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GEOGRAPHICAL DISTRIBUTION OF MOSQUITOES IN PAKISTAN

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Abstract

Although mosquitoes are vectors of many diseases, the biodiversity of mosquitoes is poorly studied in Pakistan. This study was conducted to determine the recent trends in diversity and distribution of mosquito species. For this purpose, total of 14,013 specimens were collected from 14 districts of Pakistan during 2018-19. Both immature and adult mosquitoes were collected by knockdown, aspirator, and sweep net from various habitats including ponds, farmhouses, animal sheds, residential areas, gutters, etc. Various taxonomic keys were used to identify them upto species level. Relative abundance and distribution of mosquitoes were calculated. A total of 29 species were identified during the study, which belongs to five genera i.e. Culex, Anopheles, Aedes, Mansonia, and Armigeres. Moreover, the Culex species were found to be the most abundant and constant genus across Pakistan among the collected mosquitoes, which were followed by Anopheles, and Aedes, respectively. Mansonia and Armigeres have very low diversity in Pakistan. Although, no new species were detected but this study will provide information about the current status of biodiversity, distribution and abundance of mosquitoes in Pakistan. The pattern of distribution of mosquito species could be used to predict mosquito-borne infectious diseases in Pakistan.

Keywords: Abundance, Distribution, Geographical, Mosquitoes, Pakistan

INTRODUCTION

Many arthropods act as vectors of diseases. Among them, mosquitoes are the most important as they are notoriously responsible for causing greater misery to mankind (1). Mosquitoes serve to transmit protozoans, helminths, and viruses. On the other hand, they also cause discomfort, insomnia, frustration, and blood loss in the host. Millions of individuals acquired mosquito-borne diseases such as yellow fever, dengue, and malaria each year (2). World Health Organization estimated that there were between 50 and 200 million dengue infections and roughly 20,000 fatalities worldwide in 2015. Over 225 million people worldwide suffer malaria every year, and 800,000 people die; of these, 85% are children under the age of five. Various human diseases have been reported to be transmitted by species from the genera Culex, Anopheles, Armigeres, and Aedes that act as disease vectors (3). Genus Culex is reported as the vector of Japanese encephalitis, and filariasis, while mosquitoes of genus Armigeres are responsible for the transmission of helminthic infections like lymphatic filariasis and dog heartworm (4). Malaria and dengue are caused by different species of Anopheles and Aedes mosquitoes. Anopheles contains more than 400 species of which 40 species can transmit malaria to humans (5). Malaria is among the most devastating vector-borne diseases caused by parasites (*Plasmodium spp.*). The spread of these diseases is influenced by changing meteorological factors, as well as economic, political and social conditions. In many cases the impact of these non-climatic factors is greater than that of climatic reasons (6). Due to the abundance, biodiversity, vector capability, and recurrent infections, mosquitoes are the most studied group of insects (7).

To control all mosquito-transmitted diseases in an area, it is necessary to focus on the vector to map and keep track of the mosquitoes' geographic dispersion (8, 9). For this purpose, accurate species identifications are needed to reduce the waste of limited resources on controlling those species that are not the vectors of pathogens or more resources investing in naturally resistant species and particular control methods. Different species of mosquitoes (even within a genus) vary widely in their ability to transmit







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disease, resistance mechanisms against vector control strategies, their resting and feeding behaviors, and their habitats (10). In these circumstances, accurate species-level information is required to target relevant species present in a given area by applying proper vector control measures (11). The aim of the study is to determine the distribution, diversity and abundance of the mosquitoes in different districts of Pakistan to have insights about most prevalent species found in each area, which could potentially contribute to the occurrence of any kind of outbreak in near future.

MATERIALS AND METHODS

STUDY AREA

Mosquitoes were collected from various districts of Punjab, Sindh, Baluchistan, and Khyber Pakhtunkhwa provinces of Pakistan. A total of 14 districts were surveyed to study the distribution and diversity of mosquitoes. Among these, 6 districts belong to Punjab, 4 districts to Sindh, two districts to Khyber Pakhtunkhwa, and one district to Baluchistan (Fig. 1).

COLLECTION OF ADULT MOSQUITOES

The adult mosquitoes were collected from human dwellings and animal sheds during swarming, and resting conditions. Mosquitoes were collected by using mouth aspirators, sweep nets, and knock-down methods. A mouth aspirator is used to capture adult mosquitoes resting on walls indoors and outdoors. The indoor collection was done by the knockdown method. For the knockdown of adult mosquitoes, commercial Pyrethroid spray or coils of the Mortein brand were used. The collection was done by simply spraying thoroughly inside closed rooms. Large white sheets were laid on the floor to cover all surfaces in each room before spraying. After 10-15 minutes, all immobilized mosquitoes were collected carefully with forceps. Mosquitoes were then placed in Petri dishes containing moist filter paper and then placed in a plastic cooler. For outdoor collections, sites were identified where mosquitoes swarm and congregate. Collections were carried out by sweep nets when mosquitoes exhibited swarming behaviors.

COLLECTION OF MOSQUITO LARVAE

Larvae and pupae were also collected from randomly selected larval habitats present at each site. Natural (e.g. ponds, puddles, sewerage water and garbage) and artificial (e.g. old discarded tyres) breeding sites were considered (12, 13). The larval collection was done by using a standard dipper (400ml) with a one-meter handle. They were transported to the Institute of Zoology, the University of Punjab, Lahore, in plastic containers. While waiting for the emergence of adults all larvae and pupae were kept at room temperature in plastic containers covered with a net (14, 15). Water (300- 500 ml) collected with larvae contains an adequate amount of food for larvae so no artificial food was given. A small hole was made in the net to collect adult mosquitoes and kept close by a cotton swab. Each container was labelled properly, with locality, date of collection, and collector name, counted, and recorded.

MORPHOLOGICAL IDENTIFICATIONS

Adult mosquitoes were transferred to a lethal chamber and killed by using 90% ethanol fumes (16). All the collected mosquitoes were identified up to the species level by using a stereomicroscope. The mosquitoes which were damaged or have broken body parts were not included in the study (17). For identification, various morphological keys were followed such as "The fauna of British India, including Ceylon and Burma" by Christophers (18); Barraud (19); Edwards (20); Gillies and De Meillon (21); Jupp, McIntosh (22); Harbach (23) etc. In addition, available dichotomous keys and original species descriptions were compiled through literature freely available on the WRBU website (<u>www.wrbu.org</u>) (16). All identified mosquitoes were individually transferred to labeled 1.5 ml tubes. Each tube was pierced with a mounted needle (to allow moisture escape) and placed in plastic bags already containing silica gel.





Fig. 1. Mosquito collection sites from different districts of Pakistan

DATA ANALYSIS

DISTRIBUTION OF MOSQUITOES

All the collected mosquitoes were analyzed by relative abundance (RA) and distribution (C). These were calculated following the formulas as described by Ali, Khan (24); Rydzanicz and Lonc (25); and Sengil, Akkaya (26). To calculate relative abundance (RA), the following formula was used:

$$RA = \frac{1}{L} \times 10^{\circ}$$

(Where, l= number of specimens of a species collected; L = Total number of specimens collected)

The results of RA values were interpreted as described by Trojan (27) as follows: RA > 5% = Dominant species, RA < 5% = Sub-dominant, and RA < 1% = Satellite.

The following formula was used to calculate distribution;

$$C = \frac{n}{N} \times 100$$

(Where, n = number of sites where a particular species was found; N = Total number of sites)

The values of distribution (C) were interpreted as described by (28): Sporadic species if C = 0-20%; Infrequent species if C = 20.1-40%; Moderate species if C = 40.1-60%; Frequent species if C = 60.1-80%; and Constant species if C = 80.1-100%.

RESULTS

MOSQUITO DIVERSITY IN PAKISTAN

A total of 14,013 mosquito specimens were collected from 14 districts of Pakistan (Fig. 2). These specimens belong to 29 species and 5 genera of mosquitoes viz. *Culex, Aedes, Anopheles, Armigeres,* and *Mansonia.*

From district Lahore, a total of 1629 mosquitoes were collected from 13 sites. They belonged to 13 species and 4 genera. Of which *Cx. quinquefasciatus* was the most dominant species. *Mansonia uniformis* was collected in low numbers. In terms of distribution status, *M. uniformis* and *An. nigerrimus* were sporadic, while *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* were constant species in district Lahore. *An. stephensi, An. subpictus*, and *An. albopictus* were dominant and frequent in distribution. *Cx. theleri, An. annularis, Cx. sitiens,*



and *An. pulcherimus* were dominant and infrequent. *Ae. aegypti,* and *Cx. vagans* were found to be subdominant and infrequent (Table I).

From district Gujrat, total 1966 mosquitoes were collected from 11 sites. They belonged to 14 species and 4 genera of which Cx. quinquefasciatus and Ae. albopictus were the most dominant species, followed by Ae. cogilli, Cx. tritaeniorhynchus, Cx. bitaeniorhynchus, Cx. vishnui, and An. peditaeniatus. While An. stephensi, Ae. aegypti, An. annularis, Cx. fuscocephala, An. nigerrimus and Armigeres obturbans were found to be sub-dominant. Armigeres subalbatus was the only species which was satellite in Gujrat. Cx. quinquefasciatus, Ae. albopictus Ae. cogilli, and Cx. tritaeniorhynchus were distributed throughout the district (constant in distribution). Cx. bitaeniorhynchus, Cx. vishnui, and An. peditaeniatus were frequent in distribution, while An. stephensi, Ae. aegypti, An. annularis, and Cx. fuscocephala were moderately distributed.



Fig. 2. Distribution of mosquito species in different districts of Pakistan Table I. Relative abundance and distribution of mosquito species in district Lahore, Pakistan

Sr.	Species	No. of	RA*	Status	C*	Status
190.		specimens				
1	Cx. quinquefasciatus	376	23.08	Dominant	92.31	Constant
2	Cx. tritaeniorhynchus	339	20.81	Dominant	84.62	Constant
3	An. stephensi	143	8.78	Dominant	53.85	Frequent
4	An. subpictus	109	6.69	Dominant	46.15	Frequent
5	Ae. albopictus	106	6.51	Dominant	46.15	Frequent
6	Cx. theleri	104	6.38	Dominant	38.46	Infrequent
7	An. annularis	102	6.26	Dominant	38.46	Infrequent
8	Cx. sitiens	87	5.34	Dominant	38.46	Infrequent
9	An. pulcherimus	82	5.03	Dominant	30.77	Infrequent
10	Ae. aegypti	78	4.79	Sub-dominant	30.77	Infrequent
11	Cx. vagans	62	3.81	Sub-dominant	23.08	Infrequent
12	An. nigerrimus	24	1.47	Sub-dominant	15.38	Sporadic
13	M. uniformis	17	1.04	Sub-dominant	15.38	Sporadic
	Total	1629				

*Abbreviations: RA=Relative Abundance; C=Distribution

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An. nigerrimus was infrequently distributed in Gujrat. Both species of *Armigeres* i.e. *Armigeres obturbans* and *Armigeres subalbatus* were found to be sporadic (Table II).

A total of 799 mosquitoes were collected from district Chakwal. They were identified as 12 species and 4 genera of mosquitoes. Among them *Cx. quinquefasciatus* was found to be the most dominant and constantly distributed in the Chakwal. *An. stephensi, Cx. tritaeniorhynchus, Cx. bitaeniorhynchus,* and *Cx. fuscocephala* were dominant species and frequently distributed in the area. *Ae. albopictus* and *Ae. aegypti* were also dominant but they were moderately distributed in Chakwal. *Ar. obturbans, Ae. cogilli,* and *Ar. subalbatus* were found to be sub-dominant and infrequently distributed species. *Cx. univittatus,* and *Cx. vishnui* were satellite species and sporadic in distribution (Table III).

From Narowal, 1354 mosquitoes were collected that belong to 19 species and 5 genera. Of these *Cx. quinquefasciatus, Cx. tritaeniorhynchus, An. nigerrimus,* and *Cx. bitaeniorhynchus* were dominant and constantly distributed species in Narowal. *An. stephensi, Cx. fuscocephala, An. annularis, Ae. albopictus,* and *An. maculatus* were also dominant species in the study area but they were frequently distributed. *Cx. vishnui* was the only species which was sub-dominant and frequently distributed in Narowal. *Ar. obturbans, Cx. sitiens, An. culicifacies, Cx. theileri,* and *Cx. pseudovishnui* were sub-dominant and moderately distributed species in district Narowal. *Ae. w-albus, Cx. vagans, Ma. uniformis,* and *An. subpictus* were sub-dominant and infrequently distributed species (Table IV).

Sr. No.	Species	No. of specimens	RA*	Status	C*	Status
1	Cx. quinquefasciatus	391	19.89	Dominant	100.00	Constant
2	Ae. albopictus	344	17.50	Dominant	100.00	Constant
3	Ae. cogilli	260	13.22	Dominant	81.82	Constant
4	Cx. tritaeniorhynchus	252	12.82	Dominant	81.82	Constant
5	Cx. bitaeniorhynchus	164	8.34	Dominant	72.73	Frequent
6	Cx. vishnui	126	6.41	Dominant	72.73	Frequent
7	An. peditaeniatus	100	5.09	Dominant	63.64	Frequent
8	An. stephensi	93	4.73	Sub-dominant	54.55	Moderate
9	Ae. aegypti	59	3.00	Sub-dominant	45.45	Moderate
10	An. annularis	53	2.70	Sub-dominant	45.45	Moderate
11	Cx. fuscocephala	48	2.44	Sub-dominant	45.45	Moderate
12	An. nigerrimus	42	2.14	Sub-dominant	36.36	Infrequent
13	Armigeres obturbans	20	1.02	Sub-dominant	18.18	Sporadic
14	Armigeres subalbatus	14	0.71	Satellite	18.18	Sporadic
	Total	1966				

Table II. Relative abundance and distribution of mosquito species in district Gujrat, Pakistan

*Abbreviations: RA=Relative Abundance; C=Distribution

Table III. Relative abundance and distribution of mosquito species in district Chakwal, Pakistan								
Sr. No.	Species	No. of specimens	RA*	Status	C *	Status		
1	Cx. quinquefasciatus	202	25.28	Dominant	87.50	Constant		
2	An. stephensi	119	14.89	Dominant	75.00	Frequent		
3	Cx. tritaeniorhynchus	104	13.02	Dominant	75.00	Frequent		
4	Cx. bitaeniorhynchus	94	11.76	Dominant	62.50	Frequent		
5	Cx. fuscocephala	85	10.64	Dominant	62.50	Frequent		
6	Ae. albopictus	68	8.51	Dominant	50.00	Moderate		
7	Ae. aegypti	44	5.51	Dominant	50.00	Moderate		
8	Ar. obturbans	33	4.13	Sub-dominant	37.50	Infrequent		
9	Ae. cogilli	25	3.13	Sub-dominant	37.50	Infrequent		
10	Ar. subalbatus	17	2.13	Sub-dominant	37.50	Infrequent		
11	Cx. univittatus	5	0.63	Satellite	12.50	Sporadic		
12	Cx. vishnui	3	0.38	Satellite	12.50	Sporadic		



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	Total	799				
*Abbreviat	tions: RA=Relative Abundar	ce; C=Distribution	l			
	Table IV. Relative abundation	ance and distribution	on of mosquit	o species in district Na	arowal, Pakista	an
Sr. No.	Species	No. of specimens	RA*	Status	C*	Status
1	Cx. quinquefasciatus	188	13.88	Dominant	100.00	Constant
2	Cx. tritaeniorhynchus	132	9.75	Dominant	100.00	Constant
3	An. nigerrimus	124	9.16	Dominant	88.89	Constant
4	Cx. bitaeniorhynchus	109	8.05	Dominant	88.89	Constant
5	An. stephensi	97	7.16	Dominant	77.78	Frequent
6	Cx. fuscocephala	92	6.79	Dominant	77.78	Frequent
7	An. annularis	89	6.57	Dominant	77.78	Frequent
8	Ae. albopictus	79	5.83	Dominant	77.78	Frequent
9	An. maculatus	74	5.47	Dominant	66.67	Frequent
10	Cx. vishnui	64	4.73	Sub-dominant	66.67	Frequent
11	Ar. obturbans	58	4.28	Sub-dominant	55.56	Moderate
12	Cx. sitiens	46	3.40	Sub-dominant	55.56	Moderate
13	An. culicifacies	43	3.18	Sub-dominant	55.56	Moderate
14	Cx. theileri	41	3.03	Sub-dominant	55.56	Moderate
15	Cx. pseudovishnui	31	2.29	Sub-dominant	44.44	Moderate
16	Ae. w-albus	28	2.07	Sub-dominant	33.33	Infrequent
17	Cx. vagans	25	1.85	Sub-dominant	33.33	Infrequent
18	Ma. uniformis	17	1.26	Sub-dominant	22.22	Infrequent
19	An. subpictus	17	1.26	Sub-dominant	22.22	Infrequent
	Total	1354				

Total 1030 specimens were collected from Faisalabad. They were identified as 16 species and 3 genera. *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* were found to be dominant and constant species in Faisalabad. *Ae. albopictus, An. stephensi, Ae. cogilli, An. culicifacies, Cx. theileri,* and *Ae. aegypti* were dominant and frequently distributed species in Faisalabad. *Cx. pseudovishnui, Cx. vishnui,* and *An. peditaeniatus* were dominant and moderately distributed species. The only sub-dominant and moderately distributed species was *An. pulcherrimus. An. annularis, An. nigerrimus* were sub-dominant species which were infrequently distributed in Faisalabad. *Ae. unilineatus* was sub-dominant and sporadic in distribution. *Ae. w-albus* was satellite and sporadic (Table V).

From district Bahawalpur, 905 mosquitoes were collected. They belonged to 13 species and 5 genera. *Cx. quinquefasciatus, Cx. bitaeniorhynchus, Cx. tritaeniorhynchus,* and *An. culicifacies* were dominant and frequently distributed species in Bahawalpur. *An. peditaeniatus, An. stephensi, Cx. theileri, and An. pulcherrimus* were dominant and moderately distributed species. *Ae. albopictus, Ar. subalbatus, Ae. caspius, Ma. uniformis,* and *An. subpictus* were found to be sub-dominant and infrequently distributed species in Bahawalpur (Table VI).

Total 559 mosquito samples were collected from Rahim Yar Khan. They belonged to 13 species and 5 genera. Among these, Cx. quinquefasciatus and Cx. tritaeniorhynchus were dominant and constant in distribution. Cx. bitaeniorhynchus, An. peditaeniatus, An. culicifacies, An. stephensi, An. pulcherrimus, and Ae. albopictus were also dominant species but these were frequently distributed in Rahim Yar Khan. Ae. aegypti was found to be a sub-dominant and frequently distributed species. Ar. subalbatus, and An. subpictus were sub-dominant and moderately distributed species in the study area. Ma. uniformis was sub-dominant and infrequently distributed species in the study area to be satellite and infrequently distributed species in Rahim Yar Khan (Table VII).

Sr. No.	Caradian	No. of	D A *	Chabra	C*	Chabre
	Species	specimens	KA	Status	C	Status
1	Cx. quinquefasciatus	156	15.15	Dominant	89.47	Constant
2	Cx. tritaeniorhynchus	109	10.58	Dominant	84.21	Constant
3	Ae. albopictus	99	9.61	Dominant	78.95	Frequent
4	An. stephensi	92	8.93	Dominant	78.95	Frequent
5	Ae. cogilli	87	8.45	Dominant	73.68	Frequent
6	An. culicifacies	64	6.21	Dominant	68.42	Frequent
7	Cx. theileri	63	6.12	Dominant	68.42	Frequent
8	Ae. aegypti	61	5.92	Dominant	63.16	Frequent
9	Cx. pseudovishnui	58	5.63	Dominant	57.89	Moderate
10	Cx. vishnui	56	5.44	Dominant	57.89	Moderate
11	An. peditaeniatus	53	5.15	Dominant	52.63	Moderate
12	An. pulcherrimus	49	4.76	Sub-dominant	42.11	Moderate
13	An. annularis	36	3.50	Sub-dominant	31.58	Infrequent
14	An. nigerrimus	23	2.23	Sub-dominant	21.05	Infrequent
15	Ae. unilineatus	17	1.65	Sub-dominant	15.79	Sporadic
16	Ae. w-albus	7	0.68	Satellite	10.53	Sporadic
	Total	1030				

Table V Relative abundance and	distribution of mosquite	s species in district Eaisalabad	Dakistan
Table V. Relative abundance and	ulstribution of mosquite	J Species in district raisalabau,	Farislan

Sr. No.	Species	No. of specimens	RA*	Status	C*	Status
1	Cx. quinquefasciatus	124	13.70	Dominant	77.78	Frequent
2	Cx. bitaeniorhynchus	118	13.04	Dominant	77.78	Frequent
3	Cx. tritaeniorhynchus	104	11.49	Dominant	66.67	Frequent
4	An. culicifacies	101	11.16	Dominant	66.67	Frequent
5	An. peditaeniatus	93	10.28	Dominant	55.56	Moderate
6	An. stephensi	91	10.06	Dominant	44.44	Moderate
7	Cx. theileri	79	8.73	Dominant	44.44	Moderate
8	An. pulcherrimus	78	8.62	Dominant	44.44	Moderate
9	Ae. albopictus	39	4.31	Sub-dominant	33.33	Infrequent
10	Ar. subalbatus	25	2.76	Sub-dominant	33.33	Infrequent
11	Ae. caspius	21	2.32	Sub-dominant	33.33	Infrequent
12	Ma. uniformis	16	1.77	Sub-dominant	22.22	Infrequent
13	An. subpictus	16	1.77	Sub-dominant	22.22	Infrequent
	Total	905				

*Abbreviations: RA=Relative Abundance; C=Distribution

From Quetta, 10 mosquito species belonging to 3 genera were collected. *Cx. tritaeniorhynchus,* and *Cx. quinquefasciatus* were dominant and constantly distributed species in Quetta. *An. stephensi, An. culicifacies, An. subpictus,* and *An. pulcherrimus* were dominant and frequently distributed species in the study area. Whereas, *Ae albopictus* and *An. superpictus* were dominant and moderately distributed species in Quetta. *Ae aegypti* and *Ae caspius* were sub-dominant and infrequently distributed species in the study area (Table VIII).

From Mardan, 1149 specimens were collected. They were identified as 13 species and 3 genera. *Cx. quinquefasciatus* and *Cx tritaeniorhynchus* were dominant and constantly distributed species. *Cx pseudovishnui, Cx. bitaeniorhynchus,* and *An. stephensi* were dominant and frequently distributed species. *Cx. theileri, An. maculates,* and *An. annularis* were found to be dominant and moderately distributed species in the study area. *An. culicifacies, Ae. albopictus, Ae. aegypti,* and *Ae. cogilli* were sub-dominant and infrequently distributed species in Mardan. Whereas, *An. fluviatilis* was sub-dominant and sporadic in distribution (Table IX). From district Abbottabad, 569 mosquitoes were collected. They belonged to 8 species and 3 genera. All the species were found to be dominant in the study area except *Ae. aegypti,* which was sub-dominant.



According to distribution status, *Cx. quinquefasciatus, Cx. tritaeniorhynchus,* and *An. stephensi* were constantly distributed. Whereas, *Cx. theileri, An. annularis,* and *An. culicifacies* were frequently distributed species. *Ae. albopictus* was moderately distributed and *Ae. aegypti* was an infrequently distributed species in Abbottabad (Table X).

Sr. No.	Species	No. of specimens	RA*	Status	C*	Status
1	Cx. quinquefasciatus	115	20.57	Dominant	100.00	Constant
2	Cx. tritaeniorhynchus	73	13.06	Dominant	100.00	Constant
3	Cx. bitaeniorhynchus	59	10.55	Dominant	75.00	Frequent
4	An. peditaeniatus	59	10.55	Dominant	75.00	Frequent
5	An. culicifacies	58	10.38	Dominant	75.00	Frequent
6	An. stephensi	56	10.02	Dominant	75.00	Frequent
7	An. pulcherrimus	42	7.51	Dominant	75.00	Frequent
8	Ae. albopictus	35	6.26	Dominant	75.00	Frequent
9	Ae. aegypti	24	4.29	Sub-dominant	75.00	Frequent
10	Ar. subalbatus	17	3.04	Sub-dominant	50.00	Moderate
11	An. subpictus	8	1.43	Sub-dominant	50.00	Moderate
12	Ma. uniformis	8	1.43	Sub-dominant	25.00	Infrequent
13	Ae. caspius	5	0.89	Satellite	25.00	Infrequent
	Total	559				

Table VII. Relative abundance and distribution of mosquito species in district Rahim Yar Khan, Pakistan

*Abbreviations: RA=Relative Abundance; C=Distribution

Table VIII. Relative abundance and distribution of mosquito species in district Quetta, Pakistan

Sr. No.	Species	No. of specimen	RA*	Status	C *	Status
1	Cx. tritaeniorhynchus	196	20.48	Dominant	100.00	Constant
2	Cx. quinquefasciatus	182	19.02	Dominant	100.00	Constant
3	An. Stephensi	114	11.91	Dominant	75.00	Frequent
4	An. Culicifacies	94	9.82	Dominant	62.50	Frequent
5	An. Subpictus	88	9.20	Dominant	62.50	Frequent
6	An. pulcherrimus	81	8.46	Dominant	62.50	Frequent
7	Ae albopictus	69	7.21	Dominant	50.00	Moderate
8	An. Superpictus	63	6.58	Dominant	50.00	Moderate
9	Ae aegypti	43	4.49	Sub-dominant	37.50	Infrequent
10	Ae caspius	27	2.82	Sub-dominant	37.50	Infrequent
	Total	957				

*Abbreviations: RA=Relative Abundance; C=Distribution

Table IX. Relative abundance and distribution of mosquito species in district Mardan, Pak	istan
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Sr. No.	Species	No. of specimen	RA*	Status	C*	Status
1	Cx. quinquefasciatus	179	15.58	Dominant	100.00	Constant
2	Cx tritaeniorhynchus	166	14.45	Dominant	90.00	Constant
3	Cx pseudovishnui	138	12.01	Dominant	80.00	Frequent
4	Cx. bitaeniorhynchus	115	10.01	Dominant	70.00	Frequent
5	An. stephensi	103	8.96	Dominant	70.00	Frequent
6	Cx. theileri	90	7.83	Dominant	60.00	Moderate
7	An. maculates	82	7.14	Dominant	60.00	Moderate
8	An. annularis	76	6.61	Dominant	60.00	Moderate
9	An. culicifacies	56	4.87	Sub-dominant	40.00	Infrequent
10	Ae. albopictus	47	4.09	Sub-dominant	40.00	Infrequent
11	Ae. aegypti	37	3.22	Sub-dominant	30.00	Infrequent

10	Total	1149	2.52	Sub-dominant	20.00	Sporadic
13	An flumintilis	29	2 52	Sub-dominant	20.00	Sporadic
12	Ae. cogilli	31	2.70	Sub-dominant	30.00	Infrequent

Sr. No.	Species	No. of sample	RA*	Status	C*	Status
1	Cx. quinquefasciatus	122	21.44	Dominant	100.00	Constant
2	Cx. tritaeniorhynchus	108	18.98	Dominant	100.00	Constant
3	An. stephensi	79	13.88	Dominant	85.71	Constant
4	Cx. theileri	75	13.18	Dominant	71.43	Frequent
5	An. annularis	61	10.72	Dominant	71.43	Frequent
6	An. culicifacies	54	9.49	Dominant	71.43	Frequent
7	Ae. albopictus	48	8.44	Dominant	42.86	Moderate
8	Ae. aegypti	22	3.87	Sub-dominant	28.57	Infrequent
	Total	569				

*Abbreviations: RA=Relative Abundance; C=Distribution

From Karachi, 16 species and 4 genera of mosquitoes were found during this study. The dominant species of Karachi were Cx. tritaeniorhynchus, Cx quinquefasciatus, An. subpictus, An. stephensi, An. pulcherrimus, Cx. bitaeniorhynchus, An. culicifacies, An. annularis, Cx. pseudovishnui, and Ae. unilineatus. Whereas, Ae. aegypti, Cx. fuscocephala, An. nigerrimus, An. peditaeniatus, Ma. uniformis, and An. superpictus were found to be sub-dominant species in Karachi. Moreover, Cx. tritaeniorhynchus, Cx quinquefasciatus, and An. subpictus were constant species, whereas, An. stephensi, An. pulcherrimus, and Cx. bitaeniorhynchus were frequently distributed species. Moderately distributed species were An. culicifacies, An. annularis, Cx. pseudovishnui, Ae. unilineatus, Ae. aegypti, Cx. fuscocephala, An. nigerrimus, and An. peditaeniatus. Ma. uniformis and An. superpictus were infrequently distributed species in Karachi (Table XI).

A total of 922 specimens were collected from district Badin. They were identified as 14 species and 4 genera of mosquitoes. Data shows that Cx. tritaeniorhynchus, Cx. quinquefasciatus, An. subpictus, An. peditaeniatus, Cx. bitaeniorhynchus, and An. pulcherrimus were dominant species in Badin. Whereas, Ma. uniformis, An. culicifacies, An. annularis, Cx. fuscocephala, An. stephensi, Cx. pseudovishnui, An. nigerrimus, and Ae. aegypti were found to be sub-dominant. Among these species, Cx. tritaeniorhynchus, and Cx. quinquefasciatus were found to be constantly distributed. An. subpictus, An. peditaeniatus, Cx. bitaeniorhynchus, An. pulcherrimus and Ma. uniformis were frequently distributed in the area. An. culicifacies, An. annularis, Cx. fuscocephala, An. stephensi, and Cx. pseudovishnui were moderately distributed. An. nigerrimus was infrequent in distribution and Ae. aegypti was found to be sporadic (Table XII).

Sr. No.	Species	No. of specimen	RA*	Status	C*	Status
1	Cx. tritaeniorhynchus	122	14.90	Dominant	100.00	Constant
2	Cx quinquefasciatus	89	10.87	Dominant	85.71	Constant
3	An. subpictus	83	10.13	Dominant	85.71	Constant
4	An. stephensi	69	8.42	Dominant	71.43	Frequent
5	An. pulcherrimus	53	6.47	Dominant	71.43	Frequent
6	Cx. bitaeniorhynchus	52	6.35	Dominant	71.43	Frequent
7	An. culicifacies	50	6.11	Dominant	57.14	Moderate
8	An. annularis	49	5.98	Dominant	57.14	Moderate
9	Cx. pseudovishnui	42	5.13	Dominant	57.14	Moderate
10	Ae. unilineatus	41	5.01	Dominant	57.14	Moderate
11	Ae. aegypti	39	4.76	Sub-dominant	42.86	Moderate
12	Cx. fuscocephala	34	4.15	Sub-dominant	42.86	Moderate

Table XI. Relative abundance and distribution of mosquito species in district Karachi. Pakistan



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	Total	819				
16	An. superpictus	15	1.83	Sub-dominant	28.57	Infrequent
15	Ma. uniformis	24	2.93	Sub-dominant	28.57	Infrequent
14	An. peditaeniatus	26	3.17	Sub-dominant	42.86	Moderate
13	An. nigerrimus	31	3.79	Sub-dominant	42.86	Moderate

Table XII. Relative abundance and distri	ibution of mosquito spec	cies in district Badin, Pakista
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Sr. No.	Species	No. of specimen	RA*	Status	C*	Status
1	Cx. tritaeniorhynchus	175	18.98	Dominant	100.00	Constant
2	Cx. quinquefasciatus	129	13.99	Dominant	88.89	Constant
3	An. subpictus	94	10.20	Dominant	77.78	Frequent
4	An. peditaeniatus	87	9.44	Dominant	77.78	Frequent
5	Cx. bitaeniorhynchus	73	7.92	Dominant	77.78	Frequent
6	An. pulcherrimus	65	7.05	Dominant	66.67	Frequent
7	Ma. uniformis	46	4.99	Sub-dominant	66.67	Frequent
8	An. culicifacies	44	4.77	Sub-dominant	55.56	Moderate
9	An. annularis	44	4.77	Sub-dominant	44.44	Moderate
10	Cx. fuscocephala	42	4.56	Sub-dominant	44.44	Moderate
11	An. stephensi	42	4.56	Sub-dominant	44.44	Moderate
12	Cx. pseudovishnui	36	3.90	Sub-dominant	44.44	Moderate
13	An. nigerrimus	34	3.69	Sub-dominant	33.33	Infrequent
14	Ae. aegypti	11	1.19	Sub-dominant	11.11	Sporadic
	Total	922				

*Abbreviations: RA=Relative Abundance; C=Distribution

In this study, 13 species and 3 genera of mosquitoes were collected from Naushahro Feroze. Of which *Cx. tritaeniorhynchus, An. subpictus, Cx. quinquefasciatus, Cx. bitaeniorhynchus, An. annularis, Cx. fuscocephala, An. stephensi,* and *An. pulcherrimus* were found to be the dominant species. Whereas, *An. culicifacies, An. nigerrimus, Cx. pseudovishnui, An. peditaeniatus, and Ma. uniformis* were sub-dominant species in the area. Moreover, *Cx. tritaeniorhynchus, An. annularis, and Cx. fuscocephala* were frequently distributed species. *An. stephensi, An. pulcherrimus, An. annularis, and Cx. fuscocephala* were frequently distributed species. *An. stephensi, An. pulcherrimus, and An. culicifacies* were moderately distributed. *An. nigerrimus, Cx. pseudovishnui, An. culicifacies* were infrequently distributed. *An. nigerrimus, Cx. pseudovishnui, An. peditaeniatus, and Ma. uniformis* were infrequently distributed species in the Naushahro Feroze (Table XIII).

A total of 14 species and 4 genera of mosquitoes were collected from the district of Hyderabad. The dominant species were *Cx. tritaeniorhynchus, Cx. quinquefasciatus, Ae. unilineatus, An. subpictus, Cx. fuscocephala, Cx. pseudovishnui, Cx. bitaeniorhynchus, An. pulcherrimus, An. nigerrimus, whereas An. stephensi, An. annularis, Ae. aegypti, Ma. uniformis, and An. superpictus were sub-dominant species of the Hyderabad. Cx. tritaeniorhynchus, and Cx. quinquefasciatus were constantly distributed, while <i>Ae. unilineatus, An. subpictus, An. subpictus, Cx. fuscocephala, Cx. pseudovishnui, Cx. bitaeniorhynchus, and An. superpictus*, and *An. pulcherrimus* were frequently distributed species in Hyderabad. *An. nigerrimus, An. stephensi, An. annularis, and Ae. aegypti* were found to be moderately distributed species. Moreover, *Ma. uniformis, and An. superpictus* were infrequent in distribution in Hyderabad (Table XIV).

Table XIII. Relative abundance and distribution of mosquito species in district Naushahro Feroze, Pakistan

Sr. No.	Species	No. of specimen	RA*	Status	C*	Status
1	Cx. tritaeniorhynchus	135	18.32	Dominant	90.00	Constant
2	An. subpictus	99	13.43	Dominant	90.00	Constant
3	Cx. quinquefasciatus	97	13.16	Dominant	80.00	Frequent
4	Cx. bitaeniorhynchus	74	10.04	Dominant	80.00	Frequent

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	Total	737				
13	Ma. uniformis	16	2.17	Sub-dominant	30.00	Infrequent
12	An. peditaeniatus	30	4.07	Sub-dominant	40.00	Infrequent
11	Cx. pseudovishnui	31	4.21	Sub-dominant	40.00	Infrequent
10	An. nigerrimus	32	4.34	Sub-dominant	40.00	Infrequent
9	An. culicifacies	32	4.34	Sub-dominant	50.00	Moderate
8	An. pulcherrimus	41	5.56	Dominant	60.00	Moderate
7	An. stephensi	46	6.24	Dominant	60.00	Moderate
6	Cx. fuscocephala	48	6.51	Dominant	70.00	Frequent
5	An. annularis	56	7.60	Dominant	70.00	Frequent

Table XIV. Relative abundance and distribution of mosquito species in district Hyderabad, Pakistan

Sr. No.	Species	No. of	RA*	Status	C*	Status
		specimen				
1	Cx. tritaeniorhynchus	126	20.39	Dominant	100.00	Constant
2	Cx. quinquefasciatus	87	14.08	Dominant	83.33	Constant
3	Ae. unilineatus	57	9.22	Dominant	66.67	Frequent
4	An. subpictus	56	9.06	Dominant	66.67	Frequent
5	Cx. fuscocephala	42	6.80	Dominant	66.67	Frequent
6	Cx. pseudovishnui	37	5.99	Dominant	66.67	Frequent
7	Cx. bitaeniorhynchus	33	5.34	Dominant	66.67	Frequent
8	An. pulcherrimus	31	5.02	Dominant	66.67	Frequent
9	An. nigerrimus	31	5.02	Dominant	50.00	Moderate
10	An. stephensi	30	4.85	Sub-dominant	50.00	Moderate
11	An. annularis	30	4.85	Sub-dominant	50.00	Moderate
12	Ae. aegypti	27	4.37	Sub-dominant	50.00	Moderate
13	Ma. uniformis	21	3.40	Sub-dominant	33.33	Infrequent
14	An. superpictus	10	1.62	Sub-dominant	33.33	Infrequent
	Total	618				

DISCUSSION

The present survey was carried out to examine the different mosquito species and their distribution pattern in Pakistan. The occurrence of mosquito species in different areas reflects both the habitat preferences of the mosquitoes as well as the ability of the mosquitoes to survive in various environmental conditions (29). Most mosquito species prefer high temperatures and humidity (30).

Khan, Maibach (31) reports total 104 species of mosquitoes from Pakistan and Bangladesh in the species checklist. Since then no detailed work has been done so far. Many researchers repeatedly reported the same species in different areas of Pakistan. For instance, from Murree four Anophelinae and ten Culicinae species have been reported, including *An. stephensi, An. theobaldi, An. maculatus, An. fluviatilis, Ae. albopictus, Ae. aegypti, Culex quinquefasciatus, Cx. nilgiricus, Cx. fuscitarsis, Cx. vagans Cx. raptor, Ar. obturbans and Culiseta longiareolata (Qasim, Naeem (32). From the University of Peshawar six mosquito species including <i>Ae. albopictus, Ae. unilineatus, Ae. w-albus, Ar. subalbatus, Cx. quinquefasciatus* and *An. stephensi* have been reported until now (Naz and Shabnam (33). From Swat 21 mosquito species reported up till now including *Cx. quinquefasciatus, An. maculatus, Cx. pseudovishnui, An. annularis, An. stephensi, Cx. bitaeniorhynchus, An. splendidus* and *Cx. theileri* (Ilahi and Suleman (34). From Aminkhel, Karak, and Peshawar a total of five species, including *Cx. quinquefasciatus, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. vishnui, Ae. albopictus, Ae. w-albus, Ae. w-albus, Ae. unilineatus, An. stephensi, An. annularis and <i>Ae. albopictus* were reported (Usman, Rehman (35). A total of 11 species, including *Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. vishnui, Ae. albopictus, Ae. w-albus, Ae. unilineatus, An. stephensi, An. culicifacies, An. subpictus, An. maculatus* and *Ar. subalbatus* were reported from Peshawar, KPK, Pakistan (36). Recently,



Ashfaq, Hebert (37) did a detailed study in Pakistan on the diversity of mosquitoes by DNA barcoding and they could only be able to document 32 species from Punjab and KPK, Pakistan (37).

The present study showed that three important genera *Anopheles, Culex,* and *Aedes* were found throughout the study area. While *Armigeres* and *Mansonia* were found in a few collection sites. In the current study, a total of 29 species of mosquitoes were documented in Pakistan. Of which 10 species of *Culex,* 10 species of *Anopheles,* 06 species of *Aedes,* 2 species of *Armigeres,* and 1 species of *Mansonia* were recovered in this study. Different researchers previously reported all the species however, the current study reported many species in those districts where they were not reported previously. In neighboring countries of Pakistan, scatter studies have been conducted to study the diversity of mosquitoes similarly as in Pakistan. For example, forty-four species belonging to eleven genera were reported from Alappuzha, South India (38). A checklist of mosquito species in Iran includes sixty-four species belonging to seven genera (39). Fourteen species from the *Armigeres* genus were reported from India (40); From Brazil Leal-Santos, Jacobina (41) studied 34 species belonging to 12 genera. In the current investigation, the data shows that among five genera, the most abundant and constant genus was *Culex. Culex* spp. has a wide range of preferences in their habitats as well as the seasons. *Culex* species were mostly found in stagnant water, seepage pools, field crops, houses, scrapyards, graveyards, and forests. Most of the *Culex* were found in highly humid and vegetative habitats as well as dry and low humid habitats (42).

Anopheline mosquitoes (Culicidae, Anophelinae) are of prime medical importance as human malaria vectors, yet their phylogeny is poorly known (43). In the current study, ten species of *Anopheles (An. subpictus, An. stephensi, An. peditaeniatus, An. culicifacies, An. pulcherrimus, An. annularis, An. nigerrimus, An. superpictus, An. fluviatilis, and An. maculatus*) were documented from Pakistan. The same species were recovered from Punjab and Sindh except *An. superpictus* that was not found in any district of Punjab during the current study, while it was only found in Karachi and Hyderabad districts of Sindh, Pakistan. Similarly, *An. maculatus* was not identified from Sindh. It was only found in the district of Narowal in Punjab, Pakistan. Moreover, *An. fluviatilis* was only found in district Mardan (KPK) and has not been documented anywhere else during the current study. *Anopheles* mosquitoes were recorded in the surroundings of animals, telling the zoophilic nature of most of them. Most of the *Anopheles* mosquito species were collected from animal sheds, houses and river margins. *Anopheles* species have been reported by many researchers such as Rohani, Zamree (44) from Serian, Sarawak, Malaysia; Soleimani-Ahmadi, Vatandoost (45) from South Iran; Pal and Dutta, 1992 from West Bengal Thatoi, Behera (46); Ilahi and Suleman (34) from Swat, Pakistan; Martinez (47) from South Texas; Fakoorziba and Vijayan (48) from Mysore, India; Manzoor, Shabbir (49) Lahore; Nasir, Ahmed (50) from Kasur and Sheikhupura.

Ae. albopictus was found more adaptive in nature than Ae. aegypti. Reportedly, Ae. aegypti recorded in those habitats having less humidity, vegetation, and human activities during the months with normal humidity levels (34, 51). Ae. albopictus reported comparatively in highly humid habitats during the months with high humidity July-October (52). Ashfaq et al (2014) reported that Ae. albopictus as the dominant species among other Aedes species in Punjab, but they could not detect this species at higher elevation sites (1100 m) in Swat (37). The results of our survey indicated that Ae. albopictus was more widely distributed and commoner than Ae. aegypti across the Pakistan. Akhtar et al. (2012) found that larvae of Ae. aegypti predominated in collections from water pots inside houses in Lahore during 2011 (53). Adult Ae. aegypti is unable to survive in the transition zones between the world's tropical and temperate zone, however invasive populations of Ae. albopictus can remain alive (54). Additionally, Ae. albopictus larval population growth remain unaffected by low nuitrition levels, giving it an edge over Ae. aegypti (54, 55). The current dominance of Ae. albopictus in Pakistan also supports a trend towards expansion of Ae. albopictus and a decline of Ae. aegypti. The results of the current study are also supported this phenomenon as Ae. albopictus were collected from all the districts from Punjab, while Ae. aegypti was not found in Narowal and Bahawalpur. Moreover, Ae. aegypti were less abundant as compared to Ae. albopictus. Ae. aegypti was collected from Hyderabad and Karachi. Both these species were also found in KPK, and Balochistan, Pakistan. Ae. aegypti has been previously reported from Pakistan (32-34, 37, 56, 57); Bangladesh (58), India



(38, 59, 60), Iran (61), Saudi Arabia (62) and Nepal (63). *Ae. albopictus* was reported from Pakistan (32, 37, 56, 57, 64, 65), India (38, 66), Taiwan (67) and China (68).

Ae. unilineatus was collected from Hyderabad, Karachi, and Badin from Sindh. While this species was only found in Faisalabad from Punjab. No specimen of this species was collected from KPK and Balochistan. Ae. caspius, Ae. w-albus, and Ae. cogilli were not identified from Sindh while they were found in Punjab in low numbers. In the present survey, Ae. cogilli collected from Gujrat, Faisalabad, Rahim Yar Khan, Bahawalpur, and Chakwal, while absent from Narowal. Ae. cogilli was also found in Mardan (KPK). Ae. cogilli was not found in Balochistan. Ae. w-albus was collected from Narowal and Faisalabad. Similarly, Ae. caspius was collected only from Bahawalpur and Rahim Yar Khan. Ae. caspius was also collected from Quetta, Balochistan. No specimen of Ae. w-albus was collected from KPK and Balochistan. Armigeres mosquitoes are the largest, stout-bodied and the most aggressive mosquitoes (69). Ar. obturbans was collected from Gujrat, Narowal and Chakwal districts of Punjab, while it was absent from South Punjab (Bahawalpur and Rahim and Khan) and Sindh, KPK, and Balochistan. Ar. obturbans was previously reported from Pakistan (32, 64, 65); Taiwan (67); and India (60). In the current study, Ar. subalbatus was documented from Gujrat, Chakwal, Bahawalpur, and Rahim Yar Khan. This species was not found in Sindh, KPK, and Balochistan. From the genus Mansonia, only one species could be collected in the current study. Mansonia uniformis was documented from districts Narowal, Lahore, Bahawalpur, and Rahim Yar Khan from Punjab and interestingly, it was collected from all districts (Karachi, Badin, Hyderabad, and Naushahro Feroze) of Sindh. During this study, no specimen was collected from KPK and Balochistan.

CONCLUSION

Worldwide, mosquitoes are leading vector for transmission of numerous diseases. In this study, five mosquito genera (i.e. *Culex, Anopheles, Aedes, Mansonia,* and *Armigeres*) belonging to 30 species were identified in Pakistan. In order to implement vector control programs more precisely, the abundance and distribution pattern of mosquito species found in the current study in various habitats of Pakistan could be useful. It is recommended that the molecular analysis, particularly DNA barcoding, should be used to determine the genetic diversity and species identification of mosquitoes in order to identify morphologically closely related species more accurately.

Author Contribution:

SA conducted the experiment, collected data, and wrote manuscript; AB designed and supervised the study; SSS collected data and wrote manuscript; MHL collected data and prepare all illustrations. All authors reviewed the manuscript.

Conflict of interest:

The authors declare that there is no conflict of interest.

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References:

- 1. Ganai SA, Kumar R, Basheer M, Agrawal OP. A study on sampling of mosquitos using eco-friendly mosquito trap in and around Jiwaji University Campus (India). Munis Entomology & Zoology. 2013;8(2):734-738.
- 2. Deng X, Yan R, Li Z, Tang X, Zhou Y, He H. Economic and disease burden of *Japanese encephalitis* in Zhejiang Province, 2013–2018. PLoS neglected tropical diseases. 2021;15(6):1-16.
- 3. Anoopkumar AN, Puthur S, Rebello S, Aneesh EM. Molecular characterization of Aedes, Culex, Anopheles, and Armigeres vector mosquitoes inferred by mitochondrial cytochrome oxidase I gene sequence analysis. Biologia. 2019;74(9):1125-1138.
- 4. Chakraborti S, Chhibber-Goel J, Sharma A. Drug targeting of aminoacyl-tRNA synthetases in Anopheles species and Aedes aegypti that cause malaria and dengue. Parasites & Vectors. 2021;14(1):1-11.



- 5. Norris LC, Norris DE. Phylogeny of anopheline (Diptera: Culicidae) species in southern Africa, based on nuclear and mitochondrial genes. Journal of Vector Ecology. 2015;40(1):16-27.
- 6. Khezzani B, Baymakova M, Khechekhouche EA, Tsachev I. Global warming and mosquito-borne diseases in Africa: a narrative review. Pan African Medical Journal. 2023;44(1).
- 7. Thongsripong P, Chandler JA, Green AB, Kittayapong P, Wilcox BA, Kapan DD, Bennett SN. Mosquito vector-associated microbiota: Metabarcoding bacteria and eukaryotic symbionts across habitat types in Thailand endemic for dengue and other arthropod-borne diseases. Ecology and evolution. 2018;8(2):1352-1368.
- 8. Donald CL, Siriyasatien P, Kohl A. Toxorhynchites species: a review of current knowledge. Insects. 2020;11(11):747.
- 9. Moise IK, Riegel C, Muturi EJ. Environmental and social-demographic predictors of the southern house mosquito Culex quinquefasciatus in New Orleans, Louisiana. Parasites & vectors. 2018;11(1):1-8.
- Kondapaneni R, Malcolm AN, Vazquez BM, Zeng E, Chen T-Y, Kosinski KJ, Romero-Weaver AL, Giordano BV, Allen B, Riles MT. Mosquito Control Priorities in Florida—Survey Results from Florida Mosquito Control Districts. Pathogens. 2021;10(8):947.
- 11. Chaval Y, Herbreteau V, Waengsothorn S, Cosson J-F, Hugot J-P, Morand S, Michaux J. Revisiting the taxonomy of the Rattini tribe: a phylogeny-based delimitation of species boundaries. BMC evolutionary Biology. 2010;10(1):1-27.
- 12. Muturi EJ, Shililu JI, Gu W, Jacob BG, Githure JI, Novak RJ. Larval habitat dynamics and diversity of Culex mosquitoes in rice agro-ecosystem in Mwea, Kenya. 2007.
- 13. Sattler MA, Mtasiwa D, Kiama M, Premji Z, Tanner M, Killeen GF, Lengeler C. Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. Malaria journal. 2005;4(1):1-15.
- 14. Kauffman E, Payne A, Franke MA, Schmid MA, Harris E, Kramer LD. Rearing of Culex spp. and Aedes spp. mosquitoes. Bio-protocol. 2017;7(17):e2542-e2542.
- 15. Munstermann LE. Care and maintenance of Aedes mosquito colonies, in The molecular biology of insect disease vectors. 1997; Springer. p. 13-20.
- 16. Rozo-Lopez P, Mengual X. Mosquito species (Diptera, Culicidae) in three ecosystems from the Colombian Andes: identification through DNA barcoding and adult morphology. ZooKeys. 2015(513):39.
- 17. De S, Debnath B. Prevalence of health hazards associated with solid waste disposal-A case study of kolkata, India. Procedia Environmental Sciences. 2016;35:201-208.
- Christophers SR. The Fauna of British India, including Ceylon and Burma. Diptera. Vol. IV. Family Culicidae. Tribe Anophelini. The Fauna of British India, including Ceylon and Burma. Diptera. Vol. IV. Family Culicidae. Tribe Anophelini. 1933.
- 19. Barraud PJ. The Fauna of British India, including Ceylon and Burma. Diptera. Vol. 5. Family Culieldae. Tribes Megarhinini and Culicini. The Fauna of British India, including Ceylon and Burma. Diptera. Vol. 5. Family Culieldae. Tribes Megarhinini and Culicini. 1934.
- 20. Edwards FW. Mosquitoes of the Ethiopian Region. III.-Culicine adults and pupae. Mosquitoes of the Ethiopian Region. III.-Culicine Adults and Pupae. 1941.
- 21. Gillies MT, De Meillon B. The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region). The Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region). 1968(54).
- 22. Jupp P, McIntosh B, Blackburn N. Experimental assessment of the vector competence of Culex (Culex) neavei Theobald with West Nile and Sindbis viruses in South Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1986;80(2):226-230.
- 23. Harbach RE. The mosquitoes of the subgenus Culex in southwestern Asia and Egypt (Diptera: Culicidae). Contributions of the American Entomological Institute. 1988;24(1).
- 24. Ali N, Khan K, Kausar A. Study on mosquitoes of Swat Ranizai sub division of Malakand. Pakistan Journal of Zoology. 2013;45(2).
- 25. Rydzanicz K, Lonc E. Species composition and seasonal dynamics of mosquito larvae in the Wrocław, Poland area. Journal of Vector Ecology: Journal of the Society for Vector Ecology. 2003;28(2):255-266.
- 26. Sengil AZ, Akkaya H, Gonenc M, Gonenc D, Med DOIJB. Species composition and monthly distribution of mosquito (Culicidae) larvae in the Istanbul metropolitan area, Turkey. International Journal of Biological and Medical Research 2011;2(1):415-424.
- 27. Trojan P. The analysis of the fauna's structure. Memorabilia Zoologica. 1992;47:1-121.



- 28. Dziêczkowski A. Badania ilo. ciowe. limaków buczyn po3udniowozachodniej Polski.(Quantitative researches of the beech malacofauna in south-west of Poland). Studium ekologiczno faunistyczne. Prace Komisji Biologicznej. 1972;35:243-332.
- 29. Choi G, Collins D, Ren G, Trewin B, Baldi M, Fukuda Y, Afzaal M, Pianmana T, Gomboluudev P, Huong PTT. Changes in means and extreme events of temperature and precipitation in the Asia-Pacific Network region, 1955–2007. International Journal of Climatology: A Journal of the Royal Meteorological Society. 2009;29(13):1906-1925.
- 30. Verhulst NO, Brendle A, Blanckenhorn WU, Mathis A. Thermal preferences of subtropical Aedes aegypti and temperate Ae. japonicus mosquitoes. Journal of Thermal Biology. 2020;91:102637.
- 31. Khan A, Maibach HI, Strauss WG. A quantitative study of variation in mosquito response and host attractiveness. Journal of medical entomology. 1971;8(1):41-43.
- 32. Qasim M, Naeem M, Bodlah I. Mosquito (Diptera: Culicidae) of Murree Hills, Punjab, Pakistan. Pakistan Journal of Zoology. 2014;46(2).
- Naz R, Shabnam A. Population dynamics of mosquitoes in various breeding habitats at University of Peshawar campus, Khyber Pukhtunkhwa Pakistan. Journal of Entomology and Zoology studies. 2014;2:189-195.
- 34. Ilahi I, Suleman M. Species composition and relative abundance of mosquitoes in Swat, Pakistan. International Journal of Innovation and Applied Studies. 2013;2(4):454-463.
- Usman K, Rehman HU, Pervaiz K, Khudadad S, Aslam S. A study of mosquito fauna of Amin Khel district Karak, Khyber Pakhtunkhwa, Pakistan. International Journal of Mosquito Research. 2017;4:47-49.
- Wajiha RA, Afridi H, Saeed K. Prevalence of culex, aedes, anopheles and armigers mosquitoes at selected localities of district Peshawar Khyber Pakhtunkhwa Pakistan. International Journal of Mosquito Research. 2017;4(2):128-134.
- 37. Ashfaq M, Hebert PD, Mirza JH, Khan AM, Zafar Y, Mirza MS. Analyzing mosquito (Diptera: Culicidae) diversity in Pakistan by DNA barcoding. PLoS One. 2014;9(5):1-12.
- 38. Balasubramanian R, Nikhil T. Mosquito (Diptera: Culicidae) fauna in Alappuzha and Kottayam district of the Kerala state, south India. Journal of Entomology and Zoology studies. 2013;1(16):134-137.
- 39. Azari-Hamidian S. Checklist of Iranian mosquitoes (diptera: Culicidae). Journal of Vector Ecology. 2007;32(2):235-242.
- 40. Bhattacharyya D, Prakash A, Tewari S, Mohapatra P, Mahanta J. Armigeres joloensis (Diptera: Culicidae), a rare mosquito in upper Assam: first report from India. Entomon 2000;25(1):63-66.
- 41. Leal-Santos FA, Jacobina ACM, de Oliveira MM, Santana MBA, Serra OP, Gonçalves AV, Garcêz ARS, Thies SF, Slhessarenko RD, de Oliveira Dantas ES. Species composition and fauna distribution of mosquitoes (Diptera: Culicidae) and its importance for vector-borne diseases in a rural area of Central Western-Mato Grosso, Brazil. EntomoBrasilis. 2017;10(2):94-105.
- 42. Mehmood A, Naeem M. Seasonal Occurrence and Abundance of Mosquitoes in Pothwar Plateau. Pakistan Journal of Zoology. 2022;54(3).
- 43. Krzywinski J, Wilkerson RC, Besansky NJ. Toward understanding Anophelinae (Diptera, Culicidae) phylogeny: insights from nuclear single-copy genes and the weight of evidence. Systematic Biology. 2001;50(4):540-556.
- 44. Rohani A, Zamree I, Ali WNWM, Hadi AA, Asmad M, Lubim D, Nor ZM, Lim LH. Nocturnal man biting habits of mosquito species in Serian, Sarawak, Malaysia. Advances in Entomology. 2013;2013.
- 45. Soleimani-Ahmadi M, Vatandoost H, Zare M, Turki H, Alizadeh A. Topographical distribution of anopheline mosquitoes in an area under elimination programme in the south of Iran. Malaria journal. 2015;14(1):1-8.
- 46. Thatoi H, Behera BC, Mishra RR, Dutta SK. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. Annals of Microbiology. 2013;63(1):1-19.
- 47. Martinez NH. Distribution and abundance of Anopheles spp. in the Lower Rio Grande Valley, South Texas. 2013: The University of Texas-Pan American.
- 48. Fakoorziba MR, Vijayan A. Breeding habitats of culex tritaeniorhynchus (Diptera: culicidae), A Japanese encephalitis vector, and associated mosquitoes in Mysore, India. Journal of the Entomological Research Society. 2008;10(3):1-9.
- 49. Manzoor F, Shabbir R, Sana M, Nazir S, Khan MA. Determination of Species Composition of Mosquitoes in Lahore, Pakistan. Journal of Arthropod-Borne Diseases. 2020;14(1):106.

- 50. Nasir S, Ahmed I, Hussain B, Ijaz MU, Hafeez F, Wadaan MA, Atique U, Mahboob S. A study on the role of aedes mosquitoes in arboviruses and SARS-CoV-2 infection: A new challenge. Journal of King Saud University-Science. 2022;34(6):102179.
- 51. Barrera R, Amador M, Acevedo V, Beltran M, Muñoz J. A comparison of mosquito densities, weather and infection rates of Aedes aegypti during the first epidemics of Chikungunya (2014) and Zika (2016) in areas with and without vector control in Puerto Rico. Medical and veterinary entomology. 2019;33(1):68-77.
- 52. Fakoorziba M, Eghbal F, Vijayan V. Synergist efficacy of piperonyl butoxide with deltamethrin as pyrethroid insecticide on Culex tritaeniorhynchus (Diptera: Culicidae) and other mosquitoe species. Environmental Toxicology: An International Journal. 2009;24(1):19-24.
- 53. Akhtar MS, Aihetasham A, Saeedb M, Abbassa G. Aedes survey following a dengue outbreak in Lahore, Pakistan, 2011. Dengue. 2012;36:87.
- 54. Mejía-Jurado E, Echeverry-Cárdenas E, Aguirre-Obando OA. Potential current and future distribution for Aedes aegypti and Aedes albopictus in Colombia: important disease vectors. Biological Invasions. 2024:1-19.
- 55. Deerman H, Yee DA. Competitive interactions with Aedes albopictus alter the nutrient content of Aedes aegypti. Medical and Veterinary Entomology. 2023;37(4):715-722.
- 56. Akram W, Hafeez F, Ullah UN, Kim YK, Hussain A, Lee JJ. Seasonal distribution and species composition of daytime biting mosquitoes. Entomological research. 2009;39(2):107-113.
- 57. Qutubuddin M. Mosquito studies in the Indian subregion. Part I taxonomy-a brief review Pacif. Insects. 1960;2:133-47.
- 58. Bashar K, Tuno N. Seasonal abundance of Anopheles mosquitoes and their association with meteorological factors and malaria incidence in Bangladesh. Parasites & vectors. 2014;7:1-10.
- Rajput K, Kulkarni S. Record of Culicine Mosquitoes from Bastar District (Madhya Pradesh), India.(Diptera: Culicidae.). Part III. Genus Aedes. Records of the Zoological Survey of India. 1991:309-318.
- 60. Rajput K, Singh T. Report on the occurrence of Uranotaenia (Uranotaenia) micans Leicester, 1908 in the state of Manipur, India. Mosquito Systematics. 1990;22(3).
- 61. Nikookar SH, Moosa-Kazemi SH, Yaghoobi-Ershadi MR, Vatandoost H, Oshaghi MA, Ataei A, Anjamrooz M. Fauna and larval habitat characteristics of mosquitoes in Neka County, Northern Iran. Journal of arthropod-borne diseases. 2015;9(2):253.
- 62. Khater E, Sowilem M, Sallam M, Alahmed A. Ecology and habitat characterization of mosquitoes in Saudi Arabia. Tropical Biomedicine. 2013; 30(3):409–427.
- 63. Dhimal M, Gautam I, Kreß A, Müller R, Kuch U. Spatio-temporal distribution of dengue and lymphatic filariasis vectors along an altitudinal transect in Central Nepal. PLoS neglected tropical diseases. 2014;8(7):1-13.
- 64. Rasool S, Mian Inayatullah MA, Ali M, Ali S, Rizvi SAH, Hyder S, Begum F, Raza G, Ali K. Taxonomic study of mosquitoes (Culicidae: Diptera) of district Narowal, Punjab-Pakistan. Journal of Biodiversity and Environmental Science. 2015;6(4):368-373.
- 65. Khan J, Shah M, Khan BT, Naeem M, Ismail M, Abbasi A, Khan SA. A survey of adult and larval mosquito fauna in Tehsil Daggar and Gagra of District Buner, Khyber Pakhtunkhwa, Pakistan. International Journal of Mosquito Research. 2015;2(3):170-4.
- 66. Kirti JS, Kaur S. Culicinae diversity (Culicidae: Diptera) from Punjab (India) with reference to impact of ecological changes. International Journal of Mosquito Research. 2014;1(4):10-16.
- 67. Lien J. Non-Anopheline mosquitoes of Taiwan: annotated catalog and bibliography. Pacific Insects. 1962;4(3):615-649.
- Wang L, Valderramos SG, Wu A, Ouyang S, Li C, Brasil P, Bonaldo M, Coates T, Nielsen-Saines K, Jiang T. From mosquitos to humans: genetic evolution of Zika virus. Cell host & microbe. 2016;19(5):561-565.
- 69. Liu P, Yang W, Kong L, Zhao S, Xie Z, Zhao Y, Wu Y, Guo Y, Xie Y, Liu T. A DBHS family member regulates male determination in the filariasis vector Armigeres subalbatus. Nature Communications. 2023;14(1):1-10.

