Mosquito community composition and abundance at contrasting sites in northern South Africa, 2014–2017

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ABSTRACT:

Most data on species associations and vector potential of mosquitoes in relation to arboviral infections in South Africa date back from the 1940s to late 1990s. Contextual information crucial for disease risk management and control, such as the sampling effort, diversity, abundance, and distribution of mosquitoes in large parts of South Africa still remains limited. Adult mosquitoes were collected routinely from two horse farms in Gauteng Province; two wildlife reserves in Limpopo Province, at Orpen Gate in Kruger National Park (KNP) and Mnisi Area in Mpumalanga Province between 2014–2017, using carbon dioxide-baited light and tent traps. Mosquito diversity and richness are greater in untransformed natural and mixed rural settings. In untransformed wilderness areas, the most dominant species were Culex poicilipes , Anopheles coustani, and Aedes mcintoshi, while in mixed rural settings such as the Mnisi area, the two most abundant species were Cx. poicilipes and Mansonia uniformis . However, in peri-urban areas, Cx. theileri , Cx. univittatus, and Cx. pipiens sensu lato were the most dominant. Aedes aegypti, Ae. mcintoshi , Ae. metallicus , Ae. vittatus , Cx. pipiens s.l., Cx. theileri, and Cx. u nivittatus had the widest geographical distribution in northern South Africa. Also collected were Anopheles arabiensis and An. vaneedeni , both known malaria vectors in South Africa. Arbovirus surveillance and vector control programs should be augmented in mixed rural and peri-urban areas where the risk for mosquito-borne disease transmission to humans and domestic stock is greater.

Keyword Index: Mosquitoes, landscape, distribution, sampling, diversity, species richness.

INTRODUCTION

In southern Africa, the species associations and vector potential of mosquitoes in relation to arboviral infections received particular attention during the early 1940s to late 1990s (Edwards 1941, Worth et al. 1961, Jupp and Phillips 1998). These surveys indicated that South Africa is a hotspot for arboviruses of medical importance. Despite this earlier research and the increasing recognition of the importance of mosquitoes as agents of public health threats, there is little or no information on the diversity, distribution, and relative abundance of mosquito vectors in large parts of South Africa and the sub-region (van Der Linde 1982). A recent survey (Cornel et al. 2018) attempted to partially address this void by describing the species composition, diversity, distribution, and relative abundance of mosquitoes at selected broadly distributed localities within the country, but the study was limited in time and space.

Several landscape attributes have been shown to be important in predicting mosquito abundance and composition (Reisen 2010). Land cover/vegetation, canopy cover, soil properties, elevation, and hydrology are but a few of the known environmental factors that have been associated with abundance of mosquitoes (Burkett-Cadena

et al. 2013). An understanding of the abundance and spatial distribution of mosquito vectors across different landscapes is essential in determining the risk for mosquito-borne diseases (Roche et al. 2012). In mosquito-borne disease systems, pathogen transmission may vary with changes in host and mosquito species diversity, abundance, and distribution across a landscape (Smith et al. 2004, Burkett-Cadena et al. 2013). Since vectors are not evenly distributed within their geographical range, aggregation occurs within the landscape (Smith et al. 2004, Zhou et al. 2004, Ellis 2008). Similarly, the spatial pattern of disease risk is patchy, with the highest risk being in areas with consistent or periodic high numbers of prime vector species combined with patches of pathogen reservoir (Eisen and Eisen 2008). Thus, vector diversity, density, and distribution of species are important elements of the spatial dynamics of mosquito-borne diseases (Borg et al. 2007).

The present study was undertaken to provide greater detail regarding spatial variation in community composition, diversity, distribution, and abundance of mosquito vectors in sentinel monitoring areas of northern South Africa that were known to be prone to regular occurrences of zoonotic arbovirus outbreaks in humans or animals, and assessment of the effect of sampling effort. Collected mosquitoes were also

subjected to virus assays for vector incrimination to confirm or improve on the current understanding regarding vector status. Recent outbreaks of West Nile virus (WNV) (Venter et al. 2017), Sindbis virus (SINV), Middleburg virus (MIDV) (van Niekerk et al. 2015) and Shuni virus (SHUV) (van Eeden et al. 2012) have been recorded in animals in various sites in Gauteng, Limpopo, and Mpumalanga Provinces, forming the basis for site selection for monitoring mosquito dynamics and virus prevalence.

MATERIALS AND METHODS

Study sites

Monthly mosquito collections were carried out from 2014–2017 at four core sites: two nature reserves (Marakele National Park and Lapalala Wilderness Reserve) in Limpopo Province and two horse farms of which one was at Boschkop (East of Pretoria) and the other at Kyalami (Midrand/Johannesburg) in Gauteng Province in South Africa. Opportunistic sampling was also conducted at two other sites (from November, 2015 to May, 2017): Orpen Gate in Kruger National Park (KNP) and Mnisi in the Ehlanzeni District Municipality in Mpumalanga Province (Figure 1). The ecological features associated with these sites are Highveld grassland (Boschkop and Kyalami), Middleveld Bushveld (Lapalala and Marakele) and Lowveld Bushveld (Orpen and Mnisi) ecosystems (Rutherford et al. 2006). The Lowveld Bushveld is a region which varies between 150 and 600 m in elevation, while the Middleveld Bushveld lies between 600 and 1,200 m above sea level. The largest subregion is the plateau which makes up a 1,200–1,800 m high central area known as the Highveld. The sites also occur in different climatic zones ranging from warm semi-arid, subtropical oceanic highland to humid subtropical climates (Peel et al. 2007).

Geographic and climatic attributes of sampling sites

The key geographic and climatic attributes of the various sampling areas are summarized in Table 1. These attributes are critically important in enabling interpretation and understanding of mosquito diversity and abundance at the various collection sites. Also important to note is that the regular monthly sampling sessions at Boschkop, Kyalami, Lapalala, and Marakele meant that collections were made in both dry and wet seasons over multiple years. This also means that data distortions due to infrequent collections at Orpen and Mnisi during predominantly dry periods are compensated for and enable a more accurate reflection of true diversity and abundance.

Mosquito collection

Mosquito sampling for this study commenced in January, 2014 and continued until May, 2017. Sampling took place for three consecutive nights once every month at the nature reserves and for two nights at the horse farms, with each sampling period running from 16:00 to 06:00. Mosquitoes were collected using oral suction tubes, polystyrene cups, and two types of CO_2-baited traps: Centers for Disease Control (CDC) (Newhouse et al. 1966) miniature light traps and Mosquito Tent traps (MTT). Two light and three to five tent traps were mounted each night at the nature reserves, while two light and three tent traps were mounted per night at the horse farms. At the opportunistic collection sites, two mosquito tent traps and one light trap were erected for two nights at Orpen, while two tent traps were placed at Mnisi for two nights every month for 15 months. Traps were emptied at or very soon after dawn. All collection containers were placed in cooler boxes with dry ice at -80° C until microscopically sorted and identified on cold plates (to ensure virus preservation) a few hours later, and were then immediately returned to cold storage.

Mosquito identification

A stereomicroscope was used to separate mosquitoes placed over an ice brick. Mosquitoes were sorted by locality, trap type, and date of collection, and then morphologically

Figure 1. Map of South Africa showing the six vector surveillance sites. Red stars show the four core sites with regular monthly sampling, 2014 to 2017, while blue stars show opportunistic sites periodically from 2015.

Table 1. Geographic and climatic features associated with mosquito sampling sites in northern South Africa, 2014–2017.

	Lapalala	Marakele	Boschkop	Kyalami	Orpen	Mnisi
Location (GPS coordinates)	S23°53'59.8" E28°17'50.1"	S24°17'37.4 E27°30'11.7"	S25˚49'40.2" E28˚25'12.4"	S25°59'22.3" E28°01'56.1"	S24°28'55.8" E31°23'08.9"	S24°40'41.2" E31°16'15.5"
Province	Limpopo	Limpopo	Gauteng	Gauteng	Mpumalanga	Mpumalanga
Land-use	Wildlife Reserve	Wildlife Reserve	Horse farm	Horse farm	Wildlife Reserve	Mixed rural cattle/ wildlife/human use
Landscape description	Mountains and hills with fairly dense savanna woodland, several river-courses	Open plain with moderate to sparse savanna woodland, one narrow river	Peri-urban grassland	Peri-urban grassland	Flat, natural fairly dense savanna woodland	Flat, significantly transformed open savanna woodland
Ecological zone	Middleveld Bushveld	Middleveld Bushveld	Highveld Grassland	Highveld Grassland	Lowveld Bushveld	Lowveld Bushveld
Annual rainfall (mm)	400–900 (Ben-Shahar, 1987; Hulsman et al., 2010; Ruwanza and Mulaudzi, 2018)*	556–630 (van Staden and Bredenkamp, 2005)*	677–697**	723–790**	550–600 (Gertenbach, 1980)*	600–700 (Gertenbach, 1980)*
Annual mean maximum temperature (°C)	30.9**	30.2**	27.7**	25.6**	29.6**	29.4**
Annual mean minimum temperature (°C)	16.1***	14.5***	12.5***	12.6***	17.1***	17.1***
Altitude (m)	1,163	969	1,372	1,415	452	468
Blood-meal availability	Abundant and diverse range of birds and mammals	Abundant and diverse range of birds and mammals	Horses, humans, and limited range of other mainly domestic animals and some wild birds	Horses, humans, and limited range of other mainly domestic animals and some wild birds	Abundant and diverse range of birds and mammals	Plentiful mix of cattle, humans, birds, and low abundance of wildlife species.
Sampling effort in number of trap-nights	507	432	308	299	44	64

*References
**Source: Climate-Data.org
***Based on 3-year (2014–2016) climatic data provided by the South African Weather Service (SAWS). Means were computed on data from the SAWS Station close to the study site.

identified to species level using regional keys and descriptions (Edwards 1941, Gillies and De Meillon 1968, Gillies and Coetzee 1987, Jupp 1996). Damaged specimens lacking key morphological identification characteristics were identified to genus and recorded as such. Specimens destined for PCR assay (*An. funestus* group and *An. gambiae* complex) were placed in silica gel tubes, while other species were pooled maximum 50 specimens per tube, each species separately, for virus assays.

Data analysis

Kolmogorov-Smirnov (Lilliefors modification) and Shapiro-Wilk tests were used to analyze mosquito count data for normality, while Levene's test was used to test for homogeneity of variance with Statistica 13. Due to the lack of normality of the data, large standard deviations and lack of homogeneity of variance, non-parametric tests were used to analyze mosquito densities. Since mosquito abundance data were not normally distributed, the non-parametric test, Kruskal-Wallis (> two samples) test was used to assess differences in mosquito density/trap-night across localities and ecological zones. Chi-square tests were applied to investigate whether there were any differences in abundance of known or suspected mosquito vector species between sites. Species diversity, evenness, and equitability were calculated for all localities in the three Provinces, localities compared by category; wildife (Lapalala, Marakele, and Orpen), mixed rural (Mnisi), and peri-urban (Boschkop and Kyalami). As the performance of diversity estimators varies among data, the following well-known species diversity estimators were assessed using PAST software: Shannon and Simpson diversity indices. In order to ensure that our estimates of species richness (*S*) were reliable, we computed estimates of total species number based on extrapolations from species accumulation curves using the Chao1 richness estimator for each organism in PAST, and compared them with the observed total species. To verify whether sufficient trapping effort was made during the mosquito surveys to achieve a statistically sound estimate of species diversity, rarefaction curves of the species and the number of collected mosquitoes were generated using PAST software.

RESULTS

Interpolating species richness and sampling effort with rarefaction

Using two different trap types at core and opportunistic sites in northern South Africa, a total of 42,286 mosquitoes was collected from 1,654 trap-nights between January, 2014 and May, 2017. Rarefaction curves suggest that species richness was higher at Lapalala followed by Marakele but similar at Kyalami and Boschkop (Figure 2). Estimates of total species number based on trap type showed that our observed richness values likely underestimated total richness, as the rarefaction plots for Marakele and Lapalala were almost beyond their exponential curves and thus began to level off just beyond sixty species. Overall, estimates of species richness based on rarefaction predicted higher species numbers from

Table 2. A comparison of various diversity indices based on total mosquito collections at both core and opportunistic sites sampled in South Africa, 2014–2017.

	Boschkop	Kyalami	Lapalala	Marakele	Mnisi	Orpen
Taxa_S	38	39	74	60	41	29
Total number of individuals sampled	4,130	6,342	15,387	10,593	4630	1204
Simpson dominance (*D*)	0.2179	0.2305	0.09339	0.193	0.2082	0.686
Simpson diversity (1-*D*)	0.7821	0.7695	0.9066	0.807	0.7918	0.314
Shannon *H'*	2	1.882	2.842	2.219	2.079	0.8914
Shannon Evenness (*E*)_e^H/S	0.1945	0.1684	0.2317	0.1533	0.1949	0.08409
Equitability (*J*)	0.5499	0.5138	0.6603	0.542	0.5597	0.2647
Chao1	40.63	40.11	79.63	62.55	45.2	55

Table 3. Relative abundance of mosquito species collected from all sampling localities in the northern part of South Africa, 2014–2017.

Genus	Species	Light trap	Tent trap	Light + tent trap	Relative abundance (%)
Aedeomyia	*furfurea*	27	20	47	0.111
Aedes	*aegypti*	71	86	157	0.371
	albothorax	5	2	7	0.017
	argenteopunctatus	6	13	19	0.045
	caballus	2	0	2	0.005
	cumminsii	10	15	25	0.059
	dentatus	10	70	80	0.189
	dentatus group	94	18	112	0.265
	dentatus/leesoni group	47	48	95	0.225
	durbanensis	0	1	1	0.002
	fascipalpis	1	2	3	0.007
	filicis?	5	2	7	0.017
	fowleri	83	49	132	0.312
	furcifer/cordellieri	5	2	7	0.017
	haworthi	0	2	2	0.005
	heishi	1	0	1	0.002
	hirsutus	88	94	182	0.430
	juppi	40	13	53	0.125
	ledgeri	13	0	13	0.031
	leesoni group	19	3	22	0.052
	luteolateralis	0	1	1	0.002
	marshalli	2	0	2	0.005
	mcintoshi	1,430	2,178	3,608	8.532
	metallicus	24	21	45	0.106
	microstictus	32	38	70	0.166
	ochraceus	1	18	19	0.045
	pachyurus	53	12	65	0.154
	quasiunivittatus	404	239	643	1.521
	simpsoni	6	0	6	0.014
	sudanensis	5	15	20	0.047
	unidentatus	10	5	15	0.035
	unilineatus	32	9	41	0.097
	vexans	0	2	2	0.005
	vexans group	5	0	5	0.012
	vittatus	350	284	634	1.499
	spp	850	2,026	2,876	6.801
Anopheles	*coustani*	1,622	2,486	4,108	9.715
	funestus s.l.	237	204	441	1.043
	gambiae s.l.	75	397	472	1.116
	longipalpis	0	1	1	0.002
	maculipalpis	0	4	4	0.009
	marshalli	119	66	185	0.437
	natalensis	4	2	6	0.014
	pharoensis	20	44	64	0.151
	pretoriensis	72	86	158	0.374
	rhodesiensis	2	0	2	0.005
	rufipes	158	235	393	0.929
	squamosus	1,275	2,157	3,432	8.116
	theileri	1,789	722	2,511	5.938
	ziemanni	18	222	240	0.568
	spp	1,034	573	1,607	3.800
Coquillettidia	*cristata*	48	120	168	0.397
	fuscopennata	986	219	1,205	2.850
	maculipennis	15	4	19	0.045
	spp	7	196	203	0.480

| Genus	Species	Light trap	Tent trap	Light + tent trap	Relative abundance (%)
Culiseta	*longiareolata*	1	0	1	0.002
Culex	*theileri*	1,686	2,140	3,826	9.048
	annulioris	62	110	172	0.407
	antennatus	6	55	61	0.144
	argenteopunctatus	2	0	2	0.005
	bitaeniorhynchus	0	4	4	0.009
	duttoni	3	19	22	0.052
	ethiopicus	54	107	161	0.381
	horridus	0	2	2	0.005
	neavei	2	71	73	0.173
	nebulosus	1	0	1	0.002
	pipiens s.l.	597	1,081	1,678	3.968
	poicilipes	3,160	2,633	5,793	13.700
	rubinotus	0	30	30	0.071
	simpliciforceps	1	1	2	0.005
	simpsoni	3	4	7	0.017
	sitiens	1	4	5	0.012
	terzii	8	16	24	0.057
	thalassius	0	2	2	0.005
	tigripes	4	9	13	0.031
	trifilatus	0	2	2	0.005
	trifoliatus	5	19	24	0.057
	tritaeniorhynchus	0	4	4	0.009
	univittatus	1,427	2,272	3,699	8.748
	zombaensis	0	1	1	0.002
	spp	477	171	648	1.532
Ficalbia	*circuntestacea*	1	0	1	0.002
	uniformis	39	14	53	0.125
	spp	9	4	13	0.031
Mansonia	*africana*	0	41	41	0.097
	uniformis	235	1,213	1,448	3.424
	spp	8	66	74	0.175
Mimomyia	*hispida*	17	3	20	0.047
	lacustris	68	4	72	0.170
	mediolineata	4	0	4	0.009
	mimomyiaformis	2	6	8	0.019
	pallida	15	0	15	0.035
	spp	12	3	15	0.035
Uranotaenia	spp	22	5	27	0.064
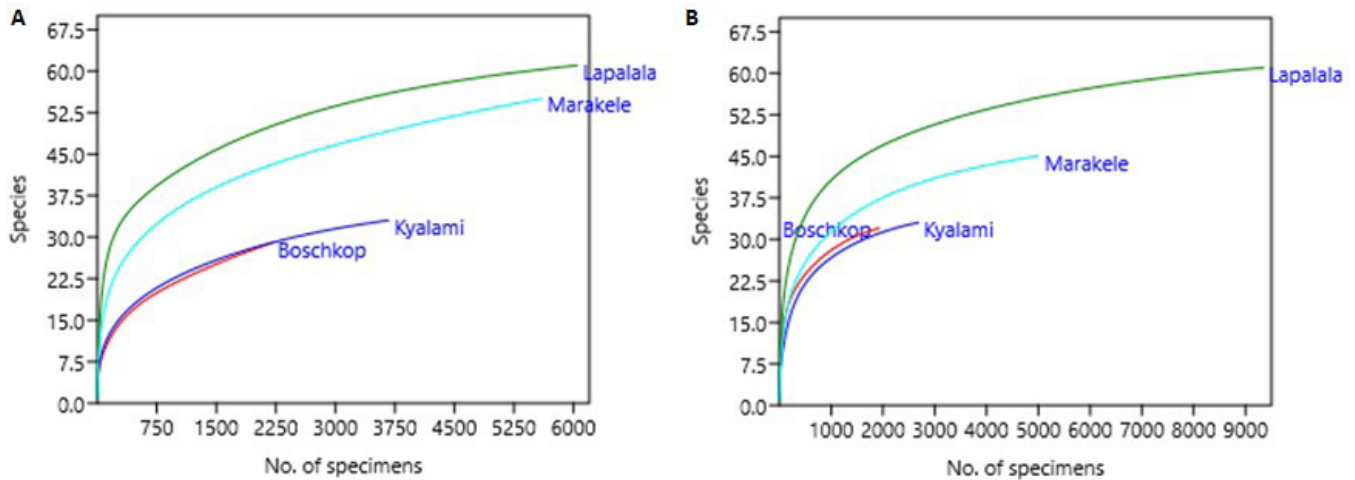
Figure 2. Sampling effort. Species-based rarefaction curves generated from tent (A) and light (B) trap collection data showing

tent than light traps at both Lapalala and Marakele for the same number of individuals, while the opposite was true at Boschkop (Figure 2 and Table 2).

Diversity indices

Mosquito species richness was highest at Lapalala Wilderness Reserve where a total of 74 species was documented over a period of three years (507 trap nights); the lowest richness of 29 species was recorded in a period of less than two years at Orpen (44 trap nights; Table 2). Biodiversity estimates on total collections of both core and opportunistic catches were calculated per locality (Table 2). A comparison of diversity using the Shannon and Simpson indices showed that Lapalala (*H'*=2.842, λ=0.9066) had the highest diversity while Orpen had the least (*H'*=0.8914, λ=0.314). The Simpson dominance index confirmed the presence of dominant species at Orpen (*D*=0.686), which is less diverse than the other sites. Species evenness (*E*) ranged from 0.08409 in Orpen to 0.2317 in Lapalala (Table 2). In this study, the mosquito community was somewhat evenly distributed at Lapalala but less so at Orpen. The Chao1 index also shows that there was a considerable underestimation of species richness at Orpen (Table 2).

Species composition and relative abundance of mosquitoes

Ninety-five species representing ten genera: *Aedeomyia, Aedes, Anopheles, Coquillettidia, Culiseta, Culex, Ficalbia, Mansonia, Mimomyia,* and *Uranotaenia* were collected in either or both the tent and light traps (Table 3). The most abundant genera were *Culex* (38.4%), *Anopheles* (32.2%), and *Aedes* (21.2%) (Table 3). This is a simplistic and generalized representation that neglects the underlying distortions of greater sampling intensity and habitat diversity at specific sites but nevertheless does facilitate some general overall impression of mosquito composition. *Culex poicilipes,* represented by 5,793 specimens, was the most abundant species caught in this survey, comprising 13.7% of the total (Table 3), but most individuals were collected from Marakele (where it represented 35.8% of the catches) and Mnisi (where

it represented 38% of the catches) (Figure 3). *Aedes aegypti, Ae. mcintoshi, Ae. metallicus, Ae. vittatus, Cx. pipiens* s.l., *Cx. theileri,* and *Cx. univittatus* had the widest geographical distribution in northern South Africa (Table 4). In the savanna regions at Middleveld and Lowveld, *An. coustani* (Lapalala), *Cx. poicilipes* (Marakele, Mnisi) and *Ae. vittatus* (Orpen) were the most dominant mosquito species. In the Central Highlands, at Boschkop and Kyalami, *Cx. theileri* and *Cx. univittatus* were the most abundant species. A sizeable population of *Cx. pipiens* s.l., accounting for approximately 20%, was among the three most dominant species at Kyalami. Species with relative abundances of less than 2.5% at a given habitat were considered as 'rare or other,' with eighty-three taxa falling into this category (Table 3), which represents 15.4% of total species recorded.

When the landscape of northern South Africa is differentiated into wildlife, mixed rural, and peri-urban locales, there are detectable differences in the species composition and relative abundances of the most dominant species (Figure 3). In mixed rural settings, the two most abundant species were *Cx. poicilipes* and *Ma. uniformis,* while in peri-urban areas, *Cx. theileri, Cx. univittatus,* and *Cx. pipiens* were the most dominant. However, in untransformed wilderness areas, the most dominant species were *Cx. poicilipes, An. coustani,* and *Ae. mcintoshi.*

Geographic distribution of mosquito species across localities and landscapes

The mean abundance of the most dominant mosquito species caught in both trap types at core opportunistic sites in northern South Africa is shown in Figure 4. The species are arranged alphabetically by genus from *Aedes* to *Culex.* Although present at all core sites, the mean abundance for *Ae. mcintoshi* was almost zero at Boschkop, Kyalami, and Orpen, but averages ranged between three and five mosquitoes/trap-night at Lapalala, Mnisi, and Marakele, respectively (Kruskal-Wallis = 115, *P*<0.001).

Anopheles coustani, although present at all sites except Orpen, attained higher abundances at Lapalala where the
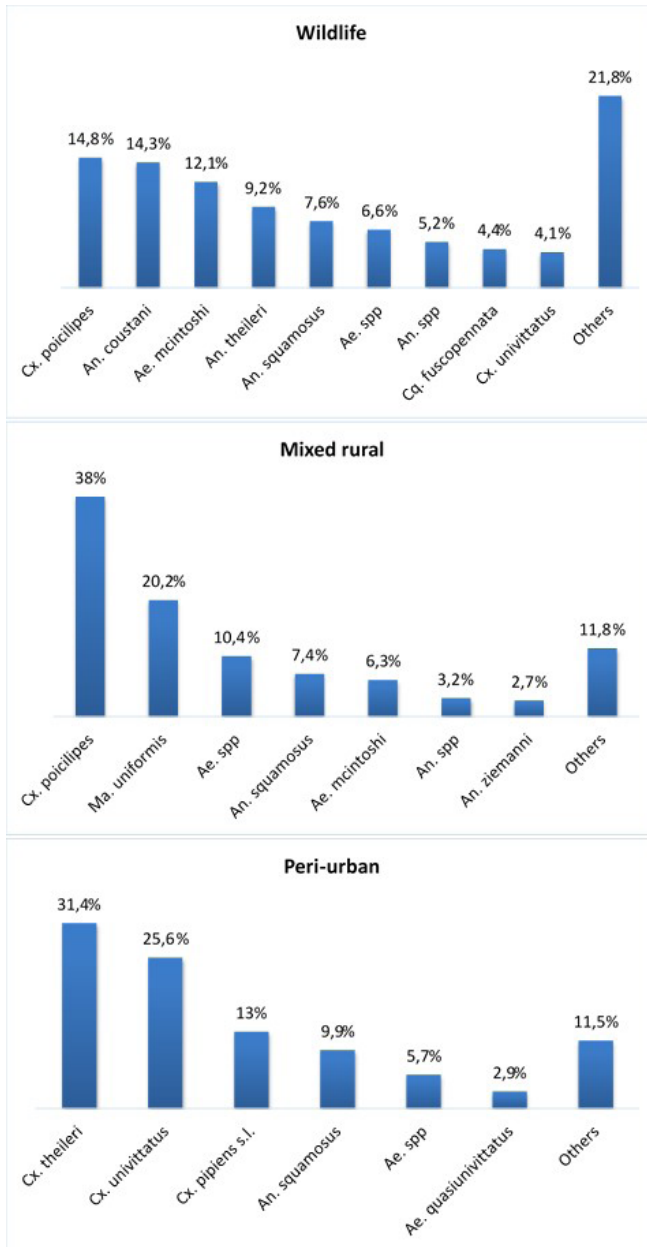
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Figure 3. Composition and relative abundance of dominant mosquito species. Mosquitoes were collected from both tent and light traps in three landscape settings within northern South Africa. A: Wildlife. B: Mixed rural. C: Peri-urban.

mean was almost six mosquitoes/trap-night (Kruskal-Wallis = 282, *P*<0.001. In the remaining three regions, this species was less abundant with the mean being ≤2 mosquitoes/trap-night. The average abundance of *Anopheles squamosus* was low at all sites, with the highest mean value recorded being slightly above five mosquitoes/trap-night in tent traps at Mnisi (Kruskal-Wallis = 126, *P*<0.001). This species was completely absent in both tent and light traps at Orpen. *Anopheles theileri,* though only recorded at Boschkop, Kyalami, Lapalala, and Marakele, only reached high numbers from light traps at Lapalala. The mean abundance of this species from light traps was nine mosquitoes/trap-night compared to less than two mosquitoes/trap-night in tent traps.

Coquillettidia fuscopennata was recorded only at Kyalami, Lapalala, Marakele, and Mnisi, with a mean of five mosquitoes/trap-night at Lapalala and almost zero at Kyalami, Marakele, and Mnisi (Kruskal-Wallis = 183, *P*<0.001).

Although present at all localities, *Cx. theileri* was the most abundant species at Kyalami with average catches reaching six and 11 mosquitoes/trap-night in tent and light traps, respectively. *Culex univittatus,* though present at all sites, was only common at Boschkop and Kyalami.

The abundance for *Culex pipiens* s.l. was generally low at all localities except Kyalami, where the mean was above three mosquitoes/trap-night. *Culex poicilipes* was the most abundant at Mnisi, where it attained an average of 26 mosquitoes/trap-night from tent traps. However, at Marakele, the mean was highest in light traps. Although there was a significant difference in mosquito density among sites for each of the species (Kruskal-Wallis test *P*-value for all species was <0.001), high standard deviations from the mean were registered for most species (Figure 4).

In the Lowveld, *Culex poicilipes* reached a peak density of 13 mosquitoes per trap from <1 mosquito per trap in the Highveld region. The mean density for *An. squamosus* increased from <2 mosquitoes per trap in the Highveld to more than two mosquitoes per trap in the Lowveld region. *Mansonia uniformis* reached a high of seven mosquitoes per trap in the Lowveld from <1 in the Highveld. The mean density for *Culex pipiens* s.l. decreased from the Highveld (>2 mosquitoes/trap) to the Middleveld (almost zero mosquitoes/trap), but then a slight increase was noticed in the Lowveld (<1 mosquitoes/trap). A clear decline in mosquito density was noticed for *Cx. univittatus* and *Cx. theileri* from the Highveld to the Lowveld. As for *Ae. mcintoshi*, there was a tendency for the mean density to increase towards the center of the distribution range for this study, which is the Middleveld. Similar tendencies were also observed for *An. coustani* and *Cq. fuscopennata*.

Abundance and distribution of mosquitoes of known medical importance

Thirty species known or suspected as vectors of arthropod-borne viruses (arboviruses) in southern Africa and elsewhere were collected during this survey. Each of the surveyed localities had more than nine species and at least three genera that are suspected vectors. The highest diversity of potential vectors was recorded at Lapalala and Marakele
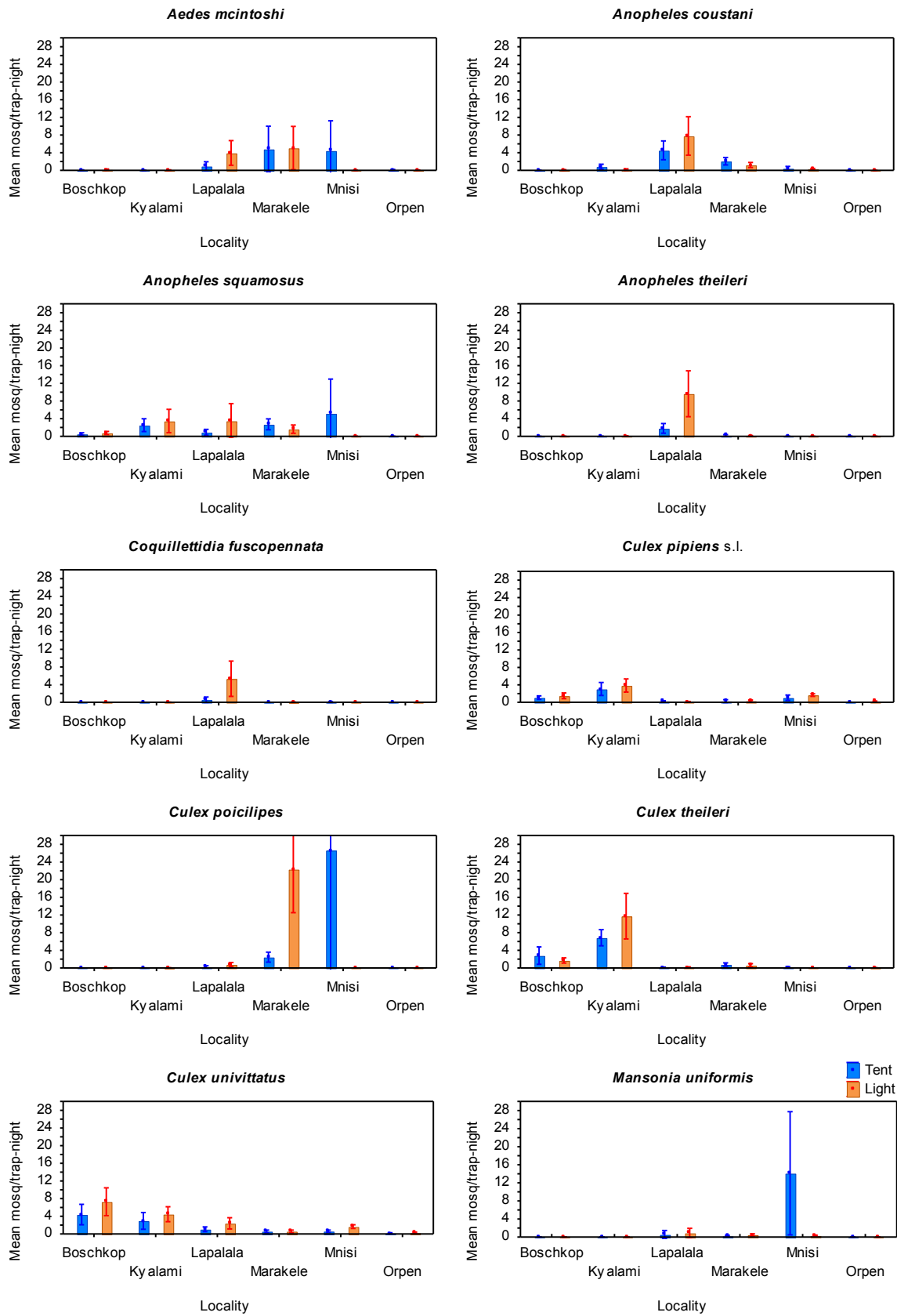
Figure 4. Mean abundance of most dominant mosquito species. These species were collected from core sites within South Africa between January, 2014 and May, 2017. The whiskers and boxes on the bars represent SDs and SEs of the mean of mosquitoes/trap-night for tent and light trap collections.

Table 4. Known or suspected vector mosquitoes and their abundances at six localities in northern South Africa.

Species	Known or suspected vector for	Boschkop	Kyalami	Lapalala	Marakele	Mnisi	Orpen
An. coustani	Malaria; Viruses (Bwamba. PGA. Uganda S & RVF)	16	185	3,053	822	32	0
An. funestus s.l.	Malaria; Viruses (Bwamba; O'Nyong-Nyong)	2	2	295	113	29	0
An. gambiae s.l.	Malaria; Viruses (Bwamba; O'Nyong-Nyong)	0	0	106	353	4	9
Ae. aegypti	CHIK, Chaoyang, Dengue (1-4), RVF, Uganda S, YF and ZIK viruses	35	42	15	14	20	31
Ae. argenteopunctatus	RVF and SF viruses	0	0	7	12	0	0
Ae. caballus	MID, RVF and WES viruses	0	0	0	2	0	0
Ae. cumminsii	RVF, Spondweni and Shokwe viruses	5	1	5	14	0	0
Ae. dentatus	MID and RVF viruses	0	80	0	0	0	0
Ae. fowleri	RVF, Spondweni and WES viruses	1	4	99	28	0	0
Ae. furcifer/cordellieri	CHIK, Dengue and YF viruses	0	0	5	1	0	1
Ae. juppi	MID, WES and RVF viruses	33	18	0	2	0	0
Ae. mcintoshi	BBK, Bunyamwera, MID, Ndumu, NRI, Pongola, RVF and WES viruses	29	6	1,063	2,212	294	4
Ae. metallicus	YF virus	2	1	21	7	1	13
Ae. ochraceus	Ndumu and RVF viruses	0	0	4	15	0	0
Ae. simpsoni	BBK, NRI and YF viruses	4	2	0	0	0	0
Ae. unidentatus	WES and RVF viruses	2	11	2	0	0	0
Ae. vittatus	BBK, CHIK, NRI, PGA, YF and ZIK viruses	2	2	544	17	3	66
Cq. fuscopennata	SIN virus	0	2	1,192	6	5	0
Cx. annulioris	SIN virus	5	3	160	0	4	0
Cx. antennatus	RVF, SIN and WN viruses	0	2	47	8	2	2
Cx. neavei	Bagaza, Mossuril, SIN, Spondweni, USU, WES and WN viruses	0	0	5	3	67	0
Cx. pipiens s.l.	OLI, RVF, SF, SIN, Uganda S, USU and WN viruses	372	992	97	138	75	4
Cx. poicilipes	RVF, Bagaza, BBK and WN viruses	1	5	234	3,794	1,759	0
Cx. rubinotus	Arumowot, Bunyamwera, Banzi, Germiston, Ndumu and Witwatersrand viruses	0	0	29	1	0	0
Cx. sitiens	Mossuril virus	0	0	3	1	0	1
Cx. thalassius	Bagaza virus	0	0	0	2	0	0
Cx. theileri	Germiston, RVF, Shuni and WN viruses	759	2528	70	329	12	1
Cx. tigripes	BBK, Mossuril and SIN viruses	0	2	6	0	5	0
Cx. univittatus	Bagaza, SIN, WES, USU and WN viruses	1,641	1,036	824	268	46	11
Cx. zombaensis	RVFV	0	0	0	1	0	0
Ma. africana	Bancroftian filariasis, Viruses (Bunyamwera, MID, Ndumu, RVF, Shokwe, SIN, Spondweni, USU)	0	0	0	0	41	0
Ma. uniformis	Bancroftian filariasis, Viruses (Bwamba, Ndumu, O'Nyong-Nyong, Spondweni, WES, ZIK)	0	2	366	147	933	0

with the least being from Orpen. While *Ae. aegypti*, known to transmit a wide range of arboviruses, was common at all six localities, it occurred in low numbers. Two species complexes known to contain vectors of malaria, *Anopheles gambiae* s.l. and *Anopheles funestus* s.l., were collected. Of the *Anopheles gambiae* complex, *An. arabiensis* was collected from Lapalala, Marakele, Orpen, and Mnisi, albeit in very low numbers except for Marakele, where it was among the most dominant vectors. Of the *Anopheles funestus* group, the species identified by PCR assays was *An. vaneedeni,* a secondary vector for malaria in the region (Burke et al. 2017). There was a significant difference between locality and mosquito abundance for the seven widely distributed potential vectors of arboviruses with the Chi-Square test *P*-value for all species being <0.001).

DISCUSSION

In this survey, estimates of species richness from the individual-based rarefaction curves reveal that the spatial distribution of mosquitoes in the selected wildlife and peri-urban sites in the northern part of South Africa is random. The sample size for the rarefaction models was large at the core sites and the curves were flattened towards the right suggesting high species turn-over. The flattening of the graph at a later stage signifies repetition of similar species at wildlife sites, Lapalala and Marakele; in other words, very little addition of new species. Despite reasonable sampling being achieved, it is possible that additional intensive sampling may continue to yield new species as suggested by the Chao1 estimate (Table 3) if larger sampling habitats are explored. Statistical approaches such as the Chao1 are widely used to estimate the number of species present, including those yet to be discovered. In this case, extra sampling is required to detect new species; however, richness estimators do not specify how much sampling effort (individuals or samples) is required (Chao et al. 2009). Nonetheless, for effective sampling to occur, special attention should be given to the species abundance distribution as it needs greater sampling effort where evenness is low (Lande et al. 2000, Yoccoz et al. 2001). Because differences in species yields between tent and light traps were observed, there is a need to use both trap types for ecological, arbovirus, and malaria vector surveillance, as found in other studies as well (Cornel et al. 2018). Although there is variation in the degree to which different mosquito species are attracted to light traps, this method is among the most widely used tools for vector surveillance due to ease of use and practicality. However, one of the disadvantages of using light traps is that they are usually biased in ways that may affect data interpretation for epidemiological studies. If used alone, light traps often fail to collect important or infected vectors and can be unproductive when competing ambient light is present (McDermott and Mullens 2017).

Mosquito species richness and diversity were higher in protected areas within the savanna, such as Lapalala Wilderness Reserve and Marakele National Park. A similar study also found high levels of diversity at Lapalala and other wildlife reserves which are located within the southern African savanna such as KNP and the Okavango

Delta in Botswana (Cornel et al. 2018). These patterns of diversity are probably related to the availability of diverse breeding habitats, ecological integrity, abundant wildlife as sources of bloodmeals, abundance of vegetation/canopy cover, landscape/topographic heterogeneity, an array of microclimates, favorable climate, and plenty of water in these areas (Gillies 1953, Rueda et al. 1990, Moncayo et al. 2000, Chaves et al. 2011, Cornel et al. 2018). The rich structural complexity at Lapalala and Marakele has led to fine partitioning of habitat space. As a result, species abundance has become more uniform with differences in the abundance of many species that coexist in untransformed areas being more gradual or evenly distributed (Verberk 2012). Our results appear to suggest that mosquito species richness decreased along landscape-level drivers such as climate, elevation, vegetation, and host availability from the Middleveld savanna portion of Limpopo Province to Lowveld savanna areas. Conversely, the decrease in species richness from Middleveld to Highveld Grassland areas in Gauteng Province reveals the presence of cold adapted species such as *Ae. dentatus, Ae. juppi, Cx. theileri,* and *Cx. univittatus.* These are mosquitoes associated with temperate or high altitude areas receiving higher amounts of rainfall (Jupp 2004).

As part of a major wildlife reserve, KNP, Orpen has a constant presence of wildlife including non-human primates but is located in a dry woodland setting with low availability of surface water for much of the year. In this study, species homogeneity and equitability were lowest at Orpen. The level of diversity was also much lower than in the two other wildlife sanctuaries at Lapalala and Marakele. Reasons for this low diversity may well have been related to relatively few collection events and the generally dry conditions which prevailed preceding all these Orpen collection periods. The importance of taking such local context into account is reflected in the findings of Cornel et al. (2018), where certain localities such as Shingwedzi, Lower Sabie, and Tshokwane had high but different diversity indices, in contrast to the low diversity findings at Orpen in this current study, despite all these locations being within the same wildlife reserve (KNP). The same need for an understanding of local contextual differences in habitats is essential to understand the low richness observed at nature reserves such as Rooipoort (Northern Cape, SA), Kogelberg (Western Cape, SA), Tswalu (Northern Cape, SA), and Vilankulo district in southern Mozambique (Cornel et al. 2018).

While the features used to describe 'urban' sites differ greatly among studies, our study found moderately high species diversity in peri-urban areas compared with mixed rural settings, such as Mnisi. This level of diversity recorded at Boschkop and Kyalami can be attributed to the availability of diverse breeding habitats both natural and artificial, plenty of surface water (ponds, dams, rivers), and animal hosts, such as domestic stock and birds. However, Mnisi has a constant presence of domestic stock, humans, and diverse birdlife but is located in a dry woodland setting prone to droughts and there were also few mosquito collection events that took place in the area during the study period. Nevertheless, this study supports previous findings suggesting that mosquito

diversity is highest in untransformed natural areas such as nature preserves (Marí and Jiménez-Peydró 2011, Reiskind et al. 2017, Cornel et al. 2018).

Our study, which was mainly focused on adult mosquitoes, found *Culex* and *Anopheles* to be the most prevalent genera across all surveyed sites in the northern part of South Africa. Other studies carried out in close proximity to the study area in the past decades have revealed different findings based on similar and different trapping techniques. In 1953, a total of 538 mosquitoes comprising 21 species in three genera was collected in a period of two weeks in the upper Limpopo River Valley not far from Lapalala (Steyn et al. 1955). A similar study conducted by Cornel et al. (2018) between late-January and early-April, 2015 collected 297 individuals comprising 19 species from five genera and found *Anopheles* and *Coquillettidia* to be the most dominant at Lapalala. Our analysis conducted over a period of 38 months in the same area recorded a total of 15,387 mosquitoes composed of 74 species from nine genera with a predominance of *Anopheles* and *Aedes*. Another survey of culicines conducted for slightly over two weeks in KNP in 1953 collected four genera comprising 25 species, with *Aedes* and *Culex* being the most abundant at multiple collection sites, including Orpen (Schulz et al. 1958). Contrastingly, we caught a total of 1,204 adult mosquitoes at Orpen which were composed of 29 species from three genera, with a predominance of *Aedes* (95%) and *Culex* (3.5%) species in a period of 15 months. Despite the limited number of breeding sites available for sampling at Orpen, the number of aedine species caught was slightly more than what Schultz et al. (1958) collected from KNP.

Previous studies have shown that there is a tendency for species to be most abundant in the center of their geographic ranges (Hengeveld and Haeck 1982, Brown 1984, Holt et al. 1997, McGill and Collins 2003). In this study, *Cx. pipiens* s.l., *Cx. theileri,* and *Cx. univittatus* were most abundant in the Highveld grassland region (Kyalami and Boschkop), confirming the findings of earlier studies (McIntosh et al. 1980, Jupp 2004). Our results also suggest that *Cx. theileri* is rare in the Limpopo-Mpumalanga Lowveld as observed by Jupp (2004). *Aedes mcintoshi*, *An. coustani*, *An. theileri,* and *Cq. fuscopennata* were more abundant in the Middleveld Bushveld region (Lapalala and Marakele), as found by others (Cornel et al. 2018). *Aedes mcintoshi* appears to be adapted to areas with intermediate temperatures and is widely distributed in northeastern Highveld, the Zimbabwean Highlands, as well as in the Lowveld, coastal region of KwaZulu-Natal, and the Karoo (McIntosh 1971, Jupp 2004). *Anopheles squamosus*, *Cx. poicilipes,* and *Ma. uniformis* were quite common in the Lowveld Bushveld region as observed by other workers (Gillies and De Meillon 1968, Cornel et al. 2018). *Culex poicilipes* appears to have a strong ecological plasticity that allows for its adaptation in the three ecological zones but was among the most widespread species (Cornel et al. 2018). *Mansonia uniformis* was one of the most dominant species we collected from Mnisi, a site in the Lowveld region just outside KNP, and was equitably abundant in some parts of Botswana, Mozambique, and South Africa, which are Middleveld and Lowveld (Cornel et al. 2018). However, in other recent

mosquito surveys conducted between March and April, 2017 at 16 sites within KNP, the most common species collected included members of the *Ae. vexans* complex, *Cx. pipiens* complex, and *Cx. univittatus* complex (Gorsich et al. 2019), the first of which was not collected in this study.

It is important to note that of the 95 species of mosquitoes documented in this work, eight have never been recorded previously in some of the study areas. This study allowed the capture of species poorly and irregularly found in northern South Africa such as *Ae. luteolateralis*, *Ae. pachyurus*, *Cx. sitiens*, *Fi. circumtestacea*, *Fi. uniformis*, *Mi. lacustris,* and *Mi. pallida*. In Gauteng Province, Highveld, several specimens were identified as *Ae. pachyurus*, a species previously only recorded from KwaZulu-Natal and the Cape Provinces (Muspratt 1955, Worth and Paterson 1961). *Aedes luteolateralis,* previously believed to only occur in Free State (Jupp 1996), KwaZulu-Natal (Huang 1985) and Cape Provinces (Jupp 1996) was recorded in our surveys in Limpopo Province. *Culex sitiens*, initially known to occur in Mozambique (Worth and Meillon 1960), was also found in Limpopo. *Ficalbia circumtestacea*, a species only found in KwaZulu-Natal in the early 1960s (Worth and Paterson 1961), is now confirmed from Limpopo Province, while *Fi. uniformis,* previously detected in Mozambique and Zimbabwe between the mid-1950s and early 1960s, is now also found in Limpopo Province, which is adjacent to the two countries (Leeson 1958, Worth and Meillon 1960). *Mimomyia lacustris* and *Mi. pallida,* previously known from KwaZulu-Natal, have now been found in Limpopo Province. It is not possible to ascertain whether these taxa have extended their geographical range or whether their finding in this work was due to a more extensive (new sites sampled) and sustained (three whole years) survey. From our study and many others, it is not easy to explain the complete absence of some species from certain localities at times and their occurrence within the same environs at other times in large numbers, suggesting that their bioecology requires further investigation, namely into their breeding/immature stages.

The high diversity of potential arbovirus vectors partly explains the periodic outbreaks of arbovirus cases recorded at the various collection sites and emphasizes such risk in the future. *Aedes mcintoshi*, *Cx. pipiens* s.l.*, Cx. theileri*, *Cx. univittatus, and Cx. poicilipes*, found in this study in considerable abundance, are medically important for their role in arboviral transmission in southern Africa and particularly in northern South Africa. *Aedes mcintoshi* is a major vector of Wesselbron virus (WESV) on the temperate inland plateau of South Africa and on the Zimbabwean Highlands (Jupp 2004) and an important maintenance vector of Rift Valley fever virus (RVFV) in Zimbabwe and possibly also on the inland plateau in South Africa (Swanepoel and Coetzer 2004). *Culex pipiens* s.l., more prevalent in the urban areas in Gauteng Province and to a lesser extent in wildlife regions such as Marakele National Park, is a known vector for WNV and RVFV, and the lesser known Olifantsvlei (OLI), Semliki Forest (SF), Sindbis (SIN), and Usutu (USU) viruses, and is known as a bridge vector for its mixed anthropophily and ornithophily habits (McIntosh 1978, Swanepoel 2003,

Braack et al. 2018, Venter 2018). *Culex theileri* is one of the main epidemic vectors of RVFV in domestic animals in South Africa and Zimbabwe, as well as a competent vector for WNV and SINV (Jupp et al. 1972, Swanepoel and Coetzer 2004). *Culex univittatus* is common in the Highveld and Karoo regions, where it is a key vector of WNV and SINV (Jupp 1971). *Culex poicilipes* is an epidemic vector for RVFV in the Lowveld (Swanepoel and Coetzer 2004), thus presenting the possibility of triggering zoonotic outbreaks of the disease in an area which has a wildlife/livestock/human interface.

   Aedes dentatus, which is a vector for Middelburg virus (MIDV and RVFV) while *Ae. unidentatus* is a vector for RVFV, were found in this study in the Highveld Grassland region, as well as in others (Jupp and Cornel 1988). *Aedes juppi*, also found in this study, is known to transmit MIDV, RVFV and Wesselsbron virus (WESV) in the same region (Jupp et al. 1987, Jupp and Kemp 1998). *Aedes vittatus*, a potential vector for Babanki (BBK), Chikungunya (CHIK), Ngari (NRI), Pongola (PGA), yellow fever (YF), and Zika (ZIK) viruses, though present at all localities, was recorded in copious numbers at Lapalala, with total catches exceeding 500 mosquitoes for the entire duration of the study. In fact, outbreaks of WNV (Venter et al. 2017), SINV, MIDV (van Niekerk et al. 2015), and Shuni virus (van Eeden et al. 2012) have recently been recorded in animals around the six surveillance spots, which highlights the presence of some known potential vectors in these sites. Current investigations are being carried out to screen these mosquito collections for the presence of such various arboviruses. Widespread distribution of *Ae. aegypti* was recorded in this study, although at low levels. This should be monitored for its epidemic potential of emerging or imported viruses.

   With regard to malaria vectors, the eastern parts of Limpopo and Mpumalanga Provinces are malaria endemic regions where the main vector is considered to be *An. arabiensis* (a member of the *An. gambiae* complex) with *An. vaneedeni* (a member of the *An. funestus* group) playing a secondary role (Burke et al. 2017). Nevertheless, this information is of importance and value from a public health perspective in creating awareness among health department officials and tourism operators regarding the potential for malaria transmission. *Anopheles gambiae* complex was recorded in high numbers in northern KNP, part of Limpopo Province (Munhenga et al. 2014), while 85% of mosquito catches at Thomo Village (also northeastern Limpopo Province) were comprised of *An. funestus* complex (La Grange and Coetzee 1997). Further evidence is that the malaria vector *An. merus* (also a member of the *An. gambiae* complex) has previously been recorded in high numbers in the southern region of Mpumalanga Province, geographically not very distant from the Orpen and Mnisi collection sites where *An. merus* was not found (Mbokazi et al. 2018). However, an assessment of anopheline mosquitoes at Mahlabaneni Village in Eswatini (south of Kruger National Park), found *An. arabiensis* to be the most abundant malaria vector in the area (La Grange 1995). These results contrast with the findings of this study, yet again emphasizing local differences and the dangers associated with extrapolating findings from one area to other localities even within the same geographic province or region.

   Our findings suggest that mosquito diversity and richness are greater in untransformed natural areas, especially the Middleveld Bushveld region and mixed rural settings, compared to peri-urban areas in the northern part of South Africa. Landscape-related factors such as land use, vegetation, host presence, and hydrological characteristics appear related to mosquito abundance, distribution, and mosquito composition. The most widely distributed known and potential arbovirus vector species detected in this study were *Ae. aegypti*, *Ae. mcintoshi*, *Ae. metallicus*, *Ae. vittatus*, *Cx. pipiens* s.l., *Cx. theileri,* and *Cx. univittatus*. The most important malaria vector in northern South Africa is *An. arabiensis,* while *An. vaneedeni* plays a lesser role in malaria transmission in the region, and our project confirmed its presence at Orpen and Mnisi. Future investigations should focus on arbovirus and vector surveillance and control programs in peri-urban and mixed rural settings where there is greater risk for arbovirus transmission to humans and domestic stock.

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