



Aedes mosquitoes in the Republic of the Sudan, with dichotomous keys for the adult and larval stages

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ABSTRACT

Descriptions of the mosquitoes of the Republic of the Sudan are mostly limited to works published more than 60 years ago. Khartoum State in central Sudan, which encompasses the capital city, has experienced many outbreaks of diseases caused by mosquito-borne pathogens, including Rift Valley fever. In this paper we focus on the composition of *Aedes* mosquitoes in high-risk areas in and around major agricultural projects. This is based on longitudinal surveillance of adults and larvae during the hot dry and rainy seasons in 2013. A total of 630 adult female mosquitoes were collected. *Anopheles* mosquitoes were the most abundant ($n = 456$, 72.4%), followed by *Culex* ($n = 96$, 15.2%) and *Aedes* ($n = 78$, 12.4%). Only three *Aedes* species were identified: *Aedes caballus* ($n = 38$, 48.7% of the *Aedes*), *Aedes vexans arabiensis* ($n = 30$, 38.5%) and *Aedes caspius* ($n = 10$, 12.8%). A total of 42,549 larvae were collected. *Aedes* larvae were the most abundant ($n = 30,936$, 72.7%), followed by *Culex* ($n = 9656$, 22.7%) and *Anopheles* ($n = 1957$, 4.6%). The *Aedes* larvae included *Ae. caspius* ($n = 21,957$, 71.0% of the *Aedes*), *Ae. vexans arabiensis* ($n = 5577$, 18.0%), *Aedes quasiunivittatus* ($n = 107$, 0.3%), *Aedes dentatus* (Theobald) ($n = 204$, 0.7%) and 3091 unidentifiable larvae (10.0%), denoted as Forms X, Y and Z. We discuss the bionomics of the four identified species of *Aedes* and provide updated keys for the identification of the mosquito genera and the larvae and adults of the *Aedes* species recorded from the Republic of the Sudan.

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Introduction

Biting insects have been studied more extensively than most other animals but our taxonomic knowledge of these arthropods is by no means complete. In the case of mosquitoes (Diptera: Culicidae), the number of described species has more than doubled since Edwards (1932) outlined the traditionally accepted system of mosquito

classification (Knight and Stone 1977; Wilkerson et al. 2015), and species new to science are still being discovered.

Taxonomic reports of mosquitoes in the Anglo-Egyptian Sudan (comprising the present countries of Sudan and South Sudan) are limited to the works of Lewis (1953, 1954, 1955, 1956a, 1956b) and the species records of Edwards (1941) and Hopkins (1952). The majority of the species belong to the subfamily Culicinae, within which the genus *Aedes* Meigen is the most species-rich and the most important because it contains vectors of several arboviruses that cause diseases in humans. After the secession of the Republic of South Sudan in 2011, the total number of mosquito species in Sudan (also known as North Sudan and officially the Republic of the Sudan) remains uncertain as many of the species previously reported were known to be confined to what is now the territory of the Republic of South Sudan.

Khartoum State in central Sudan, which includes the capital city Khartoum, has experienced many outbreaks of diseases caused by mosquito-borne pathogens, the last being an outbreak of Rift Valley fever in 2008 (World Health Organization 2008). A relative increase in malaria prevalence in recent years has also been reported (Annual Health Statistical Report 2011). After the genus *Anopheles* Meigen, which includes the species that are vectors of malarial protozoans, *Aedes* Meigen is the most important genus in Khartoum State. Lewis (1953, 1955) recorded 33 species and one variety of *Aedes* in the Anglo-Egyptian Sudan. Perusal of the collection records and distribution maps in these publications revealed that Lewis only found 22 of these species in the territory of the Republic of the Sudan, including five species that are common throughout the Afrotropical Region, i.e. *Aedes* (*Fredwardsius*) *vittatus* (Bigot), *Aedes* (*Mucidus*) *scatophagoides* (Theobald), *Aedes* (*Neomelaniconion*) *lineatopennis* (Ludlow), *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) and *Aedes* (*Stg.*) *unilineatus* (Theobald). The occurrence of a 23rd species of *Aedes* in Sudan, *Aedes* (*Aedimorphus*) *quasiunivittatus* (Theobald), was recorded by Edwards (1941).

Herein, we report the composition of *Aedes* mosquitoes in high-risk areas around major agricultural projects in Khartoum State. We also provide keys for the identification of the larvae and adults of the species of *Aedes* recorded from the Republic of the Sudan, larval and adult keys to the mosquito genera that occur in the Afrotropical Region, and data on the *Aedes* species found in typical indoor resting sites.

Materials and methods

Study area

Khartoum State lies at the junction of the White and Blue Nile Rivers in the north-eastern part of central Sudan between latitudes of 15.19 and 16.66° N and longitudes of 31.6 and 34.38° E, covering a total area of 20,736 km². Most of Khartoum State falls within the semi-desert climatic zone, but the northern part is classified as desert. Seasonal temperatures vary from hot to very hot during the summer and warm-dry to cool-dry during winter. Average temperatures range between 25 and 40°C during

the months of April to June and between 20 and 35°C during July to October. Winter temperatures range between 15 and 25°C. Annual rainfall, mostly from brief summer rains, is in the range 200–300 mm in the south, 100–200 mm in the northeast and 10–100 mm in the north.

Mosquitoes were collected in a region known as East Nile, which is located in the northeastern part of Khartoum State. It covers a total area of about 8000 km² (more than one-third of Khartoum State). East Nile is a flat plain comprised of neighbourhoods or villages covering an area of about 307 acres (1.24 km²), and agricultural land of about 400,000 acres (1618.74 km²). One of the largest dairy projects in Khartoum State, with a total area of 3200 acres (12.95 km²), is located in East Nile. A detailed geographic description and quantitative analysis of the species at all collection sites will be published at a later date.

Mosquito surveillance and collection

A longitudinal surveillance study of larval and adult mosquitoes was conducted for six months during the period of May to October 2013. Inspections of larval habitats and indoor and outdoor resting adults were carried out weekly at four study sites chosen to represent agricultural and adjacent residential areas. Mosquito larvae were collected using the standard dipping method and preserved in 70% ethanol for subsequent morphological identification. Collections of resting adult mosquitoes were performed using a knock-down procedure (World Health Organization 1995). These were carried out in early morning with prior consent from occupants. Selection of the sampled homes was random, grouped by house type (small or large), proximity to potential larval habitats (near or at a distance from such sites). This avoided sampling bias when determining mosquito densities. Also, active searches for adults in outdoor resting sites were carried out using aspirators. Adults were preserved dry for subsequent morphological identification.

Mosquito identification

Fourth-instar larvae were transferred directly from preservative into a drop of Berlese's fluid using a fine brush and were mounted dorsal side up. Slides were left to dry at room temperature for 3 days, labelled with the location and date of collection, type of habitat and species name. Specimens were identified using Hopkins (1952) for larvae and Edwards (1941) for adults. Larvae were examined under a compound microscope at magnifications of 10× and 40×. Adults were examined under a dissecting microscope and at a magnification of 25× with a compound microscope.

Preparation of identification keys

Dichotomous keys prepared for the genera of mosquitoes that occur in the Afrotropical Region (genera *Hodgesia* Theobald and *Orthopodomyia* Theobald are not recorded from Sudan and South Sudan), and the identification of *Aedes* mosquitoes recorded from the Republic of Sudan, are included after the Discussion. The

included photographs of anatomical features were taken with a Canon Power shot A650 I S-12.1. The morphological terminology of Harbach and Knight (1980, 1982), revised and updated in the Anatomical Glossary of the Mosquito Taxonomic Inventory (<http://mosquito-taxonomic-inventory.info/>), is used in the keys. The modified traditional classification of *Aedes* established by Wilkerson et al. (2015) is used herein instead of the multiple-generic classification of Reinert et al. (2009) in keeping with past and present usage in Sudan.

Results

A total of 630 adult female mosquitoes were collected. *Anopheles* mosquitoes were the most abundant ($n = 456$, 72.4%), followed by *Culex* Linnaeus ($n = 96$, 15.2%) and *Aedes* ($n = 78$, 12.4%). One *Anopheles* species was collected, *Anopheles arabiensis* Patton, whereas three *Aedes* species were identified: *Aedes caballus* (Theobald) ($n = 38$, 48.7%), *Aedes vexans arabiensis* (Patton) ($n = 30$, 38.5%) and *Aedes caspius* (Pallas) ($n = 10$, 12.8%).

A total of 42,549 mosquito larvae comprising species of *Aedes*, *Anopheles* and *Culex* were collected. *Aedes* species were the most abundant, comprising 30,936 larvae (72.7%), followed by *Culex* (9656 larvae, 22.7%) and *Anopheles* (1957 larvae, 4.6%). Identification of larvae revealed the presence of one *Anopheles* species, four *Aedes* species and three larval forms of *Aedes* that could not be identified to species. The single anopheline species, *An. arabiensis* Patton, comprised 4.6% of the total number of larvae that were collected. The aedine larvae collected include *Ae. caspius* ($n = 21,957$, 71.0%), *Ae. vexans arabiensis* ($n = 5577$, 18.0%), *Ae. quasiunivittatus* ($n = 107$, 0.3%), *Aedes dentatus* (Theobald) ($n = 204$, 0.7%) and 3091 unidentifiable larvae (10.0%).

The three unidentifiable morphological forms of *Aedes* larvae key to *Ae. vexans arabiensis* and *Ae. dentatus* using Hopkins (1952), the only (comprehensive) key available for larvae of Afrotropical *Aedes* (*sensu* Knight and Stone 1977; Wilkerson et al. 2015), but could not be identified unambiguously as either of these species, and they may possibly be new species records for Sudan or species new to science. These larval forms are henceforth referred to as Forms X, Y and Z. Only three specimens of Form Z (0.1% of the unidentifiable larvae) were collected during the study, indicating the very low density of this form in comparison to Forms X and Y. Form X (1952 larvae) comprised 63.2% and the Form Y (1139 larvae) comprised 36.8% of the unidentifiable larvae.

Discussion

This study, the first of its kind since the reports published during the mid-twentieth century, revealed the presence of four identifiable *Aedes* species and three unidentifiable larval forms in the agricultural (primarily) and residential areas of the East Nile region in central Sudan. Lewis (1953, 1955) surveyed the entire Anglo-Egyptian Sudan and speculated that the distribution of *Aedes* species is undoubtedly wider and more diverse than he reported; our findings confirm this. Edwards (1941) reported that subgenus *Aedimorphus* Theobald includes most species of *Aedes* in

Africa, with seven common species that include *Ae. dentatus* (not previously recorded from Sudan), *Ae. quasiunivittatus* and *Ae. vexans arabiensis*. This is in agreement with the finding of the present study, where we found these three species in highest densities in major agricultural areas in Khartoum State.

Aedes vexans arabiensis larvae were found in shallow pools surrounded by grass and containing algae, and in temporary rain-filled pools. Abbott (1948) reported finding larvae of this form "in great numbers breeding in temporary rain pools in the early rains". Hopkins (1952) reported the occurrence of larvae in stagnant, muddy water in sunlit or partly shaded pools in irrigation channels. The consistent presence of this species throughout 6 months of collecting during this study, which spans the hot dry season and the rainy season, indicates that agricultural lands provide temporally stable habitats for the immature stages. Lewis (1955) reported many larval collection records of this species, the adults of which seem to rarely bite humans in Khartoum State. However, it is known that *Ae. vexans arabiensis* probably vectors West Nile virus between horses and bird hosts (Fall et al. 2011).

Edwards (1941) and Lewis (1955) did not list *Ae. dentatus* as a species recorded from Anglo-Egyptian Sudan. During this study, larvae of this species were found in shallow shaded irrigation canals surrounded by grass and containing algae. Larvae are often common in all kinds of ground pools after heavy rain, particularly the edges of swamps that commonly contain grass. However, larvae have also been found in pools which, although well shaded, were devoid of vegetation (Hopkins 1952). *Aedes dentatus* is predominantly a highland species, particularly where rainfall is high. It is one of the three dominant *Aedes* species in the moist Transvaal Highveld where adult females readily attack humans and larger domestic mammals. A strain of Rift Valley fever virus was isolated from specimens during an epizootic among cattle (McIntosh 1975). Investigations have shown that *Ae. dentatus* is predominantly a daytime biter with a peak of activity around sunset. It is also zoophagic, feeding on birds, cattle and other mammals, including primates (Metselaar et al. 1973). Evidence for *Ae. dentatus* being a vector of yellow fever virus is inconclusive (Metselaar et al. 1973). Orungo virus was isolated from this species during an outbreak of human infections in Nigeria (Tomori and Fabiyi 1977). Additionally, this species has yielded isolates of Rift Valley fever virus (Meegan and Bailey 1989) and may act as a reservoir epizootic vector in South Africa (Jupp and Cornel 1988). Pongola, Semliki Forest, Shokwe and Wesselsbron viruses have also been recovered from *Ae. dentatus* (Karabatsos 1985).

Lewis (1955) reported that *Ae. caspius* is often found in the dry season, particularly in winter. This species is frequently observed biting humans at Faras, Nukheila and Zeidab (near Khartoum and northward in River Nile State). Previous studies showed that larvae of this species often occur in ditches, frequently in residual pools in irrigation channels, occasionally at the reedy sides of fast-flowing canals, and are atypically abundant in small brackish pools in oases (Hopkins 1952). *Aedes caspius* is the vector of Tahyna virus in the Mediterranean region, a potential reservoir of Rift Valley fever virus during inter-epizootic periods, harbours some microsporidia and West Nile virus, and the bacterium *Francisella tularensis*, the causative agent of tularaemia, has been detected in natural populations (Milankov et al. 2009). Reintroduction of Rift Valley fever virus into Egypt in 1993 raised concerns about the potential of endemic mosquito species to transmit this

virus. Turell et al. (1996) showed that all mosquito species experimentally tested were susceptible to infection of this virus, with *Anopheles pharoensis* Theobald and *Ae. caspius* being the most sensitive to infection. *Aedes caspius* appeared to be the most efficient vector of the Egyptian mosquitoes evaluated, including *Culex pipiens* Linnaeus and *Culex antennatus* (Becker).

In the present study, *Ae. quasiunivittatus* larvae were found in canals in agricultural areas, especially those with deep stagnant water without algae and vegetation. Larvae are usually found in temporary muddy rain-pools devoid of vegetation and in pools in riverbeds (Hopkins 1952). Lewis (1955) recorded this species from Jebel Marra in western Anglo-Egyptian Sudan. It is potentially of medical importance as Logan et al. (1991) isolated a Bunyamwera group virus from females.

The presence of high densities of indoor-resting *Aedes* mosquitoes raises the question about their possible role in transmitting pathogens of human diseases in Khartoum State. Obviously, failure to correctly identify mosquito species has implications for the study, monitoring and control of vectors. In the present study, larvae that could not be identified unambiguously as *Ae. vexans arabiensis*, denoted as Forms X and Z, and *Ae. dentatus*, denoted as Form Y, indicates the need for detailed comparative morphological investigations and molecular assays to establish the specific identity of the *Aedes* species that inhabit Sudan.

Keys for the identification of the subgenera of *Aedes* and the species of subgenera *Albuginosus* (Reinert) (nine species), *Coetzeomyia* Huang, Mathis & Wilkerson (one species), *Hopkinsius* Reinert, Harbach & Kitching (*Finlaya sensu* Huang & Rueda 2017 in part) (seven species), *Ochlerotatus* Lynch Arribálzaga (seven species), *Vansomerenis* Reinert, Harbach & Kitching (*Finlaya sensu* Huang & Rueda 2017 in part) (three species) and *Zavortinkius* Reinert (six species) in the Afrotropical Region (Huang 2001; Huang and Rueda 2014, 2015a, 2015b, 2017) are only partly useful in Sudan. The key of Huang (2001) only includes 11 of the 20 subgenera currently recognized in Africa (Wilkerson et al. 2015; Huang and Rueda 2015c) and only one species (*Ae. caspius*) of the 25 species currently known to occur in Sudan (see key below). It is anticipated that the identification keys for species of *Aedes*, and also for mosquito genera, provided below, will provide mosquito control personnel in Sudan with an enhanced capacity to more rapidly characterize mosquito populations. This in turn will enhance readiness to respond to outbreaks of diseases caused by mosquito-borne pathogens. As of this report, the following species of *Aedes* are known to occur in the Republic of the Sudan.

Ae. (Aedimorphus) alboventralis (Theobald)

Ae. (Adm.) cumminsii (Theobald)

Ae. (Adm.) dalzieli (Theobald)

Ae. (Adm.) dentatus (Theobald)

Ae. (Adm.) fowleri (de Charmoy)

Ae. (Adm.) hirsutus (Theobald)

Ae. (Adm.) ochraceus (Theobald)

Ae. (Adm.) quasiunivittatus (Theobald)

Ae. (Adm.) vexans arabiensis (Patton)

Ae. (Adm.) leesoni Edwards

Ae. (Catageomyia) argenteopunctatus (Theobald)
Ae. (Diceromyia) furcifer (Edwards)
Ae. (Dic.) taylori Edwards
Ae. (Fredwardsius) vittatus (Bigot)
Ae. (Mucidus) scatophagoides (Theobald)
Ae. (Neomelaniconion) circumluteolus (Theobald)
Ae. (Neo.) lineatopennis (Ludlow)
Ae. (Ochlerotatus) caballus (Theobald)
Ae. (Och.) caspius (Pallas)
Ae. (Stegomyia) aegypti (Linnaeus)
Ae. (Stg.) africanus (Theobald)
Ae. (Stg.) luteocephalus (Newstead)
Ae. (Stg.) metallicus (Edwards)
Ae. (Stg.) simpsoni (Theobald)
Ae. (Stg.) unilineatus (Theobald)

Dichotomous keys for mosquitoes of Sudan

The keys below are intended for the identification of the genera of mosquitoes and the species of genus *Aedes* currently known to occur in the Republic of the Sudan. *Aedes (Adm.) dentatus* and *Ae. (Stg.) africanus* are new occurrence records for the country. *Aedes (Adm.) tarsalis* (Newstead), a “doubtful” record of a female from Wad Medani in central Sudan (Lewis 1955), is not included. Species of genera *Hodgesia* Theobald and *Orthopodomyia* Theobald have not been recorded from the country, but these genera are included as a contingency. The keys to *Aedes* larvae are adapted from Hopkins (1952); keys to *Aedes* adults from Edwards (1941) and Huang (2004).

Genera: adults

1. Proboscis long, strongly bent and distinctly attenuated in distal half; wing with distinct emargination immediately distal to end of vein CuA; very large brightly adorned mosquitoes *Toxorhynchites* Theobald
 Proboscis shorter, straight or only slightly bent, not noticeably attenuated; wing without emargination distal to end of vein CuA; smaller less brightly adorned mosquitoes 2
- 2(1). Abdominal sterna, and usually terga, mainly or entirely devoid of scales; scutellum evenly rounded; maxillary palpus about same length as proboscis.....
 *Anopheles* Meigen
 Abdominal sterna and terga covered with scales; scutellum normally tri-lobed; maxillary palpus of varied length, usually shorter than proboscis 3
- 3(2). Anal vein ends before (usually) or at level of base of crossvein mcu; upper calypter without setae 4
 Anal vein ends well beyond base of crossvein mcu; upper calypter with setae ... 6
- 4(3). Microtrichia of wing membrane minute, visible only under high magnification; veins R₂ and R₃ shorter than stem (R₂₊₃)..... *Uranotaenia* Lynch Arribálzaga

- Microtrichia distinctly visible under magnification of 50x; veins R_2 and R_3 longer than stem (R_{2+3}) 5
- 5(4). Proboscis swollen distally, upturned and setose; outstanding scales on distal 0.5 of wing normal, apices not notched (emarginate) *Malaya* Leicester
 Proboscis usually slender, not upturned and setose; apices of outstanding scales on distal 0.5 of wing notched (emarginate) *Hodgesia* Theobald
- 6(3). Apices of mid- and hindfemora with large tufts of sub-erect scales; antennal flagellomeres short and thick, basal flagellomere with prominent scale-tuft; very scaly species *Aedeomyia* Theobald
 Apices of mid- and hindfemora without large tufts of scales; antennal flagellomeres slender and elongate; normal (usually) or scaly species 7
- 7(6). Postspiracular setae present; foreungues (claws) of females toothed or simple; abdomen of females blunt or pointed 8
 Postspiracular setae absent; foreungues of females simple; abdomen of female blunt. 10
- 8(7). Vertex of head, thoracic pleura and lateral margins of abdominal terga with broad silvery scales; thorax usually partly or largely yellowish; paratergite broad, bare; mesopostnotum usually with cluster of fine setae *Eretmapodites* Theobald
 Without this combination of characters 9
- 9(8). Veins of wing with very broad (spatulate) and usually asymmetrical dark and pale scales; paratergite bare; all ungues (claws) of females simple; abdomen of females blunt *Mansonia* Blanchard
 Veins of wing with narrow or broad scales, not asymmetrical if broad; paratergite with or without scales; fore and midungues (claws) of females usually toothed; abdomen of females usually pointed *Aedes* Meigen (species key below)
- 10(7). Prespiracular setae present; wing with patch of setae at base of subcosta.....
 *Culiseta* Felt
 Prespiracular setae absent; subcosta of wing without basal patch of setae 11
- 11(10). Tarsomere 1 of foreleg longer than combined length of tarsomeres 2–5, tarsomere 4 shorter than tarsomere 5 *Orthopodomyia* Theobald
 Tarsomere 1 of foreleg shorter than combined length of tarsomeres 2–5, tarsomere 4 longer than tarsomere 5 12
- 12(11). Pulvilli well developed (at least on hind leg), pad-like; alula of wing with fringe of narrow scales; maxillary palpus of males usually longer than proboscis, slender and upturned 13
 Pulvilli unapparent, appearing to be absent; alula variable, without scales, with marginal broad decumbent scales or fringe of narrow scales as in *Culex*; maxillary palpus of males not upturned 14
- 13(12). One to three lower mesepimeral setae present or absent *Culex* Linnaeus
 Four or more lower mesepimeral setae present *Lutzia* Theobald
- 14(12). Wing with few scales; veins R_2 and R_3 shorter or at most slightly longer than vein R_{2+3} ; alula without scales or with broad decumbent scales
 *Mimomyia* Theobald
 Wing with numerous scales; veins R_2 and R_3 as long as or longer than vein R_{2+3} ; alula with fringe of narrow scales 15

- 15(14). Flagellomere 1 of antenna approximately three times as long as flagellomere 2; maxillary palpus of females about as long as clypeus, maxillary palpus of males shorter than proboscis, with very few setae; very small species.....
 *Ficalbia* Theobald
 Flagellomere 1 of antenna approximately equal in length to flagellomere 2; maxillary palpus of females distinctly longer than clypeus, maxillary palpus of males setose and longer than proboscis; larger species *Coquillettidia* Dyar

Key to adult females of species of *Aedes* known to occur in Sudan

1. Wing with dark pigmentation around radiomedial crossvein (rm) and proximal segment of vein M₃₊₄ *Ae. (Mucidus) scatophagoides*
 Wing without dark pigmentation at these locations 2
- 2(1). Lower mesepimeral setae present 3
 Lower mesepimeral setae absent 6
- 3(2). Vertex and scutellum with narrow scales; tibiae without median pale band
 *Ae. (Ochlerotatus) caballus*
 Vertex and scutellum with broad scales; tibiae with or without median pale band 4
- 4(3). Each tibia with white ring near mid-length *Ae. (Fredwardsius) vittatus*
 Tibiae without white rings 5
- 5(4). Abdominal terga speckled with pale scales *Ae. (Diceromyia) furcifer*
 Abdominal terga not speckled *Ae. (Dic.) taylori*
- 6(2). Hindtarsi with basal white bands on most tarsomeres; thoracic pleura not densely scaled 7
 Hindtarsi dark-scaled or with pale bands; thoracic pleura very densely scaled 12
- 7(6). Scutum with distinct crescent-shaped patch of pale scales on scutal fossa, median longitudinal pale stripe absent or present only on anterior area of scutum 8
 Scutal fossa without crescent-shaped patch of pale scales; scutum with long median longitudinal pale stripe reaching posterior area of scutum
 *Ae. (Stegomyia) unilineatus*
- 8(7). All femora with white knee spots 9
 Knee spots present only on mid- and hindfemora or absent from all femora 10
- 9(8). Scutum with lyre-shaped white markings; tibiae without white bands
 *Ae. (Stg.) aegypti*
 Scutum without lyre-shaped white markings; tibiae with white bands
 *Ae. (Stg.) metallicus*
- 10(8). Mid- and hindfemora with white knee spots; foretibia with basal white band
 *Ae. (Stg.) simpsoni*
 All femora without knee spots; foretibia entirely dark-scaled 11
- 11(10). Hindfemur with basal pale band and two pale patches on anterior surface, one at middle and one near apex *Ae. (Stg.) luteocephalus*
 Hindfemur without basal pale band, with three pale patches on anterior surface, one beyond base, one at middle and one near apex *Ae. (Stg.) africanus*
- 12(6). Paratergite without scales; tarsi entirely dark-scaled 13

- Paratergite with scales; tarsi with or without pale bands, if basal pale bands present then scutellar scales all narrow 14
- 13(12). Abdominal sterna mainly dark-scaled; wing with numerous pale scales on veins M_2 , M_3 , R_2 and R_3 *Ae. (Neomelanoconion) lineatopennis*
Abdominal sterna almost entirely pale-scaled; wing with only dark scales on veins M_2 , M_3 , R_2 and R_3 *Ae. (Neo.) circumluteolus*
- 14(12). Scutellum with broad flat (spatulate) scales on at least one lobe (usually broad, flat and pale on all lobes); decumbent scales of vertex narrow and broad 15
Scutellum with narrow scales only; decumbent scales of vertex all narrow 17
- 15(14). Scutum with four small pale spots composed of broad scales
..... *Ae. (Catageiomyia) argenteopunctatus*
Scutum without pale spots of broad scales 16
- 16(15). Scales on ante- and postpronotum nearly all broad (spatulate) and decumbent ...
..... *Ae. (Aedimorphus) lesoni*
Scales on ante- and postpronotum all narrow *Ae. (Adm.) alboventralis*
- 17(14). Anterior surfaces of mid- and hindfemora and -tibiae with longitudinal yellow stripe; tarsi yellowish..... *Ae. (Adm.) ochraceus*
Mid- and hindfemora and -tibiae without stripes; tarsi darker or with pale bands 18
- 18(17). Hindtarsi entirely dark-scaled 19
Hindtarsi with white bands 22
- 19(18). Decumbent scales of head almost all pale; basal pale bands of abdominal terga of female usually incomplete *Ae. (Adm.) cumminsii*
Vertex with patch of dark decumbent scales; basal pale bands of abdominal terga of female usually complete 20
- 20(19). Postspiracular area with most or all scales narrow *Ae. (Adm.) dalzieli*
Postspiracular area with only broad (spatulate) decumbent scales 21
- 21(20). Scutum with a narrow border of yellowish scales; hindfemur with approximately distal third of ventral surface dark-scaled; hindungues (claws) simple
..... *Ae. (Adm.) quasiunivittatus*
Scutum with yellowish scales irregularly scattered and sometimes numerous but not forming a definite pale border; hindfemur with ventral surface largely pale-scaled, with dark scales only near tip; hindungues (claws) toothed
..... *Ae. (Adm.) dentatus*
- 22(18). Hindtarsomeres with basal and apical white bands (i.e. across the joints); hindtarsomere 5 entirely pale-scaled *Ae. (Ochlerotatus) caspius*
Hindtarsomeres with only basal white bands 23
- 23(22). Wing entirely dark-scaled, at most a few pale scales at base of costa and radius; femora and tibiae not conspicuously speckled with pale scales
..... *Ae. (Adm.) vexans arabiensis*
Wing not entirely dark-scaled; femora and tibiae conspicuously speckled with pale scales 24
- 24(23). Wing with whitish scaling on distal 0.5 of costa, wing otherwise almost entirely dark-scaled *Ae. (Adm.) hirsutus*
Wing with costa dark-scaled but other veins with numerous scattered pale scales *Ae. (Adm.) fowleri*

Genera: fourth-instar larvae

- 1. Siphon absent *Anopheles* Meigen
- Siphon present 2
- 2(1). Abdominal segment VIII with a (usually) large dorsal sclerotized plate 3
- Abdominal segment VIII without a dorsal sclerotized plate 6
- 3(2). Lateral palatal brushes (mouth brushes) modified for predation, with 6–10 strong flattened filaments; comb and pecten absent *Toxorhynchites* Theobald
- Lateral palatal brushes normal, with more numerous fine filaments; comb present; pecten present or absent 4
- 4(3). Comb a double row of long slender spine-like scales; large sclerotized plates usually present on abdominal segments VI–VIII *Orthopodomyia* Theobald
- Comb a single row of scales that may or may not be long and slender; segments VI–VIII without dorsal sclerotized plates 5
- 5(4). Antenna enlarged, markedly curved and flattened; apex of siphon with pair of seta 9-S large and hook-like *Aedeomyia* Theobald
- Antenna not enlarged, markedly curved or flattened; apex of siphon with pair of seta 9-S small and simple *Uranotaenia* Lynch Arribálzaga
- 6(2). Siphon modified for piercing plant tissues, with anterior toothed plate; head setae all much shorter than head 7
- Spiracular apparatus unmodified, without anterior toothed plate; setae 5,6-C about twice length of head 8
- 7(6). Antenna with part distal to setae 2,3-A flexible, as long or longer than proximal part; comb with 4–10 sharply pointed spine-like scales *Coquillettidia* Dyar
- Antenna with part distal to setae 2,3-A inflexible, less than 0.5 length of proximal part; comb with two or three apically blunt scales *Mansonia* Blanchard
- 8(6). Siphon with anterolateral row of four or five multi-branched setae *Malaya* Leicester
- Siphon with at most one anterolateral seta and a more lateral seta 9
- 9(8). Siphon with at least three pairs of setae 1-S, usually more 10
- Siphon with single pair of seta 1-S..... 11
- 10(9). Siphon no longer than saddle, usually shorter; pecten extending to apex of siphon *Lutzia* Theobald
- Siphon much longer than saddle; pecten only on proximal area of siphon *Culex* Linnaeus
- 11(9). Seta 1-S inserted close to base of siphon, well below 0.33 12
- Seta 1-S inserted at or distal to 0.33, usually near last pecten spine in middle part of siphon 14
- 12(11). Comb a patch of numerous scales *Culiseta* Felt
- Comb a single row of 10 or (usually) fewer scales 13
- 13(12). Head setae 5,6,7-C large and conspicuous, seta 5-C inserted mesal to 6-C, seta 6-C much longer than head; pecten with only two spines *Ficalbia* Theobald
- Head setae 5–7-C small and rather inconspicuous, seta 5-C inserted almost directly posterior to 6-C, seta 6-C much shorter than head; pecten with three or more spines *Hodgesia* Theobald

- 14(11). Antenna spiculate proximally, distally distinctly articulated and not spiculate; pecten absent or with few (up to four) spines; seta 3-X with three or more branches *Mimomyia* Theobald
Antenna otherwise; pecten present or absent; seta 3-X normally single or double 15
- 15(14). Ventral brush (seta 4-X) with four (rarely five) pairs of single or double stout and strongly aciculate setae, grid absent; pecten reduced, with 0–7 spines *Eretmapodites* Theobald
Ventral brush (seta 4-X) usually with more than four pairs of setae, each usually with several branches, grid present; pecten with eight or more spines
..... *Aedes* Meigen

Key to larvae of species of *Aedes* known to occur in Sudan

1. Mouth brushes modified for predation, lateral palatal brushes comprised of 25–30 rather short, curved filaments with strong apical pectination
..... *Ae. (Mucidus) scatophagoides*
Mouth brushes normal, with more numerous fine filaments 2
- 2(1). Antenna without spicules, surface smooth; ventral brush (seta 4-X) confined to grid (no precratal setae) 3
Antenna spiculate or precratal setae present, often both 7
- 3(2). Comb scales somewhat spatulate (squamiform), fringed all round apex
..... *Ae. (Stegomyia) africanus* and *Ae. (Stg.) luteocephalus*
Comb scales sharply pointed (spine-like), without apical fringe 4
- 4(3). Ventral brush (seta 4-X) with majority of setae single (unbranched)
..... *Ae. (Stg.) unilineatus*
Ventral brush (seta 4-X) with majority of setae branched 5
- 5(4). Pecten spines with very fine inconspicuous lateral spicules, visible only under high magnification, spines appear simple under low magnification .. *Ae. (Stg.) simpsoni*
Pecten spines with at least one distinct spicule on ventral side, visible under low magnification, one to three coarse ventral spicules and occasionally one small spicule on dorsal side visible under high magnification 6
- 6(5). Thorax with large stellate setae; comb scales with small basal denticle, scales not appearing trifid under low magnification *Ae. (Stg.) metallicus*
Thorax without large stellate setae; comb scales appearing trifid under low magnification due to strong denticles at base *Ae. (Stg.) aegypti*
- 7(2). Siphon long, index 7–8 *Ae. (Adm.) ochraceus*
Siphon short, index less than to slightly more than 4 8
- 8(7). Ventral brush (seta 4-X) without precratal setae; comb scales in a single row 9
Ventral brush with precratal setae; comb often otherwise 10
- 9(8). Siphon index > 4 *Ae. (Diceromyia) taylori*
Siphon index ≤ 3.6 *Ae. (Dic.) furcifer*
- 10(8). Surface of head obviously spiculate; integument of thorax and abdomen either spiculate or covered with small sclerotic plaques 11
Surface of head not obviously spiculate; thorax and abdomen not spiculate or covered with plaques 12

- 11(10). Integument of thorax and abdomen covered with small sclerotic plaques
 *Ae. (Adm.) cumminsii*
 Integument of thorax and abdomen densely spiculate *Ae. (Adm.) fowleri*
- 12(10). Head setae 5,6-C single or double, equal in length or 5-C much longer than
 6-C. 13
 At least one of setae 5,6-C (usually both) with three or more branches or seta 6-C
 much longer than 5-C 17
- 13(12). Pecten spines more or less evenly spaced 14
 At least one distal pecten spine more widely spaced 15
- 14(13). Comb composed of ~10 large spine-like scales with fine lateral denticles on
 proximal 0.5 or less *Ae. (Ochlerotatus) caballus*
 Comb composed of 25–30 smaller spine-like scales with strong lateral denticles
 extending from base to sides of short mid-apical spine *Ae. (Och.) caspius*
- 15(12). Antenna very sparsely spiculate; pecten spines very close-set, most with three or
 more basal denticles, most distal spine more widely spaced
 *Ae. (Fredwardsius) vittatus*
 Antenna more strongly spiculate; pecten spines less closely set, usually with
 only one basal denticle (or none), usually two or more distal spines more
 widely spaced 16
- 16(15). Siphonal seta 1-5 with seven or eight branches; pecten spines with several basal
 denticles *Ae.*
(Adm.) vexans arabiensis and *Ae. (Adm.)* Forms X and Z (**Figures 1 and 2**) (see Note 1)
 Siphonal seta 1-5 with two to four branches; or pecten spines with or without
 only one basal denticle
 *Ae. (Adm.) dentatus* (in part) and *Ae. (Adm.)* Form Y (see Note 2)
- 17(12). Comb composed of sharply pointed spine-like scales, with or without lateral
 fringe of finer spicules 18
 Comb composed of squamiform scales, with fringe of apical and lateral
 spicules 21
- 18(17). Head seta 6-C single (rarely double), much longer and usually stouter seta 5-
 C 19
 Head seta 6-C with two or more branches, shorter to slightly longer than seta
 5-C 20
- 19(18). Head seta 5-C with five or six branches *Ae. (Adm.) hirsutus*
 Head seta 5-C with two or three branches *Ae. (Adm.) dentatus* (in part) and
 *Ae. (Adm.)* form Y (see Note 1)
- 20(18). Anal papillae very narrowly lanceolate; pecten spines with only one basal
 denticle *Ae. (Neomelanicion) circumluteolus* and *Ae. (Neo.) lineatopennis*
 Anal papillae more broadly lanceolate; some pecten spines with two or three
 basal denticles *Ae. (Adm.) quasiunivittatus*
- 21(17). Antenna longer than head *Ae. (Adm.) alboventralis*
 Antenna shorter than head 22
- 22(21). Seta 2-X (upper caudal seta) with about 15 branches, more or less as long as saddle
 *Ae. (Adm.) leesoni*
 Seta 2-X with five to seven branches, distinctly longer than saddle
 *Ae. (Adm.) dalzieli*

Note 1. Specimens of Forms X and Z key to *Ae. vexans arabiensis* but the comb scales and pecten spines differ in shape and the former differs in number. The description of Hopkins (1952) applies to most specimens; however, the distal pecten spines are differently shaped in some specimens and the third distal spine often has three denticles and sometimes only two. The comb consists of 10 scales arranged in a more or less double row. For comparison, features of Form X are shown in Figures 3 and 4, and those of typical *Ae. vexans arabiensis* are shown in Figures 5 and 6. Specimens of Form Z have a comb composed of 11 scales arranged in a slightly curved row and a pecten with about 21 spines; the distal three are close-set and denticulate with very fine denticles.

Note 2. Larvae of Form Y are of two types based on the development of the three distal pecten spines.

Type 1: Pecten comprised of 21 spines, with the middle of the three distal spines smooth, without denticles. The denticles of the other spines are very fine, visible under magnification of 300x. The pecten spines are in a straight row (Figure 7). The comb of these larvae consists of nine scales (Figure 8).

Type 2: Pecten comprised of 24 spines, the most distal spine is smooth (without denticles) and the other two have very fine denticles that are only visible under high magnification. The pecten spines are in an irregular row (Figure 9). The comb of these larvae also has nine scales (Figure 10).

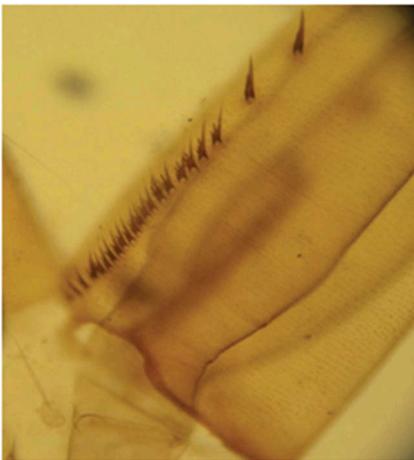


Figure 1. Pecten with two distal spines (Form X).



Figure 2. Pecten with three denticulate distal spines (Form Z).

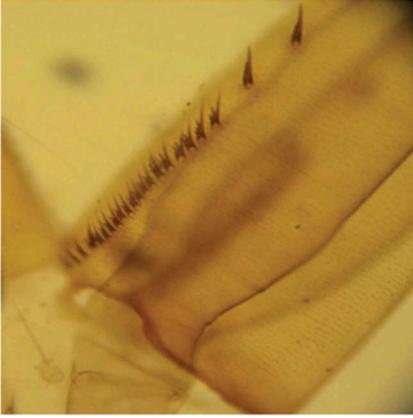


Figure 3. Pecten of Form X, consisting of about 20 spines varying in shape, a few at base often simple; central spines with one to four basal denticles of various sizes; two of the distal one to three spines are usually larger and more widely spaced, one or all are simple or all are with one to three basal denticles.

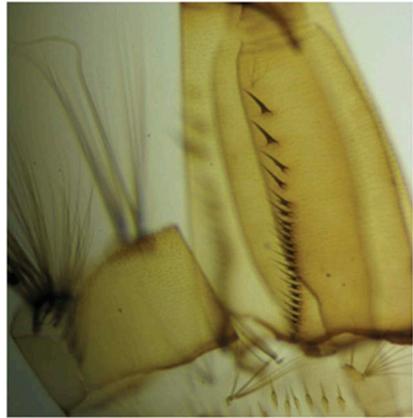


Figure 4. Pecten of *Aedes vexans arabiensis*, distal spines not so widely spaced.



Figure 5. Comb of Form X, scales in a more or less double row.

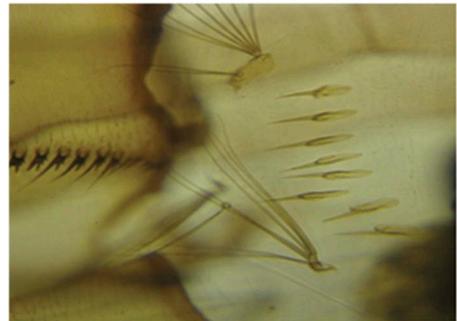


Figure 6. Comb of *Aedes vexans arabiensis*, scales in an essentially single row.

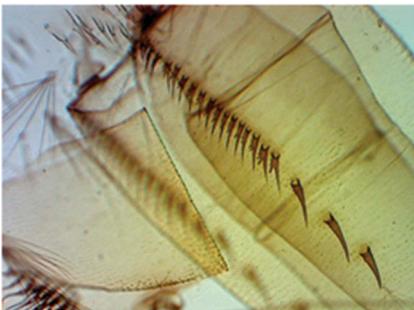


Figure 7. Siphon of Form Y Type 1 with three distal spines (the most distal with one very fine denticle, the third from end with two fine denticles and the middle spine is smooth (zoom in to see this).

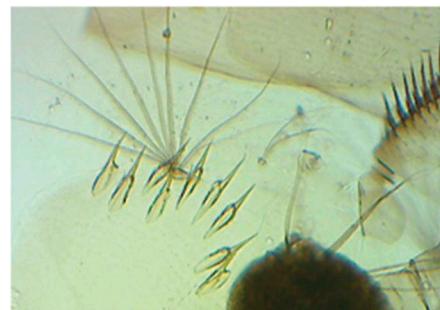


Figure 8. Comb of Form Y Type 1 with nine scales.



Figure 9. Siphon of Form Y Type 2 with 24 pectin spines, of the three distal spines the two more proximal ones have very fine denticles.

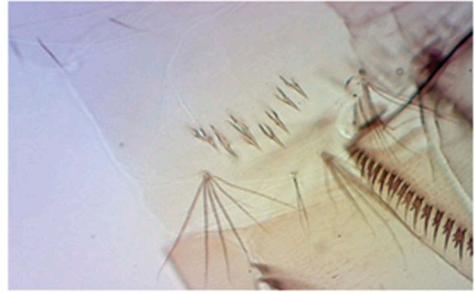


Figure 10. Comb of Form Y Type 2 with nine scales.

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References

- Abbott PH. 1948. The Culicidae (Diptera) of Darfur Province, Anglo-Egyptian Sudan, with observations on the geography and zoogeographical relationships of the region. *Proc R Entomol Soc London Ser B Taxon.* 17:37–48.
- Annual Health Statistical Report. 2011. National ministry of health. Khartoum: National Health Information Centre.
- Edwards FW. 1932. *Genera Insectorum. Diptera, Fam. Culicidae.* Fascicle 194. Brussels: Desmet-Verteneuil.
- Edwards FW. 1941. Mosquitoes of the Ethiopian Region III.—Culicine adults and pupae. London: British Museum (Natural History).
- Fall AG, Diaïté A, Etter E, Bouyer J, Ndiaye TD, Konaté L. 2011. The mosquito *Aedes (Aedimorphus) vexans arabiensis* as a probable vector bridging the West Nile virus between birds and horses in Barkedji (Ferlo, Senegal). *Med Vet Entomol.* 26:106–111.
- Harbach RE, Knight KL. 1980. *Taxonomists' glossary of mosquito anatomy.* Marlton (NJ): Plexus Publishing.
- Harbach RE, Knight KL. 1982. Corrections and additions to *Taxonomists' glossary of mosquito anatomy.* *Mosq Syst (For 1981).* 13:201–217.
- Hopkins GHE. 1952. Mosquitoes of the Ethiopian Region I.—larval bionomics of mosquitoes and taxonomy of culicine larvae. 2nd ed. London: British Museum (Natural History).

- Huang Y-M. 2001. A pictorial key for the identification of the subfamilies of Culicidae, genera of Culicinae, and subgenera of *Aedes* mosquitoes of the Afrotropical Region (Diptera: Culicidae). *Proc Entomol Soc Wash.* 103:1–53.
- Huang Y-M. 2004. The subgenus *Stegomyia* of *Aedes* in the Afrotropical Region with keys to the species (Diptera: Culicidae). *Zootaxa.* 700:1–120.
- Huang Y-M, Rueda LM. 2014. A pictorial key to the species of *Aedes* (*Ochlerotatus* and *Coetzeomyia*) in the Afrotropical Region (Diptera: Culicidae). *Zootaxa.* 3754:592–600.
- Huang Y-M, Rueda LM. 2015a. Pictorial keys to the species of the subgenera *Albuginosus* and *Aedimorphus* (Grjebinei and Apicoannulatus groups) of the genus *Aedes* Meigen in the Afrotropical Region (Diptera: Culicidae). *Zootaxa.* 3925:25–36.
- Huang Y-M, Rueda LM. 2015b. A pictorial key to the species of the *Aedes* (*Zavortinkius*) in the Afrotropical Region (Diptera: Culicidae). *Zootaxa.* 4027:593–599.
- Huang Y-M, Rueda LM. 2015c. *Pseudalbuginosus*, a new subgenus of *Aedes*, and a redescription of *Aedes* (*Pseudalbuginosus*) *grjebinei* Hamon, Taufflieb, and Maillot (Diptera: Culicidae). *Proc Entomol Soc Wash.* 117:381–388.
- Huang Y-M, Rueda LM. 2017. Pictorial keys to the sections, groups, and species of the *Aedes* (*Finlaya*) in the Afrotropical Region (Diptera: Culicidae). *Zootaxa.* 4221(1):131–141.
- Jupp P, Cornel A. 1988. Vector competence tests with Rift Valley fever virus and five South African species of mosquito. *J Am Mosq Control Assoc.* 4:4–8.
- Karabatsos N, editor. 1985. International catalogue of arboviruses, including certain other viruses of vertebrates. 3rd ed. San Antonio (TX): American Society of Tropical Medicine and Hygiene.
- Knight KL, Stone A. 1977. A catalog of the mosquitoes of the world (Diptera: Culicidae). 2nd ed., Vol. VI. The Thomas Say Foundation. College Park (MD): Entomological Society of America.
- Lewis DJ. 1953. The *Stegomyia* mosquitoes of the Anglo-Egyptian Sudan. *Ann Trop Med Parasitol.* 47:51–61.
- Lewis DJ. 1954. *Culex* mosquitoes of subgenera other than *Culex* in the Anglo-Egyptian Sudan. *Ann Mag Nat Hist.* 12:7–12.
- Lewis DJ. 1955. The *Aedes* mosquitoes of the Sudan. *Ann Trop Med Parasitol.* 49:164–173.
- Lewis DJ. 1956a. The *Culex* mosquitoes of the Sudan. *Bull Entomol Res.* 47:703–721.
- Lewis DJ. 1956b. Some mosquitoes of the Sudan. *Bull Entomol Res.* 47:723–735.
- Logan TM, Linthicum KJ, Thande PC, Wagateh JN, Roberts CR. 1991. Mosquito species collected from a marsh in western Kenya during the long rains. *J Am Mosq Control Assoc.* 7:395–399.
- McIntosh BM. 1975. A taxonomic revision of certain *Aedes* species (Diptera: Culicidae) of the subgenus *Aedimorphus* in southern Africa. *J Entomol Soc South Afr.* 38:251–287.
- Meegan JM, Bailey CJ. 1989. Rift Valley fever. *Arboviruses Epidemiol Ecol.* IV:51–76.
- Metselaar D, van Someren ECC, Ouma JH, Koskei HK, Gemert W. 1973. Some observations on *Aedes* (*Aedimorphus*) *dentatus* (Theo.) (Dipt., Culicidae) in Kenya. *Bull Entomol Res.* 62:597–603.
- Milankov V, Petrić D, Vujić A, Vapa L. 2009. Taxonomy, biology, genetic variability and medical importance of *Ochlerotatus caspius* (Pallas, 1771) and *O. dorsalis* (Meigen, 1830) (Diptera: Culicidae). *Acta Entomol Servica.* 14:195–207.
- Reinert JF, Harbach RE, Kitching IJ. 2009. Phylogeny and classification of tribe Aedini (Diptera: Culicidae). *Zool J Linn Soc.* 157:700–794 + 2 online appendices.
- Tomori O, Fabiyi A. 1977. Orungo virus: a new agent from mosquitoes and man in Uganda and Nigeria. *Niger Med J.* 7:5–8.
- Turell M, Presley S, Gad A, Cope S, Dohm D, Morrill J, Arthur R. 1996. Vector competence of Egyptian mosquitoes for Rift Valley fever virus. *Am J Trop Med Hyg.* 54:136–139.
- Wilkerson RC, Linton Y-M, Fonseca DM, Schultz TR, Price DC, Strickman DA. 2015. Making mosquito taxonomy useful: A stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. *PLoS One.* 10:e0133602.
- World Health Organization. 1995. Vector control for malaria and other mosquito-borne diseases. WHO Tech Rep Ser. 857:1–91.
- World Health Organization. 2008. World malaria report 2008. Geneva: World Health Organization.