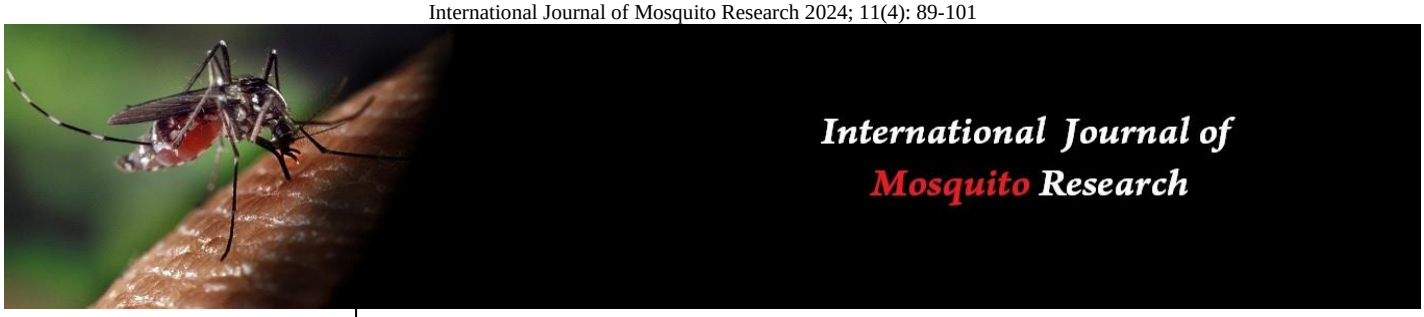

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Checklist of mosquitoes (Diptera: culcidae) in Northern Sudan, Sudan

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Abstract
Mosquitoes are considered among the most competent insect vectors to transmit pathogens to humans. Knowledge of the species of mosquitoes is crucial in designing and choosing the appropriate method to control mosquito species elsewhere. Mosquito species were surveyed and documented mosquito in northern Sudan an affected region by mosquito-borne diseases. Mosquitoes were collected in larvae and adult stages in different locations in the study area. A total of 4,803 mosquitoes including 9 species in 5 genera were recorded. Most of the mosquito collection represented *Culex* spp. (67%). The mosquito species were *Anopheles arabiensis* (n=1,473), *An. pharoensis* Theobald (n=9), *Culex quinquefasciatus* Say (n=1512), *Cx. univittatus* Theobald (n=1477), *Cx. poicilipes* Theobald (n=252), *Cx. bitaeniorhynchus* Giles (n=1), *Aedes vexans* Meigen (n=38), *Lutzia tigripes* de Grandpre & de Charmoy (n=17), and *Ochlerotatus caspius* Pallas (n = 24) This study provides a checklist of mosquitoes which is very important to design suitable control measures in northern Sudan and other regions with similar environmental settings.

Keywords: Checklist, mosquito, *Anopheles*, *Culex*, *Aedes*, Northern Sudan

1. Introduction
Despite the huge effort of control, mosquito-borne diseases (MBDs) have become a growing serious public health problem with a high global burden. This situation is largely due to the spread of mosquito vectors and pathogens into new areas as an effect of global climate change [1]. As a result, several outbreaks of MBDs such as Dengue fever (DF), yellow fever (YF), and Rift Valley fever (RVF) occurred in some regions of the world as in Sudan between the years 2005 and 2015 [2-6]. For example, an epidemic of RVF that occurred during the year 2019 in the Eldamar area, in northern Sudan has resulted in 1,129 cases and 19 deaths [6]. Furthermore, some of these MBDs such as malaria and dengue fever are endemic in different parts of Sudan [4, 5, 7, 8].

Lewis during the 1950s provided distribution maps and identification keys for Anoheline, *Aedes*, and *Culex* mosquitoes in Sudan [9-11]. These studies revealed that mosquitoes of Sudan comprise 156 species, two subspecies of mosquitoes, and seven varieties of *Culicidae* [9-11]. Then after several published reports presented comprehensive studies on the mosquito species composition in Sudan which mostly from the Afrotropical [12-15].
Some of these mosquito species were recorded in northern Sudan including *An. arabiensis* Patton, *An. pharoensis* Theobald, *An. rufipes* Gough, *Cx. pipiens* Linnaeus, *Cx. univittatus* Theobald, *Cx. bitaeniorhynchus* Giles, and *Ae. vittatus* Bigot [10, 14, 16-18].
Among the mosquito species in Nothern Sudan, only two species represent vectors of MBDs in different regions of Sudan. *Anopheles arabiensis* is the major vector responsible for the transmission of malaria in most regions of Sudan [19, 20]. *Anopheles araiensis*, and *Cx. pipien* were suspected to have a role in the transmission of RFV in Sudan [3]. These two species were found infected with RFV during an outbreak of the disease in Sudan [3]. Moreover, *Cx. univittatus* was found infected with West Nile Virus (WNV) in Sudan [21]. However, other species have been reported as vectors of malaria (i.e. *An. pharoensis*, and *An. rufipes*) [22, 23], RVF (i.e. *Cx. bitaeniorhynchus*) [24], YF as well as DF (i.e. *Ae. vittatus*) [25].
Northern Sudan is located within semi-desert and arid desert biomes, therefore, it has less

diverse species and habitats for mosquitoes than other parts of Sudan. However, this study added more species to the mosquito fauna in northern Sudan. Such studies will help to control mosquito vectors, hence controlling MBDs in the region.

2. Materials and Methods

2.1. Study Area

Longitudinal entomological surveys were done during 2015 - 2016 in the River Nile State, Northern Sudan. Generally, the study was carried out during three different seasons; dry-cold season (Nov. – Feb.), hot-dry season (March – June), and wet season (July – October). Collection, preservation, and identification of mosquitoes (adult and larval stages) were done according to standard procedures [26].

2.2. Study sites

Nine sentinel sites were selected to conduct the entomological surveillance in this study; four constant sites (Albawga, Alzidab, Soola, and Gandato) and five cross-check sites (Almikharif, New Manaseer, Abusleem, Alsyalla, and Alsheriq)

2.3. Mosquitoes Identification

Field-collected wild and adult-reared mosquitoes from different sentinel sites were identified based on morphological features as described by [27-29,47]. Briefly, adult mosquitoes were pinned on card points and examined under a dissecting microscope.

2.4. Entomological Sampling Methods

Adults were collected using Pyrethrum Spray Catches (PSCs) from indoors and aspirators (sucking tubes) from outdoors, and larvae by using the standard dipping method. The collected adults were kept dry in labeled eppendorff tubes. Larvae were reared to adults in the insectary. All procedures were as described by WHO [26].

2.5. Data analysis

The data obtained from different parts of this study were analyzed using the computer software SPSS ver. 22. Data from all parts of this study were analyzed using descriptive analysis.

3. Results and Discussion

3.1. Species Composition

A total of 4,803 mosquitoes comprising nine species belonging to five genera were recorded in this study. The mosquito species in the genus *Culex* were the most dominant (67.5%) and widely distributed in the study area. The highest proportion of mosquito catches was *Culex* (67.5%), followed by *Anopheles* species (30,9%) (Figure 1). The mosquito species included *Anopheles arabiensis* (n=1,473), *An. pharoensis* Theobald (n=9), *Culex quinquefasciatus* Say (n=1512), *Cx. univittatus* Theobald (n=1477), *Cx. poicilipes* Theobald (n=252), *Cx. bitaeniorhynchus* Giles (n=1), *Aedes vexans* Meigen (n=38), *Lutzia tigripes* de Grandpre & de Charmoy (n=17), and *Ochlerotatus caspius* Pallas (n = 24).

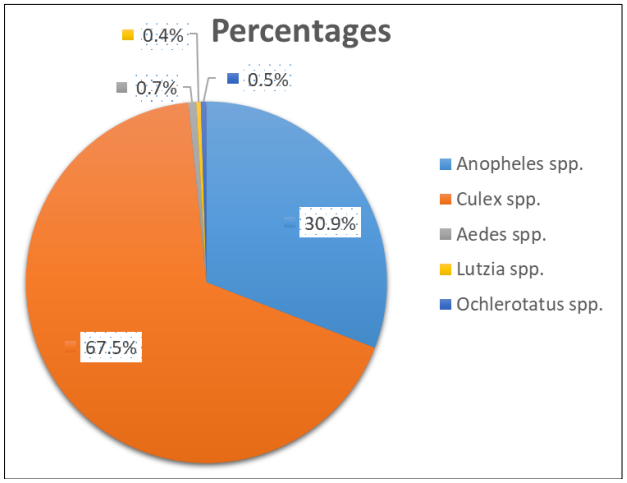

Fig 1: Percentages of mosquito genera in Northern Sudan region, Sudan.

3.2. *Anopheles* spp.

3.2.1. *Anopheles (Cellia) arabiensis* Patton, 1905

Anopheles arabiensis is a sibling species of *An. gambiae* complex [30]. It is one of the most common and major malaria vectors throughout sub-Saharan Africa [31,32]. Similarly, *An. arabiensis* is the most abundant, with a wide range of distribution in Sudan including the northern, central, and eastern parts of the country [14, 16, 17, 19, 33]. It is the potential malaria vector in Sudan [19, 34]. The presence of *An. arabiensis* in all surveyed sites in this study indicates a serious health threat in this area because it is the only malaria vector in Sudan [19, 34].

The important morphological characteristics according to

1. **Head:** palps with 3 pale bands, and hind tarsus 4 and 5 not entirely pale (Fig. 2).
2. **Legs:** speckled legs (Fig. 3).
3. **Wings:** The dark and light bands on the wings with pale bands (Fig. 3).

However, the species can be identified and confirmed based on molecular methods [35].

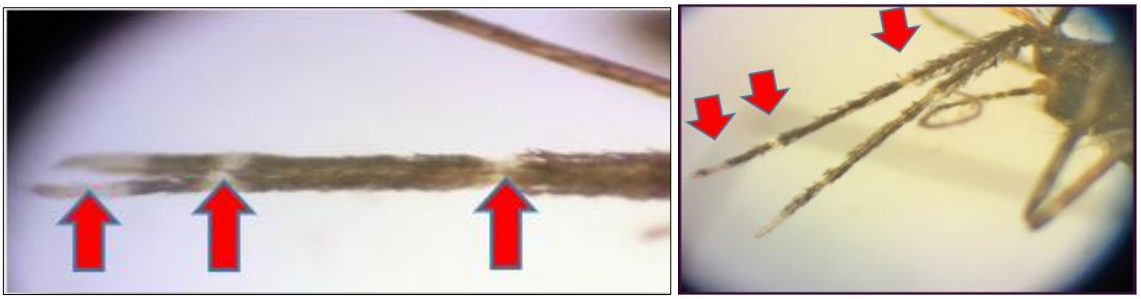

Fig 2: Palps with 3 pale bands

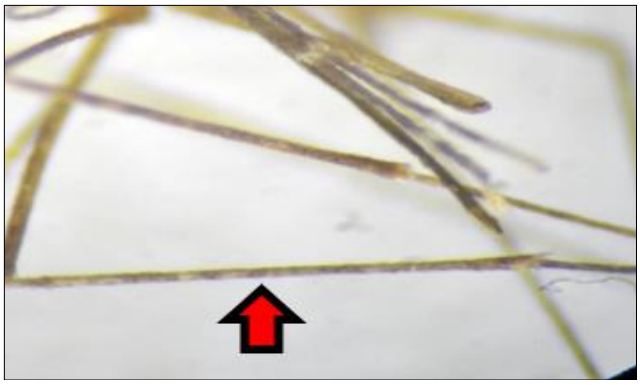

Fig 3: Legs speckled, sometimes sparsel.

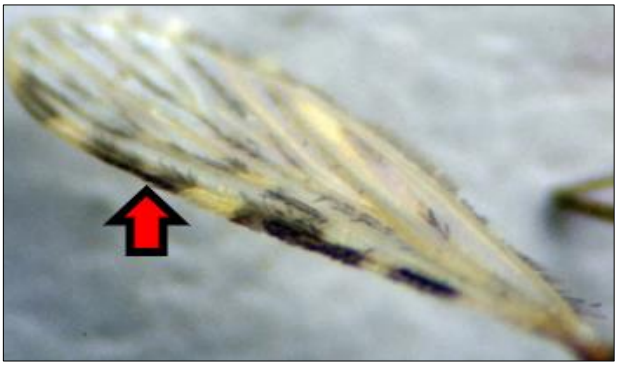

Fig 4: The 3rd main dark area of vein with a pale interruption.

3.2.2. *Anopheles* (*Cellia*) *pharoensis* Theobald, 1901

Anopheles (Cellia) pharoensis Theobald, 1901 is widely distributed in Ethiopia, Somalia, and the Sudan and also extends into Egypt [36]. It plays as a potential vector in Egypt, and found to be more *exophilic* than *endophilic*, more *exophagic* than *endophagic*, and zoophagic rather than anthropophagic [37-39] and at best it is a feeble vector of malaria in tropical Africa [40]. In the Sudan, *An. pharoensis* co-exists with *An. arabiensis* particularly in irrigated and swampy areas [41]. In the northern sudan, *An. Pharoensis* breeds in swamp and pool with vegtations, and prefer resting by day in vegtation rather in houses [42]. Moreover, *An. pharoensis* is considered a nuisance pest, especially in the irrigated area of the Gezira region where it causes a great deal of discomfort by biting humans [43]. However, it has not been incriminated as a malaria vector in the Sudan [44]. This species was found to spread in Northern, Gezira, Blue Nile, and Gedarif States [12, 44, 45, 46]. In this study, however, this species was recorded in very low densities and was recorded in only three of the surveyed sites (Alzidab, Soola, and Gandato).

The important taxonomic features of this species are

1. **Head:** Palps shaggy with 4 pale bands, some scattered pale scales which may give the palps a speckled appearance (Fig. 5).
2. **Abdomen:** Abdominal segments with laterally projecting tufts of scales (Fig. 6).
3. **Legs:** Hind tarsus 1-4, at least, with apical pale bands (Fig. 7).
4. **Wings:** with abundant pale areas, costa with at least 4 pale spots (Fig. 8).

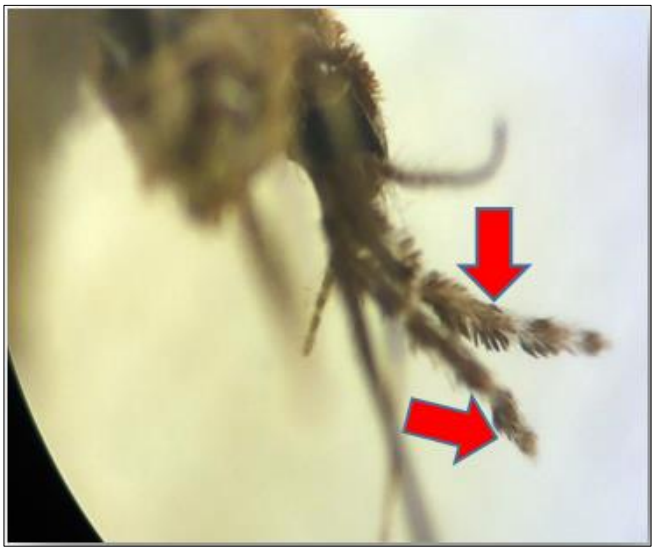

Fig 5: Palps shaggy with 4 pale bands

Fig 6: Laterally projecting tufts of abdominal scales.

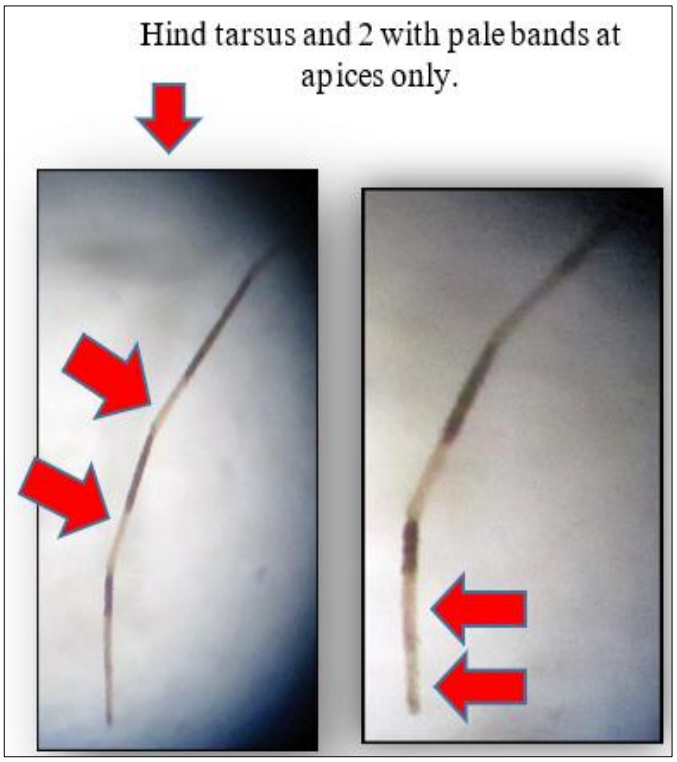

Fig 7: Hind tarsus 5 and about apical half of 4 pale

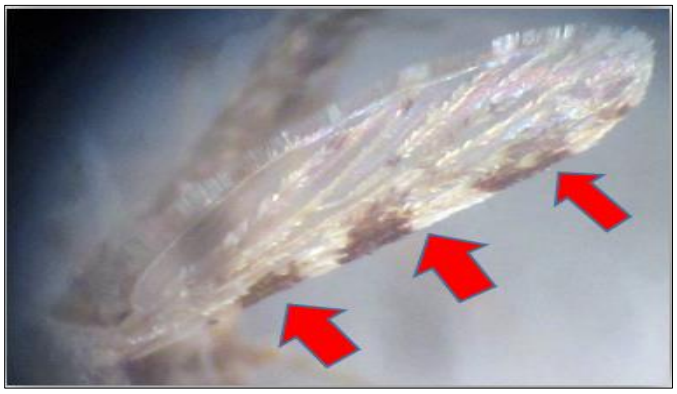

Fig 8: Wing with abundant pale areas, costa with at least 4 pale spots.

3.3. *Culex* spp.

3.3.1. *Culex* (*Culex*) *quinquefasciatus* Say, 1823

Culex quinquefasciatus Say, 1823 (originally named *Culex pipiens fatigans*) is a geographically widespread worldwide and it is responsible for the transmission of LF, avian malaria, and arboviruses including St. Louis encephalitis virus, Western equine encephalitis virus, Zika virus and WNV [48-52]. It is taxonomically considerd as a member of the *Cx. pipiens* species complex [53]. Mainly, *Cx. quinquefasciatus* occurs in tropical and sub-tropical areas and usually, it is distributed within the latitudes 36° N and 36° S. [11, 54, 55].) in the Sudan. *Cx. quinquefasciatus* is known to be a domestic annoying mosquito and it has been found to transmit lymphatic filariasis in the Blue Nile area and former southern Sudan [56].

The important Taxonomic characteristics of this species**:**

1. Head: No post-spiracular scales or bristles (Fig. 9).
2. Abdomen: Abdominal basal bands in the shape of a half-moon (Fig. 10); sterna Mostly white (Fig. 11).
3. Legs: Dark, un-banded legs (Fig. 12).

In this study, *Cx. quinquefasciatus* was recorded in all surveyed sentinel sites.

Fig 9: No post-spiracular scales or bristles.

Fig 10: Thick, half-moon-shaped, basal bands on abdominal terga.

Fig 11: Mostly white underside of abdomen (sterna).

Fig 12: Dark, un-banded. legs

3.3.2. *Culex* (*Culex*) *uninitiates* Theobald, 1901

Culex (*Culex*) *univittatus* Theobald, 1901 is a competent vector of arboviruses with public health importance, such as WNV [57-59]. In Africa, *Cx. univittatus* makes up the largest fraction of WNV-infected mosquitoes [60]. *Culex Univittatus* is the commonest and the most widely distributed Culicine in the Sudan [10, 12, 46, 61], but is scarcely ever found biting man. It occurs in almost every part of the Sudan except the desert, but in the extreme south-west and near the coast it is less common than elsewhere [11]. This species is present in 6 sites (Albawga, Alzidab, Soola, Gandato, Almikharif, and Alsyalla).

The important morphological characteristics of this species

1. **Head:** Proboscis entirely dark-scaled (Fig. 13),
2. **Thorax:** Postspiracular (Fig. 14), and prealar scales are present (Fig. 15).
3. **Legs:** presence of a pale stripe on the anterior surface of the hind tibia (Fig. 16).

Fig 13: Proboscis entirely dark-scaled.

Fig 14: Post-spiracular scales present

Fig 15: Pre-alar scales.

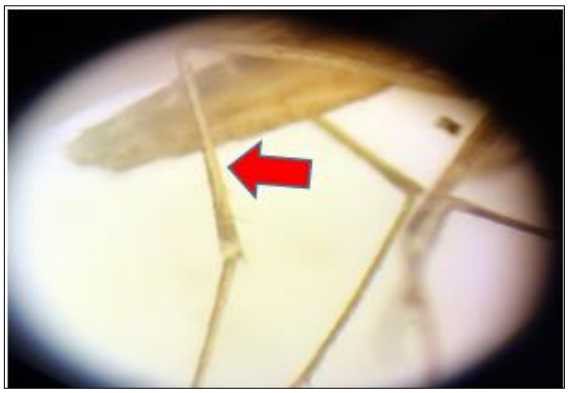

Fig 16: Presence of a pale stripe on the anterior surface of the hind tibia.

3.3.3. *Culex* (*Oculeomyia*) *poicilipes* Theobald, 1903

Culex (*Oculeomyia*) *poicilipes* Theobald, 1903 is widely distributed worldwide and is considered a potential vector of many serious human and animal diseases, such as RVF infection [62-66]. This species was recorded in all the surveyed constant sites in the present study (i.e. Albawga, Alzidab, Soola, and Gandato) as well as in two of the cross-check sentinel sites; viz. New Manaseer and Alsyalla. In a previous study in the Sudan, adults and larvae of *Cx. poicilipes* collected from Khartoum and White Nile States were found infected with the Rift Valley virus [3]. This species was also collected from different areas in the Sudan including Khartoum, White Nile, Gedarif, and Blue Nile States [3, 12, 46].

The important morphological characteristics of this species

1. **Head:** Proboscis with a median pale band (Fig. 17).
2. **Thorax:** Mesepimeral setae absent (Fig. 18).
3. **Legs:** Rows of pale spots adorn its femora and tibiae (Fig. 19), tarsi with narrow pale rings at the joints (Fig. 20).
4. **Dark:** Scaled wing (Fig. 21).

In this study recorded in 6 sites (Albawga, Alzidab, Soola, Gandato, New Manaseer, and Alsyalla).

Fig 17: Proboscis with a well-defined pale band in middle.

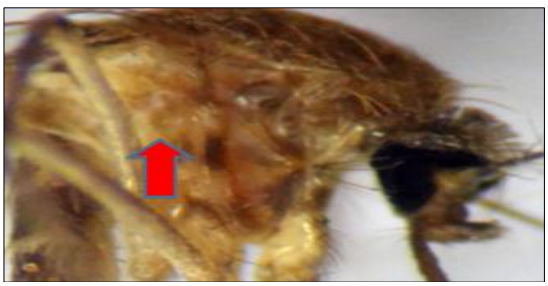

Fig 18: No lower mesepimeral bristle.

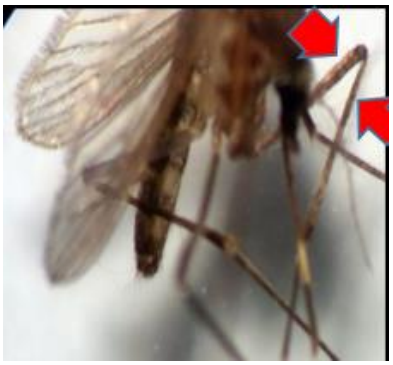

Fig 19: Femora and tibiae with rows of small pale spots

Fig 20: Tarsi with narrow pale rings at the joints

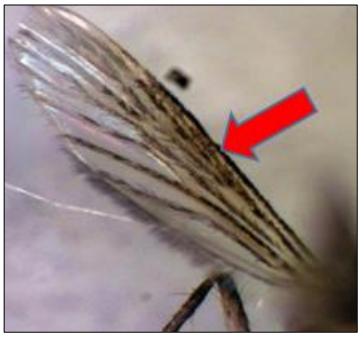

Fig 21: All Wings with scales dark, those on fork very narrow and long

3.3.4. *Culex* (*Oculeomyia*) *bitaenorhynchus* Giles, 1901

Culex (*Oculeomyia*) *bitaeniorhynchus* Giles, 1901 (formerly *Cx. ethiopicus*) is a cosmopolitan species that is an extremely common and widespread mosquito species [67-71]. *Cx. bitaeniorhynchus* is considered a vector of *Wuchereria bancrofti* in New Guinea [68]. and Japanese Encephalitis Virus (JEV) in India and Thailand [69, 71, 72]. However, this species was not found to have a role in the transmission of the disease in the Sudan. In this study, *Cx. bitaeniorhynchus* was collected as a single specimen from Alzidab site, this is consistent with the study done by Lewis [11] in the same study area and came up with same result.

The important morphological characteristics of this species

1. **Head:** Proboscis with median pale band and two dorsolateral pale spots at labellum (Fig. 22).
2. **Thorax:** Acrostichal setae present; lower mesepimeral setae absent.
3. **Abdomen:** with distinct apical bands of pale-yellow scales (Fig. 23).
4. **Wing:** with intermixed pale and dark scales, most of the scales rather broad (Fig. 24)

Fig 22: Proboscis with a pale spot at tip above (before labella).

Fig 23: Abdominal tergites with pale apical bands of even width.

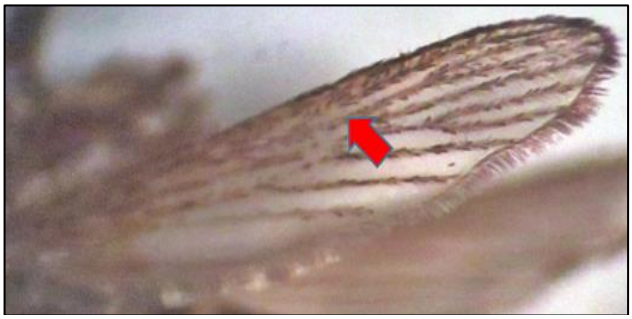

Fig 24: Wing with intermixed pale and dark scales, most of the scales rather broad.

3.4. *Aedes spp.*

3.4.1. *Aedes* (*Aedimorphus*) *vexans* Meigen, 1930.

Aedes (*Aedimorphus*) *vexans* Meigen, 1930, the inland floodwater mosquito, is a global and common pest mosquito. *Aedes vexans* has a worldwide distribution, and it is known to aggressively bite humans and is a competent vector of several arboviruses [73]. These mosquitoes are capable of transmitting WNV, St. Louis encephalitis virus, Western and Eastern equine encephalitis viruses, and RVF virus [74-76]. However, it has been suggested that *Ae. vexans* are probably vectors of *zoonotic* WNV that occur between horses and bird hosts [77]. *Aedes vexans* have also been found a competent vector of ZIKV; however, with low transmission rates for the virus [76]. Moreover, *Ae. vexans* was found infected with RVF viruses in Khartoum State, Sudan [78]. In this study, *Ae. vexans* was recorded in 3 sites (Albawga, Alzidab, and Soola,).

The important morphological characteristics of this species

1. **Head:** Proboscis is brown with numerous white scales ventrally (Fig. 25), vertex with median narrow scales. (Fig. 26).
2. **Thorax:** Scutal scales are uniformly brown, and scutellum with pale, narrow scales (Fig. 27); Paratergite and pleurae -including post-spiracular area- with broad flat whitish scales (Fig. 28).
3. **Abdomen:** with broad pale bands (Fig. 29).
4. **Legs:** The femora is pale beneath with a rather heavy sprinkling of pale scales on the dark parts (Fig. 30).
5. **Legs:** All segments of the hind tarsi with narrow white basal rings, and the last two hind tarsals are not all dark (Fig. 31).
6. **Wing:** A few pale scales at the base of Costa and first vein, no pale fringe scales along the posterior margin; wing scales are sparse, dark, and pale (Fig. 32).

Fig 25: Proboscis brown with numerous white scales ventrally.

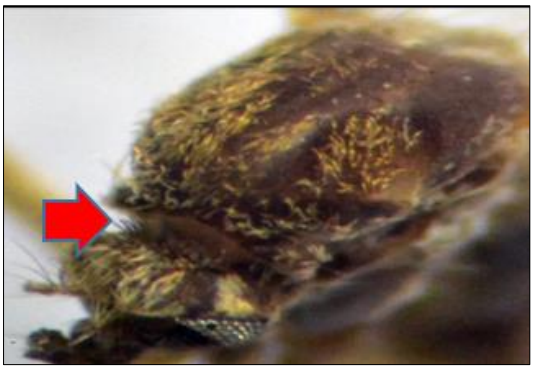

Fig 26: Decumbent scales of vertex

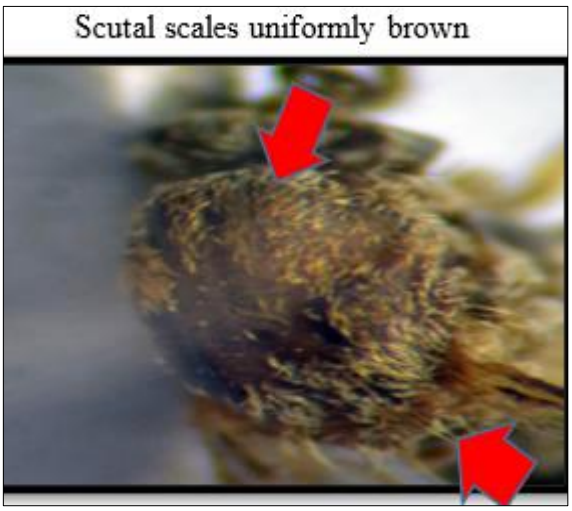

Fig 27: Scutal scales brown, and Scutellum with narrow scales only.

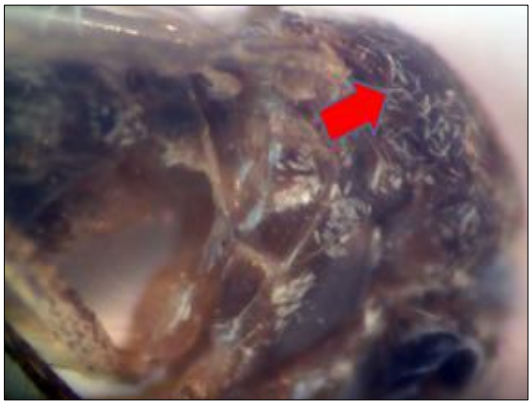

Fig 28: Paratergite and pleurae with broad flat whitish. scales

Fig 29: Abdomen with broad pale bands.

Fig 30: Femora pale beneath and with a rather heavy sprinkling of pale scales on the dark parts.

All segments of hind tarsi with narrow white basal rings

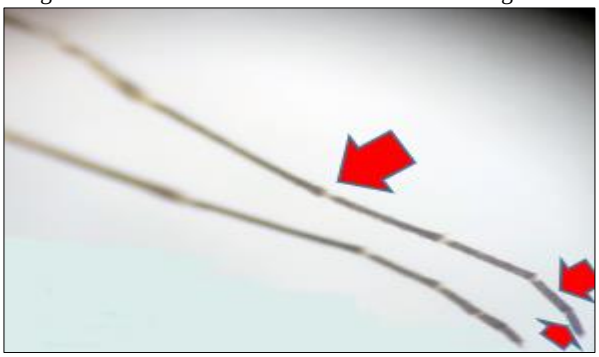

Fig 31: Last two hind tarsals not all dark.

Fig 32: Dark scaled, at most a few pale scales at base of Costa and first vein.

3.5. *Lutzia* spp.

3.5.1. *Lutzia (Meta lutzia) tigripes* de Grandpre & de Charmoy, 1901 (formerly *Cx. tigripes*)

This species was recorded in Africa and Asia [79-82]. Although it is widespread in the southern and central regions of Sudan [83], this was the first record of this mosquito species in the study area. *Lutzia tigrepis* has not been reported to transmit any disease to humans. However, larvae of *L. tigripes* act as predators that eat other mosquito species and immature stages

of aquatic insects, and more specifically, they prefer larvae of *Ae. aegypti* may [83, 84]. A similar observation was recorded in this study, where one larva of *L. tigripes* ate several larvae of mosquitoes during mosquito rearing and maintenance in the laboratory (Fig. 37).

This is the first record of this mosquito species in Northern Sudan. During this study, *L. tigrepis* was recorded in 2 sites (Soola and Almikharif).

The important morphological characteristics of this species

1. **Thorax:** No post-spiracular scales (Fig. 33); A large patch of white scales on the upper part of mesepimeron (Fig 34).
2. **Abdomen:** Apical abdominal bands (Fig. 35).
3. **Legs:** Front and middle femora and tibiae as seen from in front each with a row of about ten small pale spots on a dark ground (Fig. 36).

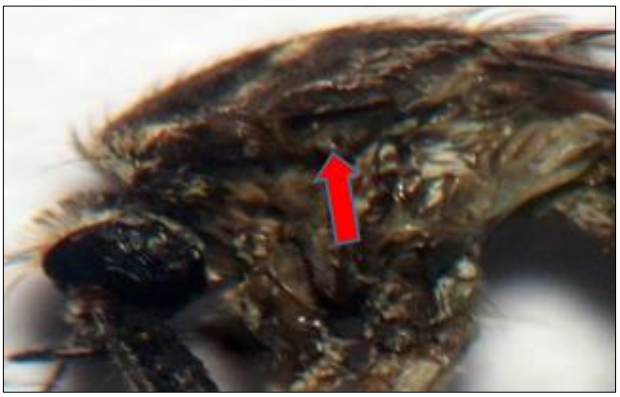

Fig 33: No post-spiracular. scales

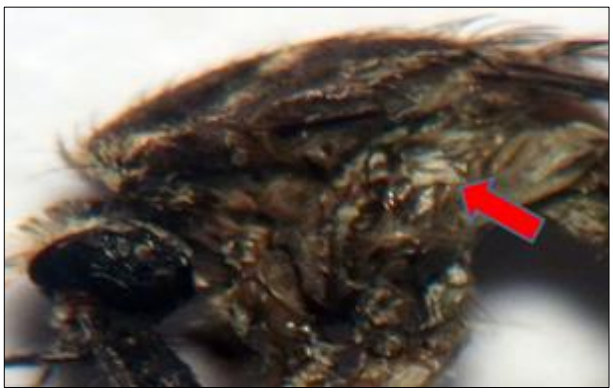

Fig 34: large patch of white scales on upper part of mesepimeron.

Fig 35: Apical abdominal bands.

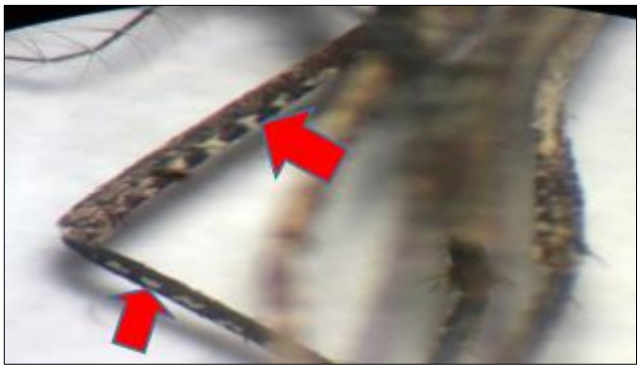

Fig 36: Front and middle femora and tibiae with a row of pale spots on a dark ground.

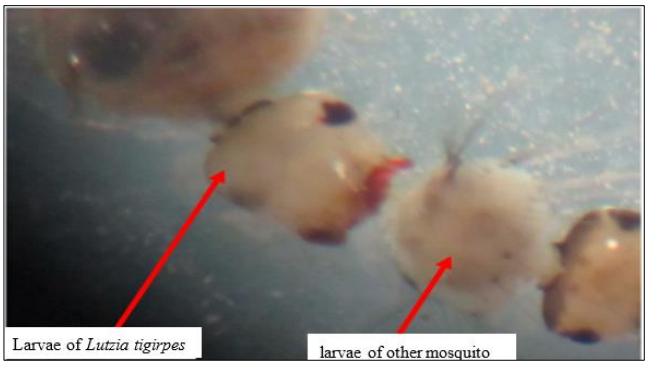

Fig 37: Larvae of *Lutzia tigirpes* feed on larvae of other mosquito

3.6. *Ochlerotatus* spp

3.6.1. *Ochlerotatus caspius* Pallas, 1771

Ochlerotatus (*Ochlerotatus*) *caspius* Pallas, 1771 (formerly *Ae. caspius*) is widely distributed worldwide and it mainly occurs in coastal areas [85-89]. It is considered as a significant nuisance biting mosquito species [90,91]. *Ochlerotatus caspius* is a vector of WNV in several countries in Europe [92-95]. In addition, *O. caspius* can also transmit microfilariae (*Dirofilaria immitis*) [96]. This species has been recorded in the Sudan, however, its role in the transmission of human disease has been discussed [83,97]. In this study, *O. caspius* was recorded in three sites **(**Albawga, Soola, and Abusleem).

The important morphological characteristics of this species

1. **Head:** Palpi is largely pale-scaled (Fig. 38); proboscis is extensively pale beneath (Fig. 39).
2. **Thorax:** Scutal scales are mostly fawn-colored, normally with two white lines running the whole length (Fig. 40); apn scales mostly broad and flat, white; ppn with narrow pale scales mostly broad and flat, white, and several lower mesepimeral bristles (Fig. 41); the dorsal surface is largely creamy (Fig. 42).
3. **Abdomen:** Sternites mainly pale the dorsal, and the dorsal surface is largely creamy (Fig. 43).
4. **Legs:** With heavily sprinkled with pale scales and creamy rings extend about equally and rather broadly (Fig. 45).
5. **Wings:** with a very heavy sprinkling of pale scales on all veins including the costa (Fig. 44).

Fig 38: Palpi largely pale scaled.

Fig 39: Proboscis extensively pale beneath.

Fig 40: Scutal scales coloured, with two white lines.

Fig 41: ppn with narrow pale scales mostly broad and flat, white.

Fig 42: Dorsal surface largely creamy.

Fig 43: Sternites mainly pale.

Fig 44: Very heavy sprinkling of pale scales

Fig 45: Heavily sprinkled with pale scales creamy rings.

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