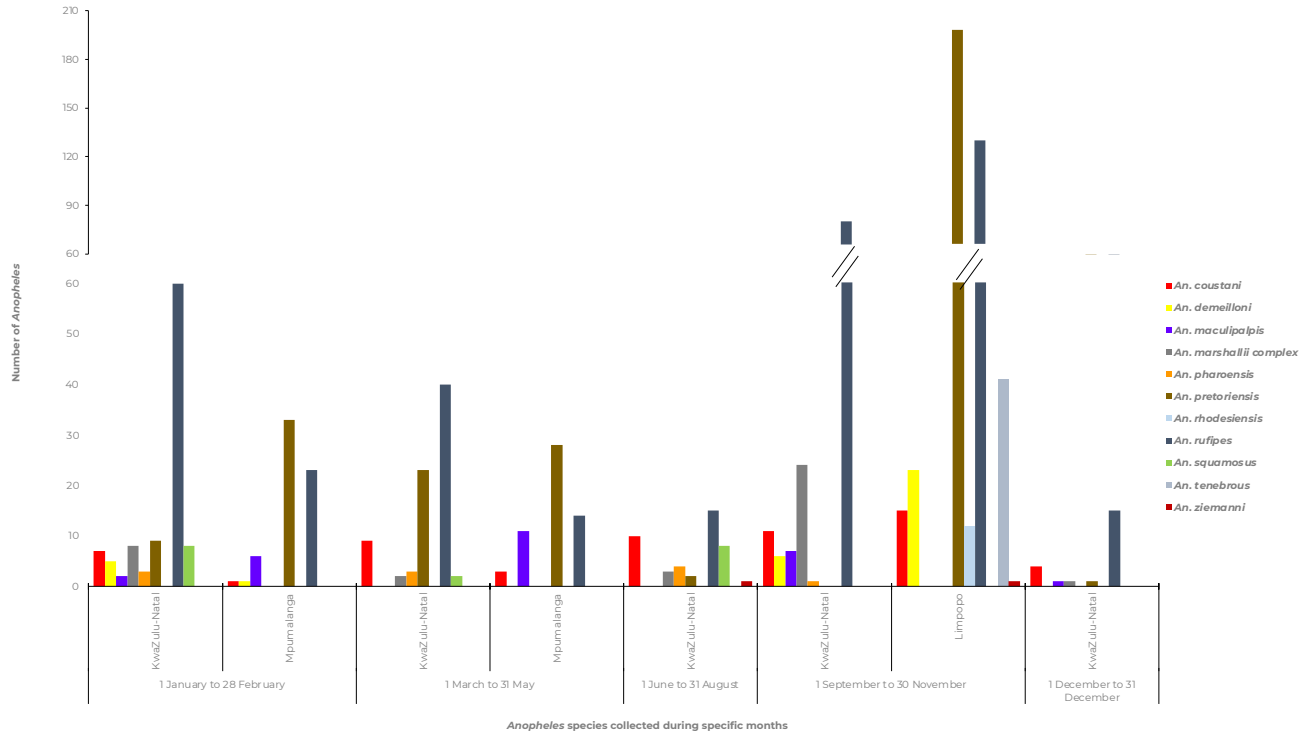

Figure 7. Distribution (in absolute numbers) of miscellaneous *Anopheles* specimens collected by species, province and season, South Africa, 2020.

DISCUSSION

Malaria vector surveillance in 2020 in the KwaZulu-Natal, Mpumalanga and Limpopo provinces of South Africa revealed 19 *Anopheles* species of interest in malaria transmission. The collections included species previously incriminated as vectors in South Africa (*An. arabiensis, An. parensis* and An. *vaneedeni*) as well as species incriminated as vectors in other African localities (*An. merus, An. leesoni, An. rivulorum, An. marshallii, An. coustani, An. demeilloni, An. pharoensis, An. pretoriensis, An. rufipes, An. squamosus* and *An. ziemanni*) [16,17,18,19, 20]

Anopheles arabiensis was the predominant species collected during 2020, accounting for 98% of the specimens collected from KwaZulu-Natal Province. This species was also present in the Mpumalanga and Limpopo collections although only one specimen of this species was collected in Limpopo Province. *Anopheles arabiensis* is currently the major malaria vector in South Africa following the near eradication of *An. funestus* by intensive IRS campaigns over the last two decades [2, 21]. Since *An. arabiensis* females are at least partially inclined to feed and rest outdoors, they are less susceptible to control by IRS [4,5]. This species is therefore considered to be the primary vector of residual malaria in South Africa [4].

Anopheles merus was collected from all three endemic provinces, with the highest numbers coming from Mpumalanga Province. Although *An. merus* has not been implicated in malaria transmission in South Africa to date, its confirmed vector status in other regions such as southern Mozambique (sporozoite rates for *An. merus* in the Boane District being 4.2%)22 suggests that it is most likely an important secondary malaria vector in South Africa as well. This species is primarily a coastal saltwater breeder, although it has also been collected from fresh water larval habitats in southern Africa including sites in South Africa[23].

Anopheles parensis and *An. vaneedeni* have been incriminated as secondary malaria vectors in South Africa [3,15], while other members of the *An. funestus* group (*An. rivulorum* and *An. leesoni*) have been implicated as secondary malaria vectors in East Africa. *Anopheles vaneedeni, An. rivulorum* and *An. leesoni* were collected from all three endemic provinces while *An. parensis* was only detected in KwaZulu-Natal Province during 2020. *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 hosts3. *Anopheles parensis* is primarily zoonotic and may rest indoors and outdoors. This species will also occasionally feed on humans[24] and can potentially contribute to residual malaria transmission in South Africa. The major vector *An. funestus s.s.*, the predominant malaria vector species in neighbouring Mozambique and Zimbabwe, was not detected in South Africa in 2020. This can be attributed to ongoing IRS programmes in the malaria-endemic provinces year on year.

Other species that occur in South Africa and that have been incriminated as malaria vectors in various African localities include *An. marshallii, An. coustani, An. demeilloni, An. pharoensis, An. pretoriensis, An. rufipes, An. squamosus* and *An. ziemanni* [16, 17, 18, 19, 20]. It is possible that one or more of these species plays a role in residual malaria transmission in South Africa. *Anopheles rufipes, An. pretoriensis, An. coustani* and *An. demeilloni* were present in all three endemic provinces in South Africa in 2020.

Anopheles population densities are expected to fluctuate between seasons. They are generally highest during the summer months, congruent with increased rainfall[4], translating into higher malaria transmission rates during summer and especially late summer. However, the highest number of malaria cases in South Africa in 2020 was recorded in the autumn months, followed by summer, spring

and winter. This disparity could be partly due to the hard lockdown imposed in March 2020 due to the COVID-19 pandemic. It was however also noted that particular species, especially *An. arabiensis* in northern KwaZulu-Natal Province, were present at comparatively high numbers during the dry winter months. This may be a consequence of continuous and intensive surveillance all year round in northern regions of that province.

The occurrence of primary and secondary vector species in all three of South Africa's malaria-endemic provinces shows that they remain highly receptive to malaria despite ongoing IRS operations each year. During 2020, the highest number of local malaria cases was recorded in Limpopo Province, from where only one *An. arabiensis* specimen was collected. This suggests that secondary vector species play an important role in ongoing malaria transmission there, which is likely true for the other endemic provinces as well.

CONCLUSION & RECOMMENDATIONS

Several malaria vector species occur in the north-eastern lowveld regions of South Africa, with their relative abundances remaining comparatively high through the dry winter months in some instances. Despite coordinated provincial IRS programmes that usually achieve high spray coverage rates (80% or more of targeted structures in endemic areas), populations of these species persist and at least three of them - *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 are multiple and certainly include outdoor-biting and outdoor-resting components of these species.

Based on this information, it is recommended that:

- Entomological surveillance be enhanced in the endemic provinces to monitor the bionomics of vectors responsible for residual transmission
- IRS based vector control be maintained at a high rate of coverage in areas of active transmission
- IRS activities should ideally be completed before the onset of each malaria season
- Consideration be given to a more targeted or reactive approach in areas where no local cases have been recorded for three or more years.
- Larval source management [25], including the treatment of winter breeding sites, be maintained to enhance the effect of IRS in high incidence areas
- Insecticide resistance management practices be maintained and periodically revised based on surveillance information
- In the context of the current COVID-19 pandemic, malaria control activities should be conducted especially timeously and efficiently. This will reduce the risk of co-infection in affected communities, reduce malaria-related hospitalisations as well as the burden of the health care system.

ACKNOWLEDGEMENTS

Entomology team members of the provincial Malaria Control Programmes of KwaZulu-Natal and Mpumalanga are thanked for the referral of surveillance specimens to the VCRL. Dr Patrick Moonasar, Dr Eunice Misiani, Prof Rajendra Maharaj, Mr Aaron Mabuza, Mr Eric Raswiswi, Mr Philip Kruger and all members of the South African Malaria Elimination Committee (SAMEC) are especially thanked for their support for vector surveillance. These activities were sponsored by the Mpumalanga and KwaZulu-Natal Malaria Control Programmes, the National Institute for Communicable Diseases, the NHLS Research Trust, the International Atomic Energy Agency, the Industrial Development Corporation and the South African Nuclear Energy Corporation (NECSA) through its Nuclear Technologies in Medicine Biosciences Initiative (NTeMBI) – a national platform funded by the Department of Science and Technology. The University of Pretoria Institute for Sustainable Malaria Control field collection is supported by UNICEF's humanitarian programme, "Generation Unlimited" coordinated by Future Africa. Prof Lizette Koekemoer is supported by a NRF/DST Research Chair Initiative grant. Prof John Frean is thanked for providing the National Department of Health malaria statistics.

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