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## Mosquitoes of Northwestern Uganda

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## Abstract

Despite evidence of arbovirus activity in northwestern Uganda (West Nile Sub-region + Murchison Falls National Park), there is very limited information on the mosquito fauna of this region. The only published study reported 52 mosquito species in northwestern Uganda but this study took place in 1950 and the information has not been updated for more than 60 years. In January and June 2011,  $CO_2$  baited-light traps were used to collect 49,231 mosquitoes from 4 different locations, Paraa (9,487), Chobe (20,025), Sunguru (759) and Rhino Camp (18,960). Overall, 72 mosquito species representing 11 genera were collected. The largest number of distinct species was collected at Chobe (43 species), followed by Paraa (40), Sunguru (34) and Rhino Camp (25). Only 8 of the 72 species (11.1%) were collected from all 4 sites: Aedes (Stegomyia) aegypti formosus (Walker), Anopheles (Cellia) funestus group, Culex (Culex) decens group, Cx. (Culex) neavei Theobald, Cx. (Culex) univittatus Theobald, Cx. (Culiciomyia) cinereus Theobald, Cx. (Oculeomyia) poicilipes (Theobald) and Mansonia (Mansonoides) uniformis (Theobald). Fifty-four species were detected in northwestern Uganda for the first time; however, these species have been detected elsewhere in Uganda and do not represent new introductions to the country. Thirty-three species collected during this study have previously been implicated in the transmission of arboviruses of public health importance.

### Keywords

Mosquitoes; species composition; Uganda; Paraa; Chobe; Sunguru; Rhino Camp; arbovirus vectors; *Coquillettidia fuscopennata*; *Coquillettidia cristata*; *Anopheles funestus* 

## Introduction

Between the mid-1930s and the early 1970s a considerable amount of information on species composition, biology and ecology of mosquitoes was compiled in Uganda (Smithburn et al. 1941, Smithburn and Haddow 1944, Smithburn and Haddow 1946, Smithburn et al. 1946, Haddow 1946, Haddow et al. 1948, Haddow and Mahaffy 1949, Haddow and van Someren 1950, Haddow et al. 1951, Smithburn and Haddow 1951,

Dick and Haddow 1952, Gillett 1972). This entomological information was gathered in conjunction with epidemiologic investigations of yellow fever (YF) conducted by the Yellow Fever Research Institute (currently the Uganda Virus Research Institute) in Entebbe. The primary aim was to study the epidemiology of yellow fever (YF) in eastern Africa. Serologic surveys in the West Nile sub-region of Uganda detected very few YF-immune human sera (Sawyer and Whitman 1936, Mahaffy et al. 1946). Yellow fever-immune sera were detected close to the northern and the western borders of the province (close to forested or wooded areas) while none were found in the vast majority of the territory in the middle and the east (Mahaffy et al. 1946) suggesting an absence of yellow fever virus (YFV) transmission in most of West Nile sub-region. As a result of these findings, the epidemiological investigations were diverted to areas with higher YF activity which led to cessation of the associated entomological studies.

In 1950 Lumsden and Buxton (1951) conducted additional epidemiological investigations in the West Nile sub-region of Uganda. The primary aim was to gain more information on the maintenance and transmission of YFV in an area with an extended dry season. Similar to previous studies (Sawyer and Whitman 1936, Mahaffy et al. 1946), the few YF antibody-positive human sera were mostly from males residing close to forested areas. In contrast, 8/27 (36%) monkey sera were positive for YF-antibodies which suggested that YF was endemic to the West Nile sub-region and maintained in transmission cycles involving monkeys. Lumsden and Buxton (1951) also conducted an entomological survey in the West Nile region in an effort to incriminate the endemic YFV vectors. During this study, 52 different species of mosquitoes were collected and identified. This study represents the only documented account of the mosquitoes of northwestern Uganda. In 2008 the US Centers for Disease Control and Prevention (CDC) and Uganda Virus Research Institute (UVRI) initiated an arbovirus surveillance program in Uganda with the primary aim of screening for and describing arboviruses of public health and veterinary importance. This study provided the opportunity to update the species composition of the mosquito fauna of northwestern Uganda. In this manuscript, we describe and discuss mosquito species composition at four locations in northwestern Uganda and the public health implications of our findings.

#### Materials and Methods

#### Study sites

Mosquitoes were collected at four study sites: Paraa and Chobe in Murchison Falls National Park (MFNP), Sunguru Village and Rhino Camp, Arua District, in the West Nile sub-region (formerly West Nile District) of Uganda (Fig. 1). The typical vegetation and landscape for each study location are presented in Fig. 2.

Paraa (2°17'N: 31°34') is located in the northwest section of MFNP approximately 15 miles south of Pakwach. Murchison Falls National Park (3,840km<sup>2</sup>) is at the northern end of the Albertine Rift Valley and extends from the northeastern shores of Lake Albert in the West to Karuma Township on the Victoria Nile in the East (Fig. 1). Paraa consists of open savannah grassland with a few isolated trees and thickets (Fig. 2A). Rainfall occurs from March to November; average rainfall at the study site is 878.2mm per annum with a range of 592–1,210.2mm (Monaghan et al. 2012). The mean annual temperature is 23.6°C with a

range of  $22.5-24.4^{\circ}$ C (Monaghan et al. 2012). The altitude at the trap site is approximately 654m (2,145ft) above sea level.

Chobe, 2°15'N: 32°08'E, is located in the northeast section of MFNP on the northern side of the Victoria Nile, approximately seven miles west of Karuma Township (Fig. 1). The ecosystem of Chobe is mostly moist semi-deciduous forest (Fig. 2B) and the altitude approximately 637m (3,716ft) above sea level. Similar to Paraa, Chobe is characterized by one wet season from March to November and one dry season from December to February. The average annual precipitation at the study site is 980mm (range 665–1,160mm) and the average daily temperature is 22.2°C (range 21.3–22.7°C) (Monaghan et al. 2012).

Sunguru, 2°48'N: 30°53'E, is located in Arua district near the border with the Democratic Republic of Congo (Fig. 1). The area is mostly wooded grassland, sparsely inhabited (Fig. 2C), approximately 27km south of the city of Arua and the altitude is approximately 1,398m (4,586ft) above sea level. Similar to Paraa and Chobe, Sunguru is characterized by one wet season from March to November and one dry season from December to February. Mean annual rainfall is 1,675.4mm (range 1,092–2,090mm) and mean daily temperature is 21.3°C (range 20.5–21.9 °C) (Monaghan et al. 2012).

Rhino Camp, 2°58'N: 31°24'E, is located approximately 32km (20mi) east of the city of Arua in Arua District and adjacent to the Albert Nile (Fig. 1). It is at an altitude of approximately 634.6m (2,082ft) above sea level (Monaghan et al. 2012). Rhino Camp is primarily a wooded savanna grassland (Fig. 2D) characterized by an extended but light rainy season, from March to November, and a short but severe dry season from December to February. The average annual precipitation is 789.5mm (range 517–1,044.4mm) and average daily temperature is 26.2 °C (range 25.1–26.8 °C) (Monaghan et al. 2012). Both Sunguru and Rhino Camp are residential areas. Chobe and Paraa are part of the MFNP, which is a protected area without human settlements.

#### **Mosquito Collections**

Mosquitoes were captured by using CDC miniature light traps (Clarke Mosquito Control, Roselle, IL) with dry ice, as a source of carbon dioxide. Dry ice was placed in an insulated modified Igloo® drink cooler (John. W. Hock Company, Gainesville, FL) with a small outlet-hole in the bottom, and suspended above the trap. The traps were hung approximately 1m from the ground between 4 and 6 pm and collected following morning between 8 and 10 am.

At Paraa, 2 collection trips were conducted from January 19–22, 2011, and June 18–21, 2011. Fifteen to 20 traps were used and all of them were hung on tree branches in thickets along trails near the student hostel and the museum approximately 1.2km north of Paraa Safari Lodge. The traps were spaced approximately 100–300m apart depending on availability of suitable sites, and sheltered from direct sunlight and wind.

Two collection trips were conducted at Chobe, January 22–25, 2011, and June 21–24, 2011. Fifteen to 20 traps were placed on the northern side of Chobe Safari Lodge approximately

0.8km away from the Victoria Nile. All traps were hung in the forest approximately 50–100m apart to minimize interference between the traps.

Two collection trips were conducted in Sunguru January 13–15, 2011 and June 13–16, 2011. Twenty traps were used, all of which were placed in homesteads or in vegetable gardens between homesteads. Traps were placed approximately 200–300m apart to minimize interference.

One collection trip was conducted in Rhino Camp between January 16 and January 19, 2011. Twenty traps were used and all were placed 100–300m apart in homesteads or in vegetable gardens between homesteads.

#### Mosquito processing

Mosquitoes were collected each morning, chilled, separated from other arthropods and counted into labeled cryotubes. Small pieces of Kimwipes tissues (Kimberly-Clark Professional\*, Roswell, GA) were included in the tubes to hold mosquito specimens in place and prevent them from rubbing against each other and losing morphological characters used in morphological identification. The tubed mosquitoes were kept frozen either on dry ice or in liquid nitrogen dry shippers and shipped frozen to the CDC laboratory in Fort Collins, CO, USA, for processing. At the CDC laboratory, the mosquitoes were identified to species on the basis of morphological characters by using the keys of Edwards (1941), Jupp (1996), Gilles and DeMeillon (1968), Gilles and Coetzee (1987) and Huang (2004), and notes by Haddow et al. (1951), Gillett (1946), Corbet (1958) and Gillett (1972). Voucher specimens for each species were kept for future reference and for identification consultations. Mosquito taxa that demonstrated morphological variation across sites (*Coquillettidia* (*Coquillettidia*) cristata (Theobald), *Cq.* (*Coquillettidia*) fuscopennata (Theobald)) as well as those representing a known species complex (*Anopheles* (*Cellia*) funestus s.1. Gilles) were further characterized using molecular methods.

From the study areas included in this manuscript, four specimens of *Cq. cristata* were analyzed molecularly: two specimens collected in Chobe on 24 January 2011, and three collected in Sunguru on 15 January 2011. Additionally, one specimen of *Cq. fuscopennata* from Chobe (25 January 2011), one from Kibale (20 June 2010), one from Lake Mburo (10 June 2010), two from Kitubulu, and one from Paraa (21 January 2011) were selected for molecular analysis. Additional specimens of both of these species collected in other parts of Uganda were analyzed simultaneously (data not shown). Eighteen specimens morphologically identified as *An. funestus* s.l. were analyzed molecularly. These mosquitoes included seven specimens from Sunguru, collected on 14 and 16 January 2011; eight specimens from Paraa collected between 19–21 January 2011 (7) and on 19 June 2011 (1); and three specimens from Chobe, collected on 24 January 2011.

For molecular characterization of the *Coquillettidia*, DNA was extracted from whole specimens frozen at  $-80^{\circ}$ C using the DNA Investigator Kit (Qiagen Inc., Valencia, CA). The tissue extraction procedure was used with the following modifications: mosquitoes were mechanically homogenized in buffer ATL prior to the addition of proteinase K, and samples were lysed overnight at 56°C. For *An. funestus* s.l., extraction methods were the same except

that a front leg was removed from mounted voucher specimens instead of homogenizing whole specimens.

From the Coquillettidia, two genetic markers were amplified for sequencing: an approximately 400bp fragment of the mitochondrial NADH dehydrogenase subunit 4 (ND4) (Simon et al. 1994), and a 311 bp fragment of the mitochondrial cytochrome oxidase 1 (CO1) (Vinogradova et al. 2003). Anopheles funestus s.l. specimens were analyzed using the species-specific multiplexed PCR described by Koekemoer et al. (2002). Specimens were also screened with the additional primer for an An. (Cellia) rivulorum Leeson-like species reported by Cohuet et al. (2003). To resolve the identity of specimens from which no PCR amplification was obtained, additional arthropod-specific CO1 primer pairs were employed: C1-J-1718/C1-N-2191 (473 bp amplicon); C1-J-1859/TL-2-3014 (1155 bp amplicon) (Simon et al. 1994). All PCR amplifications were performed on a BioRad T-100 thermal cycler (BioRad Laboratories, Hercules, CA) using previously-published amplification conditions (Simon et al. 1994). Amplicons were run on 1% agarose gels and products of the expected size were extracted and purified using a MinElute Gel Extraction Kit (Qiagen Inc., Valencia, CA). Purified amplicons were bidirectionally sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and analyzed on an ABI 3130 genetic analyzer (Applied Biosystems). Sequences were evaluated using the DNASTAR Lasergene core suite (Madison, WI), generating multiple sequence alignments and performing pairwise comparison of sequences within and between species using the ClustalW algorithm.

#### **Diversity Indices**

Species richness and species diversity were calculated for each location and collection period. Species richness was reported as the number of mosquito species at each location. Species diversity was estimated by calculating the Simpson Index (Simpson 1949). The Simpson Index (D), which accounts for both species richness and the relative abundance of each species, was calculated as  $D = \sum n(n-1) / N(N-1)$  where n = the total number of mosquitoes of a particular species and N = the total number of mosquitoes of all species collected at each site. For simplicity, we also report the Simpson's Index of Diversity (1-D), which is interpreted as the greater the index, the greater the sample diversity. An index of 0 would indicate perfect homogeneity whereas an index of 1 would indicate perfect heterogeneity.

## **Results and Discussion**

The grand total of mosquitoes collected during the study was 49,231, of these, 20,025 were collected at Chobe, 18,960 at Rhino Camp, 9,487 at Paraa, and 759 at Sunguru (Table 1). The mosquitoes belonged to 11 genera and 72 species (Table 1). 40,294 (81.6%) of the 49,393 specimen collected belonged to 2 genera *Coquillettidia* and *Mansonia* (Table 2). The number of genera varied from site to site; 10 were collected at Chobe, 9 at Paraa, 8 at Rhino Camp and 7 at Sunguru (Table 1 & 2). Six genera, *Aedeomyia, Eretmapodites, Lutzia, Mimomyia, Toxorhynchites* and *Uranotaenia*, were not detected at all study sites (Table 2). The genus *Aedeomyia* was not detected in Chobe or Sunguru, *Eretmapodites* was

only detected in Chobe and Paraa, the genus *Lutzia* was not detected in Rhino Camp, the genus *Mimomyia* was not detected in Sunguru, the genus *Toxorhynchites* was only detected in Chobe and the genus *Uranotaenia* was not detected in Paraa (Table 1). However very few individuals of these genera were collected suggesting low population density or poor response to light traps.

The highest number of different species within a genus collected from each of the sites was from the genus *Culex*; 19 species, 14 species, 16 species and 8 species collected at Chobe, Paraa, Sunguru and Rhino Camp, respectively (Tables 2–6). Overall, the most diverse genera in the collections were *Culex* (24 species) and *Aedes* (16 species) (Table 2) as observed previously in western Uganda (Mutebi et al. 2012).

In Chobe, *Coquillettidia* species made up the largest proportion of individuals collected, whereas Culex species made up the largest proportion of individuals collected at Paraa, and Mansonia species at Sunguru and Rhino Camp (Table 2–6). Mansonia species made up by far the largest proportion of the total collection (24,268 mosquitoes/49.3%) (Table 2). The second and third most frequently collected species were in the genera Coquillettidia (16,026 mosquitoes) and Culex (5,894 mosquitoes), respectively (Table 2). Coquillettidia species were the most abundant species in Chobe which is a forest ecosystem; this same association was observed between Coquillettidia species and forest ecosystems in western Uganda (Mutebi et al. 2012) and in Zika forest (Kaddumukasa et al. 2014) (Table 2). In contrast, Coquillettidia species were relatively rare at all of the other study sites (Paraa, Sunguru and Rhino Camp) (Table 2) which were predominantly open and/or wooded savannah grassland ecosystems. Mansonia species were abundant at all three sites adjacent to the River Nile (Chobe, Paraa and Rhino Camp), and were the most abundant species at both Paraa and Rhino Camp (Table 2). This is likely due to the presence of papyrus swamps along the River Nile, which are excellent larval habitats for the three Mansonia species collected in the course of this study.

Overall, a total of 72 mosquito species were identified in northwestern Uganda (Table 1). The greatest species richness was detected at Chobe (43 species), followed by Paraa (40), Sunguru (34), and Rhino Camp (25) (Table 2). Of the 72 species only 8 (11.1%) were collected from all 4 sites: *Ae. (Stegomyia) aegypti formosus* (Walker), *An. funestus* group, *Cx. (Culex) decens* group, *Cx. (Culex) neavei* Theobald, *Cx. (Culex) univittatus* Theobald, *Cx. (Culiciomyia) cinereus* Theobald, *Cx. (Oculeomyia) poicilipes* (Theobald) and *Ma. (Mansonoides) uniformis* (Theobald) (Table 1). As noted before there is a wide range of variation in species composition among different sites in Uganda despite relatively close proximity and similarity in climate and ecosystem (Mutebi et al. 2012, Mayanja et al. 2014). In the present study, Paraa, Sunguru and Rhino Camp are all wooded grasslands and the sites are within 156km (97mi) of each other, however, only 10 of the 72 species were collected from all 3 sites (Table 1).

Diversity indices for each collection are reported in Tables 3 - 6. On average, species diversity was highest in Sunguru (Simpson's Diversity Indices 1-D = 0.86 in January and 0.91 in June), followed by Rhino Camp (0.66 in January), Paraa (0.33 in January and 0.82 in June), and Chobe (0.28 in January and 0.17 in June) (Tables 3 - 6). While Chobe had

the highest species richness (43 species) (Table 2), it was the least biologically diverse. This finding is due to *Ma. (Mansonoides) africana* (Theobald) and *Cq. (Coquillettidia) fraseri* (Theobald) comprising 84.8% and 91.1% of the January 2011 and June 2011 collections from this site respectively (Table 3). Species diversity differed greatly at Paraa between the two sampling periods, ranging from 0.33 - 0.82 (Table 4). A similar temporal species diversity difference was observed in Sempaya in western Uganda (Mutebi et al. 2012). However, Sempaya is a tropical forest ecosystem whereas Paraa is mostly open grassland. While the overall number of mosquitoes collected at Sunguru (Table 5) was very low, this location had the highest species diversity and the species diversity index was consistently high (0.91 and 0.86) during both seasons (Table 5). The diversity indices presented in this manuscript were calculated based on light trap collections. Light trap collections are biased toward mosquito species that are attracted to light and CO<sub>2</sub> and may not represent an accurate picture of complete species diversity at each location.

#### Mosquito collections at Chobe

To our knowledge this is the first documented account of the mosquito fauna of Chobe. A total of 20,025 mosquitoes were collected: 3,731 in January 2011 and16,294 in June 2011 (Table 3). Forty-four mosquito species in 10 genera were collected at this site (Table 2 & 3). The largest number of species collected were in the genus *Culex* (19 species), followed by *Aedes* (7), *Coquillettidia* and *Anopheles* (5 species each), *Mansonia* (2) and 1 each in the genera *Eretmapodites*, *Lutzia*, *Mimomyia*, *Toxorhynchites* and *Uranotaenia* (Table 2 & 3). Twenty-eight and 32 species were collected in January 2011 and June 2011, respectively, and of these, only 17 (38.6%) were collected during both sampling periods (Table 3) suggesting seasonal variation in species composition. The most abundant species collected in January was *Ma. africana* (84.8%) and in June *Cq. fraseri* (91.1%) (Table 3). Interestingly, *Cq. fraseri* only made up 1.61% of the total collection in January and *Ma. africana* less than 1% of the collection in June, whereas both species are associated with the same larval habitats, swamps and marshes (Hopkins 1952).

#### Mosquito collections at Paraa

Similar to Chobe, this is the first documented account of the mosquitoes of Paraa. A total of 9,487 mosquitoes were collected at Paraa; 7,607 in January 2011, and 1,880 in June 2011. The collection included 40 species in nine genera (Table 2 & 4). The most abundant species collected was *Ma. africana* which accounted for 65.4% of the total number of mosquitoes collected at this site (Table 1) followed by *Cx. (Culex) antennatus* (8.2%), *Cx. neavei* (3.2%) and *An. rivulorum* (2.1%) (Table 1). Similar to Chobe the majority of *Ma. africana* (99.8%) were collected in January 2011 and very few in June 2011 (0.2%). The most interesting finding at Paraa was the detection of *Ae. aegypti formosus* specimens that had specific abdominal ornamentation of *Ae. (Stegonyia) aegypti aegypti* (L) (scattered white light scales on the first abdominal tergite and apical white scales on tergites 2 through 7) described by Huang (2004). To our knowledge this is the first time a form of *Ae. aegypti formosus* with the same abdominal ornamentation as *Ae. aegypti aegypti* has ever been detected in Uganda; all other specimens examined to date clearly exhibited abdominal ornamentation typical of *Ae. aegypti formosus*.

#### Mosquito Collections at Sunguru

Similar to Chobe and Paraa, the mosquito fauna of Sunguru have not previously been described. A total of 759 mosquitoes belonging to seven genera, 32 species and 2 subspecies were collected in Sunguru (374 in January 2011 and 285 in June 2011) (Tables 1 & 5). The most commonly captured species at this site were Cq. cristata (14.5%), Cx. cinereus (11.9%), Cx. (Oculeomyia) annulioris (Theobald) (10.4%), Cx. (Culex) decens group (Theobald) (9.7%), Cq. fraseri (8%) and An. (Anopheles) coustani (Laveran) (7.4%) (Table 1). There were variations in species composition between January 2011 when 23 species and one subspecies were collected, and June 2011, when 25 species and 2 subspecies were collected (Table 5). Five species: An. coustani, An. funestus s.s., Cx. (Culex) mirificus Edwards, Cx. (Kitzmilleria) moucheti Evans and Cx. poicilipes were only detected in January 2011. Eight species, Ae. (Diceromyia) furcifer (Edwards), Ae. (Stegomyia) simpsoni sl (Theobald), Cq. (Coquillettidia) aurites (Theobald), Cx. neavei, Cx. (Culiciomyia) nebulosus Theobald, Ma. uniformis, Uranotaenia (Pseudoficalbia) nivipous Theobald, Ur. (Uranotaenia) connali Edwards and one subspecies Cx. (Oculeomyia) annulioris ssp. aenescens Edwards, were only detected in June 2011. The relative abundance of some species such as An. coustani, Cq. cristata, Cx. (Culex) duttoni, Cx. cinereus, Cx. quinquefasciatus and Cx. annulioris varied dramatically from January to June. These observations demonstrate seasonal variations for these species at this site. There was very little variation in the relative abundance of some species such as Cq. fraseri, Cx. decens group, Cx. univittatus and Cx. pipiens (Table 5). The majority (93%) of the members of the Cx. pipiens complex in the present study were detected in Sunguru (Table 1) which is approximately 60 miles from Omugo, the location where WNV was first isolated from a febrile woman (Smithburn et al. 1940).

#### Mosquito Collections at Rhino Camp

This is the first description of the mosquito fauna of Rhino Camp. Only one collection was conducted at this location in January 2011 and it yielded 18,960 mosquitoes belonging to 7 genera and 25 species (Table 1). The species most frequently captured were *Ma. uniformis*, (55.8%%), *Ma. africana* (11.3%), *Cx. poicilipes* (10.7%), *An. funestus* group (3.3%), *An.* (*Cellia*) *rivulorum* Leeson (3.2%) and *Ma. (Mansonioides) africana nigerrima* Theobald (Table 1).

#### **Molecular Taxonomy Observations**

Sequences for *Coquillettidia* species and *An. funestus* have been deposited in GenBank under accession numbers MG132070, and MG190073–MG190076.

**Coquillettidia.**—ND4 sequences from *Cq. fuscopennata* and *Cq. cristata* were aligned and evaluated by pairwise comparison for this 400bp amplicon. Pairwise distances within and between these two species from multiple geographic locations were not sufficient for differentiation at the species level. Identity among *Cq. fuscopennata* from different collection sites was 99.2% - 100% whereas identity within *Cq. cristata* as well as between *Cq. fuscopennata* and *Cq. cristata* was 97.1% - 100%. Unfortunately, ND4 sequences

from other mosquitoes in the genus *Coquillettidia* are not represented on GenBank for comparison.

For the COI amplicon (using the primers of Vinogradova et al. 2003), mosquitoes morphologically identified as *Cq. cristata* from Chobe and Sunguru shared only 96.5% identity with each other, the same percentage identity observed between each of those specimens and *Cq. fuscopennata* from Lake Mburo, Kitubulu, and Kibale. Specimens collected from the same site were identical to each other. By comparison, the homologous COI sequences derived from *Cq. fuscopennata* collected from four different locations in Uganda (Sipi, Lake Mburo, Kitubulu, and Kibale) were identical to each other. Collectively, these molecular data suggest that there is some genetic structuring present within what we now consider morphologically as *Cq. cristata*, which should be investigated further.

The *Cq. cristata* specimens from Chobe and Sunguru were all morphologically identified as *Cq. cristata*. Specimens from both locations shared the same morphological property of having dark scales on the posterior corners of all tergites and mixed black and yellow scales on the sternites. These findings are in contrast to the description of Edwards (1941) which states that the abdomen of *Cq. cristata* matches that of *Cq. nigrithorax* in being wholly yellow with no darkening on the corners of the tergites. Considering these morphological variations among the *Cq. cristata* collected during this study and those described by Edwards (1941), and the divergent sequencing results at the COI locus, there appears to be some intraspecific genetic variation among *Cq. cristata* collected in different geographic locations that warrants further study. Unfortunately, no sequences from *Cq. cristata*, or the morphologically-similar *Cq. nigrithorax*, exist in the Barcode of Life (COI) database, or on Genbank for further comparison.

Anopheles funestus s.l.—Eighteen specimens morphologically identified as An. funestus s.l. were also analyzed molecularly. These mosquitoes included seven specimens from Sunguru, collected on 14 and 16 January 2011; eight specimens from Paraa collected between 19-21 January 2011 (7) and on 19 June 2011 (1); and three specimens from Chobe, collected on 24 January 2011. From Paraa, 6 of the 8 specimens tested were determined by PCR and sequencing to be An. rivulorum. The remaining two specimens from that location had a greater than 99% sequence similarity to An. funestus s.s. at the COI locus (top BLAST hit DQ287358); however, there was no amplification obtained from the multiplex assay which included an An. funestus-specific primer (Koekemoer et al. 2002). Similarly in Sunguru, 4 of 7 specimens matched An. funestus s.s. by sequencing COI after obtaining no amplification with the multiplex assay. From Chobe, 2 of 3 specimens were also identified as most like An. funestus s.s. by sequencing the COI amplicon, but for which no amplification was obtained from the multiplex assay. The remaining specimen from Chobe was confirmed as An. funestus s.s. by multiplex PCR and sequencing of both the COI amplicon and the internal transcribed spacer region 2 (ITS2) amplicon derived from the multiplex assay. These results suggest that there are multiple cryptic species within the An. funestus species complex in addition to An. funestus s.s. and An. rivulorum present at our collection sites.

Spillings et al. (2009) reported similar results from specimens collected in Malawi, specifically, 61 of 63 specimens morphologically identified as An. funestus s.l. during that study did not amplify with the multiplex assay. Sequence analysis of ITS2 identified variations within the An. funestus -specific primer binding site, and demonstrated a sequence variation of 4.5% compared with An. funestus s.s. (Spillings et al. 2009). On the basis of further molecular, cytogenetic, and cross-mating evidence, those authors concluded that the An. funestus-like specimens represented a new member of the An. funestus complex (Spillings et al. 2009). Further phylogenetic analysis of members of the An. funestus species complex, including the An. funestus-like specimens from Malawi, was carried out by Choi et al. (2012, 2013). Based on results generated from both the ND5 and COI genes, Choi et al. (2012) suggested that the Malawi An. funestus-like specimens did indeed represent a distinct lineage from the other species in the funestus group, supporting the findings of Spillings et al. (2009). Choi et al. (2012) further described two clades of An. funestuslike specimens that emerged during their analysis, and noted that further study on this group of mosquitoes is necessary to resolve phylogenetic relationships within this group. Currently, An. funestus-like specimens have only been reported from Malawi. However, our results from Uganda are consistent with the Malawian studies, and may represent additional locations from which representatives of these novel An. funestus-like lineages are present. A multiple sequence alignment of COI sequences generated from An. funestus specimens from this study and reference An. funestus sequence AY423059, demonstrated that all of the An. funestus sequenced in this study share a COI genotype consistent with lineage 1 (Choi et al. 2013) except for one specimen (#3) from Sunguru. This specimen from Sunguru is does not match the lineage 1 genotype and also differs at the locus associated with lineage 2 (Choi et al. 2013), however not enough genetic information for lineage 2 An. funestus is available on GenBank for more in-depth comparison. Further study on An. funestus complex mosquitoes in Uganda is warranted.

#### Potential medical importance

Of the 72 mosquito species collected in this study 33 have been implicated in the transmission of arboviruses of public health importance (Table 7). This suggests that there is a high potential for maintenance and transmission of arboviruses in this region. Human serological surveys (Henderson et al. 1970) showed presence of antibodies against chikungunya, Sindbis, Bunyamwera, West Nile, Wesselsbron, Banzi and Zika viruses in this region which suggests that these viruses are endemic in the region. The presence of both *Cx. pipiens* and *Cx. quinquefasciatus*, important vectors of WNV, correlates with the fact that WNV was first detected in this region of the country (Smithburn et al. 1940).

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A map of Uganda showing the locations of Chobe and Paraa in Murchison Falls National Park, Sunguru and Rhino Camp.



## Fig. 2.

Photographs illustrating the general vegetation and topology of each of the study sites A) Chobe, B) Paraa, C) Sunguru and D) Rhino Camp.

#### Table 1.

Number of mosquito species and subspecies collected at four locations in northwestern Uganda from in 2011.

Genus	Subspecies	Species	Chobe	Paraa	Sunguru	Rhino Camp
Aedes	Aedimorphus	albocephalus		33		
		alboventralis	41			
		cumminsii	7			
		stenoscutus	3	1		
		stokesi	2			
		tarsalis	8			
		spp	1			
	Diceromyia	furcifer			1	
	Mucidus	grahami		1		
	Neomelaniconion	albothorax		32		
		circumluteolus	2	42		
	Stegomyia	aegypti formosus	83	208	4	1
		metallica		1		
		simpsoni group		1	1	
Aedes		spp	5	6		
Aedomyia	Aedomyia	africana				2
	Lepiothauma	furfurea	152	1		
Anopheles	Anopheles	coustani	13	5	56	
-	-	tenebrosus		7		
		ziemanni		5		210
	Cellia	funestus s.s. *	3	3	4	
		funestus group	46	119	5	627
		gambiae group	1	8		
		gibbinsi			11	
		longinalnis				32
		maculinalnis			3	-
		rivulorum		200	2	609
		rivulorum/demeilloni		- 200	-	00,
		theileri		U		2
		wellcomei				2
		wellcomei ssn ugandae	1			-
Anonheles		species	5	77		37(
Coquillettidia	Coquillettidia	aurites	U U	185	14	128
coquinciliula	Coquincinaia	cristata	4	105	110	120
		fraseri	14905		61	4
		fusconennata	2	5	01	
		maculinennis	2	5	8	
		macanpennis	3 40	472	0	64
Comillettidie		species	40	+12	20	00
coquinettiaia		species			20	

Genus	Subspecies	Species	Chobe	Paraa	Sunguru	Rhino Camj
Culex	Culex	antennatus	308	775		65
		decens group	132	174	74	(
		duttoni	11	35	33	
		mirificus			1	
		neavei	271	299	2	278
		ornatothoracis	55			
		perfuscus	299	21		
		pipiens			12	
		pipiens complex		1		
		quinquefasciatus	1		14	
		trifilatus	1		23	
		trifilatus ssp. aenescens	6		23	
		univittatus	4	77	37	5
		watti	1			
	Culiciomyia	cinerellus		1		
		cinereus	8	6	90	
		nebulosus	36	4	1	
	Eumelanomyia	insignis	5	1		
		rubinotus			5	
	Kitzmilleria	moucheti	1		1	
	Oculeomyia	annulioris	43	29	79	
		annulioris ssp. consimilis	3		1	
		bitaeniorhynchus	2	9		
		poicilipes	1	13	1	202
Culex		species	223	82	54	5
Eretmapodites		chrysogaster	2	3		
Lutzia	Metalutzia	tigripes	45	3	2	
Mansonia	Mansonioides	africana	3308	6200		214
		africana nigerrima				58
		uniformis	69	160	2	1058
Mansonia		species	11	178		103
Mimomyia	Mimomyia	mimomyiaformis	1	1		6
Toxorhynchites	Toxorhynchites	brevipalpis	1			
Urantotaenia	Pseudoficalbia	mashonaensis			2	
		nivipous			1	
		pallidocephala	1			
	Uranotaenia	alboabdominalis				
		connali			1	
Total			20177	9487	759	1896
Grand Total	49 383					

\* identified using molecular methods (R Kading)

#### Table 2.

Number of species and total number of individuals in the different genera collected at each study site

	Chobe	Paraa	Sunguru	Rhino Camp	Total
Aedes	8(304)	10(326)	3(6)	2(3)	16(639)
Anopheles	5(69)	8(427)	6(81)	6(1,852)	14(2,429)
Coquillettidia	5(14,955)	3(662)	4(213)	3(196)	6(16,026)
Culex	19(1,411)	14(1,527)	16(451)	8(2,505)	24(5,894)
Eretmapodites	1(2)	1(3)			1(5)
Lutzia	1(45)	1(3)	1(2)		1(50)
Mansonia	2(3,388)	2(6,538)	1(2)	3(14,340)	3(24,268)
Mimomyia	1(1)	1(1)		1(62)	1(64)
Toxorhynchites	1(1)				1(1)
Urantotaenia	1(1)		3(4)	2(2)	5(7)
Total	44(20,177)	40(9,487)	34(759)	25(18,960)	72(49,383)

#### Table 3.

Mosquito species collected at Chobe, Murchison Falls National Park, Uganda, in January and June 2011. Simpson's Diversity Index (D) and Simpson's Index of Diversity (1-D) for each collection trip are presented at the bottom of the table.

			1	Number c	ollected (	%)
Genus	Subgenus	Species	Ja	n-11	Ju	n-11
Aedes	Aedimorphus	alboventralis			41	(0.25
		cumminsii	3	(0.08)	4	(0.02
		stenoscutus			3	(0.02
		stokesi			2	(0.01
		tarsalis	4	(0.11)	4	(0.02
		species	1	(0.03)		
	Neomelaniconion	circumluteolus	2	(0.05)		
	Stegomyia	aegypti formosus	3	(0.08)	80	(0.49
Aedes		species	1	(0.03)		
Culex		species	1	(0.03)	3	(0.02
Anopheles	Anopheles	coustani	13	(0.35)		
	Cellia	funestus s.s. *	3	(0.08)		
		funestus group	46	(1.23)		
		gambiae s.1.			1	(0.01
		wellcomei ssp. ugandae	1	(0.03)		
		species			5	(0.03
Coquillettidia	Coquillettidia	cristata	2	(0.05)	2	(0.01
		fraseri	60	(1.61)	14845	(91.1
		fuscopennata	2	(0.05)	1	(0.01
		maculipennis			3	(0.02
		metallica	7	(0.19)	33	(0.20
Culex	Culex	antennatus	88	(2.36)	220	(1.35
		decens group	16	(0.43)	116	(0.71
		duttoni	1	(0.03)	10	(0.06
		neavei	230	(6.16)	41	(0.25
		ornatothoracis			55	(0.34
		perfuscus	10	(0.27)	289	(1.77
		quinquefasciatus			1	(0.01
		trifilatus			1	(0.01
		trifilatus aenescens			6	(0.04
		univittatus			4	(0.02
		watti			1	(0.01
	Culiciomyia	cinereus			8	(0.05
		nebulosus	2	(0.05)	34	(0.21
	Eumelanomyia	insignis	1	(0.03)	4	(0.02
	Kitzmilleria	moucheti	1	(0.03)		

				Number co	ollected (	%)
Genus	Subgenus	Species	Ja	n-11	Ju	n-11
	Oculeomyia	annulioris	16	(0.43)	27	(0.17)
		annulioris ssp. Consimilis	3	(0.08)		
		bitaeniorhynchus			2	(0.01)
		poicilipes	1	(0.03)		
Culex		species	1	(0.03)	229	(1.39)
Eretmapodites		chrysogaster			2	(0.01)
Lutzia	Metalutzia	tigripes			45	(0.28)
Mansonia	Mansonioides	africana	3164	(84.80)	144	(0.88)
		uniformis	36	(0.96)	33	(0.20)
		species	11	(0.29)		
Mimomyia	Mimomyia	mimomyiaformis	1	(0.03)		
Toxorhynchites	Toxorhynchites	brevipalpis			1	(0.01)
Uranotaenia	Pseudoficalbia	pallidocephala	1	(0.03)		
Totals			3731		16294	
Grand total	20025					
D			0.72		0.83	
1-D			0.28		0.17	

\* identified using molecular methods (R Kading)

#### Table 4.

Mosquito species collected at Paraa, Murchison Falls National Park, Uganda, in January and June 2011. Simpson's Diversity Index (D) and Simpson's Index of Diversity (1-D) for each collection trip are presented at the bottom of the table.

			N	lumber co	llected	(%)
Genus	Subspecies	Species	Ja	n-11	Ju	ın-11
Aedes	Aedimorphus	albocephalus	1	(0.01)	32	(1.70)
		circumluteolus	1	(0.01)		
		stenoscutus			1	(0.05)
	Mucidus	grahami			1	(0.05)
	Neomelaniconion	albothorax			32	(1.70)
		circumluteolus			41	(2.18)
	Stegomyia	aegypti aegypti			44	(2.34)
		aegypti formosus	10	(0.13)	154	(8.19)
		metallica			1	(0.05)
		simpsoni group			1	(0.05)
Aedes		species	1	(0.01)	5	(0.27)
Aedomyia	Lepiothauma	furfurea			1	(0.05)
Anopheles	Anopheles	coustani	4	(0.05)	1	(0.05)
		tenebrosus	1	(0.01)	6	(0.32)
		ziemanni	1	(0.01)	4	(0.21)
	Cellia	funestus s.s. ***	2	(0.03)	1	(0.05)
		funestus group	71	(0.93)	48	(2.55)
		gambiae group			8	(0.43)
		rivulorum	67	(0.88)	133	(7.07)
		rivulorum/demeilloni	3	(0.04)		
Anopheles		species	22	(0.29)	55	(2.93)
Coquillettidia	Coquillettidia	aurites	179	(2.35)	6	(0.32)
		fuscopennata	5	(0.07)		
		metallica	336	(4.42)	136	(7.23)
Culex	Culex	antennatus	41	(0.54)	734	(39.04)
		decens group	107	(1.41)	67	(3.56)
		duttoni	16	(0.21)	19	(1.01)
		neavei	112	(1.47)	187	(9.95)
		perfuscus	4	(0.05)	17	(0.90)
		pipiens complex			1	(0.05)
		univittatus	42	(0.55)	35	(1.86)
	Culiciomyia	cinereus	3	(0.04)	3	(0.16)
		cinerellus	1	(0.01)		
		nebulosus			4	(0.21)
	Eumelanomyia	insignis			1	(0.05)
	Oculeomyia	annulioris	22	(0.29)	7	(0.37)

			ľ	Number co	llected (	(%)
Genus	Subspecies	Species	Ja	n-11	Ju	n-11
		bitaeniorhynchus	7	(0.09)	2	(0.11)
		poicilipes	11	(0.14)	2	(0.11)
Culex		species	37	(0.49)	45	(2.39)
Eretmapodites		chrysogaster			3	(0.16)
Lutzia	Metalutzia	tigripes			3	(0.16)
Mansonia	Mansonioides	africana	6190	(81.37)	10	(0.53)
		uniformis	132	(1.74)	28	(1.49)
Mansonia		species	178	(2.34)		
Mimomyia	Mimomyia	mimomyiaformis			1	
Totals			7607		1880	
Grand total	9487					
D			0.67		0.18	
1-D			0.33		0.82	

\*\*\* Identified using molecular methods (Kading)

#### Table 5.

Mosquito species collected at Sunguru, Uganda, in January and June 2011. Simpson's Diversity Index (D) and Simpson's Index of Diversity (1-D) for each collection trip are presented at the bottom of the table.

					1	(0/)
				Number col	lected	(%)
Genus	Subspecies	Species	Ja	un-11	Jı	ın-11
Aedes	Diceromyia	furcifer			1	(0.26)
	Stegomyia	aegypti formosus	1	(0.27)	3	(0.78)
		simpsoni group			1	(0.26)
Anopheles	Anopheles	coustani	56	(14.97)		
	Cellia	funestus s.s. *	4	(1.07)		
		funestus group	4	(1.07)	1	(0.26)
		gibbinsi	5	(1.34)	6	(1.56)
		maculipalpis	1	(0.27)	2	(0.52)
		rivulorum	1	(0.27)	1	(0.26)
Coquillettidia	Coquillettidia	aurites			14	(3.64)
		cristata	17	(4.55)	93	(24.16)
		fraseri	25	(6.68)	36	(9.35)
		maculipennis	2	(0.53)	6	(1.56)
Coquillettidia		species	20	(5.35)		
Culex	Culex	decens group	37	(9.89)	37	(9.61)
		duttoni	2	(0.53)	31	(8.05)
		mirificus	1	(0.27)		
		neavei			2	(0.52)
		pipiens	10	(2.67)	2	(0.52)
		quinquefasciatus	14	(3.74)		
		trifilatus	23	(6.15)		
		trifilatus ssp. aenescens	20	(5.35)	3	(0.78)
		univittatus	15	(4.01)	22	(5.71)
	Culiciomyia	cinereus	2	(0.53)	88	(22.86)
		nebulosus			1	(0.26)
	Eumelanomyia	rubinotus	4	(1.07)	1	(0.26)
	Kitzmilleria	moucheti	1	(0.27)		
	Oculeomyia	annulioris	61	(16.31)	18	(4.68)
		annulioris ssp consimilis			1	(0.26)
		poicilipes	1	(0.27)		
Culex		species	45	(12.03)	9	(2.34)
Lutzia	Metalutzia	tigripes	1	(0.27)	1	(0.26)
Mansonia	Mansonioides	uniformis			2	(0.52)
Uranotaenia	Pseudoficalbia	mashonaensis	1	(0.27)	1	(0.26)
		nivipous			1	(0.26)
	Uranotaenia	connali			1	(0.26)

			Number	collected (%)
Genus	Subspecies	Species	Jan-11	Jun-11
Totals			374	385
Grand total	759			
D			0.09	0.14
1-D			0.91	0.86

\* identified using molecular methods (R Kading)

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#### Table 6.

Mosquito species collected at Rhino Camp, Uganda, in January 2011. Simpson's Diversity Index (D) and Simpson's Index of Diversity (1-D) for each collection trip are presented at the bottom of the table.

			Number co	llected (%)
Genus	Subspecies	Species	Jan	-11
Aedes	Stegomyia	aegypti formosus	1	(0.01)
Aedomyia	Aedomyia	africana	2	(0.01)
Anopheles	Anopheles	ziemanni	210	(1.11)
	Cellia	funestus group	627	(3.31)
		longipalpis	32	(0.17)
		rivulorum	609	(3.21)
		theileri	2	(0.01)
		wellcomei	2	(0.01)
Anopheles		species	370	(1.95)
Coquillettidia	Coquillettidia	aurites	128	(0.68)
		cristata	2	(0.01)
		metallica	66	(0.35)
Culex	Culex	antennatus	65	(0.34)
		decens group	6	(0.03)
		neavei	278	(1.47)
		univittatus	55	(0.29)
	Culiciomyia	cinereus	4	(0.02)
	Eumelanomyia	insignis	9	(0.05)
		rubinotus	3	(0.02)
	Oculeomyia	poicilipes	2029	(10.70)
Culex		species	56	(0.30)
Mansonia	Mansonioides	africana	2142	(11.30)
		africana nigerrima	583	(3.07)
		uniformis	10582	(55.81)
Mansonia		species	1033	(5.45)
Mimomyia	Mimomyia	mimomyiaformis	62	(0.33)
Uranotaenia	Pseudoficalbia	mashonaensis	1	(0.01)
	Uranotaenia	alboabdominalis	1	(0.01)
Grand total			18960	
D			0.34	
1-D			0.66	

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Genus	Subgenus	Species	Arbovirus(es)
A edes	Aedimorphus	albocephalus	MIDV <sup>1</sup> , WNV <sup>1</sup> . <sup>19</sup>
		cumminsii	$DENV-2^{I,5},MIDV^{I,3,5},PGAV^{I,5},RVFV^{I,2,5,20},SHOV^{I,3},SPOV^{I,4,5},WSLV^{I,5},SINV^3CHIKV^5$
		tarsalis	$MIDV^{I,\mathcal{S}},PGAV^{I},SHOV^{I,\mathcal{S}},WSLV^{I,\mathcal{S}},ZIKAV^{I,\mathcal{S}},RVFV^{2,I7}$
	Diceromyia	taylori	DENV-2 <sup>1,5,22</sup> , yfv <sup>1,5,6</sup> , chikv <sup>5</sup> , zikav <sup>5</sup> , oruv <sup>5</sup>
	Mucidus	grahami	СНІКУ <sup>5</sup> , ZIKAV <sup>5</sup>
	Neomelaniconion	albothorax	WNV <sup>1.5</sup>
		circumluteolus	$BUNV^I, GERV^I, LEBV^I, MIDV^{I,\mathcal{A}}, PGAV^{I,\mathcal{S}}, RVFV^I, SPOV^I, WSLV^{I,\mathcal{A},\mathcal{S}}, WNV^{I,\mathcal{S},I\mathcal{P}}$
	Stegomyia	aegypti	CHIKV <sup>I, 5,</sup> DENV-1 <sup>I</sup> , DENV-2 <sup>I, 22</sup> , DENV-3 <sup>I</sup> , DENV-4 <sup>I</sup> , DUGV <sup>I</sup> , ORUV <sup>I, 5</sup> , USUV <sup>I</sup> , VEEV <sup>I</sup> , WNV <sup>I, 5, 19</sup> , YFV <sup>I, 5, 8, 21</sup> , ZIKAV <sup>I, 5, 23</sup> , SFV <sup>5</sup> , WSLV <sup>5</sup> , BBKV <sup>5</sup>
		metallicus	ΥFV <sup>5, 6</sup> , WSLV <sup>5</sup> , ZIKAV <sup>5</sup>
		simpsoni group	YFV <sup>6, I3, I4</sup> , BBKV <sup>5</sup> , NRIV <sup>5</sup>
Anopheles	Anopheles	coustani	CHIKV <sup>I, 5</sup> , pGAV <sup>I, 5</sup> , WNV <sup>I, 19</sup> , NRIV <sup>5</sup>
		funestus complex	$BWAV^{L,\mathcal{S},\mathcal{24}},CHIKV^{L,\mathcal{S}},ONNV^{L,\mathcal{S},\mathcal{9},L\mathcal{S},\mathcal{16}},ORUV^{L,\mathcal{S}},PGAV^{L,\mathcal{S}},SFV^{L,\mathcal{4}},WSLV^{L,\mathcal{S}},TATV^{\mathcal{S}},NDOV^{\mathcal{S}},TATV^{\mathcal{A},\mathcal{S}}$
		gambiae s.l.	$\mathrm{BWAV}^{L,S}, \mathrm{CHIKV}^{I}, \mathrm{ILEV}^{L,S}, \mathrm{MIDV}^{L,S}, \mathrm{ONNV}^{L,S, IS, I6}, \mathrm{ORUV}^{L,S}, \mathrm{ZIKAV}^{L,S}, \mathrm{TATV}^{S}, \mathrm{TATV}^{S}, \mathrm{NDOV}^{S}, \mathrm{BGIV}^{S}, \mathrm{TATV}^{\mathcal{A},S}$
		pharoensis	sinv <sup>1</sup> , wsly <sup>5</sup> , nriv <sup>5</sup> , bgiv <sup>5</sup>
Coquillettidia	Coquillettidia	aurites	USUV <sup>1</sup> , TATV <sup>4,5</sup>
		fuscopennata	SINV <sup>1,10</sup> , CHIKV <sup>10</sup> , YFV <sup>1,6</sup>
		maculipennis	CHIKV <sup>5</sup>
		metallica	MIDV <sup>1, 5</sup> , WNV <sup>1, 19</sup> , BBKV <sup>5</sup>
Culex	Culex	antennatus	$PGAV^{I}$ , WNV $^{I, 5, I9}$ , RVFV $^{2, 20}$ , SINV $^{I}$ , WSLV $^{5}$ , BBKV $^{5}$ , NRIV $^{5}$
		decens group	WNV <sup>5,19</sup> , CHIKV <sup>5</sup> , BBKV <sup>5</sup>
		neavei	SPOV <sup>1, 4</sup> , WNV <sup>5, 8, 12, 19</sup> , SINV <sup>8</sup> , BBKV <sup>5</sup> KOUV <sup>5</sup>

Genus	Subgenus	Species	Arbovirus(es)
		perfuscus	oruv <sup>1, 5</sup> , usuv <sup>1, 5</sup> , wnv <sup>1, 5, 19</sup> , wslv <sup>1, 5</sup> , sinv <sup>5</sup> , bbkv <sup>5</sup>
		pipiens	$\operatorname{JBEV}^I$ , LACV $^I$ , SFV $^I$ , SLEV $^I$ , TAHV $^I$ , WEEV $^I$ , WNV $^{I9}$ , BANV $^S$ , BUNV $^S$
		quinquefasciatus	$CHIKV^{I,\mathcal{S}}, EEEV^{I}, KUNV^{I}, MTBV^{I}, MURV^{I}, OROV^{I}, RRV^{I}, SLEV^{I}, SINV^{I}, VEEV^{I}, WANV^{I}, WEEV^{I}, WNV^{I,\mathcal{S},I9}, BBKV^{\mathcal{S}}$
		univittatus	sinv <sup>1, 25</sup> , spov <sup>1</sup> , usuv <sup>1, 5</sup> , wslv <sup>1</sup> , wnv <sup>1, 5, 19</sup>
	Culiciomyia	cinereus	CHIKV <sup>5</sup> , MIDV <sup>5</sup> , BBKV <sup>5</sup>
		nebulosus	MIDV <sup>5</sup> , BBKV <sup>5</sup> , BGIV <sup>5</sup>
	Eumelanomyia	rubinotus	BANV <sup>I</sup> , GERV <sup>I, 5</sup> , RVFV <sup>2</sup>
	Oculeomyia	amulioris	MIDV <sup>5</sup> , WSLV <sup>5</sup>
	Oculeomyia	poicilipes	RVFV <sup>5,11</sup> , WNV <sup>5,12,19</sup> , MIDV <sup>5</sup> , PGAV <sup>5</sup> , BBKV <sup>5</sup> , NRIV <sup>5</sup>
		chrysogaster	MIDV <sup>1,5</sup> , RVFV <sup>1,2</sup> , SFV <sup>5</sup>
Eretmapodites	Mansonioides	africana	$\mathrm{BANV}^{I}, \mathrm{BUNV}^{I}, \mathcal{5}^{I2}, \mathrm{CHIKV}^{I, \mathcal{5}}, \mathrm{LEBV}^{I}, \mathrm{MIDV}^{I, \mathcal{5}}, \mathrm{PGAV}^{I, \mathcal{5}}, \mathrm{SHOV}^{I}, \mathrm{spov}^{I, \mathcal{4}}, \mathrm{USUV}^{I, \mathcal{5}}, \mathrm{WSLV}^{I, \mathcal{5}}, \mathrm{RVFV}^{\mathcal{5}, 20}, \mathrm{WNV}^{\mathcal{5}}, \mathrm{BBKV}^{\mathcal{5}}$
Mansonia		uniformis	BUNV <sup>I, 5, 12</sup> , MIDV <sup>I, 5</sup> , RRV <sup>I</sup> , SPOV <sup>I, 4</sup> , WSLV <sup>I, 5</sup> , ZIKAV <sup>I, 5</sup> , WNV <sup>5, 11</sup> , CHIKV <sup>5</sup> , BANV <sup>5</sup> , RVFV <sup>5</sup>
Uranotaenia	Pseudoficalbia	mashonaensis	wslv <sup>5</sup>
' The Internationa	l Catalogue of Arbovi	iruses.	
Meegan & Bailey	y 1988.		
g McIntosh <i>et al.</i> 1	972.		
ARBOCAT (http	:://wwwn.cdc.gov/arb	ocat/index.asp).	
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Monath 1988.			
7 McCrae & Kirya	1982.		
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) Lutwama <i>et al.</i> 1	999.		
0 Woodhall 1964.			
<sup>11</sup> Diallo <i>et al.</i> 200	)5.		

12 Traore-Lamizana 2001. 13 Mahaffy *et al.* 1942. 14 Smithburn & Haddow 1946. 15 Williams *et al.* 1965. 16 Corbet *et al.* 1961. 17 Smithburn *et al.* 1948. 18 MacIntosh *et al.* 1948. 18 MacIntosh *et al.* 1948. 19 Hubálek & Halouzka 1999. 20 Fontenille *et al.* 1980. 21 Germain *et al.* 1980. 22 Diallo *et al.* 2003. 23 Jupp *et al.* 1969. 23 Jupp *et al.* 1969.

П Dengue type 2 virus. DEN-3 = Dengue type 3 virus. DEN4 = Dengue type 4 virus. DUGV = Dugbe virus. EEEV = Eastern Equine Encephalitis virus. GERV = Gemiston virus. ILEV = Ilesha virus. JBEV TATV = Tataguine virus. USUV = Usutu virus. VEEV = Venezuelan Equine Encephalitis virus. WANV = Wanowrie virus. WEEV = Western Equine Encephalitis virus. WSLV = Western Equine Virus. WSLV = Venezuelan Equine Virus. WNV MURV = Murray Valley virus. NDOV = Nyando virus. NRIV = Ngari virus. ONNV = Onyong-Nyong virus. OROV = Oropouche virus. ORUV = Orungo virus. PGAV = Pongola virus. RRV = Ross River virus. RVFV = Rift Valley Fever virus. SFV = Semliki Forest virus. SHOV = Shokwe virus. SINV = Sindbis virus. SLEV = St. Louis Encephalitis virus. SPOV = Spondweni virus. TAHV = Tahyna virus. BANV = Banzi virus. BBKV = Babanki virus. BGIV = Bangui virus. BUNV = Bunyamwera virus. BWAV = Bwamba virus. CHIKV = Chikungunya virus. DENV-1 = Dengue type 1 virus. DENV-2 = = Japaneese Encephalitis virus. KOUV = Koutango virus. KUNV = Kunjin virus. LACV = LaCrosse Encephalitis virus. LEBV = Lebombo virus. MIDV = Middelburg virus. MTBV = Marituba virus. West Nile virus. YFV = Yellow Fever virus. ZIKAV = Zika virus.