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ORIGINAL RESEARCH ARTICLE

First record of one Genus and four Species of mosquitoes (Diptera: Culicidae) from Pakistan

Shafia Saba[1] · Unsar Naeem-Ullah[1] · Shafqat Saeed[1] · Ishtiaq A. Rajwana[2] · Alamgir Akhtar Khan[3]

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Abstract

Mosquitoes have been ranked among the deadliest entities of world being vectors of serious viral and protozoan/ parasitic illnesses among humans and animals. Pakistan is having diverse environmental combinations, and being frontline victim of global climatic changes, has rich biodiversity including the mosquitoes that has continuously been changed. Current study was planned to investigate mosquito diversity of Dera Ghazi Khan District of Pakistan. Mosquito larvae were collected from open water bodies, ponds, and seepage water with the help of standard 250 ml dipper. Then in the lab, the specimens were boiled in hot water for few seconds to fix protein / enzyme activity and preserved in 75% ethyl alcohol in small glass vials. The preserved samples were identified to species level with the help of available taxonomic keys (Rattanarithikul 1982; Maslov et al. 1989; Rattanarithikul et al. 2005, 2006; Darsie and Pradhan 1990). Based on the microscopic examination of morphological characteristics, the current study reported one Genus i.e., "*Udaya* (Thurman, 1954)" and four species namely *Anopheles indefinitus* (Ludlow, 1904), *Culiseta bergrothi* (Edwards, 1921), *Culex alis* Theobald, 1903, and *Udaya argyrurus* (Edwards, 1934*)* for the first time from Pakistan. The said species are potential vectors of many serious viral and protozoan diseases and can be a cause of concern in future.

Keywords Anophelinae · Culicinae · *Anopheles indefinitus* · *Culiseta bergrothi* · *Culex alis* · *Udaya argyrurus* · Pakistan

Introduction

Global variations such as climate change, urbanization, land utilization patterns, and populace movements can drastically impact the mosquito faunal diversity and associated pathogens like protozoans and arboviruses (Steiger et al. 2016) etc. The environmental and ecological changes are reported to have a direct connection with diversity and abundance of mosquito species (Radhakrishnan 2019).

Pakistan possessing a prodigious range of seasons, landscape, climate and ecosystems owns rich biological diversity (Baig and Al-Subaiee 2009) including insects. Besides, Pakistan is also among the top most countries of the World that are being affected by current scenario of climate change (Chaudhry 2017). All these factors contribute / alter faunal diversity of insects especially the mosquitoes.

A comprehensive information on the mosquito fauna of the country is very limited. Following the partition of Pakistan and India in 1947, Aslamkhan (1971, 1972) published mosquito species checklists that include species found in East Pakistan (Now Bangladesh) and West Pakistan (Now

✉ Shafia Saba
sabashafia@yahoo.com

Unsar Naeem-Ullah
naeem1130@yahoo.com

Shafqat Saeed
shafqat.saeed@mnsuam.edu.pk

Ishtiaq A. Rajwana
ishtiaq.rajwana@mnsuam.edu.pk

Alamgir Akhtar Khan
alamgir.khan@mnsuam.edu.pk

1 Institute of Plant Protection, Faculty of Agriculture & Environmental Sciences, MNS University of Agriculture, Multan, Pakistan

2 Department of Horticulture, Faculty of Agriculture & Environmental Sciences, MNS University of Agriculture, Multan, Pakistan

3 Department of Agriculture Engineering, Faculty of Agriculture & Environmental Sciences, MNS University of Agriculture, Multan, Pakistan

Pakistan). These checklists mentioned 91 species as mosquito fauna of today's Pakistan. After that report, due to various anthropogenic and non-anthropogenic factors, many other species have been established in Pakistan out of which some have been reported time to time by various authors (Reisen and Milby 1986; Herrel et al. 2001; Akram et al. 2009; Naeem-Ullah et al. 2010; Shaikh et al. 2014; Gaffigan et al. 2015; Khan 2022).

Research work is a regular phenomenon, consequently discoveries of species either new to science or new to area / region are common. Keeping in view the above said facts, current study was planned to assess the mosquito diversity of the region. While working with mosquito diversity, along with various previously reported species, one genus (*Udaya* Thurman, 1954) and four species viz. *Anopheles indefinitus* (Ludlow, 1904), *Culex alis* Theobald, 1903, *Culiseta bergrothi* (Edwards, 1921), *Udaya argyrurus* (Edwards, 1934) have been reported for the first time in Pakistan, which are potentially linked with dissemination of some human diseases. So, the objective of this work is to give first hand detailed information about these new records to policy makers and researchers for future endeavors like vector incrimination, artificial infection and follow up of the development of parasites etc.

Materials and methods

Study area

Dera Ghazi Khan (D.G. Khan) district is located on the bank of Indus River in Punjab province of Pakistan at 30'03" N and 70'38" E. It is ranked as 19th largest city of Pakistan by population. The district is spread over an area of 11,294 square kilometers comprising of four tehsils (Anonymous, 2022). Weather is characterized by long, hot, clear summers and little humidity with short, mild and mainly clear winters, and it is dry the whole year. Temperature usually oscillates between 47 and 108 °F and seldom fluctuates below 41 °F or above 114 °F. The average annual precipitation is 140 mm, with the majority of it falling during summer (Weatherspark 2021). The study area consists of piedmont plains and Sulaiman hills. It runs between base of Sulaiman hills and Indus River. The soils are profound, medium-textured, calcareous, and well drained with low quantities of organic matter. Water reservoirs of the area are considered as semi-confined type which shows the fresh water zone in study area. The semi-confined aquifer consists of sand, silt and clay possessing high to moderate level of Electrical Conductivity (EC). Main freshwater aquifers are located on eastern and northern parts of study area. The western and eastern parts have fresh waters along with salt water (Shafiq et al. 2021). The map created from geographical coordinates of collection points is given in Fig. 1.

Collection of mosquito larvae

Larval collection was carried out from 2019 to 2021 in D.G. Khan District as part of a study to check mosquito fauna of the region. The samples containing mosquito larvae were taken from open water collections, ponds, and seepage water with the help of standard 250 ml dipper into 500 ml plastic bottles and shifted to the lab for identification. Collection bottles were labeled with date, sample number and address of collection sites. Proforma containing information about the collection site, GPS coordinates of the site and other information was filled on the spot (Naeem-Ullah et al. 2010).

Preservation & identification

The collected samples were brought to the Insect Biodiversity lab, MNS University of Agriculture Multan, Pakistan. In lab, the live collected specimens were put in boiling water for few seconds to fix protein/enzyme activity and preserved in 75% ethyl alcohol (Gaffigan and Pecor 1997) in small glass vials for further identification. The preserved samples were then identified to species level with the help of available taxonomic keys (Rattanarithikul 1982; Maslov et al. 1989; Rattanarithikul et al. 2005, 2006; Darsie and Pradhan 1990) in the laboratory at 25 ± 2 ºC and 65 ± 5% R.H. Only fourth instar larvae were included in identification process. Validation of species description was done by the Walter Reed Biosystematics Unit (WRBU). After identification the samples were again preserved in respective glass vials containing 75% ethyl alcohol and shifted to Insect Biodiversity lab, MNS University of Agriculture Multan, Pakistan for future reference.

Results

During identification of mosquito larvae, following species were recorded.

1. *Anopheles culicifacies* Giles, 1901
2. *Anopheles indefinitus* (Ludlow, 1904) (New record for Pakistan)
3. *Anopheles nigerrimus* Giles, 1900
4. *Anopheles stephensi* Liston, 1901
5. *Anopheles subpictus* Grassi, 1899
6. *Culex alis* Theobald, 1903 (New record for Pakistan)
7. *Culex quinquefasciatus* Say, 1823
8. *Culex sitiens* Wiedemann, 1828

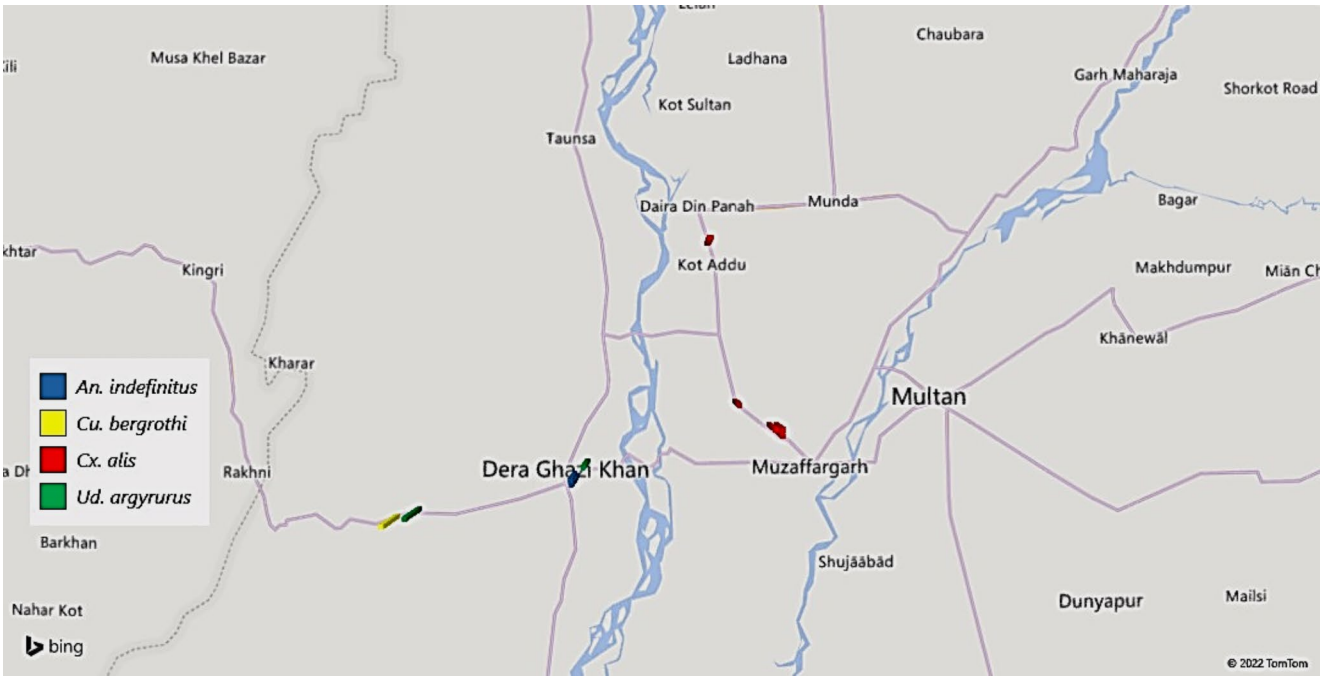

Fig. 1 Map of the study area created from geographical coordinates of collection points

9. *Culex tritaeniorhynchus* Giles, 1901
10. *Culiseta bergrothi* (Edwards, 1921) (New record for Pakistan)
11. *Udaya argyrurus* (Edwards, 1934) (New record for Pakistan)

After careful inspection based on identification of fourth instar larvae and literature review, it is declared that four mosquito species belonging to *Anopheles, Culex, Culiseta* and *Udaya* genera have been recorded for the first time from Pakistan. It is also worth mentioning that "*Udaya"* genus has not been previously reported in the country.

Detailed information and main diagnostic characteristics of all four first records are provided below along with relevant information of the species.

Anopheles indefinitus (Ludlow, 1904)

Family: Culicidae Meigen, 1818
Subfamily: Anophelinae Grassi, 1900
Genus: *Anopheles* Meigen, 1818
Subgenus: *Cellia* Theobald, 1902
Species: ***indefinitus* (Ludlow, 1904)**
Synonyms: *formosaensis II* Tsuzuki, 1902
trimaculatus Tsuzuki, 1907
malayensis Hacker, 1921

Distribution: Cambodia, China, Guam, Indonesia, Laos, Malaysia, Mariana Islands, Nepal, the Philippines, Sri Lanka, Taiwan, Thailand, Vietnam.

Biology: *Anopheles indefinitus* is generally thought to be a freshwater species. Larvae are typically linked with floating algae or other plants, and habitats can be exposed or sunny. Marshes, shallow ponds, quiet rivers, natural lakes, and banks of minor streams are natural habitats for immatures of this species. However, this species is also linked to rice fields and irrigation schemes. Small man-made containers like, earthen pots, barrels, and tanks have also been used to collect these larvae. The occurrence of this species in brackish-water coastal habitats has also been observed, indicating some level of salt tolerance (Sinka et al. 2011).

Key characters of material examined:

Total 44 larvae of 4th instar *An. indefinitus* were collected from different locations and following characteristics were observed to identify the mosquito larvae.

Head: Seta 1-A (Antennal setae) is minute i.e., not reaching to midpoint of antennae, seta 2-C is distant from 4-C and comparatively smaller than setae 3,4-C.

Thorax: Setae 9, 10-P are simple or seldom with 2–3 branches, setae 9,10-T are plumose while seta 1-P is not

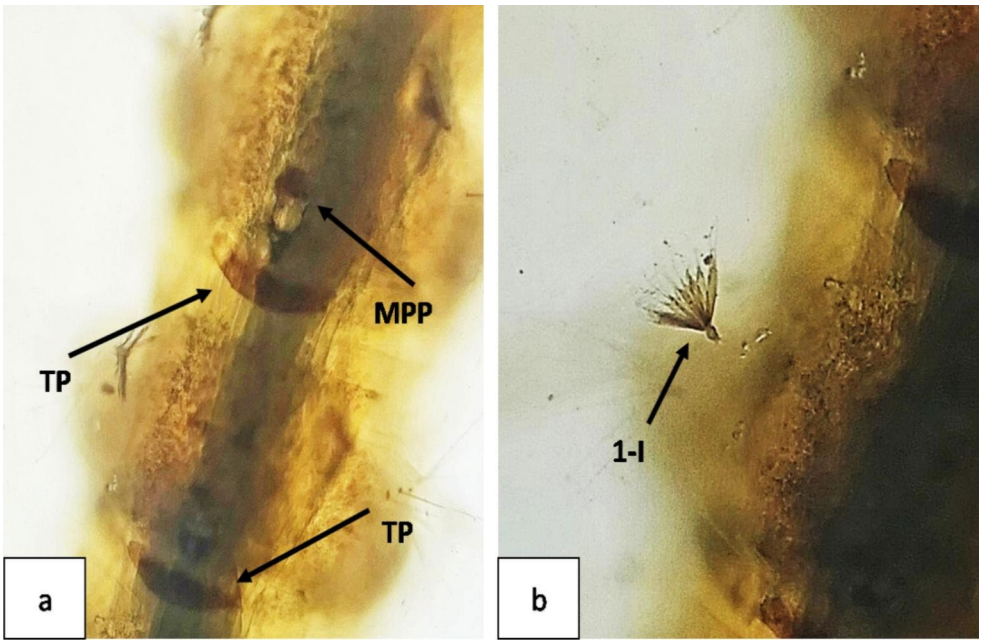

Fig. 2 *Anopheles indefinitus* **(a)** Concave Tergal Plates (TP) not enclosing median posterior plates (MPP) **(b)** Abdominal seta 1-I palmate with 7 or more leaflets

adhered to setal support plate and is generally with 13 or more branches.

Abdomen: The Larvae retain moderate sized anterior tergal plates on IV-VII abdominal segment, with concave posterior border and without enclosing small median posterior plates (Fig. 2a). Seta 1-I is typically with 7 or more branches (Fig. 2b).

Culiseta bergrothi (Edwards, 1921)

Family: Culicidae Meigen, 1818
Subfamily: Culicinae Meigen, 1818
Genus: *Culiseta* Felt, 1904
Subgenus: *Culiseta* Felt, 1904
Species: ***bergrothi* (Edwards, 1921)**
Synonyms: *borealis Shingarev*, 1927
kogievnikovi Shingarev, 1927
kanayamensis Yamada, 1932

Distribution: China, Finland, Japan, South Korea, Mongolia, Norway, Russia, Sweden.

Biology: *Culiseta* are large sized, cold adopted mosquitoes inhabiting warm climates through the colder months or lower temperatures in higher altitudes. The females prefer to feed on birds but sporadically bite humans too (Maquart et al. 2021). Another interesting feature of Culisitine mosquitoes is their variability in all morphological characters. Huge inconsistencies of larval behavior, specific morphological characters, and ecological features have been observed in *Cs. bergrothi* (Dobrotworsky 1971). This species usually breeds in open pools, the larvae dwell in swamps, pools and stagnant water especially in mountainous regions.

Key characters of material examined:

Total 16 larvae of 4th instar *Culiseta bergrothi* were collected from two locations and following characteristics were observed.

Head: Head is significantly broader than longer, with antennae about half length of head. Seta 1 of antennae (1-A) is placed slightly distal to the center of antennae (Fig. 3a), setae 5,6-C are almost parallel having numerous branches.

Thorax: The thoracic segments are entirely merged in all mosquito larvae. Setae are found on the thorax in three groups corresponding to the pro, meso and metathorax. Each group consists of 14 pairs of setae of different sizes.

Abdomen: Comb scales are arranged in triangle form, rounded apically with small fringed spines. Siphon tube is apically tapering, pecten with meager lateral denticles is followed by a line of very thin setae reaching almost 2/3rd of the siphon length (Fig. 3b).

Culex alis Theobald, 1903

Family: Culicidae Meigen, 1818
Subfamily: Culicinae Meigen, 1818
Genus: *Culex* Linnaeus, 1758
Subgenus: *Culex* Linnaeus, 1758
Species: ***alis* Theobald, 1903**
Synonyms: *neolitoralis* Bram, 1967

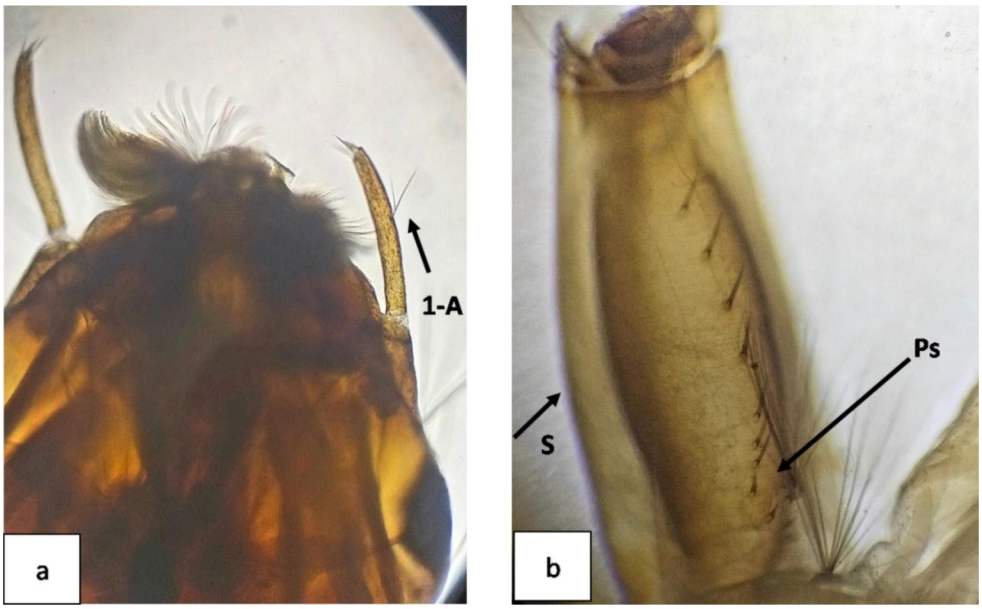

Fig. 3 *Culiseta bergrothi* **(a)** seta 1-A slightly distal to the middle of antennae **(b)** Siphon (S) and Pecten spines (Ps)

Distribution: Cambodia, Guam, Indonesia, Malaysia, New Guinea (Island), Papua New Guinea, the Philippines, Singapore, Taiwan, Thailand, Vietnam.

Biology: This species belongs to the Sitiens complex represented by two brackish water, coastal species *i.e., Culex sitiens* and *Culex alis*. Larvae of *Cx. sitiens* are exceedingly adaptable and have a high tolerance for salinity, allowing them to live in fresh, brackish, and even seawater, salt marshes and mangroves. Larvae have also been found in ponds, ditches, pits, ground pools, rock pools, crab holes, and stream margins. They have also been found in man-made containers, such as jars, cans, and boat bottoms. Adults have been collected from light traps and the cow-baited traps piercing people. Compared to *Cx. sitiens, Cx. alis* is a rare species (Rattanarithikul et al. 2005).

Key characters of material examined:

Total 181, 4th instar larvae of *Cx. alis* were collected from different locations and following characteristics were observed.

Head: The differences between larvae of *Cx. alis* and *Cx. sitiens* are the formation of saddle and position of setae 2,3-A. The seta 2 and 3 of antennae (2,3-A) are placed apically in *Cx. alis* (Fig. 4a). Setae 1 of head (1-C) are dark, stout with blunt apex in both species.

Thorax: Seta 4-P with two or more branches.

Abdomen: *Cx. alis* is a brackish water species with incomplete saddle and anal palpi smaller than saddle. Saddle is complete in *Cx. sitiens* and incomplete in *Cx. alis* (Fig. 4b). Ventral brush (seta 4-X) has 3 or more pairs of setae. Siphon is distinctly longer than saddle and more or less uniform in shape, pecten does extend to apex of siphon, comb scales are rounded apically. Seta 7-I have two or more branches.

Udaya argyrurus (Edwards, 1934)

Family: Culicidae Meigen, 1818
Subfamily: Culicinae Meigen, 1818
Genus: *Udaya* Thurman, 1954
Species: ***argyrurus* (Edwards, 1934)**
Synonyms: *Paraedes argyrurus* Edwards, 1934

Distribution: India, Malaysia, Singapore, Thailand.

Biology: Mosquitoes belonging to genus *Udaya* are small sized with eyes alienated by a spoon like spot, covered by silver scales in both sexes. Because of being uncommon, the bionomics of *Udaya* mosquitoes are not very much described. Only three species of *Udaya* viz. *Ud. subsimilis* (Barraud, 1927), *Ud. argyrurus* (Edwards, 1934), & *Ud. lucaris* (Macdonald and Mattingly ,1960) have been discovered so for globally. *Udaya* species have the most in common with *Aedes, Heizmannia*, and *Zeugnomyia* species in terms of morphology (WRBU, 2021a). This Genus has

Fig. 4 *Culex alis* (a) Setae 2,3-A (Antennae) inserted apically (b) Incomplete Saddle (Sa)

been reported for the first time in Pakistan with species *Ud. argyrurus*.

Key characters of material examined:

Total 22 larvae of 4th instar *Udaya argyrurus* were collected from two different locations and following characteristics were observed.

***Udaya* Genus:** The distinguishing characters of *Udaya* genus are the presence of 3 or more pairs of setae on ventral brush (seta 4-X). Siphon tube has pectin and a pair of seta 1-S located away from basal third of the siphon, seta 8 of siphon (8-S) is single. Hypostomal suture does not reaches posterior tentorial pit and is well developed.

Head: In *Ud. Argyrurus*, seta 1-C is short, thin and pale. Setae 5 and 6-C are bristle like, non-stout, 7-C is single, much elongated than 5 and 6-C (Fig. 5a). Apical part of antennae is not freely movable, distal portion of antennae is without joint.

Thorax: Setae 1,2,3-P are smaller in size and of same size. Setae arise from a thick blackish sclerotized base.

Abdomen: Comb scales are positioned in a single row of less than 10 spines (Fig. 5b). Siphon is small cylindrical and flattened in shape. Seta 4-X is simple and without grid.

Discussions

Ninety-three mosquito species, 24 in the subfamily Anophelinae (Grassi, 1900) and 69 in Culicinae (Meigen, 1818) are presently reported to be stirring in Pakistan (Gaffigan et al. 2015). Among these species, some are very serious vectors (*Aedes aegypti*, *Aedes albopictus*) of several fatal diseases (such as dengue) in human (Leta et al. 2018). Many Anopheline species including *An. superpictus, An. stephensi, An. fluviatilis, An. dthali*, and *An. culicifacies* are major malaria vectors. *Cx. theileri*, and *Ae. Vexans* are vectors of Dirofilariasis in Human. *Cx. modestus*, *Cx. pipienspipiens* and *Cx. quinquefasciatus* are carriers of West Nile virus. *Cx. bitaeniorhynchus*, *Cx. pipiens, Cx. tritaeniorhynchus*, and *Cx. univittatus* are possible vectors of Sindbis virus complex. Similarly Rift Valley fever virus is transmitted by *Cx. pipiens* and *Ae. Vexans* (Doosti et al. 2016).

An alien mosquito species being introduced into unfamiliar settings/areas has enormous ramifications for the environment. The species reported in the current study are not reported vectors of any serious illness in Pakistan till date, but all the blood sucking mosquitoes have a potential to serve as vectors of pathogens at any time (Chaves et al. 2010).

An. indefinitus, reported to be the carrier of *Plasmodium vivax* and confirmed as malaria vector (Arwati et al. 2018), belong to Subpictus Group and is very similar to *An. subpictus* in its larval morphology. Few differences in larvae of both species are the formation of abdominal seta 1–1, which

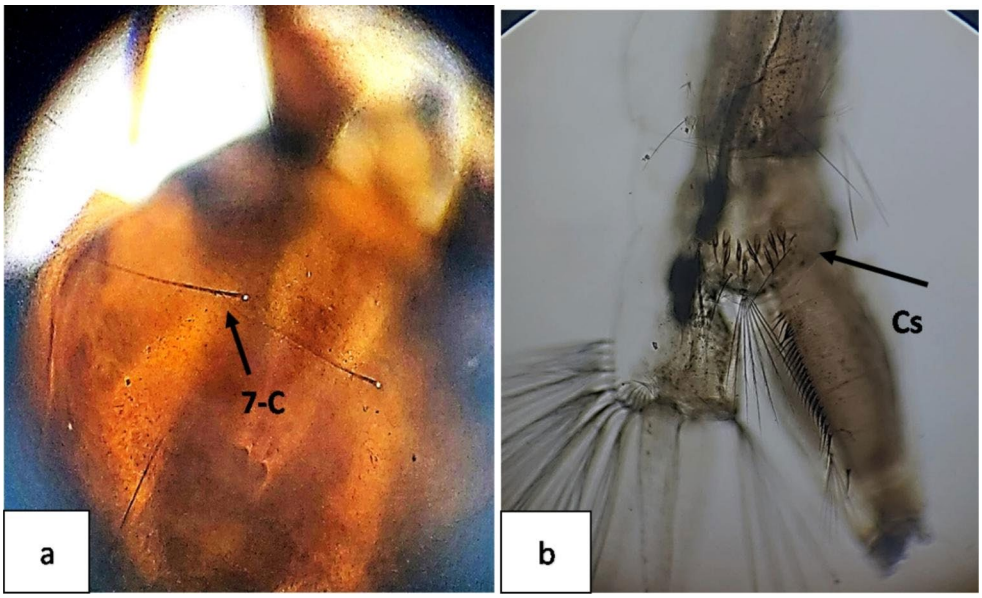

Fig. 5 *Udaya argyrurus* **(a)** 7-C single much elongated than 5 and 6-C **(b)** Comb scales arranged in a single row of less than 10 spines in triangular shape

is palmate in both species but with 6 or fewer lanceolate leaflets lacking discrete serrated shoulders in *An. subpictus* while *An. indefinitus* also have palmate seta 1-I with fewer than 7 leaflets, or most of the leaflets with discrete serrated shoulders. Seta 1-P usually have less than 13 branches in *An. subpictus* and 13 or more branches in *An. indefinitus* (Rattanarithikul et al. 2006). A detailed report on the presence and identification of this species in Malaya and the surrounding regions was presented by Reid (1966) for the first time. As described before *An. indefinitus* was confused with *An. subpictus*, Reid (1966) provided a thorough and detailed examination of specimens obtained from different countries like Philippine, India and Indonesia.

Among blood sucking mosquitoes that bite men in North America, Europe and the northern regions of Asia, especially in early summer and spring, those of the genus Culiseta (Edward) are economically important. Attack by *Culisetine* mosquitoes are sometimes reported even in temperate regions (Nilsson 1983). In Pakistan only three *Culisetine* species were reported by AslamKhan (1971), after that *Culiseta longiaerolata* was reported form Bannu, Khyber Pakhtunkhwa (Khan 2022). The differences between *Culex* and *Culisetine* larvae are the presence of Seta 1-S (pairs at base) of Siphon. Only 1 pair of Seta 1-S is present in *Culisetine* larvae. The other difference is presence of Siphonal Acus in *Culisetine* larvae, which is absent in *Culex*. Dobrotworsky (1971) gave a first-hand detailed information on the taxonomic classification and morphological description of all the life stages and differentiation of different characters in different regions of Southeast Asia. All these details were elaborated with figures and diagrams.

The associated pathogens of *Cx. alis* are Japanese Encephalitis Virus, Ross River Virus, Plasmodium spp., and Tembusu Virus (WRBU 2021b). *Culex alis* is one of six documented species in the Sitiens Subgroup, which also includes *Cx. sitiens* Theobald, *Cx. litoralis* Bohart, *Cx. palpalis* Taylor, *Cx. annulirostris* Skuse, and *Cx. whitmorei* (Giles). Bram (1967) presented detailed morphological and diagnostic characters of this species of Southeast Asia. During that era *Cx. alis* was known as *Cx. neolitoralis*. The document also described larval biology and habitat characterization of this species along with its geographical distribution i.e., from Thailand through Malaya to New Guinea. Initially *Cx. alis* was confused with *Cx. litoralis* in morphological characters and was considered the same species, but later it was distinguished as a separate species.

The Mosquitoes of the genus *Udaya* are small, exceedingly baroque species, ornated with distinctive dense areas of silvery scales. Eyes of males and females are separated by a distinctive spoon-shaped patch of silver scales, and the alula is covered by broad, flat scales. The genus *Udaya* comprises of only three described species: *Ud. argyrurus* (Edwards), *Ud. lucaris* Macdonald & Mattingly, and *Ud. subsimilis* (Barraud). Udaya species are morphologically most similar to those in Aedes, Heizmannia and Zeugnomyia species. *Ud. argyrurus* was reported to possess viruses, bacteria, and parasites that have been experimentally transported or isolated from this species, but does not indicate whether or not it is a field vector (WRBU 2021a) and was documented from Thailand by Thurman (1954) for the first time. Macdonald and Mattingly (1960) described that *Ud. argyrurus* was reported from Ulu Gombak Forest Reserve, Malaya, north of Kuala Lumpur. The differences between two confusing species *Ud. lucaris* and *Ud. argyrurus* were discussed by illustrations.

Conclusively, the species richness of an area depends mainly on its climate, and in case of mosquito larvae, characteristics of breeding site play a pivotal role. In fact, the species reported from a specific region is a depiction of species in that specific time period. It is not necessary that all the previously reported species are still present, or the species not documented before were really not present at that time or missed by the collector. The fact is dependent upon routine and region wide extensive studies along with environmental effects (Kampen et al. 2013). Numerous species are missed because of being hard to reach or less common encounters, some may be confined to specific geographical or ecological boundaries or specific weather/climatic conditions.

Conclusions

Mosquito monitoring is crucial to assess the biodiversity and ultimately add to mosquito inventories. The current study found one genus (*Udaya* Thurman, 1954) and four species viz. *Anopheles indefinitus* (Ludlow, 1904), *Culex alis* Theobald, 1903, *Culiseta bergrothi* (Edwards, 1921), *Udaya argyrurus* (Edwards, 1934) for the first time from Pakistan. The species reported in present study may be potential vector of many serious disease pathogens which can be a cause of concern in future. It is therefore recommended to conduct further country wide surveys of mosquito fauna to depict an exact analysis of the situation.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Akram W, Hafeez F, Ullah UN, Kim YK, Hussain A, LEE JJ (2009) Seasonal distribution and species composition of daytime biting mosquitoes. Entomol Res 39:107–113. https://doi.org/10.1111/j.1748-5967.2009.00204.x

Anonymous (2022) District Dera Ghazi Khan, Government of Punjab Pakistan. https://dgkhan.punjab.gov.pk/district_at_glance

Arwati H, Yotopranoto S, Rohmah EA, Syafruddin D (2018) Submicroscopic malaria cases play role in local transmission in Trenggalek district, East Java Province, Indonesia. Malar J 17:1–6. https://doi.org/10.1186/s12936-017-2147-7

Aslamkhan M (1971) The mosquitoes of Pakistan I. A checklist. Mosq syst Newsl 3:147–159. https://www.researchgate.net/publication/225291673_The_mosquitoes_of_Pakistan_I_A_checklist

Aslamkhan M (1972) The Mosquitoes of Pakistan l/II. Mosquitoes Originally Described from Pakistan. Mosq Syst 4:98–102. https://www.biodiversitylibrary.org/content/part/JAMCA/MS_V04_N4_P098-102.pdf

Baig MB, Al-Subaiee FS (2009) Biodiversity in Pakistan: key issues. Biodivers 10:20–29. https://doi.org/10.1080/14888386.2009.9712858

Bram RA (1967) Contributions to the mosquito fauna of southeast Asia-II. The genus Culex in Thailand (Diptera: Culicidae). Contr Am Ent Inst 2:1–296

Chaudhry QU(2017) Climate change profile of Pakistan. Asian Development Bank. https://www.adb.org/sites/default/files/publication/357876/climate-change-profile-pakistan.pdf

Chaves LF, Harrington LC, Keogh CL, Nguyen AM, Kitron UD (2010) Blood feeding patterns of mosquitoes: random or structured? Front Zool 7:1–11. https://doi.org/10.1186/1742-9994-7-3

Darsie RF, Pradhan SP (1990) The mosquitoes of Nepal: their identification, distribution and biology. Mosq Syst 22:69–130. https://mosquitotaxonomicinventory.myspecies.info/sites/mosquitotaxonomicinventory.info/files/Darsie%20%26%20Pradhan%201990.pdf

Dobrotworsky NV (1971) Contributions to the mosquito fauna of Southeast Asia. X. The genus Culiseta Felt in Southeast Asia. Contrib Am Entomol Inst 7:38–61. https://doi.org/10.22617/TCS178761

Doosti S, Yaghoobi-Ershadi MR, Schaffner F, Moosa-Kazemi SH, Akbarzadeh K, Gooya MM, Vatandoost H, Shirzadi MR, Mosta-Favi E (2016) Mosquito surveillance and the first record of the invasive mosquito species *Aedes* (Stegomyia) *albopictus* (Skuse) (Diptera: Culicidae) in southern Iran. Iran J Public Health 45:1064. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5139964/

Gaffigan T, Pecor J(1997) Collecting, rearing, mounting and shipping mosquitoes. The Walter Reed Biosystematics Unit, Division of Entomology, Walter Reed Army Institute of Research. Available from: http://www.wrbu.org/docs/mq_crms.pdf

Gaffigan TV, Wilkerson RC, Pecor JE, Stoffer JA, Anderson T(2015) Systematic Catalog of Culicidae. The Walter Reed Biosystematics Unit: Smithsonian Institution. http://www.mosquitocatalog.org/

Herrel N, Amerasinghe FP, Ensink J, Mukhtar M, Van Der Hoek W, Konradsen F (2001) Breeding of Anopheles mosquitoes in irrigated areas of South Punjab, Pakistan. Med Vet Entomol 15:236–248. https://doi.org/10.1046/j.0269-283x.2001.00312.x

Kampen H, Kronefeld M, Zielke D, Werner D (2013) Three rarely encountered and one new Culiseta species (Diptera: Culicidae) in Germany. J Eu Mosq Control Assoc 31:36–39. https://e-m-b.myspecies.info/sites/e-m-b.org/files/JEMCA(31)36-39_0.pdf

Khan MA (2022) Seasonal variation in species composition and relative abundance of mosquitoes in the District Bannu, Khyber Pakhtunkhwa, Pakistan: prospects of dengue fever in the study area. Int J Trop Insect Sci 42:2221–2231. https://doi.org/10.1007/s42690-022-00744-3

Leta S, Beyene TJ, De Clercq EM, Amenu K, Kraemer MU, Revie CW (2018) Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. Int J Infect Dis 67:25–35

Macdonald WW, Mattingly PF(1960) A new species of Udaya from Malaya and a description of the early stages of *U. argyrurus* (Edwards, 1934) (Diptera: Culicidae). In Proceedings of the Royal Entomological Society of London. Series B, Taxonomy. 29:22–28. https://doi.org/10.1111/j.1365-3113.1960.tb01124.x

Maquart PO, Fontenille D, Rahola N, Yean S, Boyer S(2021) Checklist of the mosquito fauna (Diptera, Culicidae) of Cambodia. Parasite 28:60. https://doi.10.1051/parasite/2021056

Maslov AV, Ward RA, Rao PM (1989) Blood-sucking mosquitoes of the subtribe Culisetina (Diptera, Culicidae) in world fauna. Smithsonian Institution Libraries and the National Science Foundation, Washington, D.C. https://library.si.edu/digital-library/book/bloodsuckingmosq00masl

Naeem-Ullah U, Akram W, Suhail A, Rana SA (2010) Grouping of different mosquito species on the bases of larval habitats. Pak J Agri Sci 47:124–131. https://pakjas.com.pk/papers/23.pdf

Nilsson C(1983) Coprophagy in larval *Culiseta bergrothi* (Diptera: Culicidae). Hydrobiologia 98:267-9. https://doi.10.1007/BF00021028

Radhakrishnan A (2019) Study on mosquito (Diptera: Culicidae) diversity in Ernakulam district of the Kerala state, South India. Int J Mosq Res 6:1–5. http://www.dipterajournal.com/pdf/2019/vol6issue1/PartA/5-6-4-136.pdf

Rattanarithikul R (1982) A guide to the genera of mosquitoes (Diptera: Culicidae) of Thailand with illustrated keys, biological notes and preservation and mounting techniques. Mosq Syst 14:139–208. https://www.biodiversitylibrary.org/content/part/JAMCA/MS_V14_N3_P139-208.pdf

Rattanarithikul R, Harrison BA, Harbach RE, Panthusiri P, Coleman RE, Panthusiri P (2006) Illustrated keys to the mosquitoes of Thailand. IV Anopheles Southeast Asian J Trop Med Public Health 37:1–128

Rattanarithikul R, Harrison BA, Panthusiri P, Coleman RE (2005) Illustrated keys to the mosquitoes of Thailand I. Background; geographic distribution; lists of genera, subgenera, and species; and a key to the genera. Southeast Asian J Trop Med Public Health 1:1–80

Reid JA (1966) A Note on Anopheles Subpictus Grassi and A. Indefinitus Ludlow (Diptera: Culicidae). J Med Entomol 3:327–331. https://doi.org/10.1093/jmedent/3.3-4.327

Reisen WK, Milby MM(1986) Population dynamics of some Pakistani mosquitoes: changes in adult relative abundance over time and space. Ann Trop Med Parasitol 80:53–68. https://doi.10.1080/00034983.1986.11811984

Shafiq W, Ullah H, Zaheer M, Mehmood M, Farooq U, Khan MJ, Mashwani SA, Ullah S (2021) Aquifer Characterization and Physiochemical Analysis of District Dera Ghazi Khan Punjab, Pakistan. Rudarsko-geološko-naftni zbornik. 36:47–57. https://doi.org/10.17794/rgn.2021.3.4

Shaikh S, Kazmi SJ, Qureshi S(2014) Monitoring the diversity of malaria and dengue vector in Karachi: studying variation of genera and subgenera of mosquitoes under different ecological conditions. Ecol Process 3:1–9. https://doi.10.1186/s13717-014-0012-y

Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, Temperley WH, Gething PW, Elyazar IR, Kabaria CW, Harbach RE, Hay SI(2011) The dominant Anopheles vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. Parasit Vectors 4:1–46. https://doi.10.1186/1756-3305-4-89

Steiger DB, Ritchie SA, Laurance SG (2016) Land Use Influences Mosquito Communities and Disease Risk on Remote Tropical Islands: A Case Study Using a Novel Sampling Technique. Am J Trop Med 94:314–321. https://doi.org/10.4269/ajtmh.15-0161

Thurman DCJr (1954) The discovery of *Paraedes (Udaya) argyrurus* Edwards. 1934, in Thailand (Diptera: Culicidae). Mosquito News 14 : 83–86

Walter Reed Biosystematics Unit (WRBU) (2021a) *Udaya* genus page. Walter Reed Biosystematics Unit Website, http://wrbu.si.edu/vectorspecies/genera/udaya, accessed on 08 December 2021

Walter Reed Biosystematics Unit (WRBU) (2021b) *Culex sitiens* species page. Walter Reed Biosystematics Unit Website, http://wrbu.si.edu/vectorspecies/mosquitoes/sitiens, accessed on 08 December 2021

Weatherspark (2021) Climate and Average Weather Year Round in Dera Ghazi Khan. Ghazi-Khan-Pakistan-Year-Round. https://weatherspark.com/y/106985/Average-Weather-in-Dera-