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Original article

## Distribution of mosquito species in various agro-ecological zones of Punjab



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## ABSTRACT

**Background:** The presence and behavior of mosquitoes in various agro-ecological zones of Punjab are influenced by a combination of seasonal and topographical patterns, cropping schemes, and human settlement. These modifications have a significant impact on the occurrence of diseases. Therefore, determining the distribution of these species could help in halting their spread and disease outbreaks.

**Methods:** Under these prevailing conditions, surveillance studies were carried out at six zones of Punjab, to know the distribution of mosquitoes. Samples were collected in various seasons (once in winter and rainy seasons, and twice in summer), from all possible habitats, having stagnant water.

**Results:** Out of twenty-four (24) recorded species, three were included in Anophelinae and Culicinae, eleven from tribe Aedini, eight from Culicini and one each from Ficulbiini and Mansoniini. Sampling was done from 91 m (Ahmed Pur East) elevation to 1759 m (Fort Monroe). High and diversified population was recorded during rainy season. The Anopheline mosquitoes were found in rural as well as urban habitats, including rainfed, wet mountains and irrigated plains whereas Aedine species were confined to northern irrigated plains and mostly recorded from Changa Manga National Forest. Culicine species were more diversified and abundant, in all seasons. However, *M. chamberlaini* was collected from urban (rainfed lands) and rural (northern irrigated plains) settings. *C. crassipes* was recorded only from rainfed lands.

**Conclusion:** Twenty-four mosquito species from the Anophelinae and Culicinae subfamilies were observed in the current investigation. Anophelinae subfamily contained three species. Eleven species of Culicinae belonged to the tribe Aedini, eight to the Culicini, and one each to the Mansoniini and Ficulbiini.

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## 1. Introduction

The association between wetlands and malaria has been acknowledged by humans since the eras of the Romans and Greeks (Andrade Jr, 1995). The association between wetlands and malaria has been acknowledged for an extended period. The ancient civilizations of Greece and Rome understood the correlation between malaria and marsh environments, and this knowledge has been transmitted over generations. Currently, this comprehension remains pertinent in the context of disease preventive and control

endeavors inside wetland regions (Demetrioff, 2020; Sallares, 2002).

Malaria is an infectious disease, mostly transferred to human hosts through the bites of female mosquitoes belonging to the *Anopheles* genus. Malaria affects a global population of over 500 million individuals and results in an annual mortality rate of 1 million individuals (Holt et al., 2002; Sangbakembi-Ngounou et al., 2022). Presently, malaria is prevalent in > 80 countries, where it is spread by anopheline mosquitoes (Dahmana & Mediannikov, 2020). The effective maturation of the malaria parasite inside the mosquito is contingent upon several variables (Janson et al., 2023). There are 3,500 distinct mosquito species classified into 41 genera (Kain et al., 2022). However, it is important to note that just a subset of 30–40 species belonging to the *Anopheles* genus are responsible for the transmission of malaria within natural ecosystems. The knowledge on the distribution of mosquito species has significant value in the formulation and implementation of efficient approaches aimed at mitigating the spread and impact of diseases.

Historical origins of vector-borne diseases in Pakistan may be traced back to ancient times like other regions around the globe (Jabeen et al., 2022). Nevertheless, with the passage of time and changes of environmental conditions, there has been a persistent increase in the diversity of vector species and the severity of diseases caused by these vectors. Mosquito-borne diseases like Dengue Fever and Dengue Hemorrhagic Fever (*Aedes aegypti*, *Ae. albopictus* and other *Aedes* spp.), West Nile Fever (*Culex tritaeniorhynchus*, *Cx. quinquefasciatus*), Sindbis Fever (*Cx. pseudovishnui*, *Cx. tritaeniorhynchus*), Japanese Encephalitis (*Cx. tritaeniorhynchus*, *Cx. pseudovishnui* and other species of *Culex* and *Anopheles*), Chikungunya Fever (*Ae. aegypti*, *Cx. tritaeniorhynchus*), Malaria (*An. culicifacies*, *An. stephensi*, *An. subpictus* and other species), Brugian Filariasis (*Mansonina* spp.) and Bancroftian Filariasis (*Cx. quinquefasciatus* and other species of *Culex* and *Aedes*) have been recorded from Pakistan (Ashfaq et al.,

2014; Attaullah et al., 2023; Khan et al., 2015; Manzoor et al., 2020; Qasim et al., 2014).

Knowledge about the distribution patterns of mosquito species has significant importance in the development of effective measures aimed at preventing and controlling diseases (Becker et al., 2020; Ferraguti et al., 2023). The transmission of vector-borne diseases is impacted in varying ways by human-induced landscape modification, particularly in the context of overall biodiversity decline resulting from increased human pressures on the environment (Glidden et al., 2021). Determination of mosquito species' diversity, distribution, and relative abundance might provide valuable insights about the ecological characteristics of various locations (Dahmana & Mediannikov, 2020; Moreno-Madriñán & Turell, 2018). The data on the composition and genetic diversity of mosquito species might provide valuable insights for further research and the development of effective management approaches.

The implementation of mosquito control measures is often conducted by regulatory bodies, who mostly depend on their knowledge rather than a systematic approach backed by spatio-temporal data (DeGroot et al., 2007). Understanding the determinants of spatio-temporal distribution patterns in animal or plant species is a fundamental aspect of ecological study. This assessment includes temporal abundance as well as the climatic factors and spatio-temporal study of the landscape in relation to mosquito populations (DeGroot et al., 2007). The breeding of several mosquito species is known to be enhanced by recent changes resulting from agricultural operations and urbanization (Adeleke et al., 2008). Moreover, complex, and interconnected nature of ecological variables has a significant role in influencing population growth over time, as shown by the observed changes in vector behavior (Barik et al., 2009). The effectiveness of mosquito control efforts can be improved by expanding our understanding of both environmental factors and biological processes that impact mosquito populations.

**Table 1**  
Association of different mosquito species with seasons and agro-ecological zones of Punjab.

Species	Seasons					Agro-Ecological Zones							
	1	2	3	$\chi^2$	p-value	1	2	3	4	5	6	$\chi^2$	p-value
<i>An. nigerrimus</i>	0	0	1	1.943	0.379	0	0	1	0	0	0	2.728	0.742
<i>An. stephensi</i>	4	11	6	1.134	0.567	1	1	4	0	10	5	11.69	0.039
<i>An. subpictus</i>	3	12	19	8.936	0.011	4	5	0	0	23	2	15.978	0.007
<i>Ae. pseudotaeniatu</i>	0	2	2	1.376	0.503	0	0	0	4	0	0	164.385	0.000
<i>Ae. vittatus</i>	1	26	38	27.801	0.000	2	4	54	5	0	0	131.634	0.000
<i>Ae. lineatopennis</i>	0	0	4	7.799	0.02	0	0	0	0	4	0	5.084	0.406
<i>Ae. caspius</i>	0	1	6	8.54	0.014	0	0	4	0	3	0	4.462	0.485
<i>Ae. aegypti</i>	1	7	10	5.285	0.071	0	5	1	0	12	0	15.582	0.008
<i>Ae. albopictus</i>	1	21	40	33.717	0.000	0	0	30	11	21	0	97.133	0.000
<i>Ae. unilineatus</i>	0	0	4	7.799	0.02	0	0	0	0	4	0	5.084	0.406
<i>Ae. w-albus</i>	0	0	1	1.943	0.379	0	0	0	0	1	0	1.266	0.938
<i>Ar. subalbatus</i>	0	3	8	8.203	0.017	0	0	0	5	6	0	92.858	0.000
<i>Ve. yusafi</i>	0	0	3	5.842	0.054	0	0	0	0	3	0	3.808	0.577
<i>Ve. indica</i>	1	0	5	6.973	0.031	0	0	0	0	6	0	7.644	0.177
<i>Cx. fuscocephala</i>	2	5	3	0.334	0.846	0	0	2	0	8	0	6.057	0.301
<i>Cx. pseudovishnui</i>	13	48	61	22.39	0.000	4	17	21	0	66	14	26.984	0.000
<i>Cx. quinquefasciatus</i>	66	121	64	12.257	0.002	34	25	44	11	121	16	22.008	0.001
<i>Cx. tritaeniorhynchus</i>	5	49	52	28.592	0.000	4	8	41	0	53	0	24.031	0.000
<i>Cx. malayi</i>	3	23	19	8.399	0.015	0	0	31	0	10	4	47.439	0.000
<i>Cx. minutissimus</i>	1	1	4	2.938	0.23	0	0	1	0	5	0	4.087	0.537
<i>Cx. bitaeniorhynchus</i>	0	1	4	4.924	0.085	0	0	5	0	0	0	13.704	0.018
<i>Lt. fuscus</i>	4	0	0	12.173	0.002	0	0	0	0	4	0	5.084	0.406
<i>Mi. chamberlaini</i>	2	1	5	3.54	0.17	0	0	4	0	4	0	4.017	0.547
<i>Cq. crassipes</i>	0	2	4	3.485	0.175	2	0	4	0	0	0	10.283	0.068

In the seasons column, 1 = winter, 2 = summer and 3 = monsoon (rainy).

**Agro-Ecological Zones:** 1. Sandy Desert A (Cholistan) 28° 45.991–29° 25.251; 071° 20.040–071° 40.479; 91–140. 2. Sandy Desert B (Thal) 32° 04.443–32° 34.400; 071° 29.554–072° 23.471; 179–213. 3. Rain-fed Lands (Barani) 32° 06.580–33° 47.995; 072° 21.659–074° 52.266; 219–720. 4. Wet Mountains 33° 46.746–33° 50.598; 073° 06.641–073° 18.821; 640–1222. 5. Northern Irrigated Plains 29° 30.770–32° 20.959; 070° 53.343–074° 28.753; 100 – 238. 6 Suleiman Piedmont 29° 55.218–30° 28.668; 069° 59.279–070° 43.455; 107–1759.

The Punjab province in Pakistan was divided into six distinct agro-ecological zones (Ahmad et al., 2019). The province has a wide range of terrain types, including arid deserts as well as verdant woods, accompanied by a diversified array of agricultural practices. The cropping patterns in the region include wheat-cotton, rice-wheat, vegetable, and fodder crops (Mujtaba et al., 2022). The province hosts a variety of seasons throughout its varied locations. These include intense heat during May and June, monsoon rains occurring between July and September, and a hard winter characterized by both dry and rainy conditions, with snow-fall occurring in some places. The seasonal diversity and topography offer a range of environments that support the growth and development of both juvenile and adult stages of vector mosquitoes in the province. This, in turn, helps to control the numbers of these insects across different locations and periods. Understanding population patterns may be crucial in effectively controlling population growth and implementing measures to reduce mosquito-borne diseases such as malaria and dengue.

Therefore, the current study was conducted to map the distribution of mosquito species in different agro-ecological zones of Punjab province. It was hypothesized that the regions would differ

for the distribution of the species. The results would provide valuable insights for the management of these species in the province.

## 2. Materials and methods

### 2.1. Study area

The current study was conducted in different agro-ecological zones of Punjab. The zones included in the study were sandy desert A (Cholistan), sandy desert B (Thal), rainfed (Barani) Lands, wet mountains, northern irrigated plains, and Sulaiman piedmont. The climatic features and major uses of soil for these zones are presented in Table 1.

### 2.2. Sampling time

The samples of immature mosquitoes were collected from October to February (winter), March to June (hot summer), and July to September (monsoon season). There are significant differences in the beginning of various seasons throughout the nation and during

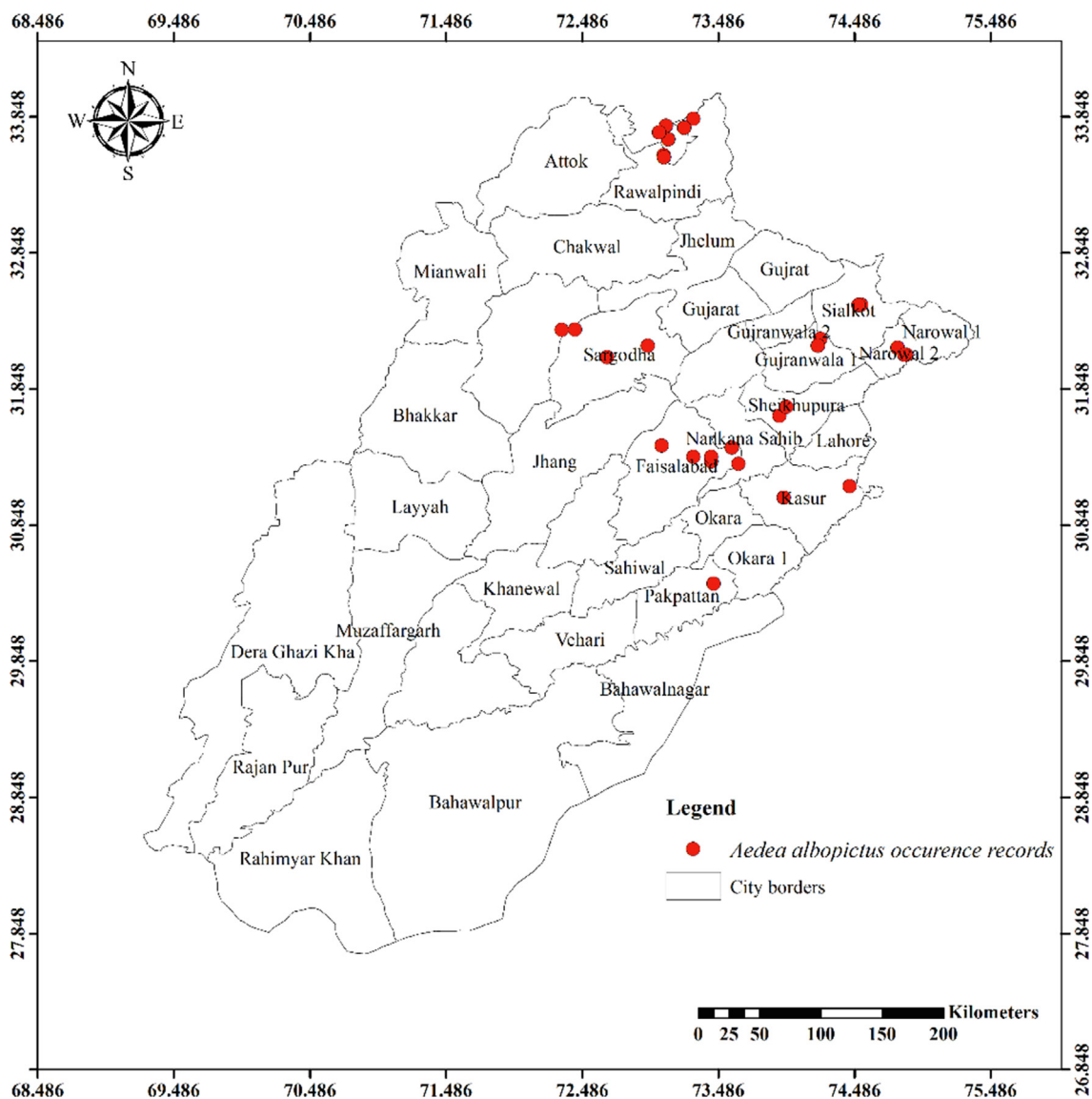


Fig. 1. Distribution of *Aedes albopictus* in different districts of Punjab province.

our study. The start of different seasons varies significantly across the country. The samples were collected once during winter, twice during summer, and once in the monsoon season.

### 2.3. Sampling procedure and data collection

Samples were collected from the Punjab between the X coordinates of 2935380.53 m (Fort Monroe, Suleiman Piedmont) and 3390600.51 m (Narowal, Rain fed Areas) and Y coordinates of 504329.4 m (Derawar Fort, Sandy Desert A) and 1076189.5 m (Murree Road, Wet Mountains). The sampling locations varied from Ahmed Pur East (91 m) in Sandy Desert A (Cholistan) to Fort Monroe (1759 m) in Suleiman Piedmont. Suleiman Piedmont has considerably higher heights and steeper slopes. However, although covering a sizable portion of the province, the Northern Irrigated Plains have a height that ranges from 100 m to 238 m above sea level, which implies less slope, a feature of these alluvial plains of the Punjab.

To determine the importance of the interactions between the various species of collected mosquito larvae found in samples, seasons, and agro-ecological zones, the chi-square test of association

was used. Regarding the occurrence of larvae of different species, the obtained p-values for samples with Seasons and Agro-Ecological Zones reveal diverse degrees of significance, ranging from non-significant to highly significant.

Elevation above sea level and geographic coordinates of sampling sites were recorded with the help of Magellan GPS (Global Positioning System) (Explorist 660). The distribution maps of the most frequently observed were created in ArcGIS 9.10 using Arc-Map (ESRI (Environmental Systems Resource Institute), 2012). The values of longitudes and latitudes were converted into X coordinates (m) and Y coordinates (m), respectively for preparing the map. Table 1 shows the distribution of mosquito species.

The samples were collected from a variety of habitats, including sewage near houses, catch basins (rain or tap), irrigation channels, rice fields, roadside pools, seepage pools, rock pools, irrigated fields, tree holes, wetlands, ground waters, fish farms, parks, tires, sewerage drains, streams, etc., were used to collect immature mosquitoes (Attaullah et al., 2023; Khan et al., 2015; Manzoor et al., 2020; Qasim et al., 2014). Characteristics of different habitats, their associations with collected immature and method of collection in detail are discussed elsewhere (Naeem-Ullah et al., 2010). Col-

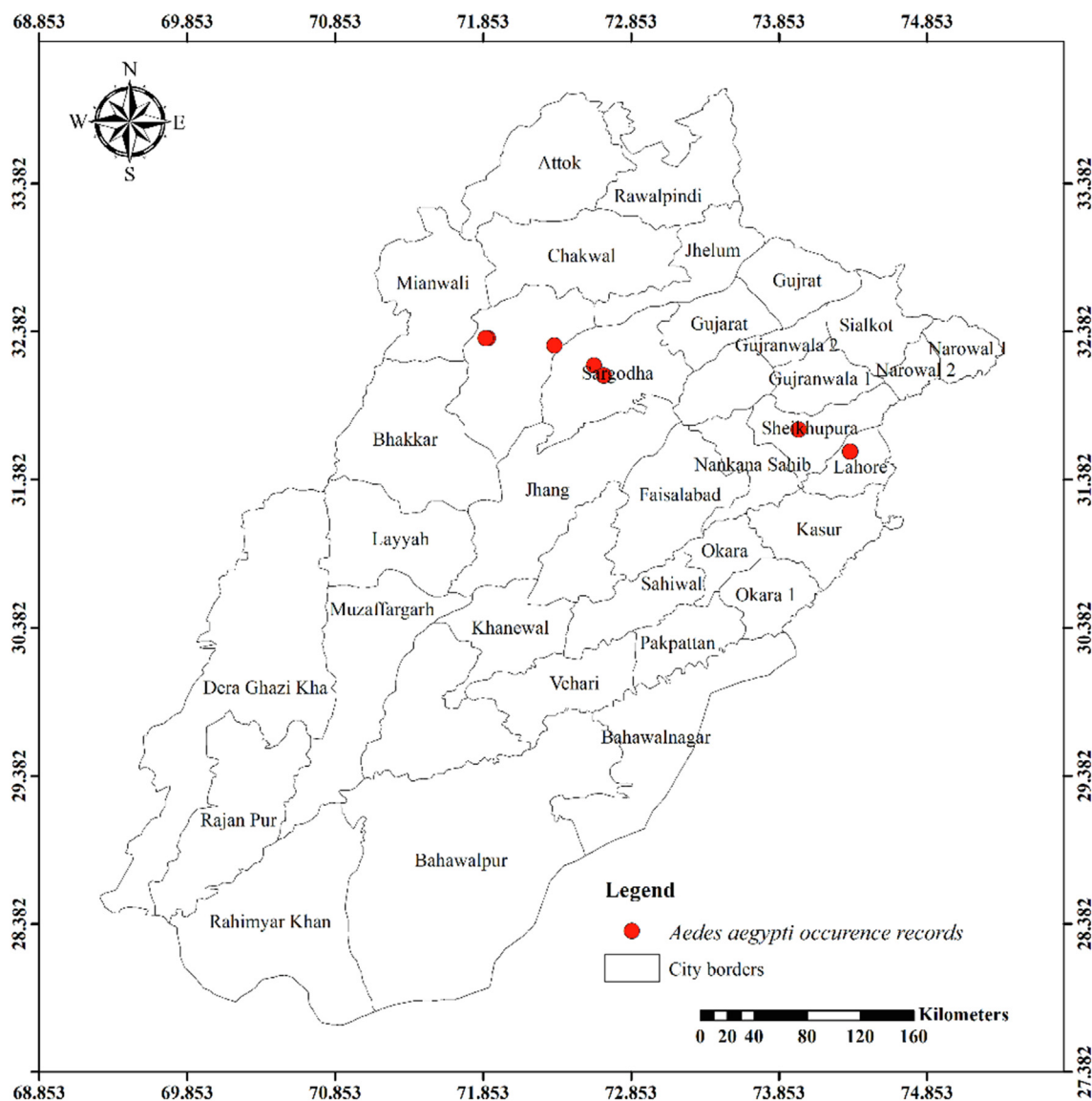


Fig. 2. Distribution of *Aedes saegypti* in different districts of Punjab province.

lected samples were brought to the laboratory of Department of Entomology, University of Agriculture, Faisalabad, Pakistan for an identification.

The Swift microscope was used to identify the species using the existing taxonomic keys (Cockerell, 1925; Hoare, 1940; Seth-Smith, 1942). This manuscript’s nomenclature, hierarchy, and abbreviations have been followed the Systematic Catalogue of Culicidae 2001, the Walter Reed Biosystematics Unit (WRBU), and Herbach (2004).

Since summer season is long enough, mosquito larvae were collected twice a year. The number of samples collected from Wet Mountains as the zone is small. Northern irrigated plains provided more samples, which cover a large region in the Punjab owing to the existence of the Indus River and its four tributaries, as well as a complex canal system developed from them. These irrigated plains contain a wide range of cropping patterns and cropping

methods. This zone contained some of Pakistan’s most populated urban areas along a wide farmed area. As a result, the necessity to examine these zones more completely and comprehensively was realized.

### 3. Results

Twenty-four mosquito species from the Anophelinae and Culicinae subfamilies were observed in the current investigation. Anophelinae subfamily contained three species. Eleven species of Culicinae belonged to the tribe Aedini, eight to the Culicini, and one each to the Mansoniini and Ficalbiini. The distribution maps of the most abundantly recorded species are presented in Figs. 1-8.

Only *An. subpictus*, an anopheles mosquito, exhibits a substantial correlation with seasons (0.011) and agro-ecological zones (0.007). *Ae. albopictus*, *Ar. subalbatus*, and *Ae. vittatus* are members

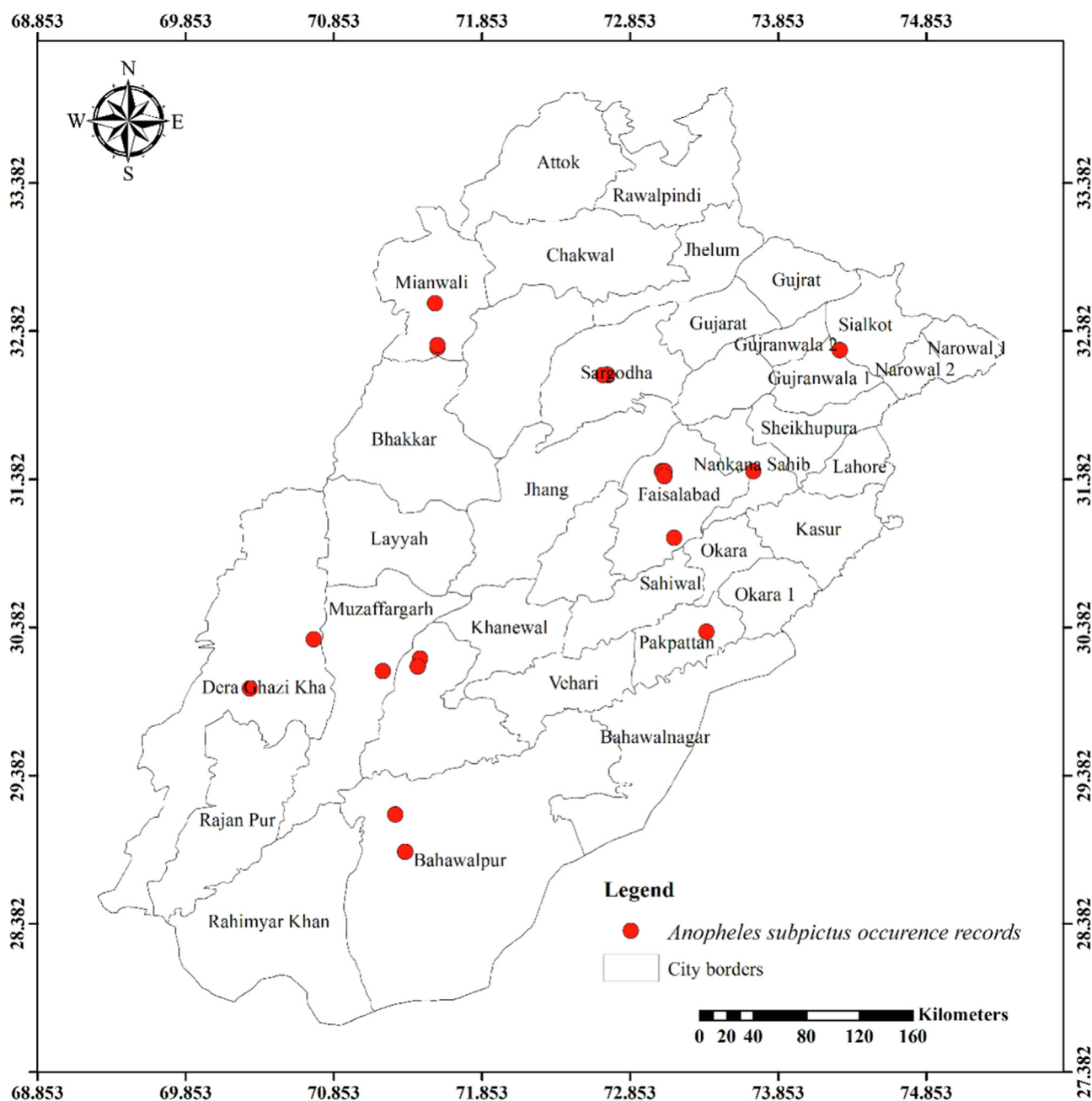


Fig. 3. Distribution of *Anopheles subpictus* in different districts of Punjab province.

of the tribe Aedini and exhibit a significant relationship with both Seasons (p-values of 0.000, 0.017, and 0.000, respectively) and Agro-Ecological Zones (having p-values of 0.000, 0.000 and 0.000, respectively). The seasons are significantly associated with *Ae. caspius* (0.014), *Ae. unilineatus* (0.020), and *Ae. lineatopennis* (0.020). Additionally, *Ae. aegypti* and *Ae. pseudotaeniatu*s are demonstrating a strong correlation with agro-ecological zones (p-values are 0.000 and 0.008, respectively). *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Cx. malayi*, and *Cx. quinquefasciatus* are culicine mosquitoes that are demonstrating very significant association with both the seasons (p-values of 0.000, 0.000, 0.015, and 0.002, respectively) and agro-ecological zones (having p-values of 0.000, 0.000, 0.000, and 0.001, respectively). With a p-value of 0.002, *Lutzia fuscans* has a strong correlation with the seasons, while *Cx. bitaeniorhynchus* has a considerable amount of interaction with agro-ecological zones (having 0.018p-value). On the other hand, *Mi. chamberlaini* and *Cq. crassipes* representing tribes Ficalbiini and Mansoniini do not exhibit any appreciable correlations with the seasons or agro-ecological zones (Table 1).

Anopheline mosquito larvae, *An. nigerrimus*, were found in urban areas during the rainy season, but *An. stephensi* and *An. subpictus* were found in both rural and urban environments throughout the year. Even though *An. subpictus* was more frequently observed than *An. stephensi*, it was not found in wet mountains or rainfed lands. So, compared to the earlier, it occupied a wider variety of agro-ecological zones. Species found only in a few agro-ecological zones and classified under aedine mosquitoes. *Ae. lineatopennis*, *Ae. unilineatus*, *Ve. indica*, *Ve. yusafi*, and *Ae. w-albus* were documented only in rural environments of the Northern Irrigated Plains, mostly in Changa Manga National Forest. Despite *Ve. indica* being recorded in the winter as well, high populations were only observed during the monsoon season. It is the only species of aedine mosquito of those described above that has been discovered in an urban area. *Ae. pseudotaneatus* is another illustration of an animal that lives in a small space. It was only taken from the Wet Mountains at an elevation of 1222 m above sea level from Pir Sohawa, even though *Ae. pseudotaneatus* captured during the summer and monsoon seasons.

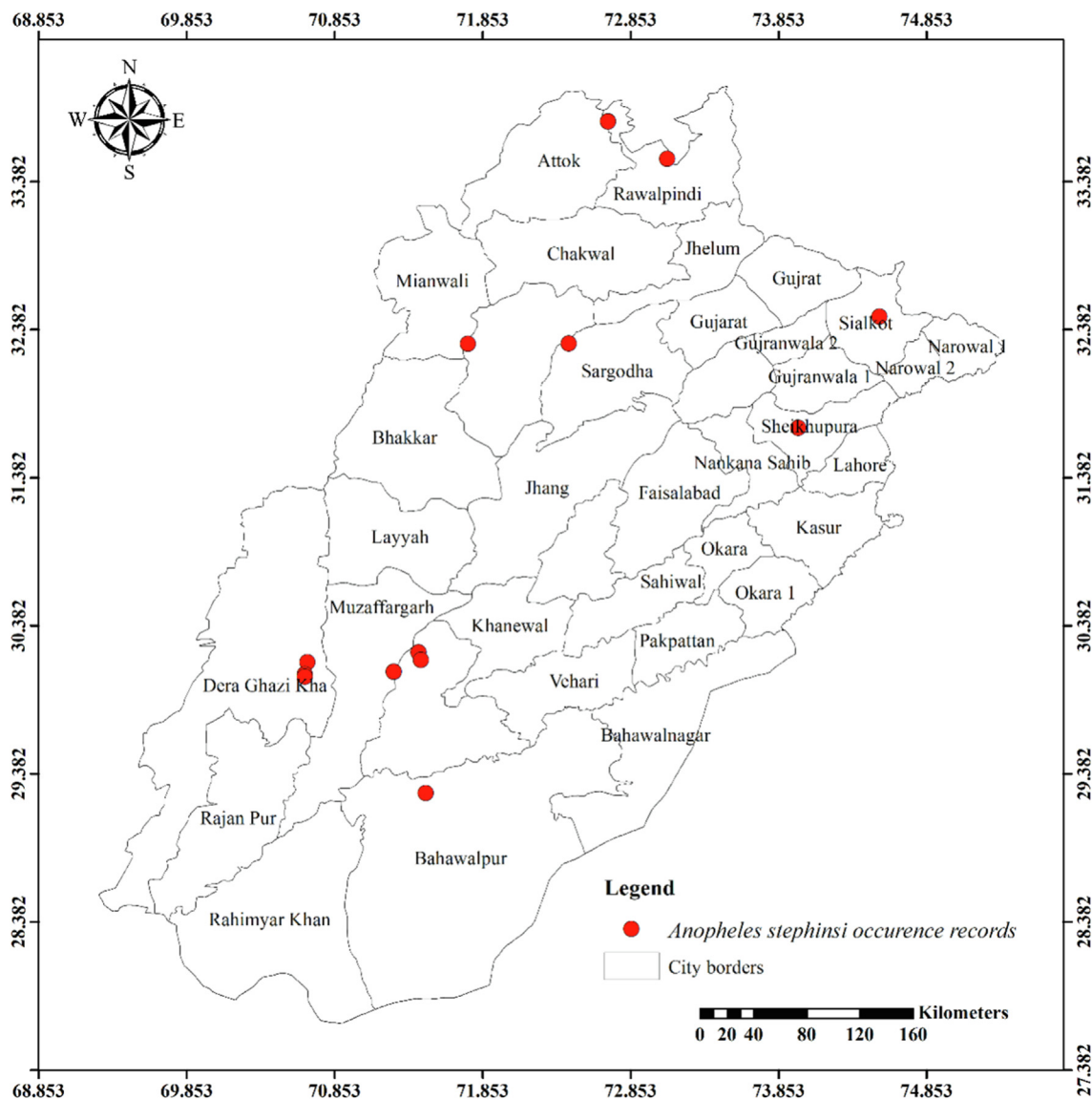


Fig. 4. Distribution of *Anopheles stephensi* in different districts of Punjab province.

Except for the northern irrigated plains and Suleiman Piedmont, *Ae. vittatus* is the only aedine species that has been discovered in all four agro-ecological zones. Rarely sampled, but it was found on rainfed lands in both rural and urban areas, including sandy desert A (Fort Derawar) and Sandy Desert B. The majority of *Ae. aegypti* specimens were collected from urban habitats, however few were also found in rural Khushab and Quidabad. Urban habitats in Lahore (northern irrigated plains), Gujrat (rainfed lands), and Sheikhupura were sampled throughout the summer and during the rainy season. It wasn't discovered from south of Khushab and Quidabad and was also not found in the Wet Mountains. However, *Ae. albopictus* was not seen in the south of Faisalabad. It was discovered in rural areas, with city parks and recreational places being the most likely locations. Except for *Cx. bitaeniorhynchus*, which was exclusively urban dweller and found during the summer and rainy seasons from rainfed lands, whereas other culicine mosquito larvae were discovered during all three seasons of the year. Only winter observations of *Lu. Fuscanus* was made in Faisalabad (northern irrigated plains). Only two culicine species, *Cx. fuscanus* and *Cx. quinquefasciatus* was discovered in the Wet Mountains. All agro-ecological zones recorded *Cx. quinquefasciatus*

population, followed by *Cx. pseudovishnui* and *Cx. tritaeniorhynchus*, while the second last was absent from wet mountains and the last from Suleiman Piedmont. They were discovered in a variety of habitats during all three seasons, from both rural and urban areas. In all seasons, *Cx. malayi* was primarily discovered in urban areas from Suleiman Piedmonts, northern irrigated plains, and rainfed lands, while it was occasionally discovered in rural areas as well. Throughout the year, *Mmomyia chamberlaini* (Tribe Ficalbiini) was found in both rural (northern irrigated plains) and urban areas (rainfed lands). In both rural and urban settings, *Coquillettidia crasipes* (Tribe Mansoniini) was found from rural as well as urban areas of Rain Fed Lands in the summer and monsoon seasons.

Table 1 presents the findings for several mosquito species in relation to latitudes (Y coordinates), longitudes (X coordinates), and altitudinal ranges. The range of latitude and longitude for some species' existence in the Punjab is limited because they were hardly sampled. *An. nigerrimus*, *Ae. pseudotaeniatu*s, *Ae. lineatopenis*, and *Ae. w-albus*, for instance, were all discovered at the same location. *An. subpictus* and *An. stephensi* have nearly identical locations, with the former occupying somewhat more northern coordinates and being discovered in some elevated regions (437 m) than

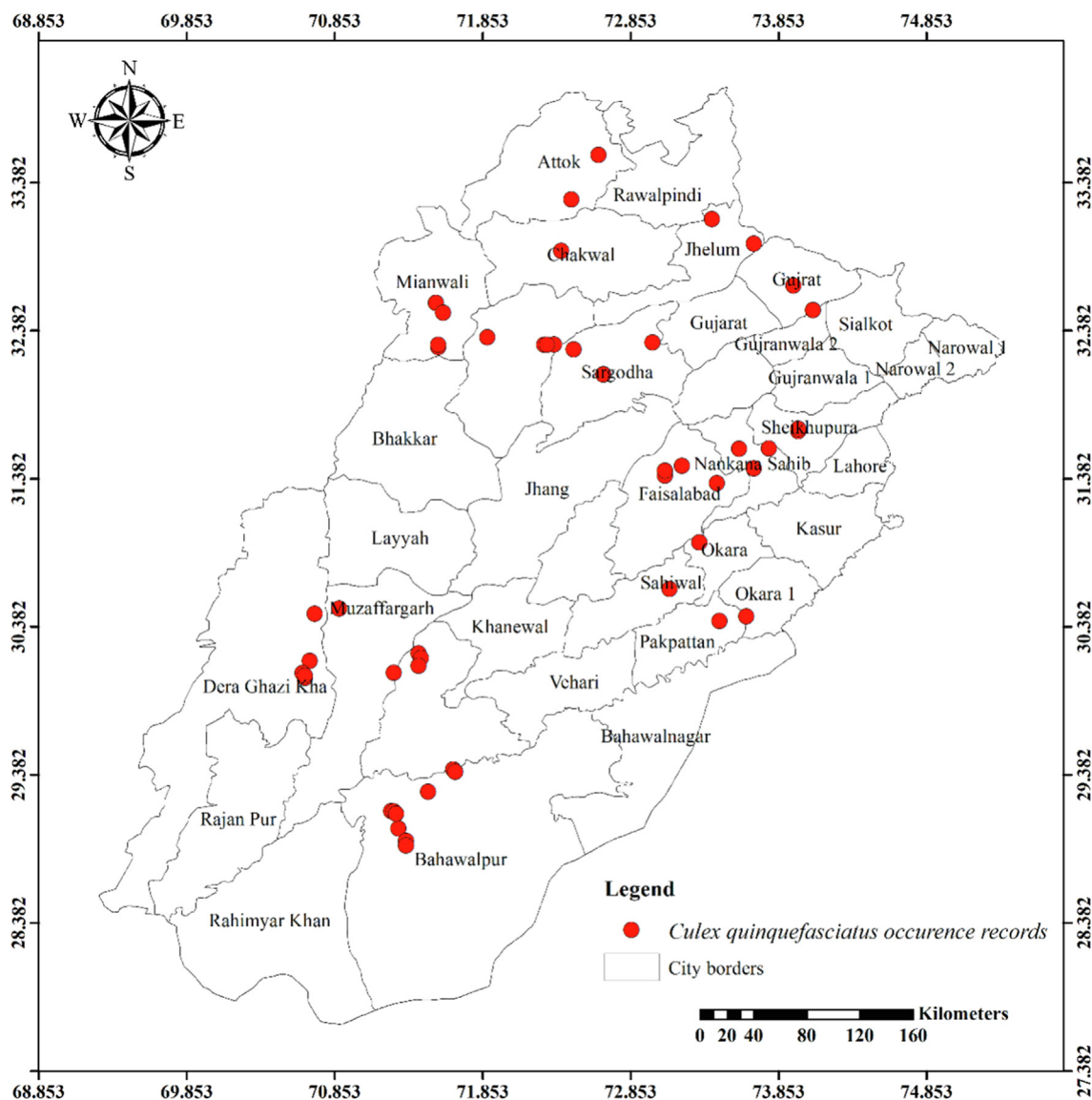


Fig. 5. Distribution of *Culex quinquefasciatus* in different districts of Punjab province.

the latter (290 m). *Ae. unilineatus*, *Ve. indica*, and *Ve. yusafi* likewise had nearly identical positions and elevations. Whereas *Ae. caspius* had a moderate range. The largest range of Y coordinates among the aedine species belongs to *Ae. vittatus* (505868.83–1069193.90) followed by *Ae. albopictus* (699720.37–1076189.50), *Ar. subalbatus* (771811.36–1076189.50) and *Ae. aegypti* (831167.55–904583.31). But as far as X coordinates are concerned, *Ae. aegypti* (3108922.47–3344090.94) has the widest range followed by *Ae. albopictus* (3160652.63–3389771.21), *Ae. vittatus* (3069393.74–3228562.68) and *Ar. subalbatus* (3184486.76–3313329.16). These four species are listed in descending order according to their range of elevation: *Ar. subalbatus*, *Ae. vittatus*, *Ae. aegypti*, and *Ae. albopictus* (Table 1). The only mosquito species (*Cx. quinquefasciatus*) recorded from 1222 m with the broadest range of Y coordinate (520976.32–1069193.90), X coordinate (2936816.47–3390234.75), and altitude was (94 m – 1222 m). *Cx. bitaeniorhynchus*, in comparison, had the smallest range of coordinates and altitude. Almost similar altitude and coordinates have been shared by *Cx. tritaeniorhynchus* and *Cx. pseudovishnui* (Table 1). *Cx. malayi* has a very extensive range, whereas *Lt.*

*fuscans* has a relatively narrow range followed by *Cx. fuscocephala*, and *Cx. minutissimus*. Compared to *Mi. chamberlaini*, *Cq. crassipes* has a greater range of latitude.

#### 4. Discussion

##### 4.1. Anopheline species

The results for *Anopheles nigerrimus* are consistent with previous study reporting its presence in agricultural areas, particularly in rice fields and around cities under an open sunlight to moderate shade up to 300 m elevation above sea level (Harrison & Scanlon, 1975). *An. stephensi* was found in rural areas throughout the summer in Noorpur (sandy desert 2, Thal). An earlier investigation from South Punjab, Pakistan did not discover *An. stephensi* from such areas (Herrel et al., 2001). The findings addressing the existence of *An. subpictus* larvae differ as the earlier study sampled waterways and fishponds, but these habitats were not used for the current assessment (Herrel et al., 2001).

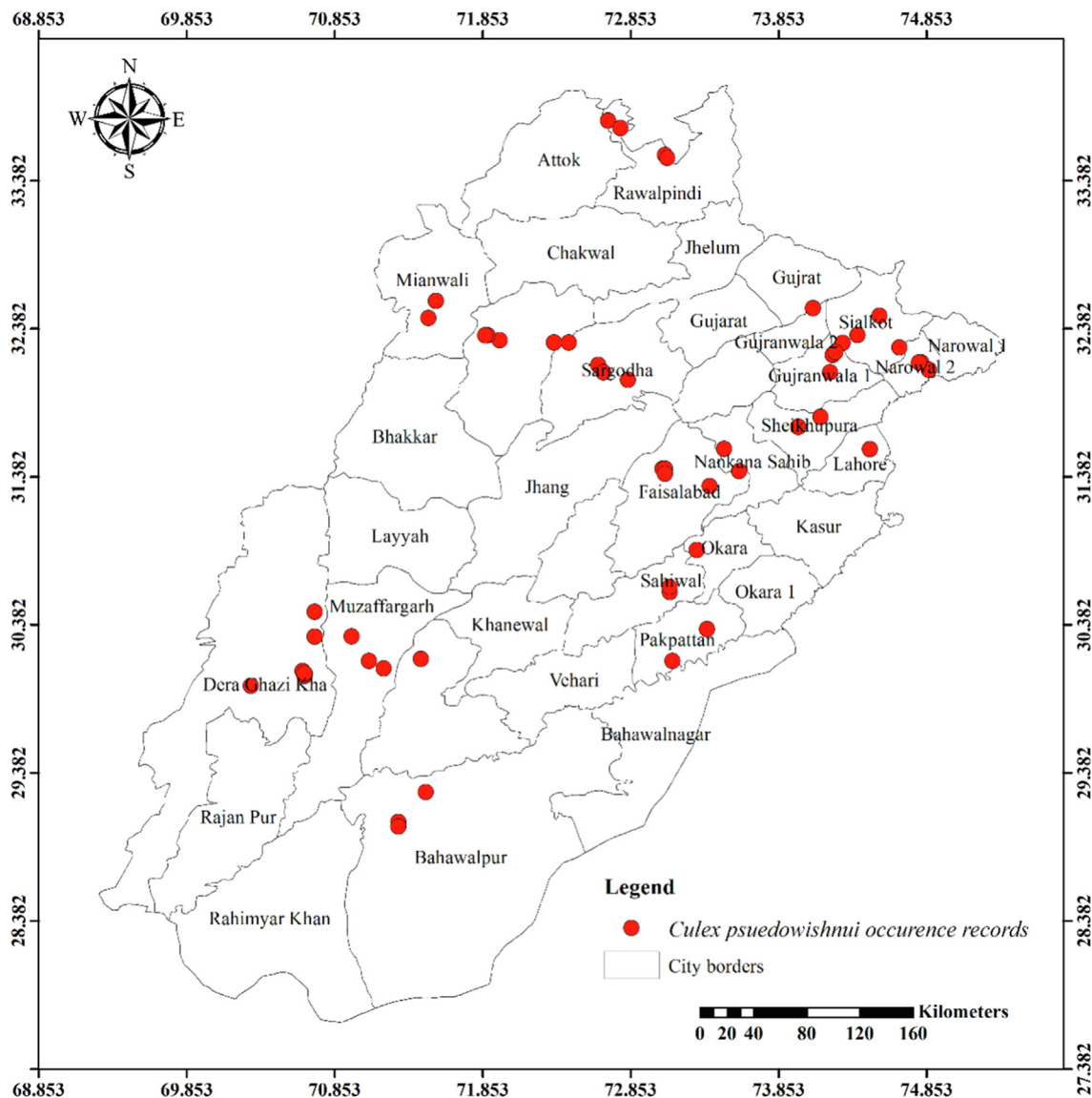


Fig. 6. Distribution of *Culex pseudovishnui* in different districts of Punjab province.



### 4.2. Aedine species

*Aedes pseudotaeniatus* was documented during the current investigation from an altitude of 1222 m, which is consistent with earlier studies reporting these species from altitudes of 420–3200 m and 340–3230 m, respectively (Rao et al., 1973). The major vector of dengue hemorrhagic fever (DHF) and dengue fever is *Ae. aegypti* (Ashfaq et al., 2014; Becker et al., 2020; Manzoor et al., 2020). Previously this species has not been reported from Punjab. However, it is widely spread throughout the Indian subcontinent. It was captured by Qutubuddin in the Kohat-Hangu valley in KPK, Pakistan, in 1960. As a result, it was initially reported from the Punjab about specific places covered in our study. (Chareonviriyaphap et al., 2003) reported this species from southern latitudes in Thailand; however, it was observed at northern latitudes in our study. Globally, *Ae. albopictus* is the most invasive mosquito species and a carrier of several viral diseases, also known as the Asian tiger mosquito (Benedict et al., 2007). In the present study, *Ae. aegypti* reported from Changa Manga National Forest, Punjab, Pakistan. It was also found in northern Punjab, including Sargodha, Sialkot, Gujrat, Mianwali, Lahore, Gujranwala, Faisalabad, Rawalpindi, and Pakpattan. A somewhat large species of

mosquito is *Ar. subalbatus*, observations regarding its biting habits are in line with earlier studies (Aslamkhan & Salman, 1969; Suleman et al., 1993). During the rainy season, immatures were collected from the water present in old tires located in the center of Sargodha. Even though *Ae. caspius* was frequently reported from Pakistan, earlier researchers did not record it from the Punjab (Mukhtar et al., 2003; Reisen & Boreham, 1979). It was documented in our study from south to north from cities in Punjab such as Bahawalpur and Rawalpindi.

### 4.3. Culicine species

The larvae of *Culex fuscocephala* and *Cx. pseudovishnui* were recorded in all seasons under present study but larvae of former species were not recorded from winter and of later species from winter and summer under a previous study conducted from Changa Manga National Forest, Punjab (Reisen & Boreham, 1979). This species was classified as a subspecies of *Cx. pipiens* as *fatigans* (Reisen et al., 1981). It is now considered a distinct species from *pipiens*, and *quinquefasciatus* is now regarded as an acceptable and legitimate name in place of *fatigans*. In our survey, *Cx. tritaeniorhynchus* was recovered from a variety of ground pools. Only

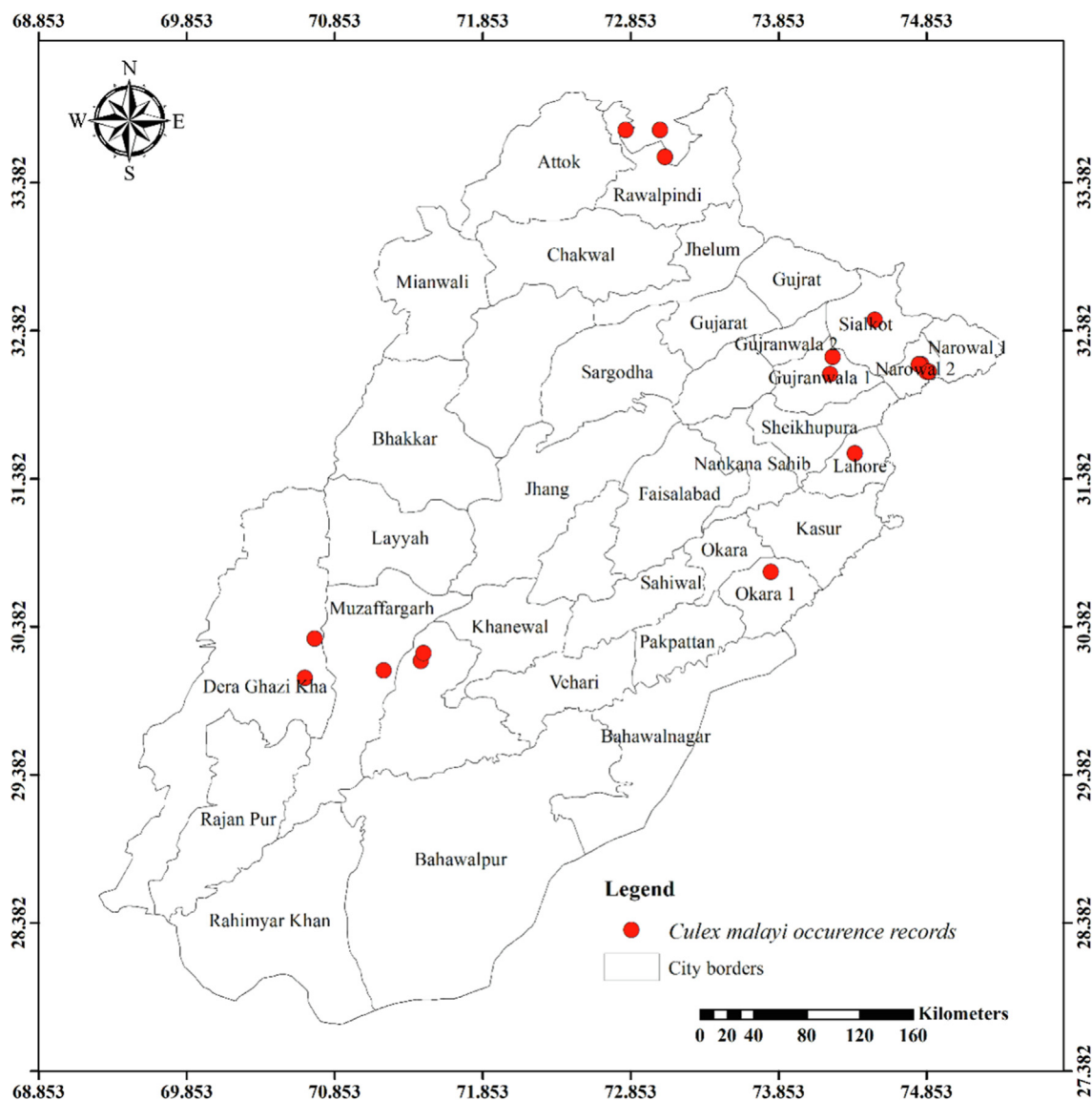


Fig. 7. Distribution of *Culex malayi* in different districts of Punjab province.

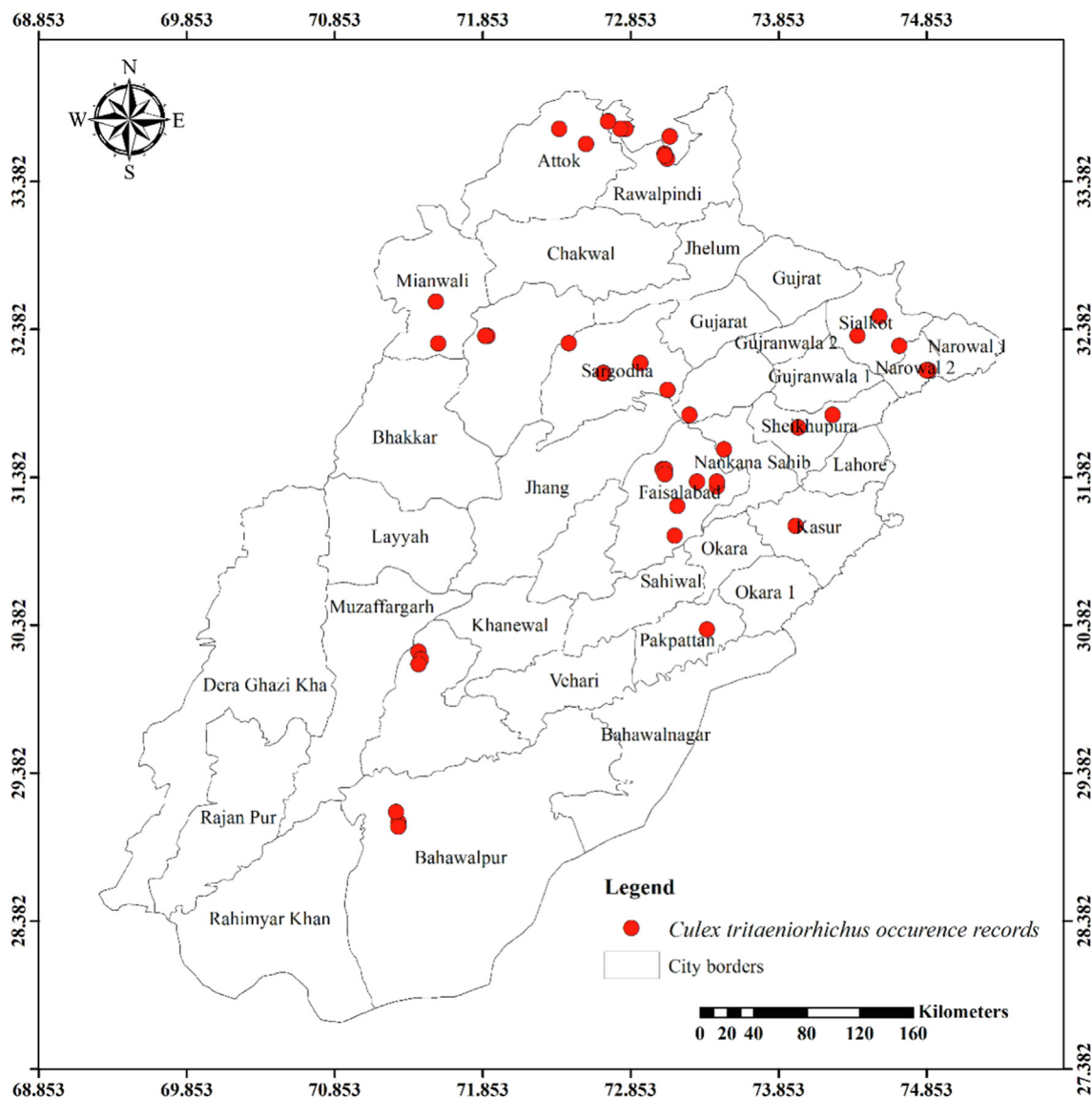


Fig. 8. Distribution of *Culex tritaeniorhynchus* in different districts of Punjab province.

Faisalabad was the source of *Lt. fuscans* in the current survey. The observations made in relation to its relationship are adjacent to those made with *Cx. quinquefasciatus* by (Reisen et al., 1981).

**5. Conclusion**

In the present study, a total of twenty-four species of mosquitoes belonging to the Anophelinae and Culicinae subfamilies were recorded. The subfamily Anophelinae had a total of three species. There were a total of eleven species of Culicinae that were classified under the tribe Aedini, while eight species were categorized under the tribe Culicini. Additionally, there was one species each that belonged to the tribes Mansoniini and Ficalbiini. The distribution maps of the species provide valuable insights for their site-specific management.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**References**

Adeleke, M.A., Mafiana, C.F., Idowu, A.B., Adekunle, M.F., Sam-Wobo, S.O., 2008. Mosquito larval habitats and public health implications in Abeokuta, Ogun State, Nigeria. *Tanzan. J. Health Res.* 10 (2). <https://doi.org/10.4314/thrb.v10i2.14348>.

Ahmad, A., Khan, M., Shah, S. H. H., Kamran, M., Wajid, S. A., Amin, M., Khan, A., Arshad, M. N., Cheema, M. J. M., & Saqid, Z. A. 2019. Agro-ecological zones of Punjab, Pakistan.

Andrade Jr, H.F., 1995. Bruce-Chwatt's essential malariology. *Rev. Inst. Med. Trop. Sao Paulo* 37 (1). <https://doi.org/10.1590/s0036-46651995000100019>.

Ashfaq, M., Hebert, P.D.N., Mirza, J.H., Khan, A.M., Zafar, Y., Mirza, M.S., 2014. Analyzing mosquito (Diptera: Culicidae) diversity in Pakistan by DNA barcoding. *PLoS One* 9 (5). <https://doi.org/10.1371/journal.pone.0097268>.

Aslamkhan, M., & Salman, C. 1969. the Bionomics of the Mosquitoes of the Changa Manga National Forest, West Pakistan. *J&V*, July 1969.

Attullah, M., Gul, S., Bibi, D., Andaleeb, A., Ilahi, I., Siraj, M., Ahmad, M., Ullah, I., Ali, M., Ahmad, S., Ullah, Z., 2023. Diversity, distribution and relative abundance of the mosquito fauna (Diptera: Culicidae) of Malakand and Dir Lower, Pakistan. *Braz. J. Biol.* 83. <https://doi.org/10.1590/1519-6984.247374>.

- Barik, T. K., Sahu, B., & Swain, V. 2009. A review on Anopheles culicifacies: From bionomics to control with special reference to Indian subcontinent. In *Acta Tropica* (Vol. 109, Issue 2). <https://doi.org/10.1016/j.actatropica.2008.09.017>.
- Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M.B., Dahl, C., Kaiser, A., 2020. *Mosquitoes : Identification, Ecology and Control. Fascinating. Life Sci.*
- Benedict, M.Q., Levine, R.S., Hawley, W.A., Lounibos, L.P., 2007. Spread of the tiger: Global risk of invasion by the mosquito *Aedes albopictus*. *Vector-Borne and Zoonotic Diseases* 7 (1). <https://doi.org/10.1089/vbz.2006.0562>.
- Chareonviriyaphap, T., Akwatanakul, P., Nettanomsak, S., Huntamai, S., 2003. Larval habitats and distribution patterns of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), in Thailand. *Southeast Asian J. Trop. Med. Public Health* 34 (3).
- Cockerell, T.D.A., 1925. The Fauna of British India, including Ceylon and Burma. Oligochaeta. Science. <https://doi.org/10.1126/science.67-b>.
- Dahmana, H., & Mediannikov, O. 2020. Mosquito-borne diseases emergence/resurgence and how to effectively control it biologically. In *Pathogens* (Vol. 9, Issue 4). <https://doi.org/10.3390/pathogens9040310>.
- DeGroot, J., Mercer, D. R., Fisher, J., & Sugumaran, R. 2007. Spatiotemporal investigation of adult mosquito (diptera: Culicidae) populations in an Eastern Iowa County, USA. *Journal of Medical Entomology*, 44(6). [https://doi.org/10.1603/0022-2585\(2007\)44\[1139:SIOAMD\]2.0.CO;2](https://doi.org/10.1603/0022-2585(2007)44[1139:SIOAMD]2.0.CO;2).
- Demetrioff, J., 2020. Miasma: Malaria's Breeding Grounds and Effects on Ancient Rome. *Past Imperfect* 22 (1), 3–32.
- ESRI (Environmental Systems Resource Institute). 2012. ArcMap 10.1. In ESRI.
- Ferraguti, M., Martínez-de la Puente, J., Bruguera, S., Millet, J.P., Rius, C., Valsecchi, A., Figuerola, J., Montalvo, T., 2023. Spatial distribution and temporal dynamics of invasive and native mosquitoes in a large Mediterranean city. *Sci. Total Environ.* 896. <https://doi.org/10.1016/j.scitotenv.2023.165322>.
- Glidden, C. K., Nova, N., Kain, M. P., Lagerstrom, K. M., Skinner, E. B., Mandle, L., Sokolow, S. H., Plowright, R. K., Dirzo, R., De Leo, G. A., & Mordecai, E. A. 2021. Human-mediated impacts on biodiversity and the consequences for zoonotic disease spillover. In *Current Biology* (Vol. 31, Issue 19). <https://doi.org/10.1016/j.cub.2021.08.070>.
- Harrison, B.A., Scanlon, J.E., 1975. Medical entomology studies - II. The subgenus *Anopheles* in Thailand (Diptera: Culicidae). *Contribution of the American Entomology Institute* 12 (1334).
- Herrel, N., Amerasinghe, F.P., 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 (3). <https://doi.org/10.1046/j.0269-283X.2001.00312.x>.
- Hoare, C.A., 1940. The Fauna of British India, including Ceylon and Burma. *Nature* 146 (3699). <https://doi.org/10.1038/146384a0>.
- Holt, R.A., Mani Subramanian, G., Halpern, A., Sutton, G.G., Charlab, R., Nusskern, D. R., Wincker, P., Clark, A.G., Ribeiro, J.M.C., Wides, R., Salzberg, S.L., Loftus, B., Yandell, M., Majoros, W.H., Rusch, D.B., Lai, Z., Kraft, C.L., Abril, J.F., Anthouard, V., Hoffman, S.L., 2002. The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science* 298 (5591). <https://doi.org/10.1126/science.1076181>.
- Jabeen, A., Ansari, J. A., Ikram, A., Khan, M. A., & Safdar, M. 2022. Impact of climate change on the epidemiology of vector-borne diseases in Pakistan. In *Global Biosecurity* (Vol. 4). <https://doi.org/10.31646/gbio.163>.
- Janson, K.D., Carter, B.H., Jameson, S.B., de Verges, J.E., Dalliance, E.S., Royse, M.K., Kim, P., Wesson, D.M., Veisoh, O., 2023. Development of an automated biomaterial platform to study mosquito feeding behavior. *Front. Bioeng. Biotechnol.* 11. <https://doi.org/10.3389/fbioe.2023.1103748>.
- Kain, M.P., Skinner, E.B., Athni, T.S., Ramirez, A.L., Mordecai, E.A., van den Hurk, A.F., 2022. Not all mosquitoes are created equal: A synthesis of vector competence experiments reinforces virus associations of Australian mosquitoes. *PLoS Negl. Trop. Dis.* 16 (10), e0010768.
- Khan, J., Tarin Khan, B., Naeem, M., Ismail, M., Ali Khan, S., Shah, M., Abbasi, A., 2015. A survey of adult and larval mosquito fauna in Tehsil Daggar and Gagra of District Buner, Khyber Pakhtunkhwa, Pakistan. *International Journal of Mosquito Research IJMR* 170 (23).
- Manzoor, F., Shabbir, R., Sana, M., Nazir, S., Khan, M.A., 2020. Determination of species composition of mosquitoes in Lahore, Pakistan. *J. Arthropod. Borne Dis.* 14 (1) <https://doi.org/10.18502/jad.v14i1.2717>.
- Moreno-Madrinán, M.J., Turell, M., 2018. History of mosquito-borne diseases in the United States and implications for new pathogens. *Emerg. Infect. Dis.* 24 (5). <https://doi.org/10.3201/eid2405.171609>.
- Mujtaba, A., Nabi, G., Masood, M., Iqbal, M., Asfahan, H.M., Sultan, M., Majeed, F., Hensel, O., Nasirahmadi, A., 2022. Impact of Cropping Pattern and Climatic Parameters in Lower Chenab Canal System—Case Study from Punjab Pakistan. *Agriculture (Switzerland)* 12 (5). <https://doi.org/10.3390/agriculture12050708>.
- Mukhtar, M., Herrel, N., Amerasinghe, F.P., Ensink, J., Van Der Hoek, W., Konradsen, F., 2003. Role of wastewater irrigation in mosquito breeding in south Punjab, Pakistan. *Southeast Asian J. Trop. Med. Public Health* 34 (1).
- Naeem-Ullah, U., Akram, W., Suhail, A., Rana, S.A., 2010. Grouping of different mosquito species on the bases of larval habitats. *Pak. J. Agric. Sci.* 47 (2).
- Qasim, M., Naeem, M., Bodlah, I., 2014. Mosquito (Diptera: Culicidae) of murree hills, Punjab, Pakistan. *Pakistan Journal of Zoology* 46 (2).
- Rao, T.R., Dhanda, V., Bhat, H.R., Kulkarni, S.M., 1973. A survey of haematophagous arthropods in Western Himalayas, Sikkim and hill districts of West Bengal. *A general account. Indian J. Med. Res.* 61 (10).
- Reisen, W.K., Boreham, P.F.L., 1979. Host selection patterns of some Pakistan mosquitoes. *Am. J. Trop. Med. Hyg.* 28 (2). <https://doi.org/10.4269/ajtmh.1979.28.408>.
- Reisen, W.K., Siddiqui, T.F., Aslamkhan, M., Malik, G.M., 1981. Larval interspecific associations and physico-chemical relationships of the ground-water breeding mosquitoes of Lahore [Includes *Ficalbia chamberlaini* clavipalpus]. *Pak. J. Sci. Res.*
- Sallares, R., 2002. 6 The Pontine Marshes. In: *Malaria and Rome*. Oxford University Press/Oxford, pp. 168–191. <https://doi.org/10.1093/acprof:oso/9780199248506.003.0006>.
- Sangbakembi-Ngounou, C., Costantini, C., Longo-Pendy, N. M., Ngoagouni, C., Akone-Ella, O., Rahola, N., Cornelie, S., Kengne, P., Nakouné, E. R., Komas, N. P., & Ayala, D. 2022. Diurnal biting of malaria mosquitoes in the Central African Republic indicates residual transmission may be “out of control.” *Proceedings of the National Academy of Sciences of the United States of America*, 119(21). <https://doi.org/10.1073/pnas.2104282119>.
- Seth-Smith, D., 1942. The Fauna of British India: including Ceylon and Burma. *Nature* 149 (3770). <https://doi.org/10.1038/149122a0>.
- Suleman, M., Khan, K., Khan, S., 1993. Ecology of mosquitoes in Peshawar Valley and adjoining areas: Species composition and relative abundance. *Pak. J. Zool.* 25 (4).