RESEARCH ARTICLE

Mosquitoes then and now: culicine mosquito research in and around Ndumo Game Reserve, KwaZulu-Natal

Peter N. Thompson[1,2]* ()[§], **Milehna M. Guarido**[1] () & **Alan Kemp**[3,‡] ()
[1]*Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110 South Africa*
[2]*Centre for Veterinary Wildlife Research, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110 South Africa*
[3]*Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases, Sandringham, 2192 South Africa*

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The exceptional biodiversity of the Ndumo area and Maputaland in general, with its tropical biota, is reflected in the high diversity of culicine mosquitoes occurring in the area and, along with their vectors, a high diversity of arthropod-borne viruses (arboviruses). Research interest in the area was driven by the need to identify risks associated with the occurrence of arboviruses potentially affecting human and livestock health in the area, as well as the risk of introduction of arboviruses from elsewhere. We give an overview of the mosquito and arbovirus-related research that has taken place at Ndumo and elsewhere along the lower Phongolo River floodplain since the first expedition in 1955. Intensive work was done on mosquitoes and arboviruses for about 15 years, after which a period of 45 years saw only one focused mosquito study (1989–1993). More recently, interest was inspired once again by the threat of emerging and re-emerging arboviruses and we have been studying culicine mosquitoes and their associated viruses in the Ndumo area since 2016. We describe our recent findings with respect to occurrence and abundance of culicine mosquito species, compare them to historical findings, discuss possible reasons for apparent changes observed, and propose priorities for future research in the area.

Keywords: *Culicinae*, arboviruses, vector-borne diseases, entomological surveillance, Phongolo River floodplain.

INTRODUCTION

A serological survey to detect antibodies to a variety of viruses in humans in various parts of South Africa was undertaken in 1954 (Kokernot, Smithburn & Weinbren, 1956). One locality, namely the 'Lake Simbu' area on the Phongolo River floodplain, stood out from the others in terms of both the diversity of viruses present and the prevalence of human exposure to infection. This led to an extensive investigation of the area from 1955 to 1963 by the Rockefeller Foundation, the South African Institute for Medical Research (SAIMR) and the Poliomyelitis Research Foundation in order to detect viruses in humans and livestock and to gain information on their arthropod vectors, primarily mosquitoes (Smithburn & De Meillon, 1957). Seventeen virus strains were isolated, including three previously unknown viruses. This set the stage for successive studies of the mosquitoes of the lower Phongolo River floodplain and their associated viruses over the following 2–3 decades. Since the motivation for studying mosquitoes was to learn more about their associated arthropod-borne viruses (arboviruses), the focus was on those of the subfamily *Culicinae* (culicines), rather than on the subfamily *Anophelinae* (anophelines). While some anophelines are of particular importance as vectors of malaria, which is also present in the area, it is primarily the culicines which transmit arboviruses, and which are the subject of this paper. We briefly describe the history of culicine mosquito research in the Ndumo and Phongolo River floodplain area and compare the results of early studies with our findings in the same area during the past 7 years (2017–2023), primarily focusing on the occurrence and apparent abundance of culicine mosquito species. For this review, we followed the species list according to Harbach (2023a,b) for the

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[‡] Present address: Quarryside, Stamford Road, Stamford, PE9 3EZ, United Kingdom

*To whom correspondence should be addressed.
E-mail: peter.thompson@up.ac.za

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taxonomy of *Culicinae*, except for *Aedini* where the classification of Wilkerson *et al*. (2015) was used.

EARLY MOSQUITO STUDIES, 1955–1990

The SAIMR expedition in April–May 1955 (Smithburn & De Meillon, 1957) used as a base camp and laboratory the Game Ranger's house at Ubombo, with samples and supplies being transported twice-weekly to and from Johannesburg *via* the airfield at Mkuze. A forward camp was established at Lake Simbu (likely Nsimbi Pan, 27.176°S 32.239°E, near Mboza), from which teams conducted their work (Fig. 1). Mosquito collections were done primarily by recruiting young men to catch mosquitoes by hand using test tubes as they rested in vegetation (De Meillon, Paterson & Muspratt, 1957). Human landing catches (trapping mosquitoes attracted to human bait) and use of livestock as bait were also done, and some trapping (presumably human landing catches) was done on a 10 m high tree platform. A total of 41 culicine and 7 anopheline species was identified during this 4-week expedition. The most abundant species collected was *Aedes* (*Neomelaniconion*) *circumluteolus*, a floodwater-breeding species that survives dry periods as desiccation-resistant eggs laid at the base of vegetation in temporary pools. Precipitin tests on blood-fed *Ae*. *circumluteolus* revealed that domestic livestock (cattle and goats) were the preferred hosts of this species. *Mansonia* (*Mansonioides*) *africana*, and to a lesser extent *Mansonia* (*Mansonioides*) *uniformis*, were also found to be abundant and readily bit humans, including on the tree-canopy platform. Other common species recorded were *Culex* (*Lasioconops*) *poicilipes*, *Culex* (*Culex*) *antennatus* and *Culex* (*Culex*) *neavei*. The latter was identified as *Culex* (*Culex*) *univittatus* but was subsequently split from that species (Jupp, 1971).

Inoculation of suspensions from pooled mosquitoes collected during this expedition, performed at the Ubombo base camp, resulted in the isolation of 17 virus strains. Two novel orthobunyaviruses, namely Simbu virus (Weinbren, Heymann, Kokernot & Paterson, 1957) and Pongola virus (Kokernot, Smithburn, Weinbren & De Meillon, 1957), were isolated from a pools of *Ae*. *circumluteolus*; in addition, Wesselsbron virus (Smithburn, Kokernot, Weinbren & De Meillon, 1957), Bunyamwera virus and Rift Valley fever (RVF) virus (Kokernot, Heymann, Muspratt & Wolstenholme, 1957) were also isolated from *Ae*. *circumluteolus*, and a novel flavivirus, named Spondweni virus, was isolated from *Ma*. *uniformis* (Kokernot, Smithburn, Muspratt & Hodgson, 1957). During the same expedition, sera were collected from domestic animals and tested for the presence of antibodies to various arboviruses. Evidence of the circulation of 10 viruses was detected, with the highest seroprevalences being found for Wesselsbron, Pongola, Middelburg, Bunyamwera and RVF viruses in the Phongolo floodplain area (Kokernot, Smithburn & Kluge, 1961). Human serum samples collected during the 1955 expedition, as well as follow-up surveys over a wider area showed the presence of antibodies to Middelburg, Sindbis, Chikungunya, RVF, Pongola, Bunyamwera, Spondweni, Wesselsbron, Banzi and West Nile viruses (Smithburn, Kokernot, Heymann, Weinbren & Zentkowsky, 1959).

Following these findings, a permanent field station was then established in 1956 in Ndumo Game Reserve, at the so-called NRC camp, by the Arthropod-borne Virus Research Unit (ABVRU), SAIMR, with funding from the Rockefeller Foundation. The aim was primarily to identify arboviruses, firstly through the collection and testing of mosquitoes and secondly through the identification and follow-up of cases of fever of unknown origin and possible viral aetiology in humans (Worth, Paterson & De Meillon, 1961). During that time, humans and domestic animals were resident in the reserve and wildlife numbers were low. Between 1956 and 1960, 46 species of mosquito were trapped using hand collection off vegetation, and using humans and livestock as bait, with some trapping using a tree canopy platform (Worth *et al*., 1961). In contrast to *Ae*. *circumluteolus*, which accounted for >60% of all the mosquitoes caught and was numerous every year, some species such as *Aedes* (*Polyleptiomyia*) *albocephalus* showed marked annual variations in number, with explosive increases in some years. Overall, the second most abundant mosquito species was *Cx*. *neavei*, followed by *Ma*. *africana*, *Ma*. *uniformis* and *Ae*. *albocephalus*, and *Culex* (*Eumelanomyia*) *insignis*, *Culex* (*Culex*) *zombaensis* and *Aedes* (*Aedimorphus*) *cumminsii* were also found to be common. The majority of mosquitoes, over 171 000, were pooled by species and screened for arboviruses by intracerebral inoculation of mice. During this period, 59 arbovirus isolations were made, including Spondweni, Wesselsbron, Pongola, Bunyamwera, Sindbis, Middelburg, Ndumu, Simbu and West Nile viruses (Worth *et al*.,

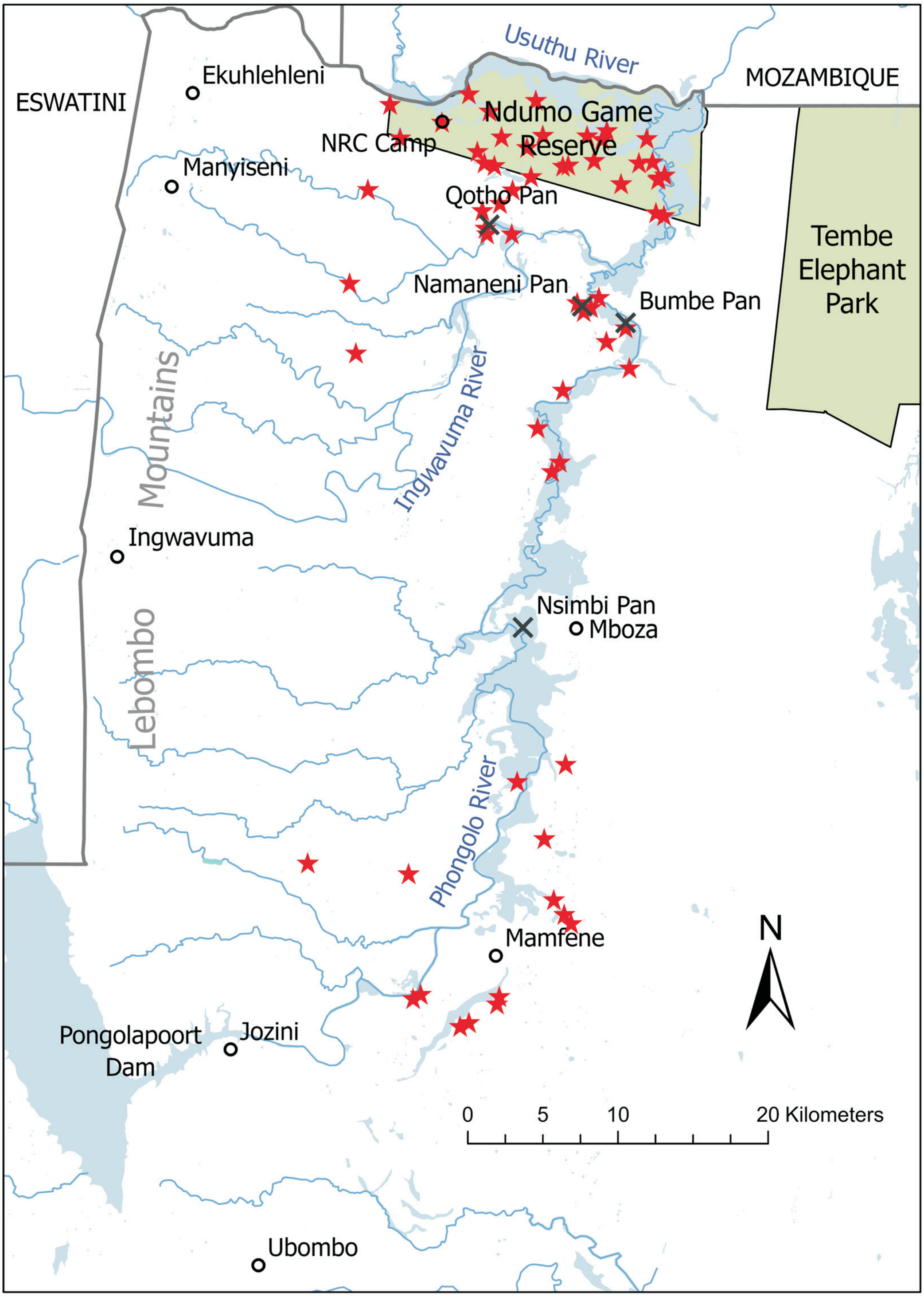

Fig. 1. Map of the study area showing the game reserves and major rivers, with the maximum extent of their associated floodplain pans. Circles show towns, villages and other places referred to, crosses show the pans mentioned in the text, and red stars indicate mosquito trapping sites used since 2017.

1961), and a virus later described as Usutu virus (McIntosh, 1985). Kedougou virus was also later identified from a 1958 *Ae*. *circumluteolus* pool (Jansen van Vuren, Parry, Khromykh & Paweska, 2021). The majority of virus isolations were from *Ae*. *circumluteolus* and the rate of virus isolations was notably higher during late summer to early winter (March to June). However, the frequency of isolation of any particular virus was highly variable from year to year (Worth *et al*., 1961). Spondweni, Wesselsbron and Pongola viruses were the most frequently recovered, and there was a correlation between the abundance of a mosquito species and its viral infection rate, with the highest rates of virus isolation being from *Ae*. *circumluteolus*, *Cx*. *neavei*, *Ma*. *africana* and *Ma*. *uniformis*.

Worth & Paterson (1961) published a comprehensive list of culicine mosquitoes known to occur in the Province of Natal, updating previous lists (Muspratt, 1955, 1956) on the basis of the intensive mosquito collecting being carried out in the Ndumo area. This also served as a checklist of culicine mosquitoes from Ndumo, listing 75 species for the locality.

During the period 1960–1968, a further 168 000 mosquitoes were trapped in Ndumo GR and screened for arboviruses (McIntosh, Jupp & de Sousa, 1972a). The latter 1.5 years of this study period comprised a detailed study of the population dynamics and ecology of *Ae*. *circumluteolus*, in an effort to better understand its potential role as an arbovirus vector (Jupp & McIntosh, 1987). During this time the human and domestic animal population decreased in the reserve as local farmers and their livestock were moved out, and wildlife numbers gradually increased. Trapping methods included hand collection off vegetation, the use of live baits, and portable suction traps sweeping through low vegetation, the latter method being used during the final part of the study period and aimed specifically at collection of *Ae*. *circumluteolus*. The relative abundance of the most common mosquito species remained similar to the 1956–1960 period, except that the abundance of *Ma*. *africana* increased markedly relative to that of *Ma*. *uniformis*, and *Cx*. *insignis* was no longer common at all. Pongola and Middelburg viruses were the most commonly isolated, and several new viruses were found, including Shokwe, Ingwavuma, Mossuril and Lebombo viruses (McIntosh *et al*., 1972a). Although *Ae*. *circumluteolus* yielded the vast majority of virus isolations, there was strong evidence that the infection rate in this species declined significantly over the study period (1960–1968).

A study of the blood-feeding preferences of mosquitoes at Ndumo was done using the blood-fed mosquitoes trapped during 1956–1963 (Paterson, Bronsden, Levitt & Worth, 1964). It was found that *Ae*. *circumluteolus* (along with *Ae*. *albocephalus*, *Ae*. *cumminsii*, *Mansonia* spp. and *Cx*. *zombaensis*) had a broad host range with a preference for large mammals including man, domestic ruminants and antelope, whereas *Cx*. *neavei* preferred avian hosts but also fed on mammals, including man. In contrast, 98% of blood meals in *Ae*. *circumluteolus* collected in 1967–1968 were from antelope (Jupp & McIntosh, 1987), reflecting the transformation of the area into a wildlife reserve. Interestingly, this may also partly explain the reduction in the arbovirus isolation rate over the same period.

Trapping of vervet monkeys (*Chlorocebus pygerythrus*) at Ndumo, in order to test their sera for exposure to arboviruses, started in 1964 and continued until 1969 (McIntosh, 1970). Exposure to several arboviruses was detected, including Sindbis, West Nile and Middelburg viruses. Unexpectedly, a very high prevalence of exposure to Chikungunya virus, close to 60%, was found in 1964/5 (McIntosh, 1970). In an effort to identify the mosquito vector, collections using human, monkey and chicken baits were made in the canopy and at ground level in riverine forest near the Usuthu–Phongolo confluence between 1964 and 1967 (McIntosh, Jupp & de Sousa, 1972b). Although no Chikungunya virus was ever isolated from mosquitoes during that study, or anywhere else in the area, the likely vectors, based on canopy feeding habits, monkey host preference and vector competence were thought to be *Aedes* (*Diceromyia*) *furcifer* and *Ma*. *africana*. The seroprevalence of Chikungunya virus in monkeys declined to around 10% by 1969, suggesting that circulation of the virus likely ceased around 1964 (McIntosh, 1970).

It appears that systematic mosquito collection and arbovirus screening at Ndumo largely ceased after 1968, or at least was not published, and there was an almost 20-year lull in mosquito-related research in the area. Jupp & McIntosh (1987) make passing reference to mosquito 'collections for arboviral vector research' subsequent to 1973 but do not cite any publications. Jupp (1996) in his landmark reference with illustrated key and distribution table for culicine mosquitoes of southern

Africa, cites three additions to the Natal list based on 'Unpublished records of mosquitoes collected in southern Africa, 1960–1993' at the National Institute for Virology, namely *Culex* (*Culex*) *tritaeniorhynchus*, *Culex* (*Eumelanomyia*) *wigglesworthi* and *Mimomyia* (*Mimomyia*) *pallida*, but their origin within the province is not stated.

Then in 1989, Ndumo was selected as a study site for the investigation of tree hole breeding *Aedes* spp., particularly the subgenera *Stegomyia* and *Diceromyia*, as potential vectors of dengue virus (Kemp & Jupp, 1991). This virus had caused a large epidemic in Durban in 1927 (Edington, 1927), but recently one confirmed and two suspected cases had occurred in travelers returning to Durban from India in 1985 (Blackburn & Rawat, 1987), which, along with the first report of dengue from Mozambique (Abreu *et al*., 1987), prompted fears of its possible reintroduction to South Africa. Although human-baited collections were also done, collection of mosquitoes was primarily *via* the use of bamboo pots used to simulate tree holes, from which eggs, larvae and pupae of mosquitoes were collected, reared and identified (Kemp & Jupp, 1991). This method resulted in the collection of, amongst others, a variety of tree hole breeding *Aedes* spp., which are generally poorly represented using other methods. The sampling was continued for at least 5 years, during which time a morphological study was also done of the *Diceromyia* subgenus, including *Ae*. *furcifer* and *Aedes* (*Diceromyia*) *cordellieri* (Jupp & Kemp, 1993; Jupp, 1998). To our knowledge, until recently these were the last published references to mosquito research at Ndumo.

Table 1 presents a list of mosquito species identified in Ndumo and the Phongolo River floodplain area until approximately 1990, as far as could be determined from published literature. However, this is not intended to be a complete list, and in some cases two or more species have been combined in order to reflect either current taxonomic uncertainty or the difficulties in separating certain species based on female specimens, and/or in order to enable comparison with recent collections, when mosquitoes were sometimes not identified to species level. We also indicate those species which were specifically mentioned as the most common or abundant species collected in those early studies.

RECENT MOSQUITO STUDIES, 2017–2022

The emergence and re-emergence of arboviral diseases in recent decades has renewed research interest in these viruses and their vectors (Smith, 2017; Wilson, Mitzel, Savini, Zientara & Richt, 2020), and due to its historical reputation as an arbovirus 'hotspot', the Phongolo River floodplain and Ndumo area have attracted attention from researchers at the University of Pretoria, in collaboration with other local and international research institutions. The studies mentioned below were approved by the relevant institutional research ethics committees, including Ezemvelo KZN Wildlife (OP1376/2021, OP1824/2021, OP2030/ 2021), University of Pretoria (REC105-19, REC038-21) and Department of Agriculture, Land Reform and Rural Development (12/11/1/1(1827SS), 12/11/1/1/4/MG(2590)).

Near Mamfene, an established malaria research site on the Phongolo River floodplain about 25 km downstream of the Pongolapoort Dam, culicine mosquitoes were sampled once in each season from January 2017 to May 2018 as part of a study to investigate the presence of zoonotic arboviruses in the northeastern parts of South Africa. Mosquitoes were collected using CO_2-baited CDC miniature light traps and CO_2-baited BG sentinel traps, and then pooled and screened for Alphavirus, Orthoflavivirus and Orthobunyavirus using PCR assays (Guarido *et al*., 2023). Sindbis and Middelburg viruses were detected in *Aedes* (*Aedimorphus*) *durbanensis*. Ndumu virus was also detected in a pool of *Aedes* (*Neomelaniconion*) *mcintoshi*. Ndumu virus had not been detected in mosquitoes since 1959/1960, when it was detected in *Ma*. *uniformis* and *Ae*. *circumluteolus* (Worth *et al*., 1961; McIntosh *et al*., 1972a). Additionally, Shuni virus was detected in a pool of *Aedes* (*Stegomyia*) *subargenteus* (Guarido, Motlou, *et al*., 2021), adding to the list of arboviruses detected on the Phongolo River floodplain.

As part of a research focus on RVF, entomological and serological studies began in 2016 and are currently ongoing, mainly focused on the communal farming areas along the Phongolo and Ingwavuma River floodplains but also including a wider study area extending to the top of the Lebombo Mountains at Manyiseni and Ekuhlehleni, as well as inside Ndumo and Tembe reserves (Fig. 1). With respect to arboviral epidemiology, the most significant finding to date was the 're-discovery' of RVF virus circulating at high levels amongst domestic livestock (Van den Bergh, Venter, Swanepoel & Thompson, 2019) along the Phongolo River floodplain and wildlife in

Table 1. Culicine mosquitoes collected at Ndumo or along the Phongolo River floodplain, northeastern KwaZulu-Natal, historically (1950–1990) and during recent studies (2017–2023), indicating the species most commonly trapped. Species indicated in bold are those from which arboviruses have been isolated, either in the study area or elsewhere.

Genus	Subgenus	Species	1950–1990	2017–2023	References
Aedeomyia	*Aedeomyia*	*Ad. africana* Neveu-Lemaire, 1906	x		Worth & Paterson (1961)
	Lepiothauma	*Ad. furfurea* (Enderlein, 1923)		x	Guarido, Riddin *et al*. (2021)
Aedes	*Aedimorphus*	*Ae. bevisi* (Edwards, 1915)	x		McIntosh (1975)
		Ae. cumminsii (Theobald, 1903)	**XXX**	x	De Meillon *et al*. (1957); Guarido, Riddin *et al*. (2021); McIntosh (1975); McIntosh *et al*. (1972a, 1972b); McIntosh, Kokernot, Paterson & De Meillon (1961); Muspratt (1961); Van den Bergh *et al*. (2022); Worth & Paterson (1961); Worth *et al*. (1961)
		***Ae. dentatus* grp.**	x	x	Guarido, Riddin *et al*. (2021); McIntosh (1975); McIntosh *et al*. (1972a); Van den Bergh *et al*. (2022); Worth & Paterson (1961)
		Ae. durbanensis (Theobald, 1903)	x	**XXX**	De Meillon *et al*. (1957); Guarido, Riddin *et al*. (2021); McIntosh *et al*. (1972a, 1972b); Muspratt (1961); Van den Bergh *et al*. (2022); Worth & Paterson (1961)
		Ae. fowleri (de Charmoy, 1908)	x	x	McIntosh *et al*. (1972b); Van den Bergh *et al*. (2022); Worth & Paterson (1961)
		Ae. hirsutus (Theobald, 1901)		x	Guarido, Riddin *et al*. (2021); Van den Bergh *et al*. (2022)
		***Ae. leesoni* grp.**	x	x	McIntosh (1975); McIntosh *et al*. (1972a); Muspratt (1959); Van den Bergh *et al*. (2022); Worth & Paterson (1961)
		Ae. ochraceus (Theobald, 1901)	x	x	De Meillon *et al*. (1957); Guarido, Riddin *et al*. (2021); Muspratt (1961); Van den Bergh *et al*. (2022); Worth & Paterson (1961)
		Ae. vexans (Meigen, 1830)		x	Guarido, Riddin *et al*. (2021)
	Albuginosus	*Ae. capensis* (Edwards, 1924)	x		Kemp & Jupp (1991); Worth & Paterson (1961)
		Ae. haworthil (Edwards, 1923)	x		Worth & Paterson (1961)
		Ae. kennethi (Muspratt, 1956)	x		De Meillon *et al*. (1957); Kemp & Jupp (1991); McIntosh *et al*. (1972a, 1972b); Worth & Paterson (1961)
		Ae. marshalli (Theobald, 1901)	x		McIntosh *et al*. (1972a, 1972b); Worth & Paterson (1961); Worth *et al*. (1961)

Continued on p. 99

Table 1 (continued).

Genus	Subgenus	Species	1950–1990	2017–2023	References
	Catageiomyia	*Ae. argenteopunctatus* (Theobald, 1901)	x	x	McIntosh (1975); Muspratt (1959); Worth & Paterson (1961)
		Ae. bedfordi (Edwards, 1936)	x		McIntosh (1975); Muspratt (1959); Worth & Paterson (1961)
		Ae. filicis (Ingram & de Meillon, 1927)	x		McIntosh (1975); McIntosh *et al.* (1972a); Worth & Paterson (1961)
		Ae. minutus (Theobald, 1901)	x	x	De Meillon *et al.* (1957); McIntosh (1975); McIntosh *et al.* (1972a); Muspratt (1961); Worth & Paterson (1961); Worth *et al.* (1961)
		Ae. tarsalis (Newstead, 1907)		x	McIntosh (1975)
		Ae. veeniae (McIntosh, 1975)		x	Guarido, Riddin *et al.* (2021); Van den Bergh *et al.* (2022)
	Coetzeemyia	*Ae. fryeri* (Theobald, 1912)	x		Worth & Paterson (1961)
	Diceromyia	***Ae. cordellieri*** (Huang, 1986)	x		Jupp (1998); Jupp, McIntosh & Kemp (1993); Kemp & Jupp (1991)
		Ae. fascipalpis (Edwards, 1912)	x		Muspratt (1959); Worth & Paterson (1961)
		Ae. furcifer (Edwards, 1913)	x	x	De Meillon *et al.* (1957); Jupp (1998); Jupp *et al.* (1993); Kemp & Jupp (1991); McIntosh *et al.* (1972a, 1972b); Muspratt (1961); Worth & Paterson (1961)
	Mucidus	*Ae. lucianus* (Muspratt, 1959)	x	x	McIntosh *et al.* (1972a, 1972b); Worth & Paterson (1961)
		Ae. mucidus (Karsch, 1887)	x		Muspratt (1959); Worth & Paterson (1961)
		Ae. sudanensis Theobald, 1908	x	x	Guarido, Riddin *et al.* (2021); McIntosh *et al.* (1972a, 1972b); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
	Neomelaniconion	*Ae. aurovenatus* Worth, 1960	x	x	Mcintosh (1971); Van den Bergh *et al.* (2022); Worth (1960); Worth & Paterson (1961)
		Ae. circumluteolus (Theobald, 1908)	**XXX**	**XXX**	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); Jupp & McIntosh (1987); Kokernot, McIntosh & Worth (1961); Mcintosh (1971); McIntosh *et al.* (1972a, 1972b); McIntosh *et al.* (1961); Muspratt (1961); Smithburn *et al.* (1957); Van den Bergh *et al.* (2022); Worth (1960); Worth & Paterson (1961); Worth *et al.* (1961)
		Ae. luteolateralis (Theobald, 1901)	x		De Meillon *et al.* (1957); Mcintosh (1971); Worth (1960); Worth & Paterson (1961)

Continued on p. 100

Table 1 (continued).

Genus	Subgenus	Species	1950–1990	2017–2023	References
		Ae. mcintoshi Huang, 1985	x	**XXX**	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); Mcintosh (1971); McIntosh *et al.* (1972a, 1972b); Van den Bergh *et al.* (2022); Worth (1960); Worth & Paterson (1961)
	Polyleptiomyia	*Ae. albocephalus* (Theobald, 1903)	**XXX**	x	Jupp & McIntosh (1987); McIntosh (1975); McIntosh *et al.* (1972a, 1972b); McIntosh *et al.* (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961); Worth *et al.* (1961)
	Pseudarmigeres	*Ae. natalensis* Edwards, 1930	x		McIntosh *et al.* (1972a); Worth & Paterson (1961)
	Stegomyia	***Ae. aegypti*** (Linnaeus, 1762)	x	x	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); Kemp & Jupp (1991); McIntosh *et al.* (1972a); Muspratt (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Ae. calceatus Edwards, 1924	x		De Meillon *et al.* (1957); McIntosh *et al.* (1972a); Worth & Paterson (1961)
		Ae. demeilloni Edwards, 1936	x		De Meillon *et al.* (1957); Worth & Paterson (1961)
		Ae. dendrophilus Edwards, 1921	x		Kemp & Jupp (1991); Worth & Paterson (1961)
		Ae. heischi van Someren, 1951	x		De Meillon *et al.* (1957); Kemp & Jupp (1991); Worth & Paterson (1961)
		Ae. ledgeri Huang, 1981	x	x	Guarido, Riddin *et al.* (2021); Kemp & Jupp (1991)
		Ae. metallicus (Edwards, 1912)	x	x	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); Kemp & Jupp (1991); McIntosh *et al.* (1972a); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Ae. simpsoni (Theobald, 1905)	x	x	Kemp & Jupp (1991); McIntosh *et al.* (1972a); Muspratt (1959); Worth & Paterson (1961)
		Ae. soleatus Edwards, 1924	x		Kemp & Jupp (1991); Worth & Paterson (1961)
		Ae. subargenteus Edwards, 1925	x	x	Guarido, Riddin *et al.* (2021); Worth & Paterson (1961)
		Ae. unilineatus (Theobald, 1906)	x	x	Kemp & Jupp (1991); Worth & Paterson (1961)
	Zavortinkius	*Ae. fulgens* (Edwards, 1917)	x		De Meillon *et al.* (1957); Kemp & Jupp (1991); Muspratt (1961); Worth & Paterson (1961)
Culex	*Culex*	***Cx. antennatus*** (Becker, 1903)	**XXX**	**XXX**	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); Jupp & McIntosh (1987); McIntosh *et al.* (1972a); Muspratt (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961)

Continued on p. 101

Table 1 (continued).

Genus	Subgenus	Species	1950–1990	2017–2023	References
		Cx. decens Theobald, 1901 / *Cx. trifoliatus* Edwards, 1914	*x*	**XXX**	Guarido, Riddin *et al.* (2021); Muspratt (1955); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Cx. duttoni Theobald, 1901	x	x	Guarido, Riddin *et al.* (2021); Worth & Paterson (1961)
		Cx. guiarti Blanchard, 1905	x		De Meillon *et al.* (1957); McIntosh *et al.* (1972a); Muspratt (1959); Worth & Paterson (1961)
		Cx. neavei Theobald, 1906*	**XXX**	**XXX**	Guarido, Riddin *et al.* (2021); Jupp (1971); Jupp & McIntosh (1987); Jupp, McIntosh & Blackburn (1986); McIntosh *et al.* (1972a, 1972b); Muspratt (1959); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Cx. perfuscus Edwards, 1914	x	**XXX**	McIntosh *et al.* (1972a); Muspratt (1959); Van den Bergh *et al.* (2022)
		***Cx. pipiens* cx.**	x	**XXX**	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); McIntosh *et al.* (1972a); Muspratt (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Cx. simpsoni Theobald, 1905	x	x	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); Muspratt (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Cx. sitiens Wiedemann, 1828		x	Guarido, Riddin *et al.* (2021); Van den Bergh *et al.* (2022)
		Cx. telesilla de Meillon & Lavoipierre, 1945		x	Makhanthisa (unpubl. data 2023)
		Cx. thalassius Theobald, 1903	x	x	McIntosh *et al.* (1972a, 1972b); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Cx. theileri Edwards, 1912	x	x	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); McIntosh *et al.* (1972a); Muspratt (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Cx. tritaeniorhynchus Giles, 1901		**XXX**	Guarido, Riddin *et al.* (2021); Van den Bergh *et al.* (2022)
		Cx. vansomereni Edwards, 1926		x	Van den Bergh *et al.* (2022)
		Cx. zombaensis Theobald, 1901	**XXX**	x	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); Jupp & McIntosh (1987); Kemp & Jupp (1991); McIntosh *et al.* (1972a, 1972b); Muspratt (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961); Worth *et al.* (1961)
	Culiciomyia	*Cx. cinerellus* Edwards, 1922	x		Kemp & Jupp (1991); Worth & Paterson (1961)

Continued on p. 102

Table 1 (continued).

Genus	Subgenus	Species	1950–1990	2017–2023	References
		Cx. cinereus Meigen, 1818	x		Kemp & Jupp (1991)
		Cx. nebulosus Theobald, 1901	x	x	De Meillon *et al*. (1957); Guarido, Riddin *et al*. (2021); Kemp & Jupp (1991); McIntosh *et al.* (1972a); Muspratt (1961); Worth & Paterson (1961)
		Cx. pseudocinereus Theobald, 1901	x		De Meillon *et al*. (1957)
	Eumelanomyia	*Cx. horridus* Edwards, 1922	x		Kemp & Jupp (1991); McIntosh *et al*. (1972a); Muspratt (1955)
		Cx. inconspicuosus (Theobald, 1908)	x		De Meillon *et al*. (1957); Muspratt (1961); Worth & Paterson (1961)
		Cx. insignis (Carter, 1911)	**XXX**		De Meillon *et al*. (1957); Jupp & McIntosh (1987); McIntosh *et al.* (1972a, 1972b); Muspratt (1961); Worth & Paterson (1961); Worth *et al*. (1961)
		Cx. rubinotus Theobald, 1906	x		Worth & Paterson (1961)
	Lasioconops	***Cx. poicilipes*** (Theobald, 1903)	**XXX**	**XXX**	De Meillon *et al*. (1957); Guarido, Riddin *et al*. (2021); Jupp & McIntosh (1987); McIntosh *et al*. (1972a); Muspratt (1961); Van den Bergh *et al*. (2022); Worth & Paterson (1961)
	Maillotia	*Cx. avianus* de Meillon, 1943	x		Worth & Paterson (1961)
		Cx. salisburiensis Theobald, 1901	x		Worth & Paterson (1961)
	Oculeomyia	*Cx. annulioris* Theobald, 1901	x	x	De Meillon *et al*. (1957); Guarido, Riddin *et al*. (2021); McIntosh *et al.* (1972a); Muspratt (1961); Van den Bergh *et al*. (2022); Worth & Paterson (1961)
		Cx. aurantapex Edwards, 1914	x		De Meillon *et al*. (1957); McIntosh *et al*. (1972b); Muspratt (1959, 1961); Worth & Paterson (1961)
		Cx. bitaeniorhynchus Giles, 1901	x	x	Guarido, Riddin *et al*. (2021); McIntosh *et al*. (1972b); Muspratt (1959); Worth & Paterson (1961)
		Cx. ethiopicus Gillies & Coetzee, 1987	x	x	De Meillon *et al*. (1957); Guarido, Riddin *et al*. (2021); Muspratt (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
Lutzia	*Metalutzia*	*Lt. tigripes* (de Grandpre & de Charmoy, 1901)	x	x	De Meillon *et al*. (1957); Guarido, Riddin *et al*. (2021); McIntosh *et al*. (1972a); Muspratt (1961); Van den Bergh *et al*. (2022); Worth & Paterson (1961)

Continued on p. 103

Table 1 (continued).

Genus	Subgenus	Species	1950–1990	2017–2023	References
Mansonia	*Mansonioides*	***Ma. africana*** (Theobald, 1901)	**XXX**	**XXX**	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); McIntosh *et al.* (1972a); McIntosh *et al.* (1961); Muspratt (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961); Worth *et al.* (1961)
		Ma. uniformis (Theobald, 1901)	**XXX**	**XXX**	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); Kokernot, Smithburn, Paterson & De Meillon (1960); McIntosh *et al.* (1972a); McIntosh *et al.* (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
Coquillettidia	*Coquillettidia*	*Cq. aurea* (Edwards, 1915)	x		McIntosh *et al.* (1972a); Muspratt (1959); Worth & Paterson (1961)
		Cq. chrysosoma (Edwards, 1915)	x	x	McIntosh *et al.* (1972a, 1972b); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Cq. fuscopennata (Theobald, 1903)	x		McIntosh *et al.* (1972a, 1972b)
		Cq. metallica Edwards, 1912	x	x	De Meillon *et al.* (1957); McIntosh *et al.* (1972a); Muspratt (1961); Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Cq. microannulata (Theobald, 1911)	x		McIntosh *et al.* (1972a); Worth & Paterson (1961)
		Cq. wahlbergi (Edwards, 1936)	x		McIntosh *et al.* (1972a); Worth & Paterson (1961)
Eretmapodites		*Er. quinquevittatus* Theobald, 1901	x	x	De Meillon *et al.* (1957); Guarido, Riddin *et al.* (2021); McIntosh *et al.* (1972a); Muspratt (1961); Worth & Paterson (1961)
		Er. silvestris Ingram & de Meillon, 1927	x		De Meillon *et al.* (1957); McIntosh *et al.* (1972a, 1972b); McIntosh *et al.* (1961); Worth & Paterson (1961); Worth *et al.* (1961)
		Er. subsimplicipes Edwards, 1914	x		De Meillon *et al.* (1957); McIntosh *et al.* (1972a); Muspratt (1961); Worth & Paterson (1961)
Mimomyia	*Etorleptomyia*	*Mi. mediolineata* (Theobald, 1904)	x	x	De Meillon *et al.* (1957); Muspratt (1961); Worth & Paterson (1961)
	Mimomyia	*Mi. hispida* (Theobald, 1910)	x		Muspratt (1959); Worth & Paterson (1961)
		Mi. lacustris (Edwards, 1935)	x		Worth & Paterson (1961)
		Mi. mimomyiaformis (Newstead, 1907)	x	x	De Meillon *et al.* (1957); McIntosh *et al.* (1972a); Muspratt (1961); Worth & Paterson (1961)

Continued on p. 104

Table 1 (continued).

Genus	Subgenus	Species	1950–1990	2017–2023	References
		Mi. pallida Edwards, 1925		x	Makhanthisa (unpubl. data 2023)
		Mi. plumosa (Theobald, 1901)	x	x	Van den Bergh *et al.* (2022); Worth & Paterson (1961)
		Mi. splendens Theobald, 1903		x	Makhanthisa (unpubl. data 2023)
Uranotaenia	*Pseudoficalbia*	*Ur. mashonaensis* Theobald, 1901	x	x	Worth & Paterson (1961)
		Ur. nivipous Theobald, 1912	x		Worth & Paterson (1961)
	Uranotaenia	*Ur. balfouri* Theobald, 1904	x	x	Worth & Paterson (1961)
		Ur. pallidocephala Theobald, 1908	x		Worth & Paterson (1961)
Ficalbia		*Fi. circumtestacea* (Theobald, 1908)	x	x	Worth & Paterson (1961)
Malaya		*Ml. taeniarostris* (Theobald, 1911)	x		Muspratt (1959); Worth & Paterson (1961)
Toxorhynchites	*Afrorhynchus*	*Tx. lutescens* (Theobald, 1901)	x		Muspratt (1961); Worth & Paterson (1961)
	Toxorhynchites	*Tx. brevipalpis* (Giles, 1902)	x	x	De Meillon *et al.* (1957); Kemp & Jupp (1991); Muspratt (1961); Worth & Paterson (1961)

x = occurred; **XXX** = recorded as common or abundant.

*Prior to 1970, *Cx. neavei* was included with *Cx. univittatus*, its higher-altitude counterpart, until it was recognized as a separate species (Jupp, 1971); therefore, it can be assumed that early records of *Cx. univittatus* in the lowlands refer to *Cx. neavei*.

Ndumo and Tembe reserves (Van den Bergh, Venter, Swanepoel, Hanekom & Thompson, 2020). Further serological studies in livestock and humans in the area are ongoing. Concurrently, regular mosquito trapping has been done using CO_2-baited tent traps and CO_2-baited CDC miniature light traps (operated without the light) at sites close to two large pans (Namaneni and Bumbe Pans) on the Phongolo River floodplain within 10 km of Ndumo GR, and at Qotho Pan near the Ingwavuma River. Aspiration of mosquitoes in vegetation has also been done in order to collect blood-fed females, using a CDC backpack aspirator (Makhanthisa *et al.*, 2024). The objective is to describe the population dynamics and host preferences of potential RVF virus vectors and to detect the virus in mosquitoes. In addition, trapping at numerous sites across the area, including inside the two reserves, was done using tent and CDC traps in order to investigate the genetic variation in several potential arbovirus vectors across the landscape. Almost all trapping in recent studies has been done at ground level, although occasionally CDC traps have been used at higher levels (5–8 m) off the ground in trees.

Most of the above-mentioned studies are still in progress or have not yet been published. However, in Table 1 we indicate the species we have recorded since 2017, restricting the list to those trapped in the lowlands from the base of the Lebombos (approx. 31.13°E) to just east of the Phongolo River (approx. 32.33°E), including Ndumo Game Reserve but not including Tembe Elephant Park. We also indicate which species have been most common or abundant in our collections.

The majority of mosquitoes collected to date are being screened for RVF virus, and some for other arboviruses. Large-scale screening for multiple arboviruses has not yet been done, although such a study is currently in progress. To date, RVF virus has been detected in *Ae. durbanensis* (Van den Bergh *et al.*, 2022), a floodwater-breeding species which shows extreme variations in abundance, sometimes erupting in large numbers during late summer. Table 2 shows the range of arboviruses known to have been detected in mosquitoes from the Ndumo area, including both historical and recent studies. Additionally, mosquito species from which one or more arboviruses have been isolated, either in the study area or elsewhere, are indicated in bold in Table 1, showing the potential for the detection of additional arboviruses in the Ndumo area.

COMPARISON OF EARLY AND RECENT FINDINGS

Of the 106 culicine mosquito species historically recorded from the Ndumo area, we have encountered at least 76 species since 2017. The lower species diversity recently collected likely largely reflects the difference in trapping methods and sites, since the majority of recent collections were made at ground level using CO_2-baited tent and CDC traps, with very little trapping being done in the tree canopy. Most importantly, the recent collections have largely targeted host seeking females, using only CO_2 as bait, and have also mainly targeted floodwater and permanent water breeding species. Relatively less aspiration was done in vegetation which collects resting mosquitoes of both sexes, including blood-fed females, and no sampling was done using artificial tree hole containers. The latter largely explains the relative absence of most arboreal tree hole breeding species from recent collections, particularly the aedine subgenera *Albuginosus*, *Diceromyia* and *Stegomyia*, and some *Culex* (*Culiciomyia*) spp. In addition, many mosquito species are known to be poorly attracted to CO_2 bait, resulting in few of such species being represented in recent collections.

Importantly, variation in mosquito abundance at the time of the collection is certainly influenced by climatic conditions impacting breeding and survival of various species. Annual and seasonal variations in rainfall were noted to profoundly affect numbers and species of mosquitoes trapped in early studies (De Meillon *et al.*, 1957; Worth *et al.*, 1961; Jupp & McIntosh, 1987), and this is likely an important reason for differences between historical and recent collections. Although no long-term trend in total annual rainfall was identified for 1921–2015 for northeastern KZN (Kruger & Nxumalo, 2017), there were indications of seasonal trends, with increased spring (September–November) and decreased autumn (March–May) rainfall, as well as increased extreme daily rainfall events and daily rainfall intensity, reduced wet spell duration and increased dry season duration. Average annual temperatures in South Africa have also increased by about 1.5°C since the 1960s (World Bank Group, 2021). Such medium to long-term climate changes can be expected to influence mosquito population composition and abundance. Our current studies began in 2017, when conditions were generally very dry, water releases from the Pongolapoort Dam had ceased,

Table 2. Arboviruses known to have been isolated or detected in mosquitoes at Ndumo or along the Phongolo River floodplain, northeastern KwaZulu-Natal.

Family	Genus	Virus	Host	References
Togaviridae	*Alphavirus*	Middelburg	*Ae. circumluteolus*, *Ae. durbanensis*, *Ae. albocephalus*, *Ae. leesoni/dentatus*, *Ae. cumminsii*	Guarido *et al.* (2023); McIntosh *et al.* (1972a); Worth *et al.* (1961)
		Ndumu	*Ma. uniformis*, *Ae. durbanensis*, *Ae. circumluteolus*	Guarido *et al.* (2023); Kokernot, McIntosh, *et al.* (1961); McIntosh *et al.* (1972a); Worth *et al.* (1961)
		Sindbis	*Cx. neavei*, *Ae. durbanensis*, *Ae. cumminsii*, *Ma. africana*	Guarido *et al.* (2023); McIntosh *et al.* (1972a); Worth *et al.* (1961)
Flaviviridae	*Orthoflavivirus*	Spondweni	*Ma. uniformis*, *Ma. africana*, *Ae. circumluteolus*, *Cx. neavei*, *Ae. cumminsii*, *Er. silvestris*	Kokernot, Smithburn, Muspratt *et al.* (1957); McIntosh *et al.* (1972a); McIntosh *et al.* (1961); Worth *et al.* (1961)
		Usutu	*Cx. neavei*	McIntosh (1985)
		Wesselsbron	*Ae. circumluteolus*, *Ae. minutus*, *Ma. uniformis*, *Cx. neavei*	McIntosh *et al.* (1972a); Smithburn *et al.* (1957); Worth *et al.* (1961)
		West Nile	*Cx. neavei*	Worth *et al.* (1961)
		Kedougou	*Ae. circumluteolus*	Jansen van Vuren *et al.* (2021)
Peribunyaviridae	*Orthobunyavirus*	Shuni	*Ae. subargenteus*	Guarido, Motlou *et al.* (2021)
		Shokwe	*Ae. circumluteolus*	McIntosh *et al.* (1972a)
		Ingwavuma	*Ae. circumluteolus*	McIntosh *et al.* (1972a)
		Bunyamwera	*Ae. circumluteolus*, *Ma. africana*	Kokernot, Heymann *et al.* (1957); McIntosh *et al.* (1972a); Worth *et al.* (1961)
		Germiston	*Ae. circumluteolus*	McIntosh *et al.* (1972a)
		Pongola	*Ae. circumluteolus*	Kokernot, Smithburn, Weinbren *et al.* (1957); McIntosh *et al.* (1972a); Worth *et al.* (1961)
		Simbu	*Ae. circumluteolus*	McIntosh *et al.* (1972a); Weinbren *et al.* (1957); Worth *et al.* (1961)
Phenuiviridae	*Phlebovirus*	Rift Valley fever	*Ae. circumluteolus*, *Ae. durbanensis*	Kokernot, Heymann *et al.* (1957); Van den Bergh *et al.* (2022)
Reoviridae	*Orbivirus*	Lebombo	*Ae. circumluteolus*	McIntosh *et al.* (1972a)
Rhabdoviridae	*Vesiculovirus*	Mossuril	*Cx. neavei*, *Ae. circumluteolus*	McIntosh *et al.* (1972a)

and several large pans such as Bumbe, Namaneni and Qotho had dried up completely; until 2021 these held only very small areas of inundation during the rainy season. However, beginning in late January 2021, the large pans rapidly filled and have remained at very high levels since, due to high rainfall and water releases from the dam. This has had a large effect on the abundance of *Culex* and *Mansonia* spp. relative to *Aedes* spp. at our trapping sites.

The other major factor which may have caused changes in the mosquito population since the 1950s is the change in human and livestock populations along the Phongolo River floodplain and surrounding areas. Outside the reserves, both human and livestock population densities have increased markedly, resulting in changes to mosquito host availability. In addition, human agricultural and water use activities have modified the landscape, and notably the construction of the Pongolapoort Dam in 1973 reduced the extent and frequency of inundation of the floodplain pans. This has likely had a major impact on mosquito populations.

In contrast to the majority of early studies, *Ae. circumluteolus* was not the most abundant species recorded recently. However, this is likely at least partly due to the difference in trapping methods and sites. Historically a lot of hand collecting was done from vegetation in riverine areas where *Ae. circumluteolus* rests in large numbers, and it was specifically targeted for collection in some studies. Our recent collections also found high numbers shortly after partial flooding of riverine areas and pans, and at such times large numbers of resting mosquitoes were seen at ground level in shaded, grassy areas. Jupp & McIntosh (1987) remark that, subsequent to the damming of the Phongolo River, population densities of *Ae. circumluteolus* at Ndumo appeared to have decreased, presumably due to reduced frequency of flooding. It is therefore possible that there has indeed been a general reduction in the population of this species. Nevertheless, given the early reports of isolation of a wide variety of arboviruses from this species (Kokernot, Heymann, *et al.*, 1957; Kokernot, Smithburn, Weinbren, *et al.*, 1957; Smithburn *et al.*, 1957; Weinbren *et al.*, 1957; Worth *et al.*, 1961; McIntosh *et al.*, 1972a), and its incrimination as a vector in a 1981 RVF outbreak at Mtubatuba, KZN (Jupp, McIntosh & Thompson, 1983), it remains a species of interest for arbovirus screening, particularly in areas where a variety of domestic and wild hosts, as well as humans, are prevalent.

Another closely-related floodwater-breeding species, also in the subgenus *Neomelaniconion*, *Ae. mcintoshi*, was apparently not commonly collected in early studies in the area. In contrast, we recently found fairly high numbers, comparable to those of *Ae. circumluteolus*. *Aedes mcintoshi* is found in large numbers in the interior plateau and northern parts of South Africa and is regarded as one of the most important vectors of RVF virus in East Africa (Sang *et al.*, 2010). In South Africa and elsewhere in Africa, this species has been associated with several other arboviruses including Ndumu, Wesselsbron, Pongola and Bunyamwera viruses (Braack, Gouveia de Almeida, Cornel, Swanepoel & de Jager, 2018; Guarido *et al.*, 2023); therefore its apparent increase in abundance on the Phongolo River floodplain deserves further investigation.

The sporadic occurrence in recent collections of very large numbers of *Ae. durbanensis*, which varied a lot from year to year, is also of interest, since this species is nowhere indicated as common in previous studies in the area. However, it was reported in large numbers on the Hluhluwe River near Lake St. Lucia in January 1984 (Sharp, Le Sueur & Ridl, 1988). Although our detection of RVF virus from a pool of *Ae. durbanensis* at Qotho Pan in March 2017 (Van den Bergh *et al.*, 2022) does not necessarily incriminate it as a vector, it is possible that it may at times play a role in the endemic circulation of the virus in the area. This could also be true for Middelburg and Sindbis viruses which have been also detected in pools of this species (Guarido *et al.*, 2023). Sharp *et al.* (1988) studied the host preferences of *Ae. durbanensis*, showing that it was strongly zoophilic, being attracted most to goat and bovine baits, but that it also fed on man.

Amongst the other *Aedes* spp., apart from the tree hole breeding species mentioned above, apparent differences in abundance were seen for *Ae. cumminsii* and *Ae. albocephalus*, which in early studies were second in abundance only to *Ae. circumluteolus*. In recent collections, *Ae. cumminsii* was not uncommon in small ephemeral wetlands, but *Ae. albocephalus* was rarely collected. Differences in collection methods may at least partly explain these apparent changes, although, as mentioned above, early studies described extreme inter-annual variations in the numbers of *Ae. albocephalus*, possibly not unlike we found recently for *Ae*. *durbanensis*. Apparent changes in abundance for such species are therefore difficult to interpret.

Culex antennatus, *Cx*. *neavei* and *Cx*. *poicilipes*, previously reported to be common or abundant, were similarly found in large numbers in our recent collections, particularly near large pans, and the latter two species were also trapped higher up in trees. Elsewhere, RVF virus has been isolated from all three species, including from *Cx*. *neavei* in the 1981 Mtubatuba outbreak (McIntosh, Jupp, dos Santos & Rowe, 1983). However, it is noteworthy that *Cx*. *zombaensis*, the main vector implicated in this outbreak which occurred about 160 km south of Ndumo (McIntosh *et al*., 1983), now appears to be less common around Ndumo than it was historically.

Probably the single biggest change that we have found in the mosquito species mix at Ndumo, and one that is unlikely to be explained purely by differences in methodology or short-term climatic fluctuation, is the recent occurrence of very large numbers of *Cx*. *tritaeniorhynchus*. It appears that the species was unknown in South Africa until at least the 1960s, having not been included in the updated list of species known to occur in Natal by Worth & Paterson (1961), nor detected at all in collections on the Phongolo River floodplain or at Ndumo between 1955 and 1968 (De Meillon *et al*., 1957; Worth *et al*., 1961; McIntosh *et al*., 1972a). However, it is listed for Natal by Jupp (1996) on the basis of unpublished records (1960–1993) at the National Institute of Virology, Johannesburg. It was reported from coastal Mozambique as far south as the Beira area in 1959 (Worth & De Meillon, 1960). *Culex tritaeniorhynchus* is widely distributed across Asia and tropical Africa and has been associated with several arboviruses, being the primary vector of Japanese encephalitis virus (Van den Hurk, Ritchie & Mackenzie, 2009). It is also regarded as a potentially invasive species (Samanidou & Harbach, 2003; Juliano & Lounibos, 2005). It was found to be one of the main vectors of RVF virus during the large outbreak in 2000 in Saudi Arabia and Yemen by Jupp *et al*. (2002), who surmised that the reason that it had not been implicated as a vector of RVF virus in Africa was because it did not occur there in high densities, and particularly not in South Africa. Our finding of consistently high numbers of *Cx*. *tritaeniorhynchus* along the Phongolo River floodplain since 2017 strongly suggests a southerly range expansion of the species in recent decades, and indicates that further work is required to assess its potential as an arbovirus vector in the region.

Several other *Culex* spp., notably the subgenera *Culiciomyia*, *Eumelanomyia* and *Maillotia*, were poorly represented or absent in our recent collections. There may be several reasons for this, including the strong possibility that some of them were overlooked, misidentified, or identified simply as *Culex* spp. due to their strong resemblance to other *Culex* spp. and the large numbers of mosquitoes being processed. In many cases, accurate identification to species level, particularly in the genus *Culex*, depends on detailed examination of male genitalia, whereas most of our recent collections targeted mainly host-seeking females.

To date, recent efforts to describe the diversity of arboviruses circulating in mosquitoes in the Ndumo area have been limited. Nevertheless, of the arboviruses historically described from the area, RVF, Sindbis, Middelburg and Ndumu viruses have been found again recently – in the case of RVF virus, after having not been detected in the area for more than 50 years. This highlights the value of entomological surveillance in monitoring the occurrence of important pathogens. In addition, Shuni virus was recently added to the list of arboviruses known to occur along the Phongolo River floodplain, reaffirming its historical reputation as an arbovirus ‘hotspot’.

DIRECTIONS FOR FUTURE RESEARCH

Given its historical reputation, confirmed by recent and ongoing studies, as an arbovirus ‘hotspot’, the Ndumo area should be an important target area for entomological and arbovirus surveillance. This is particularly true in the context of the current range expansions of several emerging and re-emerging arboviruses such as Dengue, Chikungunya and RVF viruses. The threat of the re-emergence of such diseases is certainly enhanced by the increasing density and mobility of human and livestock populations in the area, increasing the risk of pathogen spill-over at the wildlife–livestock–human interface, thus increasing the need for intensified surveillance.

Since the full range of competent mosquito vectors of certain arboviruses, notably RVF virus, is unknown, particularly in southern Africa, further studies focusing on vector competence experiments of the viruses detected on the Phongolo River floodplain should be done using mosquito species collected in the area. In addition, the ecology of both known and potential arbovirus vectors is poorly known, including many key parameters necessary in order to model arbovirus transmission dynamics, such as host preference, thermal tolerance, biting rate, etc. There is therefore a need for ecological studies of vectors and of their interactions with host populations and response to changing environmental variables.

Accurate identification of mosquito vector species is essential for a proper understanding of arbovirus transmission dynamics, as well as to provide guidance to species-specific control programmes and interventions, to correctly identify disease risks and exposures, and to monitor the introduction of invasive species. There is also a need to further develop taxonomic methods, combining morphological and molecular data for example, which would improve identification, particularly when specimens are damaged or only females are collected. This highlights the necessity for further research to understand mosquito taxonomy and ecology in the region.

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§ORCID iDs

P.N. Thompson: orcid.org/0000-0002-2268-9748
M.M. Guarido: orcid.org/0000-0002-0737-1483
A. Kemp: orcid.org/0000-0002-2051-467X

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