

Population and Community Ecology

Mosquito Diversity in an Experimental Township in Tamil Nadu, India

P. Visa Shalini,¹ A. N. Shriram,^{1,✉} A. Elango,¹ R. Natarajan,¹ B. Vijayakumar,¹
K. H. K. Raju,¹ Lucas Dengel,² K. Gunasekaran,¹ and Ashwani Kumar¹

¹ICMR-Vector Control Research Centre, Department of Health Research, Ministry of Health & Family Welfare, GOI, Medical Complex, Indira Nagar, Puducherry 605 006, India, ²EcoPro, Aurosarjan Complex, Auroshilpam, Auroville, Tamil Nadu 605 101, India, and ³Corresponding author, e-mail: anshshriram@gmail.com, shriram.an@icmr.gov.in

Subject Editor: Richard Wilkerson

Received 19 July 2021; Editorial decision 20 April 2022.

Abstract

To glean more information on mosquito diversity and distribution in Auroville, a cross-sectional study was carried out by mapping the distribution of water bodies and habitats supporting immature stages on the one hand and the distribution of water bodies/habitats supporting mosquito immature stages on the other. A satellite image covering an area of 8.08 km² was overlaid with a grid of 500 × 500 m. Fifteen modules were selected and the area of each module served as the sampling site for the entomological survey. Adult and larval stages were sampled. Diversity indices were analyzed to compare mosquito diversity. Rarefaction estimations were used to compare abundance and richness of the mosquito species between different zones. In total, 750 mosquito larvae and 84 resting adults were sampled. Eighteen species of mosquitoes belonging to 11 subgenera and 7 genera were documented. Genera included *Aedes* (Johann Wilhelm Meigen 1818, Diptera, Culicidae), *Anopheles* (Johann Wilhelm Meigen 1818, Diptera, Culicidae), *Armigeres* (Theobald 1901, Diptera, Culicidae), *Culex* (Carl Linnaeus 1758, Diptera, Culicidae), *Lutzia* (Theobald 1903, Diptera, Culicidae), and *Mimomyia* (Theobald 1903, Diptera, Culicidae). Of the 18 mosquito species identified, 8 species are new records for Auroville. The Alpha (α) biodiversity indices show that the mosquito fauna is diverse ($S = 18$; $D_{Mg} = 2.732$ [95% CI: 2.732–2.732]). The Shannon-Weiner ($H' = 2.199$ [95% CI: 2.133–2.276]) and Simpson indices ($\lambda = 0.8619$ [95% CI: 0.8496–0.8723]) measured species richness, evenness, and dominance. The values of these indices suggest high species richness, evenness, and dominance. Prevailing conditions can provide suitable environment for establishment of different mosquito species in this ecosystem. Given the sociodemographic characteristics of this area, research on mosquito diversity and risk of vector-borne diseases will be of great use.

Key words: biodiversity, rarefaction, Culicidae, Auroville, India

Mosquitoes (Diptera: Culicidae) are a main group of arthropods in terms of public health significance, and have a direct relationship with environmental factors, diversity in habitats or host preferences (Becker et al. 2010). Certainly, different types of immature mosquito habitats provide more opportunities or niches resulting in the transmission of different pathogens (Adebote et al. 2008, Emidi et al. 2017). However, some of the highest abundances of mosquitoes come from particular habitats, or specific habitats, e.g., mangroves, swamps, or irrigated fields like rice crops. Climate change and human activity may influence mosquito abundance and biodiversity (Multini et al. 2020, Schrama et al. 2020, Chaves et al. 2021), with

uncertain effects on vector-borne diseases (VBDs) risk (Eveline et al. 2005, Hanafi-Bojd et al. 2012, Mwangangi et al. 2012, Versteirt et al. 2013, Nikookar et al. 2015). Mosquito diversity studies could be of much help in understanding this complex interaction between environment and risk to human health.

Environmental and landscape changes due to human intervention can lead to search for novel breeding habitats of mosquito vectors. For example, Krishnamoorthy et al. (2005) observed that as seawater line extended further inland following the Tsunami, habitat and vector species came along. These changes can influence the epidemiology of diseases (Enayati and Hemingway 2010, Keesing

et al. 2010, Kweka et al. 2016). Deforestation leads to changes in abundance and feeding behavior of vector mosquitoes (Manga et al. 1995, Kamdem et al. 2012, Burkett-Cadena and Vittor 2018), life table attributes of mosquitoes (Kweka et al. 2016), development of pathogen in the vector due to shifts in environmental and aquatic temperatures, and opportunities for finding new breeding and resting sites (Tuno et al. 2005, Afrane et al. 2012).

Auroville ('The City of Dawn') is an experimental city, envisaged to be an international society, where citizens from all over the world can live in harmony, rising above creeds and nationalities, with the ultimate aim of realizing human unity (<https://en.wikipedia.org/wiki/Auroville>). Prior to the establishment of Auroville, this area was an open dry land covered mostly by casuarina groves and there used to be farming operation during monsoon. With the establishment of this city, afforestation and organic agriculture could have created mosquito larval habitats, namely tree holes, cement tanks, ponds, large water storage earthen tanks, small pools, artificial fountains, and water drainage systems. As a result, the changes in the environment, like aquatic habitats, provide humidity, and tree shadow all over Auroville provides a favorable environment for mosquito diversity. All this is bound to influence the adjoining areas of Puducherry too. Earlier studies were undertaken with a view to preparing 'A check-list of mosquitoes of Puducherry (Rajavel et al. 2004) covered only a small area of Auroville'. Seven aedine species and one species of the genera *Culex* and *Aedes* were recorded (Rajavel et al. 2004). Over two decades have elapsed since these observations were documented. A lot of infrastructural development, changes in the demography, and in-land use including water resource management within the Auroville area have taken place. Such vicissitudes can have a great impact on the ecological balance where mosquitoes breed, develop, and transmit diseases. There is an increased risk of introducing parasites and vectors, along with increased connectivity of our world and the study region in particular. In these circumstances, it becomes imperative to further our understanding of the risk of VBDs and vector introductions to Auroville and the adjoining Puducherry. So this study was undertaken as a first step to document mosquito abundance and diversity. Information along these lines would enable us to better consider vector control options to prevent possible VBD risks.

Materials and Methods

Study Area

Auroville is an experimental township spanning an area of about 20 km², encompassing larger areas in Villupuram district, Tamil Nadu and some parts within the Union Territory of Puducherry. Over the last half a century, environmental rehabilitation and afforestation work have helped create a relaxed lifestyle for inhabitants in and around the township area.

Demography, Climate, Developmental Activities, Crops, Tourism, and VBDs

As of January 2018, the population was 3,218 with people from 59 nations registered as residents. The climate is hot and humid with minor fluctuations in temperature during different seasons of the year (minimum 24.2°C in January and February and maximum 31.1°C in May and June). Average annual rainfall recorded by the Auroville meteorological station was 1,141 mm (8 mm in March and 274 mm in November) (<https://auroville.org/contents/135>). Construction of residential buildings, maintenance of existing buildings, engagement of media persons, waste water treatment plants, research teams,

town planning, afforestation activities, water table management, printing, and manufacturing clothing and jewelry form some of the core activities in Auroville. Rice is cultivated as an annual crop in 9% and vegetables in 6% of the cultivable land. Millet is also grown in Auroville. Tourism constitutes an important source of commerce. During the chikungunya outbreak in 2006, 441 cases were reported in Kulilapalyam, a village within the periphery of Auroville (Auroville Wikipedia 2020) (<https://research.auroville.org>). Subsequently 20 cases of dengue were reported (<http://wiki.auroville.org.in>). Auroville has red, brown, and white soil, providing a red sandy loam structure (<https://auroville.org—contents Land and Nature>).

Study Design

In Environmental Systems Research Institute's (ESRI) ArcGIS ArcMap (ArcGIS Desktop: release 10.2; ESRI, Redlands, CA), Auroville area map was opened covering areas of Puducherry and Tamil Nadu. The satellite image was added using add basemap function from ArcGIS online. This satellite image covering an area of 8.08 km² of Auroville was overlaid with a grid of 500 × 500 m. Fifteen proportionate modules were selected in consultation with residents of Auroville. The area within each of the 15 modules served as the sampling site for the entomological survey (Fig. 1). This area was divided into four zones, i.e., zone 1, zone 2, zone 3, and zone 4 in order to make the calculations mosquito species diversity. With the Matrimandir globe at the center of Auroville and based on latitudes and longitudes, four zones/directions were derived to understand mosquito species diversity within the precincts of Auroville (Fig. 2).

Entomological Survey and Mapping

A transect entomological survey was carried out during cold season (between 1 February and 4 March 2020). Immature stages were collected from all available types of habitats, ranging from man-made and artificial to natural habitats. Each larval habitat was georeferenced and its coordinates were recorded using Global Positioning System (GPS) (Garmin GPSMap 76Cx). The datum used was D_WGS_1984, plotted using ArcGIS software. Sampling devices such as dippers, pipettes, siphons, and buckets, as appropriate to the type of larval habitat, were used. Adult mosquitoes were collected from indoor and outdoor resting habitats such as cattle sheds, human dwellings, tree holes, and bushes in the morning between 0900 and 1100 h using mechanical aspirator/oral aspirator.

Identification of Field-Collected Mosquitoes

Larvae were reared to the adult stage in the Center's laboratory and pinned using minuten pins with corresponding habitat numbers. Locations from where each of the species was collected are denoted by alphabetic codes, which provide information on geo-coordinates, followed by larval and adult habitats. Adults mounted on minuten pins were given a unique number. Wherever necessary, larvae, larval and pupal exuviae, and male and female genitalia were mounted on glass microscope slide in Hoyer's mounting medium. Larval, pupal exuviae, and male and female genitalia were assigned unique number after the adult was mounted. Species identification was based mainly on adult characteristics and wherever necessary, associated larval and pupal skins and male and female genitalia were used for confirmation. Both adults and larval/pupal identifications were carried out using standard taxonomic keys (Christophers 1933, Barraud 1934, Bram 1967). The records of mosquitoes were listed alphabetically by genus, subgenus, and species, and the classification of Wilkerson et al. (2015) was followed. All the voucher specimens from the study area were deposited in the mosquito museum at the ICMR-Vector

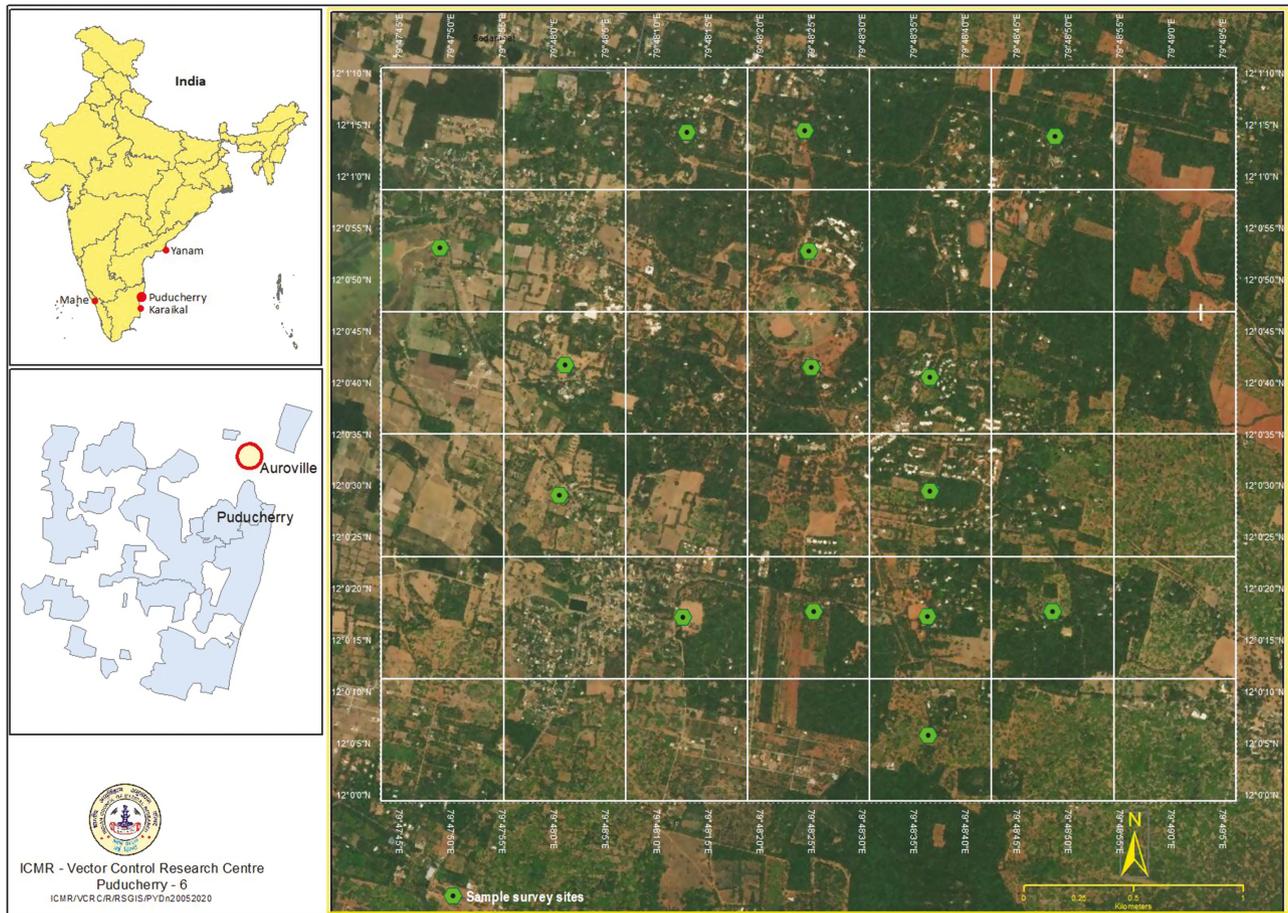


Fig. 1. Location of mosquito sampling sites in Auroville.

Control Research Centre, Puducherry. The complete collection has been catalogued with relevant information of collection for each specimen.

Statistical Analysis

Diversity Indices Species diversity was analyzed following Shannon-Weiner index, which characterizes abundance and evenness of the existing species and measures species diversity within the community of an ecosystem (Shannon and Weaver 1949). The degree of strength of dominance, on the likelihood of any two species drawn at random from a community belong to the same species, was assessed by using Simpson's index (Shannon 1948). Evenness with which individuals are divided among the taxa present was measured by Margalef's index (Death 2008). A higher number of species and even distribution were measured by Pielou index (Pielou 1984).

Rarefaction and Canonical Analysis (CA) Rarefaction-centered estimations were derived using EcoSim (Gotelli and Entsminger 2001) to estimate and collate the respective abundance and richness of mosquito species between environs and analyze sampling efficiency. The application of rarefaction permits differentiation of the number of species in different sizes of samples, as rarefaction simulates anticipated number of species, with a given number of mosquitoes sampled. Rarefaction trajectories typically rise early and, as a maximum number of mosquito species are detected, then plateau when unusual mosquito species continue to be sampled. Rarefaction curves were constructed in Past 4.0.3 software. CA was performed

to investigate differences in clusters of *Aedes aegypti*, *Anopheles*, and *Culex* between the zones, using STATA 14.2 (StataCorp, 2015 College Station, TX).

Results

Larval Sampling

Sixty-two mosquito-positive larval habitats spread in 15 locations within Auroville were sampled. Mosquito larvae were variously distributed in the following habitats: discarded tyres (DTs), artificial ponds (APs), dug well (DWL), ground pools (GPs), cement tanks (CTs), lake margins (LMs), grinding stones (GS), plastic water storage tanks (PWSTs), ant trap holders (ATHs), earthen pots (EPs), and soak pits (SPs). *Anopheles* larvae were found in five habitat types (AP, DWL, GP, CT, and LM), of which four were in combination with culicines, and *Anopheles* (GP) (Table 1). Culicine larvae were found in 11 habitat types (DT, AP, DWL, GS, ST, GP, CT, ATH, EP, LM, and SP) and 7 were for culicines only (DT, GS, PWST, GP, ATH, EP, and SP). Both anopheline and culicine larvae were found in nine habitats (17.6%), suggesting that the larvae from the subfamilies Culicinae and Anophelinae coexist in the majority of the habitats.

Species Composition

During the study period, 750 mosquito larvae and 84 resting adults were collected from 15 sampling locations, spanning an area of 8.08 km². Out of 750 larvae, 610 emerged, of which 106 were damaged. Consequently, 504 mosquitoes could be identified under 18

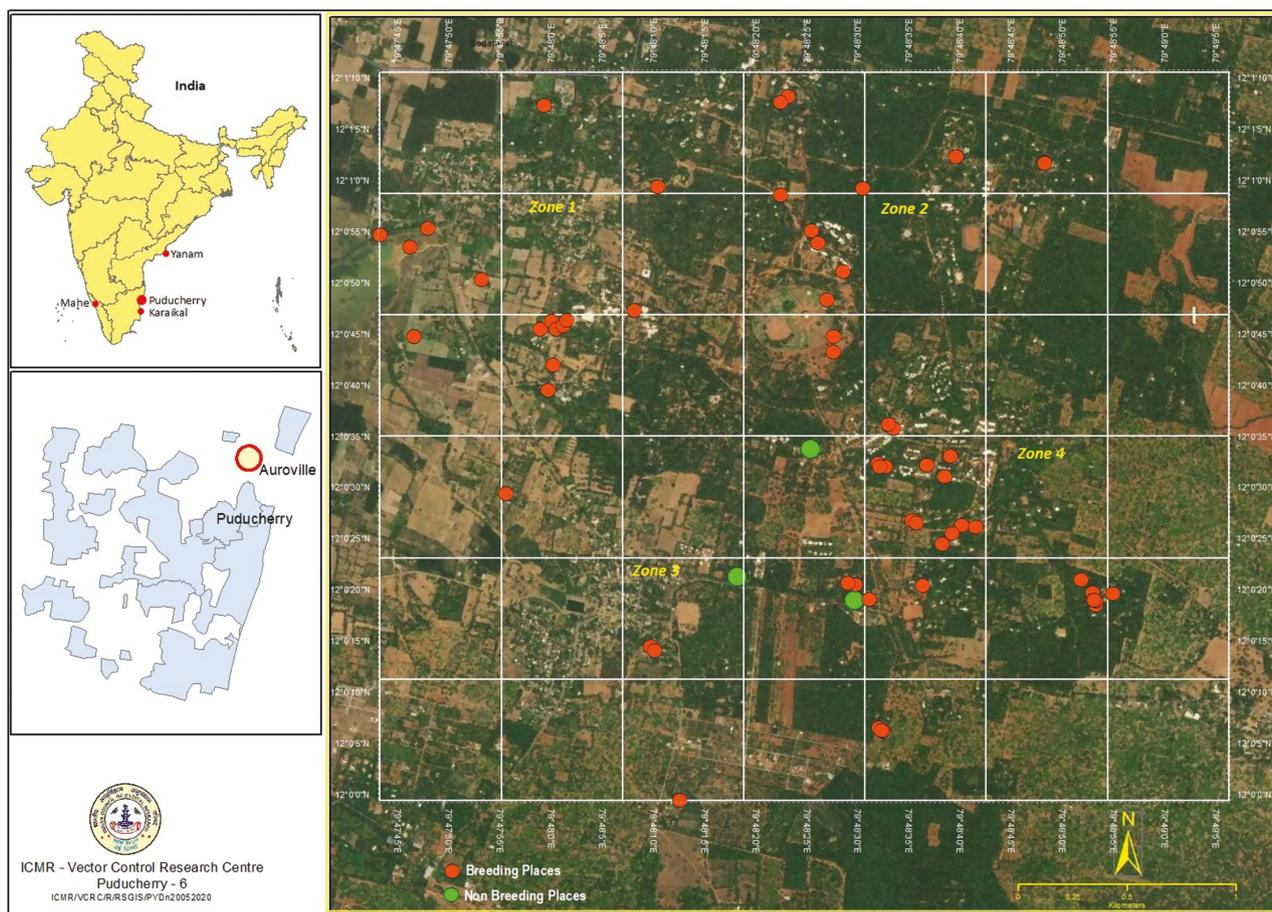


Fig. 2. Spatial distribution of mosquito larval habitats in Auroville.

Table 1. Distribution of anopheline and Culicine mosquito larvae in habitats sampled in Auroville (February–March 2020)

	Number of larval habitats (%)	Larval habitats										
		DT	AP	DWL	GS	PWST	GP	CT	ATH	EP	LM	SP
Anopheline vs Culicine												
Presence of anopheline only	1 (1.9)	0	0	0	0	0	1	0	0	0	0	0
Presence of culicine only	41 (80.4)	1	2	0	2	1	5	25	2	1	1	1
Presence of both anophelines and culicines	9 (17.6%)	0	2	1	0	0	0	5	0	0	1	0
Frequency of larval habitats	51 (100.0)	1	4	1	2	1	6	30	2	1	2	1
		1	4	1	2	1	7	37	2	1	2	1

AP, artificial pond; ATH, ant trap holders; CT, cement tank; DT, discarded tyres; DWL, dug well; EP, earthen pot; GP, ground pool; GS, grinding stone; LM, lake margins; PWST, plastic water storage tank; SP, soak pit.

species, belonging to 11 subgenera and 7 genera were identified. The genera included *Anopheles*, *Armigeres*, *Culex*, *Aedes*, *Lutzia*, and *Mimomyia*. Of the 18 mosquito species, 8 were new records for Auroville (Table 2).

Among the seven genera, genus *Culex* was predominant comprising 58.1% (293/504) followed by *Aedes* (22.6%, 114/504), *Armigeres* (10.5%, 53/504), and *Anopheles* (3.7%, 19/504). This included eight species belonging to three medically important genera viz. *Anopheles*, *Culex*, and *Aedes*.

By species *Aedes (Stegomyia) albopictus* Skuse (114/504, 22.6.0%) was the most common, followed by *Culex (Culex) gelidus*

(Theobald) (64/504, 12.7%) and *Culex (Culex) quinquefasciatus* (63/504, 12.5%). *Anopheles (Anopheles) barbirostris* Van der Wulp and *Anopheles (Cellia) subpictus* Grassi constituted 2.4 and 1%, respectively.

Habitat and Species Diversity in Auroville

The larval habitats were grouped into 11 categories (Table 3). Anophelines were found in relatively cleaner waters, while the culicines were found in muddy waters. Five permanent mosquito breeding habitats, i.e., CTs, GPs, APs, DWLs, and PWSTs, and

Table 2. List of culicid species recorded in Auroville

1	<i>Anopheles (Anopheles) barbirostris</i> Van der Wulp 1884
2	<i>Anopheles (Cellia) pallidus</i> Theobald 1901 ^a
3	<i>Anopheles (Cellia) subpictus</i> Grassi 1899
4	<i>Anopheles (Cellia) vagus</i> Doenitz 1902 ^a
5	<i>Armigeres (Armigeres) subalbatus</i> Coquillett 1898
6	<i>Culex (Oculeomyia) bitaeniorhynchus</i> Giles 1901 ^a
7	<i>Culex (Culex) gelidus</i> Theobald 1901 ^a
8	<i>Culex (Culex) mimulus</i> Edwards 1915 ^a
9	<i>Culex (Culex) pseudovishnui</i> Colless 1957 ^a
10	<i>Culex (Culex) quinquefasciatus</i> Say 1823 ^a
11	<i>Culex (Culex) tritaeniorhynchus</i> Giles 1901
12	<i>Culex (Eumelanomyia) brevipalpis</i> Giles 1902
13	<i>Culex (Culiciomyia) nigropunctatus</i> Edwards 1926
14	<i>Culex (Lophoceraomyia) minutissimus</i> Theobald 1907
15	<i>Fredwardsius vittatus</i> Bigot 1861
16	<i>Lutzia (Metalutzia) fuscana</i> Weidemann 1820
17	<i>Mimomyia (Mimomyia) chamberlaini</i> Ludlow 1904 ^a
18	<i>Aedes (Stegomyia) albopictus</i> Skuse 1895

^aA species reported from Auroville for the first time.

six temporary breeding habitats, i.e., GS, cattle farm, EPs, LMs, discarded tires, and SPs, were found across Auroville. CTs were the predominant breeding habitat, which constituted 57% (39/62) of the habitats sampled. This was followed by GPs (8.7%, 6/62) and APs (5.8%, 4/62). The remaining habitats had a negligible number of mosquitoes. An array of 18 species was found to breed in 12 categories of habitats. The species diversity with respect to the individual habitats is presented in Fig. 3. The maximum number of mosquito species was recorded in CTs (n = 12), followed by GPs (n = 9) and APs (n = 8). Six species of *Culex*, two species of *Anopheles*, and one species each of *Lutzia*, *Armigeres*, and *Aedes* were detected from 62 CTs. *Aedes albopictus* was the predominant among the 12 species that were found to breed in CTs. This mosquito species was also found in GPs but in smaller numbers. *Culex (Culex) mimulus* (Edwards) was the predominant species breeding in ground and artificial pools. *Culex gelidus*, *Culex (Eumelanomyia) brevipalpis* (Giles), *Cx. quinquefasciatus*, *Lutzia (Metalutzia) fuscana* (Wiedmann), and *An. barbirostris* were observed to breed in all these habitats.

Species Richness and Evenness

Overall, 504 mosquitoes were identified to 18 species. The species diversity was calculated for 15 sampling locations, where larval collections were made during the study period. The alpha (α) biodiversity estimates for Auroville are furnished in Table 4. Examination of α biodiversity indices indicated that the Auroville environment was diverse (S = 18; D_{Mg} = 2.732 [95% CI: 2.732–2.732]). The Shannon-Weiner (H' = 2.199 [95% CI: 2.133–2.276]) and the Simpson indices (λ = 0.8619 [95% CI: 0.8496–0.8723]) highlighted species richness, evenness, and dominance. However, the Pielou index (J' = 0.5011 [95% CI: 0.4688–0.5408]) indicated evenness.

Zone-wise α biodiversity estimates for Auroville are furnished in Table 5. In zone 1, there were 12 sampling sites, out of which 10 sites showed evidence of species richness (S = 2–10), evenness (J' = 0.4615–1.0), and dominance (H' = 0–0.2082; λ = 0–0.6). In zone 2, there were four sampling sites, of which two sites showed evidence of species richness (S = 2–5), evenness (J' = 0.5567–1.0), and dominance (H' = 0.6058–1.024; λ = 0.4152–0.6122). In zone 3, also four sites were sampled, of which three showed evidence of species richness (S = 1–7), evenness (J' = 0.6707–1.0), and dominance

Table 3. Mosquito species recorded in different habitats (indicated by an 'x' in Auroville)

Species name	DT	AP	DWL	GS	PT	GP	CT	ATH	EP	LM	SP	TH ^a	CF ^a	OC ^a	HD ^a
<i>Anopheles barbirostris</i>		x				x	x						x		
<i>Anopheles pallidus</i>			x												
<i>Anopheles subpictus</i>															
<i>Anopheles vagus</i>															
<i>Armigeres subalbatus</i>					x										
<i>Culex bitaeniorhynchus</i>						x	x								
<i>Culex brevipalpis</i>						x	x								
<i>Culex gelidus</i>						x	x								
<i>Culex mimulus</i>						x	x								
<i>Culex minutissimus</i>						x	x								
<i>Culex nigropunctatus</i>															
<i>Culex pseudovishnui</i>															
<i>Culex quinquefasciatus</i>															
<i>Culex tritaeniorhynchus</i>															
<i>Fredwardsius vittatus</i>															
<i>Lutzia fuscana</i>															
<i>Mimomyia chamberlaini</i>															
<i>Aedes albopictus</i>															

AP, artificial pond; ATH, ant trap holders; CF, cattle farm; CT, cement tank; DT, discarded tyres; DWL, dug well; EP, earthen pot; GP, ground pool; GS, grinding stone; HD, human dwellings; LM, lake margins; OC, outdoor collection; PT, plastic water storage tank; SP, soak pit; TH, tree hole. – refers to not present/absence.
^aResting collections.

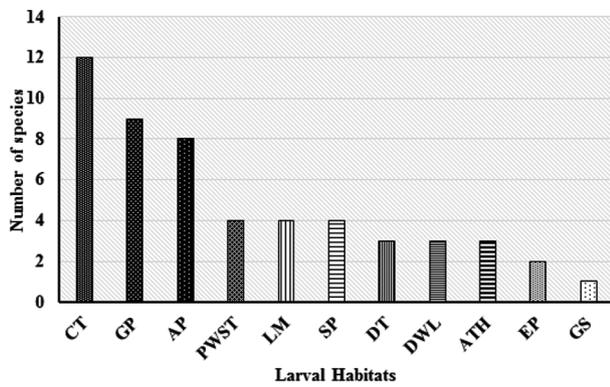


Fig. 3. Species diversity in different habitats.

Table 4. Alpha (α) biodiversity estimates, Auroville

Individuals	504
Specific richness (S)	18
Shannon-Weiner index (H')	2.199 (95% CI: 2.133–2.276)
Margalef index (D_{Mg})	2.732 (95% CI: 2.732–2.732)
Simpson index (λ)	0.8619 (95% CI: 0.8496–0.8723)
Evenness of Pielou index (J')	0.5011 (95% CI: 0.4688–0.5408)

($H' = -1.546$; $\lambda = 0-0.7273$). In zone 4, 11 sampling sites were checked, of which 9 showed evidence of species richness ($S = 1-6$), evenness ($J' = 0.462-1.0$), and dominance ($H' = 0-1.139$; $\lambda = 0-0.6111$).

Species Accumulation Curve

Rarefaction curves were accomplished with the objective of observing the asymptotic trends of the number of mosquito species and an assessment of the comparability between the four zones in Auroville (Fig. 4). The rarefaction curves indicated the strength of the number of species in each of the four zones. In zones 1 and 4, the curve tended to stabilize with 17 and 11 species, respectively. But in zones 2 and 3, the tendency to stabilize was at 7 and 9 species, respectively. Furthermore, the curve of zone 3 appears to still be rising, forecasting an increase in the number of mosquito species with larger sampling effort/sites. In zones 1 and 4, an increase in the number of sampling sites resulted in an increase in the number of species in the curve without a detectable limit. On the strength of this proposition, overall species accumulation is probably foreseen to rise with a higher sampling effort within the environs of Auroville.

Canonical Analysis

The CA ordination indicated an emerging segregation between a cluster of zone 1/zone 3, and that of zone 2 and zone 4. Axes 1 and 2 showed 40.1 and 38.2%. Three clusters were noticeable, one related to zone 1/zone 3 with *Anopheles (Celia) pallidus* Theobald, *An. subpictus*, *Anopheles (Cellia) vagus* (Donitz), *Armigeres (Armigeres) subalbatus* (Coquillett), *Culex (Oculeomyia) bitaeniorhynchus* Giles, *Culex (Lophoceraomyia) minutissimus* (Theobald), *Culex (Culiciomyia) nigropunctatus* (Edwards), *Culex (Culex) Pseudovishnui* Colless, *Culex (Culex) quinquefasciatus*, *Culex (Culex) tritaeniorhynchus* Giles, *Lutzia (Metalutzia) fuscana* (Wiedmann), and *Mimomyia chamberlaini* (Ludlow). The second cluster related to zone 2 with *Cx. gelidus* and *Aedes (Fredwardsius) vittatus* (Bigot). The last cluster related to zone 4 with *Ae. albopictus*, *An. barbirostris*, *Cx. brevivalpis*, and *Cx. mimulus* (Fig. 5).

Discussion

The current study revolved on the distribution of water bodies/habitats supporting mosquito breeding, and documentation of mosquito fauna and biodiversity of culicine mosquitoes in Auroville. It was possible to segregate larval habitats in terms of selection of sites for oviposition by anopheline and culicine mosquitoes. Although there was overlapping habitat preference, the habitat selectivity was noticeably different between the anophelines and culicines (Bentley and Day 1989). The mosquito larval habitats included a mixture of container and GP types. It is evident that mosquito larval habitats have a spatial pattern. However, maximum numbers of habitats occurred towards the northeast and southeast direction of Auroville, which was probably due to the presence of permanent water bodies in these areas.

Culicine mosquito larvae were found in a variety of man-made, artificial, and natural habitats across Auroville. The most noteworthy observation was the preference of *Ae. albopictus* observed in seven different types of habitats. This indicates a catholic preference of this mosquito species in choosing habitats compared to *Aedes (Stegomyia) aegypti* Linnaeus. Although, container breeding is more common for *Ae. albopictus*, it has been recorded in other types of habitats too. The mosquito larval habitats are diverse and different classifications have been documented (Bates 1949, Colless 1957, Mattah et al. 2017, Amarasinghe and Ranasinghe 2019). Among the man-made breeding sites, CTs were the predominant site which supported diverse mosquito species belonging to five genera, including *Aedes*. It can be inferred that the sylvan species, *Ae. albopictus*, is displaying the phenomenon of invasion to urbanized settings. Such phenomena have been observed elsewhere (Shriram et al. 2018, Brennan et al. 2021). In addition, the natural larval sites, GPs and APs, also supported breeding of species viz. *Ae. albopictus*, *An. barbirostris*, *Cx. brevivalpis*, *Cx. gelidus*, *Cx. mimulus*, *Cx. quinquefasciatus*, and *Lt. fuscana*, overlapping the three habitats, namely CTs, GPs, and APs. This indicated the adaptability of these species to colonize the available habitats for oviposition.

Rajavel et al. (2004) listed the following species as associated species, although it is not possible to identify which are from Auroville and which are from the other localities listed for the primary species: *Ae. albopictus*, *Ae. jamesi*, *Ae. krombeini*, *Ae. lineatopennis*, *Ae. novalbopictus*, *Ae. pallidostriatus*, *Ae. ramachandrai*, *Ae. reginae*, *Ae. thomsoni*, *Ae. vittatus*, *Ae. w-albus*, *An. barbirostris*, *An. subpictus*, *Ar. subalbatus*, *Cx. brevivalpis*, *Cx. fuscatus*, *Cx. fuscocephala*, *Cx. malayi*, *Cx. minutissimus*, *Cx. nigropunctatus*, *Cx. pallidothorax*, *Cx. tritaeniorhynchus*, *Oc. scatophagoides*, *Tx. splendens*, and *Ve. indica*.

Of the 18 species recorded in the present study, none of the above 8 species from Rajavel et al. (2004) for Auroville were recollected in the present study and only 10 of the 18 were listed by Rajavel et al. (2004) as known associates of the species they listed for Auroville. Consequently, all 18 species are new records for Auroville. Three species reported here (*Cx. mimulus*, *Ae. vittatus*, and *Lutzia fuscana*) were not recorded by Rajavel et al. (2004) and appear new to the Puducherry region. So it appears that either the mosquito fauna at Auroville is different from those of other locations around Puducherry or it has changed drastically from when Rajavel et al. (2004) carried out their study.

Among the mosquito species recorded under the three medically important genera viz. *Anopheles*, *Culex*, and *Aedes* in the current study, *Ae. albopictus* was predominant followed by *An. subpictus*, *Cx. gelidus*, and *Cx. quinquefasciatus*. Notable dominance of *Ae. albopictus* renders Auroville receptive to potential transmission of different arboviral pathogens. This mosquito species has been

Table 5. Alpha (α) biodiversity estimates in different zones (1–4), Auroville

Zones	Diversity indices			
	Shannon-Weiner index (H')	Margalef index (D_{Mg})	Simpson index (λ)	Evenness_Pielou index (J)
Zone 1				
Site 1	0.6365	0.9102	0.4444	0.9449
Site 5	1.168	1.303	0.64	0.8041
Site 16	0.4506	0.5581	0.2778	0.7846
Site 17	0	0	0	1
Site 18	0.4087	0.6002	0.1964	0.5016
Site 19	0.5623	0.7213	0.375	0.8774
Site 20	0.6931	1.443	0.5	1
Site 21	2.082	2.531	0.8555	0.8019
Site 23	0.9369	0.9102	0.5679	0.8507
Site 34	1.018	1.517	0.4774	0.4615
Site 35	1.121	1.251	0.6116	0.7669
Site 36	1.532	1.299	0.7578	0.7713
Zone 2				
Site 2	0.6931	1.443	0.5	1
Site 3	0.6058	0.353	0.4152	0.9164
Site 4	1.004	1.028	0.6122	0.9099
Site 24	1.024	1.228	0.4941	0.5567
Zone 3				
Site 8	0	0	0	1
Site 9	1.546	1.914	0.7221	0.6707
Site 10	1.376	1.516	0.7041	0.7919
Site 22	1.342	1.251	0.7273	0.9568
Zone 4				
Site 6	1.011	0.8049	0.6111	0.9165
Site 7	1.02	1.406	0.5127	0.462
Site 11	1.181	1.063	0.6101	0.6512
Site 12	0.4851	0.7385	0.24	0.5414
Site 25	0.6096	1.019	0.2825	0.4599
Site 26	0.8853	1.059	0.4637	0.6059
Site 27	1.139	1.535	0.5385	0.5206
Site 28	0.9003	0.7213	0.5313	0.8201
Site 29	0	0	0	1
Site 30	0.5456	0.353	0.3599	0.8628
Site 31	0.6547	0.692	0.3642	0.6415

implicated as vector of outbreaks of dengue in India (Kumari et al. 2011, Sivan et al. 2016) and several parts of the world (Effler et al. 2005, Xu et al. 2007, Ratsitorahina et al. 2008, Leroy et al. 2009, Issack et al. 2010). Although *An. subpictus* is not an established urban malaria vector, this species has been reported to transmit both *Plasmodium falciparum* and *P. vivax* in Western and Eastern India, Maldives, and certain areas of South-East Asia (Roy 1943, Panicker 1981, Kulkarni 1987, Amerasinghe et al. 1992, Kumari et al. 2009, Chandra et al. 2010, Surendran et al. 2010, Surendran et al. 2013, Kumar et al. 2016). Even though it was not very common in Auroville, constant surveillance is still necessary. In Nepal, 21 species from five genera were reported in four districts (Maharjan et al. 2014), while Irish et al. (2016) in Bangladesh have documented an updated list with records of 123 species.

Appropriate larval source management measures are central to sustaining a low larval density at low levels. It is recommended that the PWSTs be provided with tightly closed lids, and CTs equally with hermetically covered lids. Larger CTs could be stocked with top water minnow fishes (*Gambusia affinis*, S. F. Baird & Girard, 1853, Cyprinodontiformes, Poeciliidae, mosquito fish), and guppies (*Poecilia reticulata*, W. Peters, 1859, Cyprinodontiformes, Poeciliidae, million fish and rainbow fish) could be introduced in water bodies with minimal pollution at a rate of five fish/m² (WHO

2013). Reduction of water-holding receptacles such as GS, EPs, and discarded tires at monthly intervals would help keep the *Aedes* spp. firmly under check. It would also be beneficial to have vegetation around LMs trimmed periodically (WHO 2013).

The mosquito biodiversity was assessed through three diversity indices: 1) (Shannon-Weiner richness and evenness, 2) Margalef, Simpson richness, and 3) Pielou evenness. The rarefaction analysis depended on the trends of the number of species. The abundance of mosquito species, based on a cross-sectional spatio-temporal sampling, showed the largest number of specimens in the collections. Based on the Shannon index, the temporal samples during the study period indicated diversity and evenness. Zone-wise analysis of mosquito biodiversity estimates indicates that zone 1 and zone 4 had the highest species diversity, dominance, and evenness. This was probably due to the availability of maximum number of larval habitats that were exploited by different mosquitoes in these zones of Auroville.

Rarefaction curves are useful for estimating species richness. Mosquito species counts, which were used to create accumulation curves, are comparable when species richness has reached a clear asymptote. Rarefaction curves provide smooth lines that enable point-to-point or complete dataset comparisons (Meerman 2004). The objective of rarefaction is to make direct comparisons among

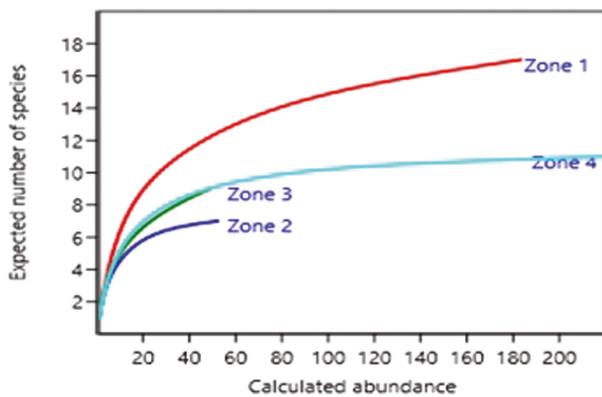


Fig. 4. Species accumulation curve using the rarefaction analysis of the Culicine samples.

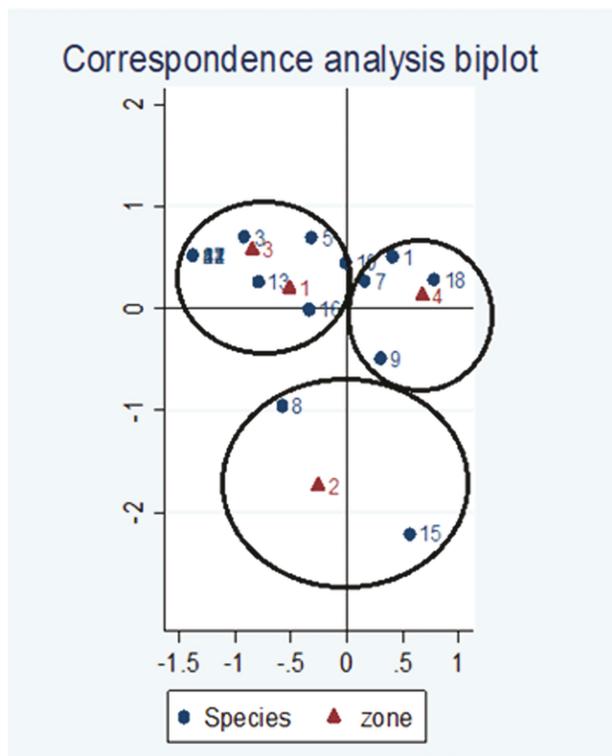


Fig. 5. Canonical analysis biplot of the mosquito fauna in Auroville with species located where they are abundant.

species where sampling effort is unequal and reduces the sample data to a common abundance level (the same number of individuals) so that direct comparisons between species richness of a particular area is possible. The comparison of species richness by rarefaction curves illustrated that species richness in zone 1 and zone 4 was higher than that of the other two zones due to more sampling. Rajavel et al. (2004) reported mosquitoes of 64 species, based on surveys conducted over 2 yr in Puducherry region. Further sampling covering different seasons would probably increase the overall species accumulation.

Spatial biodiversity to judge the relationship among the zones and species occurrence were predicted by CA technique. A distinct segregation between zone 1/zone 3 and zone 2/zone 4 was revealed. The presence of *An. barbirostris*, *Cx. brevipalpis*, *Lt. fuscana*, and *Ae. albopictus* in zone 1, *Cx. brevipalpis* in zone 4,

and *Cx. mimulus* and *Ae. vittatus* in all the four zones indicates a fragmented landscape of Auroville, where all these zones are interconnected by different water bodies, man-made, artificial, and natural.

Conclusions

In sum, the current study showed that larval habitat sites of anopheline and culicine are distinguishable in Auroville. The culicine mosquitoes are found to breed in a variety of man-made, artificial, and natural habitats. *Aedes albopictus* displays wide preference in choosing oviposition sites and dominance of this species renders Auroville receptive to transmission of different arboviral pathogens. Although, *An. subpictus* constitutes a low proportion, a constant vigilance is necessary. In view of the potential risks and continuing changes in climate and land use, it would be prudent to monitor vector populations from time to time, and carry out additional research in this ecosystem. Analysis and mapping of larval habitats are the first steps to understand the mosquito species distribution. Changes in the environmental conditions can offer convenient habitats for the establishment of diverse species of mosquitoes in this ecosystem. Considering the sociodemographic characteristics of Auroville, research into mosquito biodiversity and risk of VBDs will be of great help.

Acknowledgments

We gratefully acknowledge the Auroville Working Committee's permission to carry out this study on the premises of Auroville. We are grateful to Mr. S. Krishnamurthy of EcoPro for extending support to reach various areas within Auroville. The authors acknowledge the technical assistance rendered by Mr. R. Krishnamoorthi, Technical Officer 'C'; Mr. S. Gopalakrishnan, Technical Assistant; and Mr. N. Krishnaraj, Technician from the Division of Vector Biology and Control. This study is a part of Ms. P. Visa Shalini's dissertation in partial fulfillment of her M.Sc. degree in Public Health Entomology from Pondicherry University (Puducherry, India).

Funding

This study was supported by the internal funds of the ICMR-Vector Control Research Centre.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

References Cited

- Adebote, D., D. Abolude, S. Oniye, and O. Wayas. 2008. Studies on some physicochemical factors affecting the breeding and abundance of mosquitoes (Diptera: Culicidae) in phytotelmata on *Delonix regia* (Leguminosae: Caesalpinioidea). *J. Biol. Sci.* 8: 1304–1309.
- Afrane, Y. A., B. W. Lawson, R. Brenya, T. Kruppa, and G. Yan. 2012. The ecology of mosquitoes in an irrigated vegetable farm in Kumasi, Ghana: abundance, productivity and survivorship. *Parasit. Vectors* 5: 233.
- Amarasinghe, L. D., and H. A. K. Ranasinghe. 2019. Diversity and species composition of microbiota associated with mosquito breeding habitats: a study from Kurunegala district in Sri Lanka. *Biomed. Res. Int.* 2019: 5897317.
- Amerasinghe, P. H., F. P. Amerasinghe, R. A. Wirtz, N. G. Indrajith, W. Somapala, L. R. Pereira, and A. M. Rathnayake. 1992. Malaria transmission by

- Anopheles subpictus* (Diptera: Culicidae) in a new irrigation project in Sri Lanka. *J. Med. Entomol.* 29: 577–581.
- Barraud, P. J. 1934. *The fauna of British India, including Ceylon and Burma. Diptera: family Culicidae, tribes Megarhinini and Culicini*, vol. 5. Taylor & Francis, London, United Kingdom.
- Bates, M. 1949. The adaptations of mosquitoes to the tropical rain forest environment. *Proc. Am. Phil. Soc.* 93: 340–346.
- Becker, N., D. Petrić, M. Zgomba, C. Boase, M. Madon, C. Dahl, and A. Kaiser. 2010. Mosquito research techniques, pp. 43–61. *In Mosquitoes and their control*. Springer, Berlin, Germany.
- Bentley, M. D., and J. F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. *Annu. Rev. Entomol.* 34: 401–421.
- Bram, R. A. 1967. Contributions to the mosquito fauna of southeast Asia. II. The genus *Culex* in Thailand (Diptera: Culicidae). *Contributions of the American Entomological Institute* 2: 1–296.
- Brennan, S. A., I. C. Grob, C. E. Bartz, J. K. Baker, and Y. Jiang. 2021. Displacement of *Aedes albopictus* by *Aedes aegypti* in Gainesville, Florida. *J. Am. Mosq. Control Assoc.* 37: 93–97.
- Chandra, G., I. Bhattacharjee, and S. Chatterjee. 2010. A review on *Anopheles subpictus* Grassi—a biological vector. *Acta Trop.* 115: 142–154.
- Chaves, L. S. M., E. S. Bergo, J. E. Conn, G. Z. Laporta, P. R. Prist, and M. A. M. Sallum. 2021. Anthropogenic landscape decreases mosquito biodiversity and drives malaria vector proliferation in the Amazon rainforest. *PLoS One* 16: e0245087.
- Christophers, S. R. 1933. *The fauna of British India, including Ceylon and Burma. Diptera: family Culicidae, tribe Anophelini*, vol. 4. Taylor & Francis, London, United Kingdom.
- Colless, D. H. 1957. Components of the catch curve of *Culex annulus* in Singapore. *Nature* 180: 1496–1497.
- Death, R. 2008. *Margalef's index*. Doi: [10.1016/B978-008045405-4.00117-8](https://doi.org/10.1016/B978-008045405-4.00117-8).
- Effler, P. V., L. Pang, P. Kitsutani, V. Vorndam, M. Nakata, T. Ayers, J. Elm, T. Tom, P. Reiter, J. G. Rigau-Perez, et al. 2005. Dengue fever, Hawaii, 2001–2002. *Emerg. Infect. Dis.* 11: 742–749.
- Emidi, B., W. N. Kisinza, B. P. Mmbando, R. Malima, and F. W. Mosha. 2017. Effect of physicochemical parameters on *Anopheles* and *Culex* mosquito larvae abundance in different breeding sites in a rural setting of Muheza, Tanzania. *Parasit. Vectors* 10: 1–12.
- Enayati, A., and J. Hemingway. 2010. Malaria management: past, present, and future. *Annu. Rev. Entomol.* 55: 569–591.
- Eveline, K., P. J., I. M. McCall, M. D. Hastings, F. P. Wilson, M. J. Amerasinghe, and Donnelly. 2005. Malaria and irrigated crops, Accra, Ghana. *Emerg. Infect. Dis.* 11(8): 1290–1293.
- Gotelli, N. J., and G. L. Entsminger. 2001. Swap and fill algorithms in null model analysis: rethinking the knight's tour. *Oecologia* 129: 281–291.
- Hanafi-Bojd, A., H. Vatandoost, M. Oshaghi, Z. Charrahy, A. Haghdoost, M. Sedaghat, F. Abedi, M. Soltani, and A. Raeisi. 2012. Larval habitats and biodiversity of anopheline mosquitoes (Diptera: Culicidae) in a malarious area of southern Iran. *J. Vector Borne Dis.* 49: 91–100.
- Auroville-Wikipedia. 2020. Odisha-Wikipedia, the free encyclopedia. <<https://research.auroville.org>>.
- Irish, S. R., H. M. Al-Amin, M. S. Alam, and R. E. Harbach. 2016. A review of the mosquito species (Diptera: Culicidae) of Bangladesh. *Parasit. Vectors* 9: 559.
- Issack, M. I., V. N. Pursem, T. M. Barkham, L. C. Ng, M. Inoue, and S. S. Manraj. 2010. Re-emergence of dengue in Mauritius. *Emerg. Infect. Dis.* 16: 716–718.
- Kamdem, C., B. Tene Fossog, F. Simard, J. Etoua, C. Ndo, P. Kengne, P. Bousses, F. X. Etoa, P. Awono-Ambene, D. Fontenille, et al. 2012. Anthropogenic habitat disturbance and ecological divergence between incipient species of the malaria mosquito *Anopheles gambiae*. *PLoS One* 7: e39453.
- Keesing, F., L. K. Belden, P. Daszak, A. Dobson, C. D. Harvell, R. D. Holt, P. Hudson, A. Jolles, K. E. Jones, and C. E. Mitchell, et al. 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* 468: 647–652.
- Krishnamoorthy, K., P. Jambulingam, R. Natarajan, A. N. Shriram, P. K. Das, and S. C. Sehgal. 2005. Altered environment and risk of malaria outbreak in South Andaman, Andaman & Nicobar Islands, India affected by tsunami disaster. *Malar. J.* 4: 32.
- Kulkarni, S. 1987. Feeding behaviour of anopheline mosquitoes in an area endemic for malaria in Bastar district, Madhya Pradesh. *Indian J. Malariol.* 24: 163–171.
- Kumar, A., R. Hosmani, S. Jadhav, T. de Sousa, A. Mohanty, M. Naik, A. Shettigar, S. Kale, N. Valecha, L. Chery, et al. 2016. *Anopheles subpictus* carry human malaria parasites in an urban area of western India and may facilitate perennial malaria transmission. *Malar. J.* 15: 124.
- Kumari, R., K. Kumar, and L. S. Chauhan. 2011. First dengue virus detection in *Aedes albopictus* from Delhi, India: its breeding ecology and role in dengue transmission. *Trop. Med. Int. Health* 16: 949–954.
- Kumari, S., S. K. Parida, N. Marai, A. Tripathy, R. K. Hazra, S. K. Kar, and N. Mahapatra. 2009. Vectorial role of *Anopheles subpictus* Grassi and *Anopheles culicifacies* Giles in Angul District, Orissa, India. *Southeast Asian J. Trop. Med. Public Health* 40: 713–719.
- Kweka, E. J., E. E. Kimaro, and S. Munga. 2016. Effect of deforestation and land use changes on mosquito productivity and development in western Kenya highlands: implication for malaria risk. *Front. Public Health* 4: 238.
- Leroy, E. M., D. Nkoghe, B. Ollomo, C. Nze-Nkoghe, P. Becquart, G. Gard, X. Pourrut, R. Charrel, G. Moureau, A. Ndiyo-Mbiguino, et al. 2009. Concurrent chikungunya and dengue virus infections during simultaneous outbreaks, Gabon, 2007. *Emerg. Infect. Dis.* 15: 591–593.
- Maharjan, M., S. K. Pant, and D. K. Pant. 2014. Distribution of mosquito species in Kathmandu, Rupandehi, Kapilabastu and Morand districts of Nepal. *Zoonoses Food Hyg. News* 20.
- Manga, L., J. C. Toto, and P. Carnevale. 1995. Malaria vectors and transmission in an area deforested for a new international airport in southern Cameroon. *Ann. Soc. Belg. Med. Trop.* 75: 43–49.
- Mattah, P. A., G. Futagbi, L. K. Amekudzi, M. M. Mattah, D. K. de Souza, W. D. Kartey-Attipoe, L. Bimi, and M. D. Wilson. 2017. Diversity in breeding sites and distribution of *Anopheles* mosquitoes in selected urban areas of southern Ghana. *Parasit. Vectors* 10: 25.
- Meerman, J. 2004. *Rapid ecological assessment Columbia River Forest Reserve, past hurricane Iris. Report commissioned by Yaaxché Conservation Trust and Toledo Institute for Development and Environment*.
- Multini, L. C., A. L. da Silva de Souza, M. T. Marrelli, and A. B. BrunoWilke. 2020. The influence of anthropogenic habitat fragmentation on the genetic structure and diversity of the malaria vector *Anopheles cruzii* (Diptera: Culicidae). *Sci. Rep.* 10: 18018.
- Mwangangi, J. M., J. Midega, S. Kahindi, L. Njoroge, J. Nzovu, J. Githure, C. M. Mbogo, and J. C. Beier. 2012. Mosquito species abundance and diversity in Malindi, Kenya and their potential implication in pathogen transmission. *Parasitol. Res.* 110: 61–71.
- Burkett-Cadena, N. D., and A. Y. Vittor. 2018. Deforestation and vector-borne disease: forest conversion favors important mosquito vectors of human pathogens. *Basic Appl. Ecol.* 26: 101–110.
- Nikookar, S., S. Moosa-Kazemi, M. Oshaghi, H. Vatandoost, M. Yaghoobi-Ershadi, A. Enayati, F. Motevali-Haghi, S. Ziapour, and M. Fazeli-Dinan. 2015. Biodiversity of culicid mosquitoes in rural Neka township of Mazandaran province, northern Iran. *J. Vector Borne Dis.* 52: 63–72.
- Panicker, K. N. 1981. *Anopheles subpictus* vector of malaria in coastal villages of South-East India. *Curr. Sci.* 50: 694–695.
- Pielou, E. C. 1984. The interpretation of ecological data: a primer on classification and ordination. John Wiley and Sons, New York.
- Rajavel, A. R., R. Natarajan, and K. Vaidyanathan. 2004. A checklist of mosquitoes (Diptera: Culicidae) of Pondicherry, India with notes on new area records. *J. Am. Mosq. Control Assoc.* 20: 228–232.
- Ratsitorahina, M., J. Harisoa, J. Ratovonjato, S. Biacabe, J. M. Reynes, H. Zeller, Y. Raelina, A. Talarmin, V. Richard, and J. Louis Soares. 2008. Outbreak of dengue and chikungunya fevers, Toamasina, Madagascar, 2006. *Emerg. Infect. Dis.* 14: 1135–1137.
- Roy, D. N. 1943. The role of *Anopheles subpictus* Grassi as a carrier of malaria. *J. Mal. Inst. India* 6: 117–121.
- Schrama, M., E. R. Hunting, B. R. Beechler, M. M. Guarido, D. Govender, W. Nijland, M. van 't Zelfde, M. Venter, P. M. van Bodegom, and E. E. Gorsich. 2020. Human practices promote presence and abundance of disease-transmitting mosquito species. *Sci. Rep.* 10: 13543.
- Shannon, C. E. 1948. A mathematical theory of communication. *Bell Sys. Tech. J.* 27: 379–423.

- Shannon, C. E., and W. Weaver (eds.). 1949. *The mathematical theory of communication*. University of Illinois Press, pp. 1-117. Urbana, IL.
- Shriram, A. N., A. Sivan, and A. P. Sugunan. 2018. Spatial distribution of *Aedes aegypti* and *Aedes albopictus* in relation to geo-ecological features in South Andaman, Andaman and Nicobar Islands, India. *Bull. Entomol. Res.* 108: 166–174.
- Sivan, A., A. Shriram, A. Sugunan, M. Anwesh, N. Muruganandam, C. Kartik, and P. Vijayachari. 2016. Natural transmission of dengue virus serotype 3 by *Aedes albopictus* (Skuse) during an outbreak in Havelock Island: entomological characteristics. *Acta Trop.* 156: 122–129.
- StataCorp. 2015. Stata statistical software: release 14. College station, TX: StataCorp LP. <https://www.stata.com/resources>.
- Surendran, S. N., O. P. Singh, P. J. Jude, and R. Ramasamy. 2010. Genetic evidence for malaria vectors of the *Anopheles sudaicus* complex in Sri Lanka with morphological characteristics attributed to *Anopheles subpictus* species B. *Malar. J.* 9: 343.
- Surendran, S. N., D. K. Sarma, P. J. Jude, P. Kempainen, N. Kanthakumaran, K. Gajapathy, L. B. Peiris, R. Ramasamy, and C. Walton. 2013. Molecular characterization and identification of members of the *Anopheles subpictus* complex in Sri Lanka. *Malar. J.* 12: 304.
- Tuno, N., W. Okeka, N. Minakawa, M. Takagi, and G. Yan. 2005. Survivorship of *Anopheles gambiae* sensu stricto (Diptera: Culicidae) larvae in western Kenya highland forest. *J. Med. Entomol.* 42: 270–277.
- Versteirt, V., S. Boyer, D. Damiens, E. De Clercq, W. Dekoninck, E. Ducheyne, P. Grootaert, C. Garros, T. Hance, and G. Hendrickx. 2013. Nationwide inventory of mosquito biodiversity (Diptera: Culicidae) in Belgium, Europe. *Bull. Entomol. Res.* 103: 193–203.
- Wilkerson, R. C., Y. M. Linton, D. M. Fonseca, T. R. Schultz, D. C. Price, and D. A. Strickman. 2015. Making mosquito taxonomy useful: a stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. *PLoS One* 10(7): e0133602. Doi: <https://doi.org/10.1371/journal.pone.0133602>
- World Health Organization (WHO). 2013. *Larval source management: a supplementary measure for vector control: an operational manual*.
- Xu, G., H. Dong, N. Shi, S. Liu, A. Zhou, Z. Cheng, G. Chen, J. Liu, T. Fang, H. Zhang, et al. 2007. An outbreak of dengue virus serotype 1 infection in Cixi, Ningbo, People's Republic of China, 2004, associated with a traveler from Thailand and high density of *Aedes albopictus*. *Am. J. Trop. Med. Hyg.* 76: 1182–1188.