

Mosquito community composition in Central District, Botswana: insights from a malaria endemic to non-endemic gradient

M Buxton^{1,2}, C Nyamukondiwa^{2,3}, M Kesamang² and RJ Wasserman^{3,2}

¹Department of Biological Sciences, Physical and Chemical Sciences, Botswana University of Agriculture and Natural Resources, Gaborone, Botswana

²Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology, Palapye, Botswana

³Department of Zoology and Entomology, Rhodes University, Makhanda, South Africa

Spatial distribution of vector mosquitoes plays a critical role in the dynamics of associated diseases' spread across diverse landscapes. In Botswana, six Districts are implicated as malaria endemic zones, one of which is the Central District comprising both malaria endemic and non-endemic sub-districts. Despite being the biggest in the country, mosquito diversity in this District is under-explored, more so in the malaria non-endemic sub-districts. Here, we thus sampled mosquito adults and larvae from the malaria endemic sub-district of Bobirwa and non-endemic sub-districts of Palapye and Serowe, to determine spatial mosquito abundance and diversity in the District. Overall, all the sub-districts had a representation of key mosquito taxa of medical and economic importance (*Aedes*, *Culex* and *Anopheles*), irrespective of malarial endemicity status. Bobirwa had the highest number of mosquitoes sampled (429) although the greatest species richness (0.8511) was observed from Palapye. Moreover, Palapye also recorded a species from another genus, *Culiseta longiareolata*, a known natural vector of avian malaria parasites. Given global climate shift projections for the region, there is a need for continuous area-wide surveillance for vector mosquitoes and associated parasites in curbing the risk of emerging and re-emerging infections. While the *Anopheles*-centric approach to mosquito control is still necessary, a holistic approach, incorporating other vector incriminated mosquito species is warranted, particularly given shifting climates and the presence of invasive disease associated vector mosquito species.

INTRODUCTION

Through the transmission of parasites such as protozoans, helminths and viruses, mosquitoes facilitate incapacitating diseases such as malaria, Zika, dengue, West Nile fever, chikungunya (Lee et al. 2018; Ralapanawa & Kularatne 2020). Globally, the introduction of invasive vector mosquitoes in new environments are on the rise (Akiner et al. 2016; Wilke et al. 2020) and so are numbers of other vector incriminated mosquito species and associated parasites (Braack et al. 2018). This has led to a concomitant surge in cases of vector-borne diseases (WHO 2014), with disease vector mosquitoes contributing increasingly to human morbidity and mortality worldwide (Ahmed et al. 2020; Boyer et al. 2020). Similarly, although less well documented, vector mosquitoes also contribute significantly to livestock and wildlife disease transmission (Mroz et al. 2017). The distribution of mosquitoes has been well studied in tropical and temperate regions worldwide (Atoni et al. 2019; Foster & Walker 2019; Martinet et al. 2019) with biotic and abiotic factors largely shaping mosquito community composition (Biteye et al. 2018; Akpan et al. 2019; Cane et al. 2020; Moutassem et al. 2020). There is, however, continued scope for further exploration in this regard, given that changing environmental conditions and biological invasions are resulting in shifts in distribution of known vectors, and the presence of new vector mosquito species and associated pathogens (Weyl et al. 2019; Pereira dos Santos et al. 2018).

Climatic and human-mediated factors are regarded as crucial drivers of mosquito population dynamics (Monaghan et al. 2018; Lee et al. 2020; Wang et al. 2020). Given the recent increase in shifting climates and anthropogenic activities facilitating vector mosquito species (Schrama et al. 2020; Wang et al. 2020), it is highly likely that certain mosquitoes (Liu-Helmersson et al. 2019) and indeed their associated pathogens (Braack et al. 2018) may proliferate and translocate to novel environments. Many mosquitoes have also been reported as being invasive (Vaux et al. 2019; Fălcută et al. 2020) with potential of dispersing across different landscapes (Kampen et al. 2017; Strickman 2020). This necessitates the need for continual and revised spatio-temporal monitoring of known and potential vector species (Ludwig et al. 2019; El-Sayed & Kamel 2020). The geographic distribution and composition of mosquito communities is well documented in Africa (Jupp & McIntosh 1990; Njabo et al. 2009; Alves et al. 2010; Johnson et al. 2020) albeit biased towards malaria-burdened areas (Coetzee et al. 2000; Wiebe et al. 2017). As such, anopheline mosquitoes are the focus of much of the biological, autecological and even community ecology studies in the region, particularly in southern Africa (Ferguson et al. 2010; Zengenene et al. 2020). As a result, little work has focused on culicine mosquito species in southern Africa (but see Pachka et al. 2016; Matowo et al. 2019; Kahamba et al. 2020).

Botswana mosquito research is largely reflective of that of the region, with mosquito work mainly explored within parts of the country that experience human malaria cases (Chirebvu & Chimbari 2015; Pachka et al. 2016; Tawe et al. 2017; Cornel et al. 2018; Kgoroebutswe et al. 2020). Malaria is currently the most apparent mosquito-borne infection in the country resulting in anopheline

CORRESPONDENCE

M. Buxton

EMAIL

lodybuxton@hotmail.co.uk

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SUPPLEMENTARY MATERIAL

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species research prioritisation, particularly within districts referred to as “malaria endemic”, where historical malaria cases have been high. In contrast, the “non-endemic” malaria areas are largely neglected (Bango et al. 2020). Furthermore, the vector control monitoring programmes are also focused on anopheline species within the endemic localities. Areas that were previously known to be malaria free (e.g. Palapye, Serowe, Ghanzi, Kweneng) have, however, recently recorded both indigenous and imported malaria cases (Chihanga et al. 2016; Bango et al. 2020). For example, in recent years, malaria cases and deaths have been reported persistently across sub-districts in the Central District, with Bobirwa having the highest cases, Serowe exhibiting the least while Palapye region remained intermediate (Table S.1). But in addition to malaria, there are other mosquito-borne diseases in the region, vectored by non-anopheline mosquitoes, but are overlooked by the vector control monitoring programmes. For example, *Culex* spp. are known vectors of diseases for livestock and wild animals, although very little work has been conducted on this in the region. Similarly, globally invasive vector species have been detected in surrounding countries (Masaninga et al. 2014; Noden et al. 2014; Weyl et al. 2019), and very recently from a single sub-district in Central District of Botswana (Buxton et al. 2019), although no further investigation of their prevalence in Botswana has been explored. As such, nation-wide vector mosquito monitoring data remains scant. This has hindered understanding of mosquito species distribution and predictive modelling of vector-borne disease outbreaks. This also has critical implication on modelling vector borne disease risk in areas or for taxa regarded of ‘less concern’. An understanding of available mosquito species would facilitate development of policy for a move from the *Anopheles*-centric approach to a more inclusive vector management strategy, including screening for associated human, domestic and wildlife associated pathogens and parasites.

Given its primacy and centrality, and attracting national developmental attention, Central District is an area requiring research prioritisation in Botswana. Owing to the confluence of so many trade and touristic routes, the risk of introduction of new vectors and or associated pathogens/parasites in this district may be high. The consequential effects of these introductions to trade and tourism could also be highly significant. The aim of the study was to conduct an assessment of the mosquito species composition in human inhabited areas in the district. More specifically, species composition was contrasted across a malaria endemic to non-endemic sub-district gradient. We hypothesised that (i) abundance of *Culex* spp. would be more than other mosquito species due to the influence of favourable anthropogenic-degraded habitats in highly urbanised sub-districts (ii) anopheline species would dominate in the malaria endemic Bobirwa where malaria and associated vectors and pathogens are indigenous and historically prevalent, and that (iii) invasive *Aedes* spp. would be present in the Palapye and Serowe sub-districts given their increased urban size and proximity to South Africa where this species has been reported (Weyl et al. 2019), and their previous presence being recorded in Palapye (Buxton et al. 2019). The results of this study will serve as important findings for future work for these understudied regions (Tawe et al. 2017; Bango et al. 2020; Kgorobutswa et al. 2020) and enlighten communities, researchers and practitioners of potential risk vectors, and associated disease transmission. The findings will also have implications in the monitoring of vector proliferation due to anthropogenic and climate driven factors prevailing in the region that favour mosquito success and associated infections (e.g. Endo & Eltahir 2020).

MATERIALS AND METHODS

Mosquito community collection

Mosquito samples were collected from understudied sub-districts in Central District, Botswana. This District straddles a malaria-endemicity gradient, consisting of three malaria-endemic and three non-endemic zones (Figure 1). Samples were collected from both the malaria endemic (Bobirwa) and the non-endemic sub-districts (Palapye and Serowe) between November and January austral summer of 2019/2020 (Figure 1; Table S.2). Sampling was conducted during this period as it falls within the rainy season for the area, the time of year when mosquito abundances are highest and where communities identified risk of bites to be the greatest (Buxton et al. 2020). Nine collection points were identified per sub-district based on proximity to human habitation (six for adults) and the presence of breeding sites (three for larvae). Adult and larval mosquito species were sampled from a total of nine collection points per sub-district, monitored repeatedly over 3 days (adults). The adults were trapped using a Biogents BG-GAT (Model: 10719, Biogents AG, Germany) baited with decaying hay. Samples were stored individually in 2 ml Eppendorf tubes with desiccating silica gel beads. Mosquito larvae were sampled with a 1000 µm mesh net (40 cm × 60 cm) from three separate aquatic habitats within each sub-district with standardised search times of 10 min per sampling site (Lee et al. 2020). Each larval specimen was preserved in 2 ml Eppendorf tubes using 80% alcohol.

Identification

All adult and larval mosquito specimens were first identified using morphological features and adults were further sexed male and female based on the antenna (Folmer et al. 1994; Gillies & Coetzee 1987; Jupp 1996; Rueda et al. 2004). Modified protocols of Buxton et al. (2019) were subsequently used to confirm species identity with molecular techniques. Five individuals of both adults and larvae were randomly selected across species and subjected to polymerase chain reaction

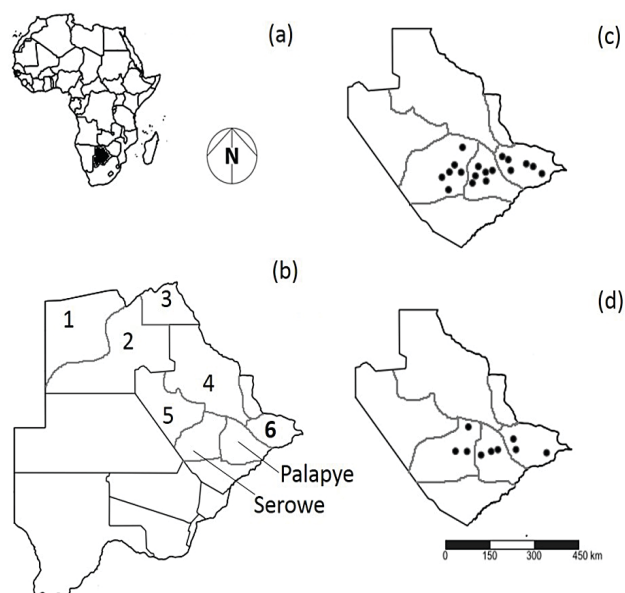


Figure 1. A map showing (a) the location of Botswana in Africa, (b) malaria endemic districts and/ sub-districts [Okavango: 1, Ngamiland: 2, Chobe: 3, Tutume: 4, Boteti: 5, and Bobirwa: 6] with the Central District indicating (c) adult and (d) larval sampling sites across malaria endemic sub-district (Bobirwa) and non-endemic sub-districts (Palapye and Serowe). For coordinates of sampling sites see Table S 2.

(PCR) for the amplification of the cytochrome oxidase subunit I (COI) using the universal primers LCO 1490 and HCO 2198. For adults, a leg was used in the amplification whilst for larval specimens, a portion of the abdomen was utilised (Bass et al. 2007). Dissected mosquito parts were added directly to the 50 µl PCR reaction mix consisting of 25 µl of 2× Phusion U Multiplex PCR Mix (Thermo Fisher Scientific, U.S.A.), 9 µl nuclease-free water (VWR International LLC, U.S.A.) and 0.8 µM of each primer. The PCR conditions were: initial denaturation at 98 °C for 30 s, 35 cycles of denaturation at 98 °C for 10 s, annealing at 48 °C for 30 s and extension at 72 °C for 30 s. Subsequently, a final extension at 72 °C for 7 min was performed. Visualisation of amplicons was done under 1% agarose gel stained with ethidium bromide. Purification of 40 µl PCR product was performed using a JET PCR purification kit (Thermo Fisher Scientific, U.S.A.) according to manufacturer's recommended protocols, followed by elution with 20 µl nuclease free water (VWR International LLC, U.S.A.). Amplicons were sequenced in Inqaba Biotechnical Industries (Pretoria, South Africa). The sequences were trimmed and contigs were assembled using a CodonCode Aligner 8.0.2 software package and subjected to nucleotide blast for species homology identification. Sequences were further deposited in Genbank to obtain species accession numbers.

Statistical analyses

Data on mosquito community composition were analysed using the Primer version 6 statistical software package, within which the total species number (*S*), total number of individuals (*N*), Margalef's species richness (*d*), species diversity (*H'*) and Pielou's evenness (*J'*) were determined per site. The same software package was then used to assess similarities in community composition. Although mixed methods were employed, given that the effort was standardised across all three sub-districts (6 and 3 sites each for adult and larval collection, respectively), a combined species list along with abundances per species was created per sub-district. With this matrix, a Bray-Curtis similarity matrix was used to construct a dendrogram to assess similarities across sub-districts. The Simper analysis in Primer version 6 was then used to assess the levels of dissimilarity between the sites (sub-districts) and the species contributing the greatest towards these differences.

Mosquito abundances (adults) data were analysed in STATISTICA, version 13.5.0.17 (Statsoft Inc., Tulsa, OK, U.S.A.). The data were checked for normality using Shapiro-Wilks test and fulfilled assumptions of analysis of variance (ANOVA). Factorial ANOVA was then used to analyse data, with abundances as dependent variables while the species, sex, sub-district were the independent factors.

RESULTS

A total of 1013 mosquito samples were collected across the three sub-districts sampled (754 adults and 259 larvae). Of these, 10 species from 4 genera (*Aedes*, *Anopheles*, *Culex*, *Culiseta*) were identified in either adult, larval stage, and/or both (Table 1). Aedine species identified similar to the partial COI gene isolate were *Ae. vittatus* and *Ae. aegypti* with the nucleotide sequence homology of 99.4 and 99.64% respectively. *Aedes aegypti* was identified and reported in all sub-districts however, *Ae. vittatus* was only found in Bobirwa. The anopheline species were identified as *An. rhodesiensis* (93%), *An. quadriannulatus* (98.51%), *An. gambiae sensu stricto* (s.s.) (98.95%), *An. rufipes* (95.53%) and an unidentified specimen with homology of 100% with an *Anopheles* sp. Bobirwa had one anopheline species (*An. quadriannulatus*) as compared to more than one species reported for the non-endemic sub-districts of Serowe (*An. quadriannulatus* and *An. rufipes*) and Palapye (*An. rhodesiensis*,

An. gambiae s.s. and *Anopheles* sp.). Thus, the highest number of anopheline spp. Palapye were documented from Palapye from sampled sub-districts. *Culex* mosquito species identified comprised *Cx. pipiens* and *Cx. sinaiticus* with 99.85 and 99.23% homology respectively while *Cs. longiareolata* were identified at homology of 96.59% (Table 1). Mosquito species identified as adults were *Ae. aegypti*, *Ae. vittatus*, *Cx. sinaiticus* and *Cx. pipiens*. For the larval samples, *Ae. aegypti*, *Ae. vittatus*, *An. rhodesiensis*, *An. quadriannulatus*, *An. gambiae* s.s., *An. rufipes*, *Anopheles* sp., *Cx. sinaiticus*, *Cx. pipiens* and *Cs. longiareolata* were identified. Overall, larval sampling yielded more species and genera (*Aedes*, *Anopheles*, *Culex* and *Culiseta*) compared to adult (*Aedes* and *Culex*) collection. Subsequently, the sequences of all species identified here, were deposited in the Genbank and accession numbers confirmed for each species (Table 1).

Out of the 1013 total mosquito samples, Bobirwa had the highest number of mosquitoes (429) while Serowe produced the least (228), with 356 individuals collected from Palapye (Table 2). A total of four, five and six different species were reported in Serowe, Bobirwa and Palapye respectively (Table 2). The highest species richness was reported for Palapye (0.8511), the least Serowe (0.5526) while Bobirwa was intermediate (0.6599) (Table 2). Palapye exhibited the highest species diversity (1.304), while Serowe reported the least (0.7554) with Bobirwa intermediate (0.9433). The highest evenness was observed for Palapye while both Bobirwa and Serowe reported the least species evenness (Table 2).

Average pairwise dissimilarity between Bobirwa and Serowe was 31.8%, Bobirwa and Palapye was 30.7% while Palapye and Serowe dissimilarity was 27.7% (Table 3). The dominant species contributing to dissimilarity between Bobirwa and Palapye was *Cx. pipiens* at 40.3%. Similarly, *Cx. pipiens* contributed to dissimilarity (59.8%) between Bobirwa and Serowe. For Palapye and Serowe sub-districts, *Ae. aegypti* contributed to the dissimilarity (21%) (Table 3). Overall, Palapye and Serowe (non-endemic) exhibited similar level of resemblance as opposed to Bobirwa (malaria endemic) (Figure 2).

Table 1. A summary of mosquito species identification information as sampled from across endemic and non-endemic malaria sub-districts.

Mosquito species	Accession No	Homology ID	ID%
<i>Aedes vittatus</i>	MT741508	MG242527.1	99.40
<i>Culex sinaiticus</i>	MT741509	KU380450.1	99.23
<i>Culex pipiens</i>	MT741514	MK300247.1	99.85
<i>Anopheles quadriannulatus</i>	MT741513	DQ792581.1	98.51
<i>Anopheles gambiae sensu stricto</i>	MT741510	MK300234.1	98.95
<i>Anopheles</i> sp.	MT741511	MF372931.1	100.00
<i>Culiseta longiareolata</i>	MT741512	JQ388785.1	96.59
<i>Anopheles rhodesiensis</i>	MT830930	MK047667.1	93.00
<i>Aedes aegypti</i>	MK571449	MK300221.1	99.64
<i>Anopheles rufipes</i>	MT741515	MK586028.1	95.53

Table 2. Summary of community diversity measures from samples of communities in the Bobirwa, Palapye and Serowe sub-districts. *S* = total species number; *N* = total number of individuals; *d* = Margalef's species richness; *H'* = species diversity and *J'* = Pielou's evenness.

	<i>S</i>	<i>N</i>	<i>d</i>	<i>H'</i>	<i>J'</i>
Bobirwa	5	429	0.6599	0.9433	0.5861
Palapye	6	356	0.8511	1.304	0.7281
Serowe	4	228	0.5526	0.7554	0.5449

Table 3. SIMPER test results showing the level of pairwise dissimilarities between sub-districts.

Sub-districts	Average dissimilarity (%)	Dominant dissimilarity Contributing species	Dominant dissimilarity contributions (%)
Bobirwa vs Palapye	30.7	<i>Culex pipiens</i>	40.3
		<i>Anopheles quadriannulatus</i>	17.8
		<i>Culiseta longiareolata</i>	11.2
		<i>Anopheles gambiae s. s</i>	10.8
Bobirwa vs Serowe	31.8	<i>Culex pipiens</i>	59.8
		<i>Aedes aegypti</i>	15.8
		<i>Culiseta longiareolata</i>	14.4
		<i>Culex sinaiticus</i>	4.3
Palapye vs Serowe	27.7	<i>Aedes aegypti</i>	21.0
		<i>Culex pipiens</i>	17.3
		<i>Culiseta longiareolata</i>	16.7
		<i>Anopheles rhodesiensis</i>	16.1

For the adults sampled, the abundances were significantly

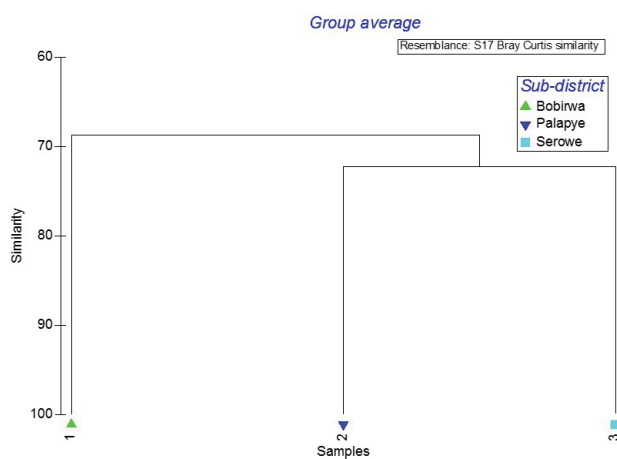


Figure 2. Dendrogram outline the level of mosquito community similarity between sub-districts (Bobirwa, Palapye, Serowe) across a malaria endemicity gradient. The dendrogram was constructed using by creating a Bray-Curtis similarity matrix. Data used was for both larvae and adult samples collected.

different for each species with *Cx. pipiens* exhibiting the highest abundances (Figure 3; Table 4). In addition, neither sex nor sub-districts were statistically significant factors (Table 4). Similarly, the interaction between Sex × Species, Sex × Sub-District, Species × Sub-district and Species × Sex × Sub-district were not significant (Table 4).

DISCUSSION

The study revealed that across the malaria endemic to non-endemic gradient, multiple species of potential vector significance were present with the highest species richness reported in a non-endemic sub-district (Palapye). This study also showed that *Cx. pipiens* was more abundant compared to other mosquito species sampled. Anopheline species instead, dominated in the non-malaria endemic sub-district (Palapye) while the malaria endemic Bobirwa reported only one anopheline species (*An. quadriannulatus*). The sub-districts bordering South Africa (Bobirwa and Palapye) as well as Serowe, which is further way, reported invasive *Ae. aegypti*. The native *Ae. vittatus* was, however, only reported from Bobirwa. Overall,

Table 4. A summary of factorial ANOVA showing mosquito abundances across malaria endemic (Bobirwa) and non-endemic sub-districts (Serowe and Palapye).

Effect	SS	df	MS	F	P
Sex	54.22	1	54.22	2.845	0.094
Species	302.7	1	302.7	19.2	<0.001
Sub-district	55.75	2	27.87	1.462	0.235
Sex × Species	2.9	3	1.0	0.057	0.982
Sex × Sub-district	67.17	2	33.58	1.762	0.175
Species × Sub-district	70.2	2	35.1	2.23	0.111
Species × Sex × Sub-district	37.1	2	18.5	1.18	0.311

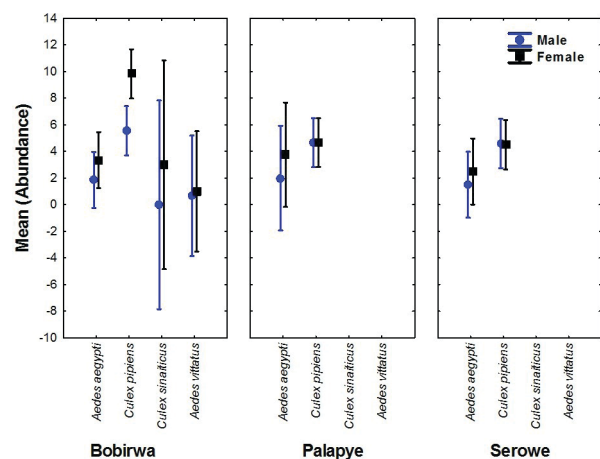


Figure 3. Mean ± 95% confidence dominant mosquito abundances (*Aedes aegypti*, *Culex pipiens*, *Culex sinaiticus*, *Aedes vittatus*) across malaria endemic (Bobirwa) and non-endemic (Palapye, Serowe) sub-districts.

the abundances of male and female mosquitoes were the same across sub-districts. Key mosquito taxa (*Aedes*, *Anopheles* and *Culex*) were recorded across all the sub-districts with *Culiseta* spp. reported only in Palapye. The species reported here, have vectorial capacity for infectious diseases such as dengue, yellow fever, Zika, chikungunya (*Aedes*), malaria (*Anopheles*) and Rift Valley, West Nile fevers, filariasis, various encephalitis (*Culex*) and avian malaria (*Culiseta* and *Culex*) (Braack et al. 2018; Lee et al. 2018; Ralapanawa & Kularatne 2020). The presence of these incriminating vectors represents a significant biosecurity threat to in the District. In particular, if associated pathogens and parasites are present in the region, this may threaten the health of the affected populations through associated vector-borne disease burdens (Ewen et al. 2012).

All three sampled sub-districts reported *Ae. aegypti*, a novel invasive species mainly across tropical and sub-tropical regions and originally from West Africa (Powell et al. 2018). The species colonises artificial water holding systems or microenvironments (e.g. used tyres and tins) at disposal of human habitation (Powell & Tabachnick 2013). Despite *Ae. aegypti* being sampled from homesteads of rural-urban lifestyle, the species has never been assessed for transmission of pathogens to humans in the country. Thus, future studies should explore pathogen incrimination to assess the risk of infection. Currently, *Ae. aegypti* is distributed worldwide (e.g. Díez-Fernández et al. 2018) as an invasive species (Alarcón-Elbal et al. 2020) breeding in a variety of habitats (e.g. rock pools, artificial containers, tree holes) (Eshag et al. 2019). The species has the potential to spread of a wide variety of arboviruses to humans, livestock and wildlife (Díez-Fernández et al. 2018). With regard to its role as an emerging threat to public health (see Lwande et al. 2020), a fine-scale distribution

of the species countrywide and regionally, is highly warranted. Botswana is a tourism hotspot contributing to the country's second largest economic sector (e.g. ~8.5% of GDP in 2014) after diamonds, receiving an increasing influx of tourists annually (Mbaiwa 2017). Should tourists arrive from regions where dengue and other *Ae. aegypti* associated diseases are prevalent, there is a chance of importing these pathogens and parasites within human hosts. The presence of healthy populations of *Ae. aegypti* should be a concern for authorities. *Aedes vittatus* was also reported in the endemic Bobirwa sub-district, although in fewer abundances compared to the invasive congeneric *Ae. aegypti*. The species is potentially a significant health threat mainly to humans. For example, *Ae. vittatus* is known to transmit important pathogens to humans viz, yellow fever, dengue, chikungunya and Zika viruses (Sudeep & Shil 2017).

The study further reported *Cx. pipiens* as the most abundant and widely distributed species from sampled areas, congruent with Karki et al. (2016). This may be mainly due to the ability of the species to colonise various habitats of diverse water quality (Geoffrey et al. 2017; Amusan & Ogbogu 2020), making them near omnipresent. Although environmental parameters play a crucial role in *Cx. pipiens* development and growth (Pachka et al. 2016), life stage physiological traits (e.g. aestivation and diapause) and seasonal variability (Ewing et al. 2019) promoting population exacerbation is less explored in the country. Despite the vectorial role of *Cx. pipiens* in humans (Mavridis et al. 2018) and livestock (Pachka et al. 2016) transmission cycles, it is a key carrier of avian malaria parasites (e.g. *Plasmodium*) (Schoener et al. 2019). To this end, no study on the local context has explored host-parasite interaction to ascertain the role of *Cx. pipiens* in avian host species. This is crucial since the country benefits from wildlife through tourism schemes. In addition, *Cs. longiareolata* reported here is associated with the spread of avian malaria parasites (Brahim et al. 2019). Botswana is a country well-known for its wetlands of importance to migratory bird species (Ramberg et al. 2006) likely to translocate parasites and pathogens in time and space. Therefore, there it is essential to disentangle and understand the functioning of the tripartite ecological interaction in curbing emerging and re-emerging disease associated with wildlife.

Anopheles spp. were reported from all sub-districts regardless of the malaria endemicity status, more so the geographical area is largely similar in terms of land cover and climatic conditions. This has potential for the burden of malaria (Janko et al. 2018), to further extend to the non-endemic areas given the possibility of parasite introduction. More so, *Anopheles* spp. were reported in Palapye (*An. gambiae* s.s., *An. rhodesiensis*, *Anopheles* sp.) and Serowe (*An. rufipes*, *An. quadriannulatus*), both formally designated as non-malarious zones. The prevalence of *An. rufipes* and *An. quadriannulatus* reported here, confirm previous studies documenting the same species in the Central District (Tawe et al. 2017; Bango et al. 2020). *Anopheles rhodesiensis* yielded relatively lower similarity of BLAST (93%) compared to other species identified. This could be due to poor sequence quality that may have resulted in several unassigned nucleotides, which may potentially affect the BLAST algorithm and overall reduced percentage identification output (see discussions in e.g. Afizah et al. 2019). Although *An. quadriannulatus* is regarded as a non-malaria vector (Lobo et al. 2008), all other *Anopheles* species sampled in this study are human malaria vectors (e.g. Abdulla-Khan 1998; Sinka et al. 2010; Tawe et al. 2017). *Anopheles quadriannulatus* was sampled along the river near homesteads, likely to exploit human habitation for dietary and resting needs, in corroboration with reports by Pates et al. (2006). This has implications for its current susceptibility status to *Plasmodium* parasites (Takken et al. 1999). The study further reports unidentified *Anopheles* sp. in non-endemic

area (Palapye). This warrants taxonomic classification and investigation regarding vector incrimination. In the country, the malaria pathogen (*Plasmodium*) is mainly vectored by *An. arabiensis* (Tawe et al. 2017) which was not reported, although other studies documented the species within the Central District and elsewhere in the country (Abdulla-Khan 1998; Bango et al. 2020; Kgorobutswe et al. 2020).

The species abundances varied across the sub-districts in the current study with *Cx. pipiens* dominated the dissimilarity. In particular, *Cx. pipiens* abundances were high in the malaria endemic area. Although seasonal abundance and fine scale distribution of this species is not investigated in Bobirwa, it remains a threat not only as a biting nuisance but also as a potential threat for disease (e.g. avian malaria, West Nile and Rift valley fevers) in the region. The study also highlights that mosquito sex ratios are similar across the sub-districts. However, other studies have demonstrated a skewed collection of significantly high numbers of females compared to males mainly associated with trapping methodology (McPhatter & Gerry 2017).

Different mosquito trapping methodology can potentially yield contrasting species results, as well as numbers (e.g. Lee et al. 2020). However, the present study only used a single Biogents BG-GAT trap type that targets adult mosquitoes. Therefore, the inclusion of other adult sampling techniques should be considered in future studies assessing mosquito diversity in the region. While hay was effectively used to lure many species of adult mosquitoes, not all species may be equally attracted to the trap type used in the study. As such, this could lead to skewed species representation in the sampled sites. An additional limitation in the present study was the lack of extended temporal sampling events and the slight mismatch in temporal synchronicity between sub-districts. This study only sampled austral summer months (November to January) and as such may not have yielded all species that occur in the study region. Similarly, even the marginal temporal separation between sampling of sub-districts could have had implications for species and abundances encountered. To this end, the interpretation of these results should be based on the methodological context presented here.

CONCLUSION

The current mosquito diversity survey of human-inhabited areas outlined the presence of key vector mosquito taxa (*Aedes*, *Anopheles*, *Culex*) across all sampled districts regardless of malaria endemicity status, including the presence of a previously unrecorded invasive species, *Ae. aegypti* (but see Buxton et al. 2019). More mosquito species were sampled from aquatic habitats (during early life stages) than were adults in the terrestrial environment, highlighting the importance of the incorporation of mosquito breeding sites into monitoring and education programmes. A recent study from the region highlighted that many members of vulnerable human communities are unaware that mosquitoes breed in aquatic habitats (Buxton et al. 2020). Given the reverence of water in this arid region, education and research programmes exploring exclusion of mosquitoes from water bodies in proximity to households could be highly effective for localised mosquito control. However, surveillance of mosquito species also remains essential in the Central District, to strengthen vector management and reduce disease spread.

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CONFLICT OF INTEREST

All authors declare no conflict of interests.

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