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Comparative study on species diversity, abudance and distribution of mosquitoes in selected habitats, Bhutan

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Abstract— Mosquitoes (Diptera: Culicidae) diversity is an integral aspect of human life as they are medically and ecologically important insects. Mosquito species diversity study in Bhutan is limited except that of malaria causing *Anopheles* species. This paper compares the mosquito diversity in selected habitat types of Punakha and Thimphu *dzongkhags*. The data was collected from purposively selected habitats over the periods of two months from September to October, 2019. The Human landing collection and sweeping net method was used. The research documented nine species belonging to two subfamilies (Culicinae and Anophelinae) and four Genera *(Aedes, Armigeres, Anopheles* and *Culex)* with total encountered of 3,171 individuals. Six of the nine collected species has never been reported in any earlier publications. The species diversity was high in Punakha (H = 1.84) than in Thimphu (H = 1.09). All the encountered species are of medical importance. Recorded species are found to have different time period for the biting activities. This study is expected to sever as future baseline data and helps in developing vector monitoring and control strategies.

Index Terms— abundance, biting habit, distribution, habitats, medicinal vector, mosquitoes, richness.

1 Introduction

Worldwide about 3,500 species of mosquitoes are estimated, belonging to family Culicidae, order Diptera [22]. They are distributed throughout the tropics and temperate regions of the world [25], [39] except for some Island and Antarctic regions [7]. They are adapted to survive in different habitats including vegetation types, water bodies, animal sheds and human settlements and other both natural and artificial habitats. The diversity of the mosquito species differs among the different geographical regions of the world. The highest diversity of mosquito species is found in the Neotropical (NT) and the Nearctic region has the lowest species diversity [39].

In Indian about 393 species of mosquito belonging to 49 genera and 41 subgenera are recorded [5]. In Jammu and Kashmir State, India alone has, about 47 species of mosquitoes [14]. In the East Nepal, 22 species of four genera (i.e., *Aedes, Anopheles, Armigeres* and *Culex*) was recorded [16]. In Bhutan, 30 species of *Anopheles* mosquitoes from limited collection site was recorded [29], [36]. Mosquitoes are considered as medically important insects as they form the main vector for transmission of various arthropod-borne diseases. Vector-borne diseases such as malaria, dengue, Japanese encephalitis and Leishmaniasis, occurs mostly in subtropical Bhutan which are bordering with India [29].

Many adult mosquito depends on flower nectars to obtain energy, only female mosquitoes need blood meals to gain protein for laying eggs. It can be attributed that without mosquito many plant species would lose group of pollinators. Mosquito larvae make important biomass in aquatic ecosystem as they feed on decaying leaves, organic detritus, microorganisms and algae and makes the nitrogen and other nutrients available for plants. Many species of insects; spider, salamander, lizards, frog, birds, dragonfly and damselfly feed on mosquito larvae and adults [20].

Mosquito species diversity study in Bhutan is limited [29]. This study however focused on malaria causing *Anopheles* mosquitoes only. There are other blood sucking female mosquitoes, except sub-family *Toxorhynchitinae,* which play vital role in transferring important and life threatening diseases [32]. Rapid urbanization, increasing deforestation, resistance among mosquitoes to different insecticides and increasing conducive habitats for breeding, both mosquitoes and mosquito borne diseases are spreading everywhere [41], [23]. People are traveling to and fro from vector born disease infected areas, within and across international borders to higher altitudes [16].

Assessment of species diversity and distribution of mosquito vector in the areas is important to develop vector monitoring program and effective implementation of control strategies, which depends on mosquito species present in different areas [7]. This study will serve as a baseline data for the mosquito diversity, abundance and its distribution in selected habitats, and helps to make strategy for control over mosquito vector diseases. It will also generate the baseline data for further scientific study on mosquitoes. Therefore, the present study aims to compare mosquito's species diversity, abundance, distribution in selected habitats and documentation of feeding habitats of collected species in different effort hours.

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2 Methods and materials

2.1 study area

Mosquito diversity was compared in selected habitats of Punkaha (27°35'28.9"N 89°52'38.8"E) and Thimphu (27°27'57.9"N 89°38'30.9"E) districts (fig.1) having different elevation coverage and different temperature, rainfall and humidity. It is one of the highly populated districts. In Punakha district, the study area was *Barp* and *Guma* gewogs (block). The two gewogs consists of 1,539 households and has a population of about 7,694 people and elevation ranged between 1200 to 2200 m [30]. These gewogs experiences hot and humid summer with heavy rainfall whereas winters are moderate [37]. In Punakha, two gewogs are selected because of the comparatively dense human population in those areas.

Chang gewog under Thimphu district was also selected for the study. The gewog consists of 245 households and has a population of about 6,752 people and elevation ranged between 2100 to 6800 m [30]. The gewog experience warm summer and cold and dry winter [43]. Thimphu being most populated district, there is high chance of spreading diseases by mosquitoes found there.

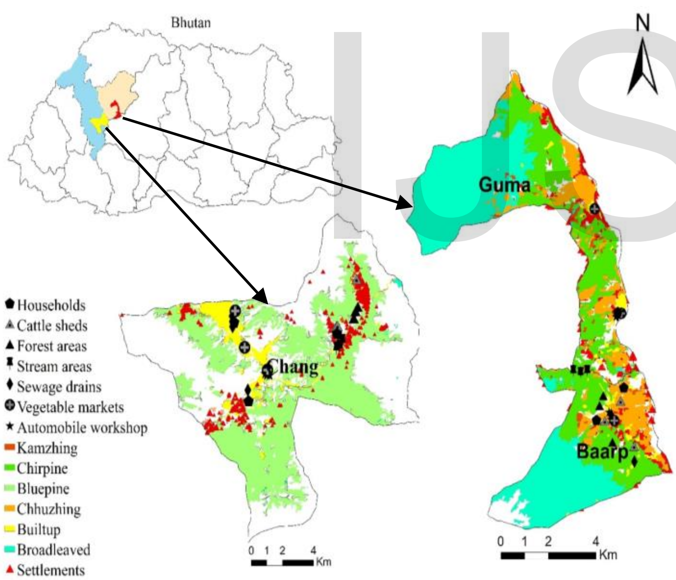

Fig. 1. Map of study area

2.2 Sampling design

The mosquito diversity was assessed in purposively selected habitats. In the selected districts and gewogs, seven different habitat types was selected such as the sewerage drain, freshwater stream, cattle-shed [29] household, automobile workshop, forest area [31], [18] and vegetable market. From the each selected habitat type, three sampling sites was randomly selected. A total of 21 sampling sites was selected from each study area. Forest and stream sites were selected at 500 m distances from human settlements and between two sites 200 m distance was maintained. Preliminary survey was done before selecting any sites.

2.3 Data collection method

The adult mosquitoes were sampled using human landing collection method (HLC) [22], [12], [29] and with the help of sweep net [3]. By HLC method, the mosquitoes that land on the hands and legs of the collectors was collected using small test tubes. Net sweeping method was also used in each site. Once the mosquitoes are caught, the test tube was covered with a cotton ball soaked in ethyl acetate. Collected mosquitoes were separated based on morphological similarities and given species Id.

Total collection was done for four hours in evening (1800 till 2200 h) and one hour in morning (0500 to 0600 h) [9]. In selected forest habitats, a square plot of 20 x 20m was laid. In every corner, an hour of collection was done in evening and 15 minutes in morning. In cattle-shed and household, both indoor and outdoor collection of sample was done (i.e., one person collected indoor and one person collected in outdoor simultaneously) [42]. Outdoor sample collection was done from four corners of selected houses. In stream and sewerage drain site, the sample was collected from stagnant water or small pool formed nearby the stream and sewerage drain. Mosquitoes in vegetable market and automobile workshop were collected within market areas. The HLC method was continuously used 45 minutes in each hour followed by 15 minutes resting period [42]. Collected mosquitoes were preserved by pinning with mini steel pin. All the plants, animals, temperature, relative humidity, suitable habitat (like any discarded container, tyres, etc.), physiochemical parameters, and vegetation around the sampling plots were recorded.

2.4 Data analysis

All the data collected from the field were compiled using MS Excel. Data were analysed using R-Studio. Spearman correlation was computed to see the association between mosquito abundance in relation to temperature humidity and elevation separately. The Shannon-Weiner index [$H = -\sum Pi \log Pi$] and Evenness index [$J=H/H\text{max}$] were used in diversity, evenness and abundance studies. Charts and graphs were produced using MS Excel. Adult mosquitoes were identified with the help of expertise and by using dichotomous key available from Thailand and India.

3 Result and Discussion

3.1 Comparision of mosquito diversity between two study areas

In total four genera and nine species in two subfamilies of mosquitoes were recorded with the total number of 3,171 individuals from 42 sampling sites (Table 1). Subfamily Culicinae dominated the number of individual recorded (n = 2,690). Genera *Culex* and *Anopheles* were the dominated genus with three species each. Among nine species recorded, eight species were identified up to species level and one species up to genus

Table 1. List of mosquito species of two areas with diversity and evenness

Place	Subfamily	Species	Count	*H*	*E*
Punakha	Culicinae	*Aedes albopictus* (Skuse, 1894)*	324	1.84	0.95
		Aedes vexans (Meigen, 1830)*	455		
		Armigeres subalbatus (Coquillett, 1899)*	393		
		Culex gelidus (Theobald, 1901)*	448		
	Anophelinae	*Anopheles peditaeniatus* (Leicester, 1908)	136		
		Anopheles nigerrimus (Giles, 1908)*	104		
		Anopheles splendidus (Koidzumi, 1920)	241		
Thimphu	Culicinae	*Armigeres subalbatus* (Coquillett, 1899)	387	1.09	0.99
		Culex quinquefasciatus (Say,1823)*	393		
		Culex sp.	290		

H = diversity; ***E*** = Evenness, * = New to Bhutan

level (i.e., *Culex* sp.). Six species among nine collected species are new to Bhutan. In checklist of *Anopheles* species, [29] *An. nigerrimus* was not recorded. Similarly, *Culex sasai* Kano. was recorded from Bhutan [36] but did not mention the species recorded in this study.

Table 2. Summary of Spearman correlation

	Altitude	Temperature	Relative humidity
Abundances	-.39*	.86**	.76**
Richness	-.49**	0.21	0.06

** = Significant at .01, * = Significant at .05

The diversity and species richness (H = 1.84; seven species) of Punakha were higher (Table 1) than that of Thimphu (H = 1.09; three species). In Punakha, the species evenness (E = 0.95) was less than that of Thimphu (E = 0.99). The possible reason could be due to difference in elevation which has effect on temperature since the elevation in Punakha was between 1200 to 1500 m and Thimphu was between 2100 to 2900 m. The Spearman correlation also showed negatively significant correlation between altitude with mosquito richness (r_s = -0.49, p =0.00) and abundance (r_s = -0.39, p = 0.01) (Table 2). Similarly, it was found that diversity, species richness and abundance of mosquitoes greatly change along the habitat-climate-elevation gradient and numerous species are predominantly encountered below 1700 m [19].

3.2 Comparision of diversity of mosquitoes in relation to habitat types

In Punakha, from seven selected habitat types, the diversity and species richness of household habitat (H = 1.84; seven species) was the highest. The lowest diversity and species richness was found at stream habitat (H = 0.93). Automobile workshop (E = 0.99), sewage drain (E = 0.99) and forest (E = 0.99) had highest evenness. Evenness of mosquitoes was lowest at stream (E = 0.84) habitat (Table 3). In Thimphu, species diversity and species richness were found highest at cattle shed and household habitat (H = 1.09; three species respectively) and lowest diversity and species richness at stream (H = 0.5; with two species). Highest evenness was found at cattle shed (E = 1) and lowest evenness was found at automobile workshop habitat (E = 0.63) (Table 3).

Similar study on species richness and diversity among three selected agroecosystem habitats (i.e., planned, unplanned and non-irrigated village) in Kenya found that non-irrigated agroecosystem with diverse habitat types supports more mosquito species [28]. In this study, the household habitat was found surrounded with more diverse habitat types which includes sewage drain, septic tank, domestic animals, barrels, discarded containers and flower pots with filled water. The presence of this diverse habitats [41], high species richness and diversity in the household habitat is possible. However, stream habitat had less diverse habitats supporting few mosquito species. It indicate that there is strong relation between species richness and diversity with diverse habitat types.

3.3 Distribution and abundance of mosquito species in different habitats

There was difference in temperature and humidity in both the areas. There was strong positive correlation between abundance and temperature r_s = 0.86, p = 0.00 and, abundance and humidity; r_s = 0.76, p = 0.00 (Table 2). Similarly, it was found a positive relation between mosquito abundance with temperature and humidity [3]. It was well studied that the temperature and humidity affects the diversity and population of mosquitoes [25], [33]. This could be the reason that more species and more abundance of mosquito were recorded from Punakha due to conducive environment than in Thimphu.

From the two study areas, *Ar. subalbatus* was the only species found in both areas and it was only the species that was encountered in all the sampling sites. In the same way this species was found to be present in all the land cover used and vegetation types [8]. This particular species was also described as a multi habitat preferring species [24]. *Ar. subalbatus* was

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Table 3. Species diversity, richness and evenness in two areas

Habitat types	Punakha				Thimphu			
	Species	**Count**	***H***	***E***	**Species**	**Count**	***H***	***E***
Automobile workshop	3	153	1.09	0.99	2	111	0.69	0.63
Vegetable market	5	299	1.6	1	2	79	0.55	0.8
Sewage drain	3	180	1.09	0.99	3	249	1.06	0.97
Cattle shed	6	276	1.68	0.94	3	195	1.09	1
Household	7	244	1.84	0.94	3	255	1.09	0.99
Forest	5	122	1.59	0.99	2	111	0.68	0.97
Stream	3	118	0.99	0.9	2	70	0.5	0.72

H = Species diversity; *E* = Species *evenness*

found relatively more abundant in cattle shed (Table 5) but there was no significant difference in abundance of *Ar. subalbatus* between habitat types ($X^2_{(6)}$ = 6.87, *p* = .33) (Table 4). This species decreases its abundance rate with increase in elevation [8]. More abundance was recorded from Punakha (*n* = 393) than in Thimphu (*n* = 387).

Table 4. Summary of Kruskal-Wallis test

Species	***X*²**	***P***
Ar. subalbatus	6.87	.33
Cx. gelidus	30.55	.00**
Ae. albopictus	13.52	.04*
An. peditaeniatus	30.97	.00**
An. nigerrimus	32.77	.00**
An. splendidus	28.32	.00**
Ae. vexans	26.07	.00**
Cx. quinquefasciatus	14.45	.03*

*X*² = Chi-square, ** = significant at .01, * = Significant at .05

Ae. vexans was recorded only in Punakha area where it was collected from the elevation ranging from 1200 to 1500 m. The elevation below 1500m was found to be suitable for *Ae. Vexans* [19]. This species may prefer warm places thus was found only in Punakha area. *Ae. vexans* was found more abundant in cattle shed and there was significant difference of abundance in different habitats ($X^2_{(6)}$ = 26.07, *p* = .00). *Ae. vexans* is described as an opportunistic feeder, taking blood meals from a variety of animals, but mostly preferring large mammals, including cattle, horse, deer and humans [49]. This could be the reason the species was found abundant in cattle shed.

Cx. quinquefasciatus was recorded only in Thimphu, where elevation ranged between 2000 to 2900 m. The species prefer to be within the elevation of 3000 m [35]. This particular species can be found within the temperature range of 12 to 32ºC but their survivorship drops with an upper lethal threshold at 35ºC [4]. The temperature in Punakha was within the optimal range of this species with less elevation range (1200 to 1500 m) but could not be recorded in this study site. This may be because of the mismatch of morphological feature of *Cx. quinquefasciatus* with other *Culex* species. The more abundance of *Cx. quinquefasciatus* was recorded from households and significant difference in abundance was found in different habitats ($X^2_{(6)}$ = 14.44, *p* = .02). *Cx. quinquefasciatus* is described as domesticated mosquito species of rural and urban areas [3]. They have anthrophilic blood feeding habits which brings them close to human dwellings and human habitations [38].

Ae. albopictus was found only in Punakha within the elevation range of 1200 to 1500 m. This result is consistent with the result of Dhimal *et al*. [15] where they found common occurrence of *Ae. albopictus* up to 1350 m in Katmandu valley. More abundance was recorded from automobile workshop and there was significant difference among different habitat types ($X^2_{(6)}$ = 13.52, *p* = .03). The discarded tyres, damaged metallic parts of vehicle, other plastic bottles and cans were also found, in which some were filled with water. This could provide better place for breading as explained by Harrington [21]. Radhakrishnan [38] also found *Ae. albopictus* breeding in almost all types of fresh water holding containers.

All the three *Anopheles* species were recorded from Punakha study area. *An. peditaeniatus* and *An. nigerrimus* were recorded within the elevation range of 300 to 2000 m but found most abundant at the range of 300 to 1000 m asl [35]. *An. splendidus* was recorded at elevation range of 850 to 1250 m [27]. *An. Peditaeniatus* and *An. nigerrimus* were recorded abundantly in household and cattle shed and *An. splendidus* was abundant in cattle shed. There was significant difference between abundance and habitat types ($X^2_{(6)}$ = 30.97, *p* = .00, $X^2_{(6)}$ = 32.77, *p* = .00 and $X^2_{(6)}$ = 28.31, *p* = .00 respectively). It may be because that they are nocturnal and prefer to feed on cattle and human [13]. Dida *et al*. [17] and Ukonze *et al*. [46] also reported that *Anopheles* mosquitoes prefer habitat proximity to human settlements with presence of live stocks and wildlife for their blood meals.

In this study *Cx. gelidus* was recorded only from Punakha within the elevation ranged from 1200 to 1500 m and it

Table 5. Distribution of species in different habitats

Study area	Species	Habitat types						
		Aw	Vm	Sd	Cd	Hd	Ft	Sm
Punakha	*Armigeres subalbatus*	+(47)	+(51)	+(53)	+(73)	+(58)	+(56)	+(55)
	Culex gelidus	-	+(62)	+(74)	+(173)	+(139)	-	-
	Anopheles peditaeniatus	-	-	-	+(50)	+(50)	+(36)	-
	Anopheles nigerrimus	-	-	-	+(52)	+(52)	-	-
	Anopheles splendidus		+(67)	-	+(83)	+(56)	+(35)	-
	Aedes albopictus	+(62)	+(52)	+(53)	-	+(51)	+(59)	+(47)
	Aedes vexans	+(44)	+(67)	+	+(141)	+(136)	+(51)	+(16)
Thimphu	*Armigeres subalbatus*	+(60)	+(19)	+(68)	+(65)	+(74)	+(45)	+(56)
	Culex quinquefasciatus	+(51)	+(60)	+(66)	+(72)	+(78)	+(66)	-
	Culex sp.	-	-	+(115)	+(58)	+(103)	-	+(14)

Aw = automobile workshop,Vm = vegetable market, Sd = sewage drain, Cd = cattle shed, Hd = house hold, Ft = forest, Sm = stream, + = Presence of species, - = absence of species, abundance in bracket ().

was consistent with the finding of Anwar *et al*. [1] where, they have also found this species at elevation ranging from 736 to 1477 m. The habitat preference and occurrence within certain elevation range [41] could have contributed to its occurrence at Punakha. *Cx. gelidus* found more abundant in cattle shed and there was significant difference of abundance in different habitats ($X^2_{(6)}$ = 30.552, p = .00). The host preference experiment found that *Cx. gelidus* preferring cow was significantly higher than with other hosts [44]. The washed animal sheds were observed creating polluted ground pools which might serve as breeding sites for *Cx. gelidus.*

3.4 Relationship between species abundance and time effort

The abundance of the species collected was recorded for each time effort. Collection time was divided into five ranges i.e., 1800-1900 h, 1900-2000 h, 2000-2100 h, 2100-2200 h and 0500-0600 h [9]. In this study, *Ar. subalbatus* was the only species that overlapped in both study areas. The highest number of *Ar. subalbatus* was recorded at 1800-1900 h followed by 0500-0600 h of the sampling effort hour in both the areas (Figure 2). Since the peak biting period of *Ar. subalbatus* was between 1800-1900 h and 0600 to 0800 h [10] and it had a crepuscular biting behavior with peak biting hour during dusk [34] which slightly extend up to mid night and minor peak hour was observed during dawn period.

The highest abundance of *Ae. albopictus* in this study was recorded at 1800-1900 h followed by 0500-0600 h (Figure 3). Similarly, peak biting activity of *Ae. albopictus* was reported between 1800-1900 h and 0600-0800 h [9], [10], [16]. This particular species was also described as aggressive and day time biting insect [6]. The *Ae. vexans* was abundant at 2100-2200 h and similar result was reported [34] that described *Ae. vexans* as night time bitter as this species exhibited restricted night time biting activity.

More abundant of *Cu. quinquefasciatus* was recorded at 2100-2200 h. Its abundance increased with time effort and was found less during morning. Uttah, *et al*. [47], reported that the peak biting activity of *Cu. quinquefasciatus* at 2000-2200 h, but the biting declines steadily to the lowest level towards morning. *Cu. gelidus* was found only in Punakha and its abundance was found highest at 2100-2200 h and was less towards morning [10].

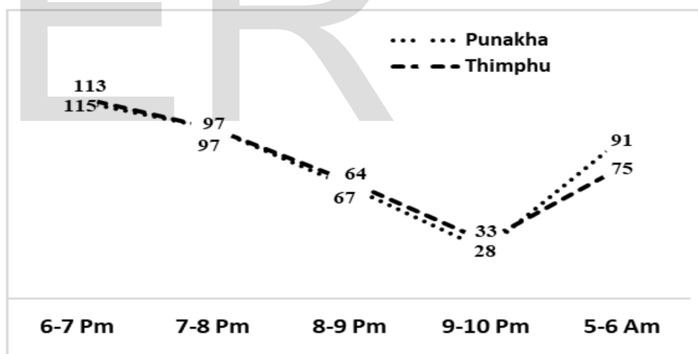

Fig. 2. *Ar. subalbatus* occurance at different time in two areas

All three *Anopheles* species had peak biting activity at 2100-2200 h. All three species were found biting starting from the sampling hour (1800 h). Their abundance increased with time effort hour, which indicates their nocturnal biting behavior but abundance decreased towards morning. The anophelinae are active throughout the night with less active towards morning [13} which confirms the nocturnal behavior of *Anopheles* species with peak biting activity at mid night.

Biting patterns of mosquito species varied, which could be due to the ability of each species to adapt in different areas and environments as reported by Asha and Aneesh [2]. However, all the mosquitoes collected started to hunt for blood meals shortly after sunset and reached their peak biting activity at night. These findings may be considered while taking any preventive measure related to mosquito vector in these two areas.

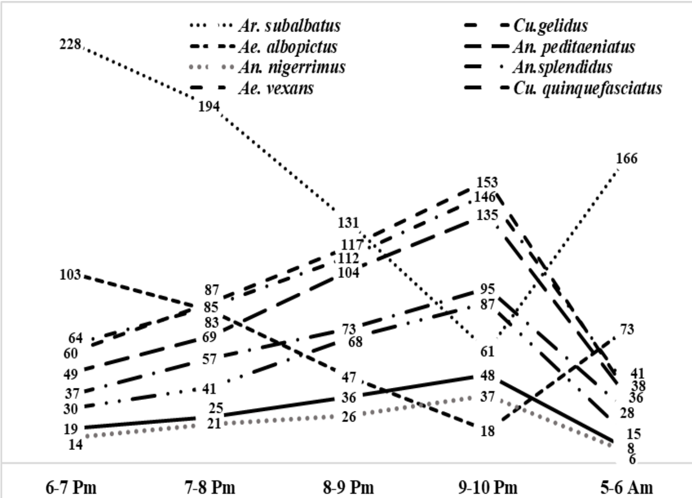

Fig. 3. Mosquito species occurance at different time effort

3.5 Public health importance of collected species

All the species encountered in this study are of medically importance and many studies have confirmed the potential of each species to transmit diseases (Table 6). The presence of these medically important species in Punakha and Thimphu indicates the potential of spreading diseases by these species. These findings can be of medical importance in developing any preventing mosquito diseases in these areas.

Table 6. Medical importance of collected species

Species	Medical importance
Aedes albopictus	Dengue and Chikungunya [6]
Aedes vexans	Rift valley virus [26]
Armigeres subalbatus	Japanese encephalitis [11]
Culex gelidus	Japanese encephalitis [40]
Culex quinquefasciatus	Bancroftian filariasis [4]
Anopheles peditaeniatus	Malaria vector [48]
Anopheles nigerrimus	Malaria vector [48]
Anopheles splendidus	Malaria vector [32]

4 Conclusion

This study documented nine species of mosquitoes belonging to four genus and two subfamilies from selected habitats of Punakha and Thimphu. In the subfamily Culicinae three genera and in subfamily anophelinae one genus was recorded. *Anopheles* and *Culex* was more diverse with three species and *Armigeres* with one species. *Ar. subalbatus* was the most dominant species and *An. nigerrimus* was least abundant. Six species are new to Bhutan as they are not reported in any published articles. Diversity was higher in Punakha (H = 1.84, seven species) then in Thimphu (H = 1.09, three species). This could be due to difference in elevation, temperature and humidity in the two areas, where more warm and humid environment was found in Punakha than in Thimphu. *Ar. subalbatus* was only the species found in the both areas, which was also found in all the selected habitat types. All the species encountered are potential pathogens of some diseases and all the species collected started to hunt for blood meals shortly from the dusk and reached their peak biting at night. This findings needs to be considered for any preventive measure and strategies that will take in future to control mosquito borne diseases. This paper however lacks the data covering different seasons and habitats other than selected habitats as described in this paper. Therefore, the study has to expand across different season and diverse habitats

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