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DIVERSITY AND LARVAL HABITATS OF MOSQUITOES (Diptera: Culicidae) IN URBAN AREAS IN HA NOI, VIETNAM

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ABSTRACT

Although some mosquito-borne diseases have been thriving in Ha Noi city, Vietnam very few studies on mosquitoes have been conducted. Determining how the species composition and abundance of mosquito vectors depend on environmental resources in different habitats addresses where different types of vector control need to be applied. The present study aims to investigate the characteristics of larval habitats and to provide a composition of mosquitoes (Culicidae) in this city. Surveys of larval and adult mosquitoes were carried out from different habitats in urban areas during rainy seasons from 2018 to 2023. Overall, in almost all water containers that were surveyed (80.9%), immature mosquitoes were present. A total of 4593 mosquitoes including 3,950 larvae and 643 adults were collected from 16 sampling sites. Twelve mosquito species belonging to 6 genera were collected and identified. *Aedes albopictus* and *Culex quinquefasciatus* were the most common and abundant species, each accounting for 34% of the total collected larval specimens. Five species, *Anopheles umbrosus, Culex cintellus, Armigeres subalbatus, Mansonia annulifera*, and *Lutzia fuscana* were the first records for Ha Noi city. The species composition varied between different habitat types. Peri-domestic habitats were the most common breeding sites for mosquitoes in the study area. Noteworthy, species belonging to genus *Aedes* were abundant in domestic habitats created by anthropogenic materials while that of genus *Culex* was mainly thriving in large water bodies in both peri-domestic and natural habitats. The current results show a variation in the distribution and abundance of mosquitoes in urban areas of Ha Noi which should be considered for effective planning and implementing mosquito control programs in the city.

Keywords: Mosquito diversity, larval habitats, *Anopheles, Aedes*, *Culex.*

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INTRODUCTION

The family of Culicidae (Diptera) includes two subfamilies, 11 tribes, 113 genera, and 3,719 described species in the world fauna (Harbach, 2023). Culicine mosquitoes are the most important vectors concerning public health, as they transmit malaria, dengue fever, and various types of arboviruses (Tolle, 2009). The recent increase of mosquito-borne diseases worldwide is also likely associated with the rapid expansion of urban areas (Norris, 2004; Wilke et al., 2019). Particularly, the density of human populations in urban areas likely enhances vector-human contact which has resulted in the diseases circulating more frequently. Further, many major mosquito vectors have been shown to be well adapted to urban environments and their larvae are often found in discarded containers in residential areas (Kolimenakis et al., 2021). That may explain the close association between high infection risk, socio-economic factors, and rapid urbanization without appropriate infrastructure. Culicine mosquitoes are known to breed in diverse habitats and occur in various environments. Therefore, information on larval habitats may help managers and residents target breeding habitats of mosquito vectors, which could have a significant impact on the prevalence of arboviruses in urban areas.

Vietnam is a tropical country where environmental conditions favor mosquitoes' diversity and circulation of mosquito-borne diseases. While the number of positive cases of malaria has decreased recently, that of dengue has been increasing in the country (Nguyen et al., 2019). Ha Noi is one of the most populous cities in Vietnam with fast urbanization in recent decades (General Statistics Office, 2020). Under rapid changes in the socio-economic and biophysical conditions, mosquito-borne diseases can become a significant threat for the local people in urban areas (Nguyen et al., 2019). For example, dengue fever, a mosquito-borne disease, is now thriving in the city, particularly, with roughly 1,000 cases in 2019 (Nguyen et al., 2023). However, the culicid fauna in the urban area of Ha Noi is still poorly understood since the surveys have been conducted in limited areas or habitats. Particularly, a study investigated insect fauna in residential areas in Ha Noi has reported only a few mosquito species, including *Culex quinquefasciatus, Culex tritaenyorhynchus, Aedes aegypti, Aedes albopictus*, and *Anopheles sinensis* (Pham, 2015). The study by Nguyen et al. (2021) aimed to assess the effects of livestock on the abundance of mosquitoes in urban households in Ha Noi has reported 10 species including *Ae. aegypti, Ae. albopictus*, *Cx. quinquefasciatus, Cx. tritaenyorhynchus*, *Culex vishnui*, *Culex pseudovishnui, Culex gelidus, Manosia* sp., *Amigeres* sp., *Anopheles* sp.. Therefore, the current study aims to investigate the diversity of mosquitoes in urban areas of Ha Noi city. We also focus on evaluating the larval characteristics, breeding habitats preference of mosquito species in different habitat types. The results from this study are expected to provide important knowledge for future vector control strategies in Ha Noi city.

MATERIALS AND METHODS

Study area

Ha Noi city is located within the Red River Delta and is the second-largest city in Vietnam. It covers an area of 3,359 km2 including 12 urban districts, one district-level town and 17 rural districts. Further, the city is the second most populous with more than 8 million people recorded in 2019 and half of them are living in urban districts where it accounted for only 307.83km2 (General Statistics Office, 2020).

Samplings were done during the period between July to September 2018 and September to November 2022, associated with rainy seasons in urban areas in Ha Noi, Vietnam (Table 1).

Table 1. Sampling localities of mosquitoes in urban areas of Ha Noi

ID	Locality (district)	Sampling date (d/m/y)	Geographic coordinate (lat, long)	Life-stages analyzed	No. of species	No. of larvae	Types of habitats
S1	Bac Tu Liem 1	11/7/2018, 20/8/2023	21.072353, 105.723094	Larvae	5	552	Canals, sewers, ponds, mud puddles
S2	Bac Tu Liem 2	11/7/2018, 20/8/2023	21.070970, 105.727829	Adult & Larvae	3	222	Used tires, plastic containers, glass containers, coconut shells
S3	Bac Tu Liem 3	11/7/2018, 20/8/2023	21.074426, 105.726044	Adult & Larvae	4	317	Flowerpot/vases, plastic containers, ceramic jars, tree holes
S4	Nam Tu Liem	30/8/2018, 20/8/2023	21.016347, 105.774877	Adult & Larvae	4	199	Used tires, plastic containers, glass containers, bonsai tank, rock holes, tree holes
S5	Cau Giay 1	30/8/2018, 20/8/2023	21.034861, 105.786359	Adult & Larvae	2	61	Flowerpots, plastic buckets
S6	Cau Giay 2	30/8/2018, 20/8/2023	21.028913, 105.790667	Adult & Larvae	2	166	Used tires, plastic pots and trashes, tree holes
S7	Cau Giay 3	30/8/2018, 15/10/2023	21.040719, 105.796991	Adult & Larvae	2	224	Used tires, plastic pots, and trashes
S8	Cau Giay 4	30/8/2018, 15/10/2023	21.031446, 105.806900	Adult & Larvae	3	127	Bonsai tanks, pagoda ornaments, ponds, and lakes
S9	Thanh Xuan 1	12/7/2018, 6/9/2023	20.996130, 105.807847	Adult & Larvae	3	185	Bonsai tanks, plastic containers
S10	Thanh Xuan 2	12/7/2018, 6/9/2023	21.004609, 105.810181	Larvae	2	53	Flowerpots, used tires, plastic containers
S11	Thanh Xuan 3	12/7/2018, 6/9/2023	20.987592, 105.808736	Larvae	2	35	Flowerpots, used tires, plastic containers
S12	Ha Dong 1	3/9/2018, 13/10/2023	20.973583, 105.792591	Larvae	9	766	Flowerpots, used tires, plastic containers, bonsai tanks, ceramic jars, rock holes, tree holes, coconut shells, ponds, mud puddles
S13	Ha Dong 2	3/9/2018	20.967884, 105.762447	Adult & Larvae	3	325	Flower vases, plastic buckets, sewer
S14	Ha Dong 3	3/9/2018	20.964183, 105.788825	Larvae	5	212	Plastic buckets, used tires, pagoda ornament
S15	Hoang Mai 1	15/10/2023	20.966738, 105.826541	Adult & Larvae	2	193	Used tires, plastic buckets
S16	Hoang Mai 2	15/10/2023	20.957115, 105.852187	Adult & Larvae	2	313	Used tires, plastic trash, tree holes

Note: ID/identification codes, d/m/y- date/month/year, lat, long/latitude, longitude.

Larval and adult collection

Both adults and larvae of mosquitoes were collected in 16 locations in urban areas of Ha Noi (Fig. 1, Table 1). Containers with water and water bodies were inspected as potential breeding sites of mosquitoes. Positive breeding sites were recorded when at least one larva or pupa was found. We surveyed various kinds of habitats such as residential houses, public areas (parks, playgrounds, car parks, pagodas, schools,…) and each site was far from each other at least 500 m. The categorization of habitat types was based on types (natural or artificial), locations (indoor or outdoor) including domestic (indoor, artificial containers), peri-domestic (outdoor, artificial containers) and natural (outdoor, natural-material containers) habitats. Depending on the volume of the containers or waterbodies, mosquito larvae were sampled using either a standard 350-mL dipper where the water volume was higher than 350 mL or a pipette where the water volume was lower than 350 mL (Medlock et al., 2018). In the former method, one to three dipper samples were taken in large water bodies such as ponds, lakes, canals, sewers, or mud puddles. The collectors scooped on the water surface and then inspected for the presence of mosquito larvae. If the cup was emptied the collector would try again in another nearby spot. If mosquito larvae were present, a pipette was used to remove them and transfer them to the holding container prior to taking another dip samples. The number of dip samples were recorded. In the latter method where a dipper could not be used, water was emptied onto a tray and all larvae and pupae were collected. The samples were then stored in labelled plastic containers and transported to the laboratory for rearing and identification.

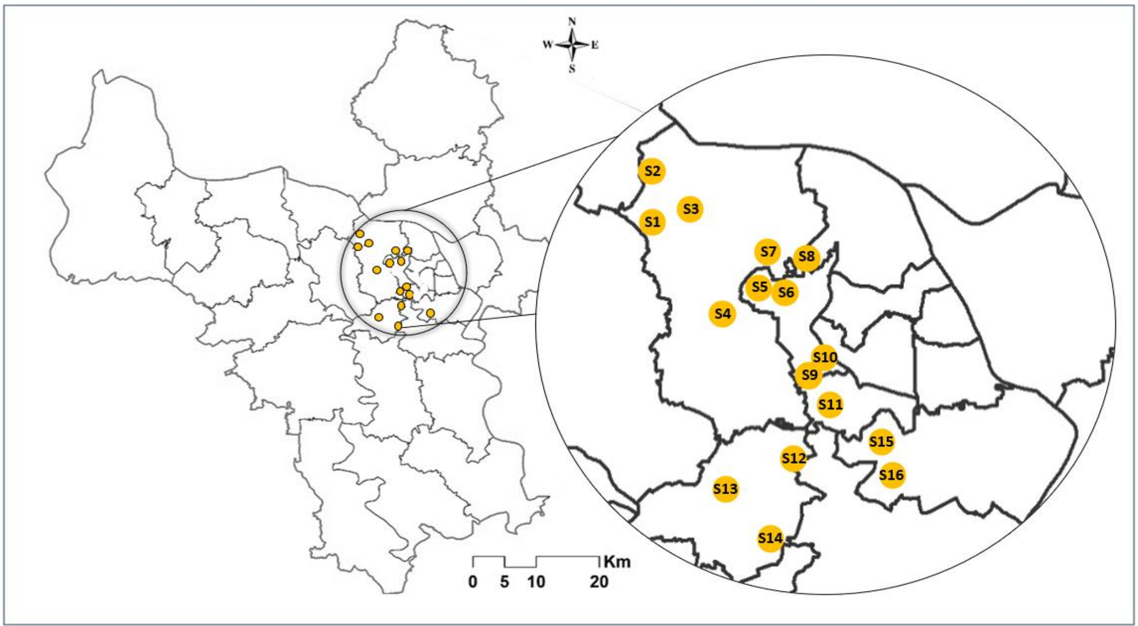

Figure 1. Map of sampling sites in urban areas of Ha Noi city (1–16: Study sites)

Some larvae and pupae were also reared until the adult stage for more reliable identification. Only the fourth and fifth larval instars were used for morphological identification and subsequent analyses. Larval density was calculated by dividing the total number of larvae per breeding site by the number of dippers collected. For samples collected by pipette, the number of larvae in each container was considered equivalent to the collection by one dipper.

Adult mosquito collections were done by several different methods, including using aspirators, sweeping nets, night landing catch on cows, and BG-sentinel trap (BioGents, Regensburg, Germany). Both larval and adult mosquitoes were identified using the keys by

Mattingly (1970) and Nguyen et al. (2008). Adult specimens were used only for determining species composition, while larval specimens were used for both determining species composition and analyzing larval habitat characteristics. Special permissions were not required for the collection of mosquitoes at any of the sites, and in the case of private residential areas, permission was obtained from owners before entry.

Data analysis

Mosquito diversity was measured using the Shannon-Weiner index (H′), species richness was analyzed by Margalef index (d) that were calculated by using the following formulas:

Shannon-Weiner diversity index (H'):

$$H' = -\sum_{i=1}^{s} \frac{n_i}{N} \log_2 \frac{n_i}{N}$$

Where: s: number of species; n_i = no. of individuals of a species; N = Total number of individuals.

Margalef species richness index (d):

$$d = \frac{S-1}{\log N}$$

Where: S = Total species; N = Total individuals.

The similarity percentage analyses (SIMPER) between habitat types were conducted to evaluate the percentage of contribution and average dissimilarity between the habitat types, in which the overall significance of the difference is measured using the Bray-Curtis similarity by using the following formula:

$$BC_{ij} = 100 * \left(1 - \left(2 * C_{ij}\right)\right) \Big/ \left(S_i + S_j\right)$$

Where: C_{ij}: The sum of the lesser values for the species found in each habitat type; S_i: The total number of specimens counted at habitat i; S_j: The total number of specimens counted at habitat j.

The Bray-Curtis dissimilarity ranges between 0 and 100% where 0 indicates that two localities have exactly the same number of each type of species while 100% indicates that two localities have complete dissimilarity (Clake, 1993). A standardization step to normalize the distribution in each habitat was applied using $\log_{10}(X+1)$ transformation. All analyses were conducted using the software Primer V.6 (Gorley & Clarke, 2006).

RESULTS

Mosquito abundance and diversity

A total of 4,593 mosquitoes including 3,950 larvae and 643 adults were collected from 16 localities in urban areas in Ha Noi city (Table 1). Twelve different species belonging to six genera of mosquitoes were identified (Table 2). The genus *Culex* is recorded with the highest number of species (five species), while the other genera (*Anopheles, Aedes, Armigeres, Mansonia* and *Lutzia*) were recorded at most two species in each genus (Table 2). Five species, *Anopheles umbrosus, Culex cintellus, Armigeres subalbatus, Mansonia annulifera,* and *Lutzia fuscana* have been recorded from Ha Noi city for the first time. For *Lt. fuscana* and *Ma. annulifera*, only larvae were collected (Table 2). Among 12 species found in the surveys, both *Ae. albopictus* and *Cx. quinquefasciatus* were the most dominant species, each accounting for 34% of total larval individuals. These dominant species were also found in wide ranges in the study area (14/16 sites, Table 2). The other 10 species were scarce with each accounting for less than 8% of total larval individuals, and several of them were recorded from only one site, including *An. umbrosus* and *Cx. cintellus* only in site S1, *An. sinensis, Cx. gelidus,* and *Lt. fuscana* only in site S12 (Table 2).

The diversity in each locality was measured as, the Shannon-Weiner diversity index (H′) varied from 0.23 (site 10) to 2.38 (site 12) with a mean value of 1.21 ± 0.56, while Margaleft species richness (d) ranged from 0.17 (S16) to 1.205 (S12) with a mean value of 0.42 ± 0.26 (Fig. 2).

Table 2. The density of larval mosquitoes at sampling locations in urban areas of Ha Noi, Vietnam. Sites codes are defined in Table 1; “-” not computed

No.	Species	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	Total no. of individual (%)
1	*Anopheles umbrosus* (Theobald, 1903)	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15 (0.4)
2	*Anopheles sinensis* Wiedemann, 1828	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3 (0.1)
3	*Aedes aegypti* (Linnaeus, 1762)	-	4	11	-	50	-	-	-	34	-	2	-	35	65	-	-	201 (5.1)
4	*Aedes albopictus* (Skuse, 1894)	-	86	77	131	11	105	203	86	42		51	41	116	35	124	235	1343 (34.0)
5	*Culex quinquefasciatus* Say, 1823	224	132	43	11	-	61	21	29	109	22	-	306	174	69	69	78	1348 (34.1)
6	*Culex vishnui* Theobald, 1901	97	-	-	-	-	-	-	-	-	-	-	195	-	12	-	-	304 (7.7)
7	*Culex tritaeniorhynchus* Giles, 1901	185	-	-	16	-	-	-	-	-	13	-	101	-	-	-	-	315 (8.0)
8	*Culex gelidus* Theobald, 1901	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	21 (0.5)
9	*Culex cintellus* Theobald, 1901	31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	31 (0.7)
10	*Armigeres subalbatus* (Coquillett, 1898)	-	-	186	41	-	-	-	12	-	-	-	13	-	-	-	-	252 (6.4)
11	*Mansonia annulifera (*Theobald, 1901)	-	-	-	-	-	-	-	-	-	-	-	54	-	31	-	-	85 (2.2)
12	*Lutzia fuscana* (Wiedemann, 1820)	-	-	-	-	-	-	-	-	-	-	-	32	-	-	-	-	32 (0.8)

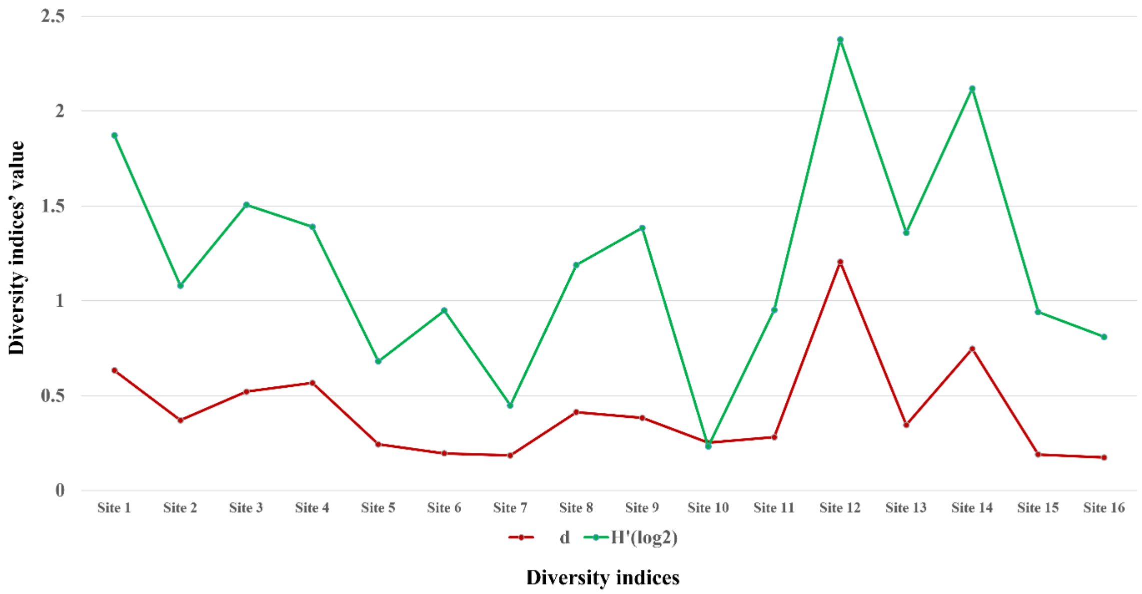

Figure 2. Diversity indexes in sampling sites in urban areas in Ha Noi, Vietnam. D: Margaleft species richness index; H′(log2), Shannon-Weiner diversity index

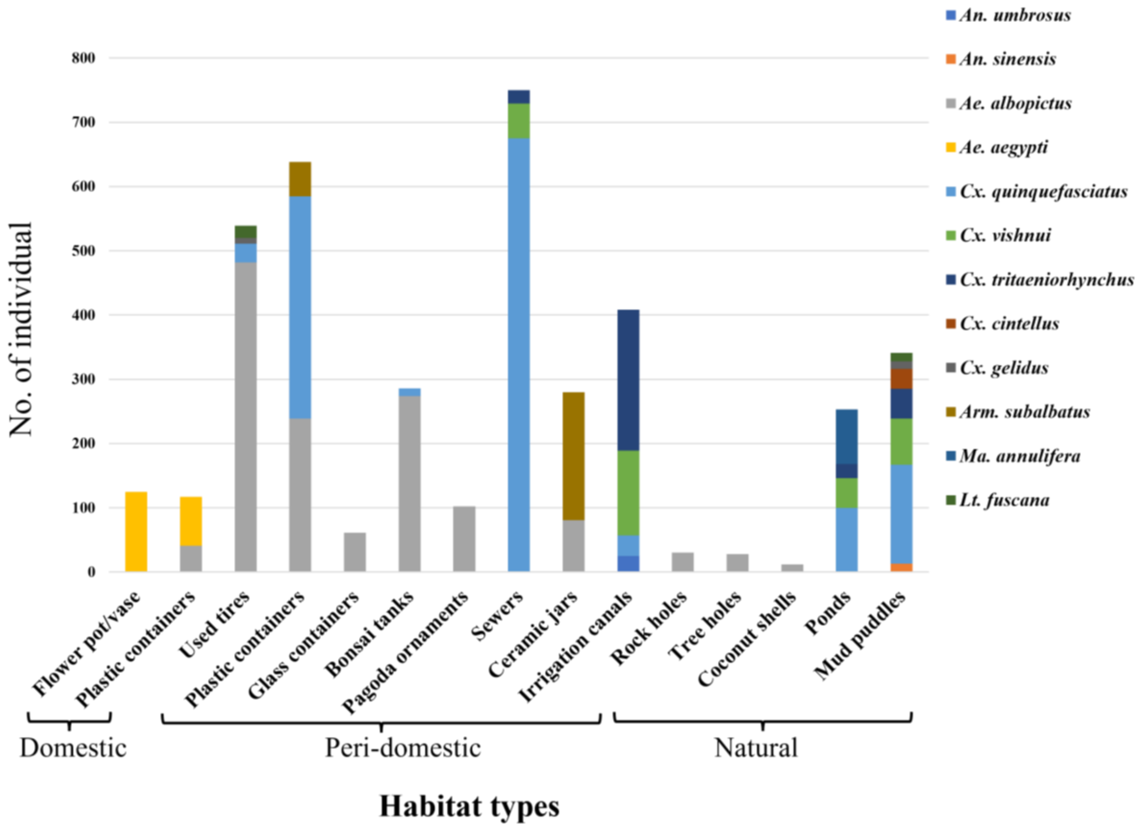

Figure 3. Mosquito species proportions in each type of breeding site across all sampling sites

Larval habitat characteristics

Out of 220 breeding sites surveyed in 16 sampling locations, 178 were positive for larval/pupae of mosquitoes, the majority being plastic containers (34.6%), followed by used tires (24.6%). Other types of breeding sites included flowerpots/ vases, glass containers, bonsai tanks, pagoda ornaments, ceramic jars, sewers, canals, rock holes, tree holes, coconut shells, ponds, and mud puddles (Fig. 3). Analysis of habitat characterization has shown that the species composition and abundance of mosquitoes vary strongly between habitat types. Natural habitats have the highest number of species with nine species, whereas peri-domestic habitats have eight species, and domestic habitats have only two species. However, peri-domestic habitats have the highest abundance of mosquito larvae, with 3054 individuals, while the abundance in natural habitats and domestic containers is much lower, with 654 individuals and 242 individuals respectively.

Most species found in the area showed strong habitat preference, except for *Ae. albopictus* which occur in all types of habitats. On the contrary, *Ae. aegypti* occurred exclusively in domestic habitats, *An. umbrosus* and *Ar. subalbatus* only occurred in peri-domestic habitats, and *An. sinensis*, *Cx. cintellus*, and *Ma. annulifera* were only found in natural habitats. Other five species were found in both natural and peri-domestic habitats (Fig. 4).

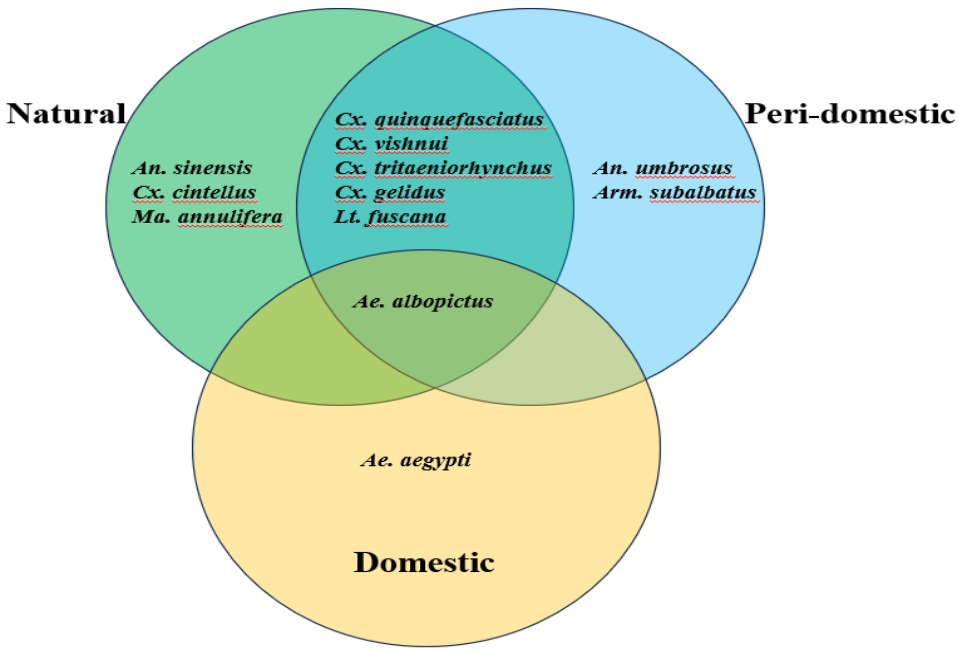

Figure 4. The diagram shows the absolute presence of mosquito species found in domestic (yellow), peri-domestic (blue) and natural (green) habitats

The SIMPER analyses, which were used to evaluate the species dissimilarity between habitats, showed a very high dissimilarity between domestic and peri-domestic habitats (84.3%), followed by that between domestic and natural habitats (82.4%). In contrast, the dissimilarity between peri-domestic and natural habitats was the lowest with 33.8% (Table 3). *Cx. quinquefasciatus* was one of the most important species contributing to the dissimilarity between domestic and peri-domestic habitats (17.5%) and between natural and peri-domestic habitats (15.9%). On the other hand, *Ar. subalbatus* contributed the highest percentage of dissimilarity (22.8%) between the domestic and natural habitats (Table 3). Noteworthy, *Ae. aegypti*, the most important vector species, was also one of the top species contributing to the dissimilarity between habitats since it was found only in domestic containers (Fig. 4, Table 3).

Table 3. SIMPER analysis showing the percentage of dissimilarity between different habitats with their top 4 contributing mosquito species (AD = average dissimilarity, PC = percentage contribution)

Domestic versus Peri-domestic AD = 84.3		Domestic versus Natural AD = 82.4		Natural versus Peri-domestic, AD = 33.8	
Species	PC (%)	Species	PC (%)	Species	PC (%)
Culex quinquefasciatus	17.5	*Culex quinquefasciatus*	15.9	*Armigeres subalbatus*	22.8
Armigeres subalbatus	13.8	*Aedes aegypti*	15.2	*Mansonia annulifera*	18.4
Culex tritaeniorhynchus	13.7	*Culex vishnui*	13.7	*Culex cintellus*	14.3
Aedes aegypti	13.2	*Mansonia annulifera*	12.8	*Aedes albopictus*	11.8
Culex vishnui	13.1	*Culex tritaeniorhynchus*	12.1	*Anopheles umbrosus*	11.4

DISCUSSION

Knowledge of larval habitat characteristics in an area and its impacts on mosquito diversity, abundance, and distribution is critical for developing integrated vector control strategies and mitigating future outbreaks of mosquito-borne diseases. The current study has reported a higher number of mosquito species than previous studies in Ha Noi (Pham, 2015; Nguyen et al., 2021). Moreover, it is worth noting that there were five new records for urban areas of Ha Noi city including *An. umbrosus*, *Ar. Subalbatus*, *Cx. cintellus*, *Ma. annulifera*, and *Lt. fuscana*. That is mainly due to the systematic planning of the survey which considered diverse habitat preferences of both adult and larvae stages. However, the geographical scope of this study was rather narrow and focused on urban areas of Ha Noi city, so the results still may not represent the true diversity of mosquitoes in Ha Noi. Therefore, surveys in larger areas may provide a more complete checklist of mosquito species in Ha Noi.

In addition, among 12 species found this this study, eight are known to transmit mosquito-borne diseases to humans such as malaria, dengue, Japanese encephalitis, Lymphatic filariasis in the world, including *An. sinensis*, *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, *Cx. vishnui*, *Cx. tritaeniorhynchus*, *Ar. subalbatus*, and *Ma. annulifera* (Jupp, 2005; Kraemer et al., 2019; Muslim et al., 2013; Pham et al., 2017). Their occurrence in the studied area presents the risks of exposure of mosquito-borne diseases to residents and potential outbreaks, therefore, the findings of this study may be useful for planning mosquito-borne disease elimination and eradication strategies.

Another significant finding of our survey is that most of the mosquito species in the area have separate niches, except *Ae. albopictus*, which was found to occur in all habitat types (Fig. 4). In domestic containers, most larvae belong to *Ae. aegypti*, which is similar to the findings by previous studies (Braks et al., 2003; Duong et al., 2022). The species is an invasive species in Vietnam and domestic containers may create favor conditions for their successful invasion because they can prevent unsuitable weather conditions in invasion regions and interspecific competition with *Ae. albopictus* (Braks et al., 2003). Since the strong affiliation of this species with households are associated with Dengue outbreaks, this poses a significant challenge to control the disease (Nguyen et al., 2019; Kolimenakis et al., 2021). On the other hand, *Culex* species were found mostly in outdoor containers in both peri-domestic and natural habitats, particularly, in the case of *Cx. quinquefasciatus* which was the dominant species in these habitats but was absent in domestic containers (Table 3). This is because *Culex* larvae are able to colonize polluted aquatic habitats with aquatic plants, while *Aedes* larvae prefer fairly clean and unpolluted water (Noori et al., 2015). This also indicates that water with rich organic nutrients may attract gravid *Culex* mosquitoes to lay eggs. Regarding *Ma. annulifera*, a primary vector of Lymphatic filariasis in Vietnam, was recorded only in ponds with the presence of an aquatic plant species *Pistia stratiotes* and it showed a strong affiliation with this type of plants. Species of the genus *Mansonia* are known to obligatorily attach their breathing tubes to the underwater roots stems, or leaves of floating aquatic plants and the vegetation further protects them from physical disturbances and provides mechanical support and favorable conditions for oviposition (Chandra et al., 2006).

The differences in mosquito species composition and variations in diversity indices among urban sites also indicate habitat preferences by mosquito species, e.g., site S12 was recorded with the highest diversity index (H′ = 2.38, *d* =1.21), sites S10 (H′ = 0.23*)* and S16 (d = 0.17) with the lowest (Fig. 2). Site 12 was found to have various types of mosquito breeding habitats, such as discarded artificial containers, ponds, mud puddles, canals which may support diverse species production. In contrast, sites S5, S6, S7, S10, S11, S15 and S16, which mostly had artificial containers, only contained at most two species in each site. In contrast to a previous study of mosquito breeding sites conducted from 1994 to1997 by Tran & Vu (1999), which reported a strong preference for big containers (tanks, clay jars, drums) as breeding habitats by mosquitoes, the findings of the current study indicate that small containers are dominant breeding sites of mosquitoes. This could be due to the development and improvement of the tap water system in the city, making it more stable in recent years, so reducing the habits by local people to spare water in large containers. Besides, people have been releasing a lot of smaller plastic containers into the environment creating favorable habitats for *Aedes* mosquitoes, particularly.

CONCLUSION

This study has provided the most comprehensive update on the mosquito fauna in urban areas of Ha Noi city based on the systematic selection of sampling habitats. Most of the sampling sites were occupied by mosquito larvae or pupae. The diversity of mosquito species varied between sites and was associated with the types of breeding habitats at each site. Only *Ae. albopictus* distributed in all kinds of habitats, while other species known to be vectors of arboviruses were predominant in more specific habitats, such as *Ae. aegypti* is mainly thriving in domestic habitats while *Culex* species are mostly found in domestic. Our current results can serve as baseline data for entomological monitoring of mosquito vectors and for designing targeted control programs. Nevertheless, further studies should be conducted in a wider geographical range, considering the complex characteristics of the physicochemical, ecological, and social factors of the study area and their interaction with various mosquito species.

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