

Research Paper

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Corresponding author:

Kannan Thiruvengadam;
Email: kannan.t@icmr.gov.in

¹ICMR – Regional Medical Research Centre, Port Blair, Andaman & Nicobar Islands, India and ²ICMR – Vector Control Research Centre, Puducherry, India

Abstract

The Culicidae mosquito family breeds in various natural and artificial water bodies. Phytotelmata are plant structures that hold water and are used by many mosquito species during their life cycle. Mosquitoes are disease vectors, and phytotelmata play an important role in harbouring them. Investigating these phytotelmata will facilitate to develop appropriate site specific vector control strategy. Approximately 750 phytotelmata in 16 locations on Car Nicobar Island was examined. Mosquito larvae were collected from different habitats such as tree holes, root holes, leaf axils, and bamboo stumps. On average, the number of larvae per 10 ml of water ranged from 0.003 to 3.833. Among the 16 mosquito species identified, there were vectors of malaria (*Anopheles sundaicus*), dengue (*Aedes aegypti* and *Aedes albopictus*) and filariasis (*Culex quinquefasciatus*), belonging to seven genera. The most common phytotelmata were coconut trees (17.5%) followed by Java apple trees (10.3%) and bamboo stumps (7.1%). The most widespread mosquito species was *Aedes edwardsi*, followed by *Aedes malayensis* and *Ae. albopictus*, and the per cent of phytotelmata that contained these species were 42.6, 22.0, and 16.8% respectively. *Anopheles* mosquitoes had the highest container index (6.1%) in root holes, while *Aedes* and *Culex* mosquitoes were more frequently found in bamboo stumps. The alpha biodiversity indices indicated that the mosquito population in Car Nicobar Island was highly diverse ($S = 16$; $DMg = 36.9$). Tree holes showed the highest diversity, as all 16 species were collected from them. Assessment of phytotelmata, there is a significant relationship observed between mosquito genus, village, habitat, and tree families, on the remote island of Car Nicobar. Considering the presence of disease-transmitting mosquito vectors in the phytotelmata, these habitats should be considered when implementing control measures to eliminate mosquito-borne infections.

Introduction

The aquatic stage plays a vital role in the mosquito life cycle, and their habitat preferences have a substantial effect on the distribution and abundance of mosquito species that transmit disease pathogens (Wang *et al.*, 2012; McKeon *et al.*, 2013). Various environmental factors can directly or indirectly influence the oviposition of mosquitoes (Wong *et al.*, 2011), thereby influencing the larval distribution. The mosquito breeding sites consist of stagnant water bodies or running water such as, surface water pools, tree holes, rock holes, and discarded containers, in the premises of human dwelling, cattle shed, mixed dwelling, and also in forest areas (Muturi *et al.*, 2009; Dutta *et al.*, 2010).

Phytotelmata referred to singularly as ‘phytotelma’ are water-retaining structures formed within hollows of plant matter. These can be cavities within tree stems or branches, water collected in bamboo internodes, or water collecting at the base of leaves, petal, or bracts (Kitching, 2009). Separate from the living plant, phytotelmata can also form in decaying plant matter (Greeney, 2001). Although they are typically ephemeral, some phytotelmata can persist for long time period and can hold water for almost twenty-years. Except for Antarctica, they are found on every continent (Derraik, 2009). Phytotelmata are a vital model system for investigating the community ecology of insects (Srivastava *et al.*, 2004; Jocque *et al.*, 2010).

Mosquitoes (Diptera: Culicidae) have received most attention among the numerous insects documented in phytotelmata (Greeney, 2001). This emphasis likely stems from the fact that mosquitoes are disease transmitting vectors and the most abundant organisms in this ecosystem. Nearly 400 species from 15 genera have been observed in phytotelmata out of more than 3000 mosquito species described (Fish, 1983). Notably, several medically significant mosquito species thrive in phytotelmata, including dengue and malaria vectors, *viz.* *Aedes aegypti* (L.) and *Anopheles bellator* Dyar and Knab. Numerous studies have analysed the total mosquito population using plant held water as larval habitat (Derraik, 2009). Understanding the structure of mosquito communities is necessary for answering questions about ecological interactions involving mosquitoes that breed in phytotelmata (Yee and Juliano, 2006).

The fauna of mosquitoes in the Andaman and Nicobar Islands consists of 109 species distributed across 25 genera and 24 subgenera, arranged into two subfamilies. The subfamily Anophelinae contains 23 species, whereas the subfamily Culicinae contains 86 species (Patel, 2016). *Anopheles sundaicus*, a vector of malaria parasite, *Ae. aegypti* and *Ae. albopictus*, responsible for transmitting dengue and chikungunya viruses, and *Culex quinquefasciatus*, a vector of filarial parasite, are among the mosquitoes responsible for transmitting pathogens. These mosquito species prefer stagnant water bodies on earth surface and in abandoned containers. There are numerous preventive measures in place to reduce the risk of pathogen transmission to humans. As a result of their pervasive distribution and inaccessibility, species that breed in tree holes or phytotelmata present a unique challenge.

Car Nicobar Island is home to a variety of phytotelmata habitats utilised by mosquito larvae for growth and development, including tree holes, bamboo stumps, and leaf axils. In addition, a variety of artificial containers can be found in the vicinity of human dwellings, particularly in rural areas. In a survey carried out at Car Nicobar Island earlier in man-made containers (Sunish *et al.*, 2014), four types of water-holding containers were consistently observed to contain *Aedes* spp. These include; plastic tank, plastic & metal drums, coconut shells and discarded containers. Among these, large plastic tanks (500–1000 l capacity) recorded the highest Breteau Index during all the four seasons. This habitat was observed to support four mosquito species, of which 52% constituted *Aedes albopictus*. The maximum species diversity was found in metal drums. In earlier survey, Das *et al.* (1998) reported 31 species of mosquitoes belonging to 10 genera, from Car Nicobar Island, i.e. *Anopheles*, *Aedes*, *Armigeres*, *Culex*, *Harpagomyia*, *Hodgesia*, *Mansonia*, *Orthopodomyia*, *Toxorhynchites*, and *Uranotaenia*. The present study aims to thoroughly characterise the mosquito communities in phytotelmata that inhabit breeds in this island, which is primarily inhabited by the Nicobarese people. The mosquito species with respect to the volume of water in the phytotelmata also was

explored. The percentage of occupied habitats, species richness, composition, abundance, immature density, as well as measures of diversity and evenness were used to evaluate these mosquito communities. In addition, we examined the significance of plant species in relation to their function in the breeding of mosquito fauna, focusing on mosquitoes which act as disease vectors.

Materials and methods

Study area

The survey was conducted in and around the villages of Car Nicobar Island, the headquarters of Nicobar district, Andaman & Nicobar archipelago. The northernmost of the Nicobar Islands, Car Nicobar is located 143 miles from Port Blair and is separated from Little Andaman by a 10-degree channel. The island is located at 9.16°N and 92.75°E and has a surface area of approximately 127 square kilometres (fig. 1). There are a few minor hilly areas within its interior, but the landform is predominantly plain. Its beautiful silvery coastlines and vast level regions distinguish Car Nicobar. Analyses of the soil have disclosed a significant presence of silt and clay, which presents cultivation challenges. The island also contains vast tropical forests.

Car Nicobar's climate is tropical due to its proximity to the equator. Both the northeast and southwest monsoon have a significant impact on precipitation between May and November. The annual average rainfall on the island is 2380 mm, with more than 120 rainy days per year. The average relative humidity on the island, according to decadal meteorological data from the Directorate of Economics and Statistics in A&N, is 79%. The average maximum and minimum temperatures are 30 and 23 degrees Celsius respectively.

Car Nicobar Island has a population of 17,125 as per the 2011 census, with a population density of 140.5 inhabitants per square kilometre. Following the 2004 tsunami, the Nicobar administration

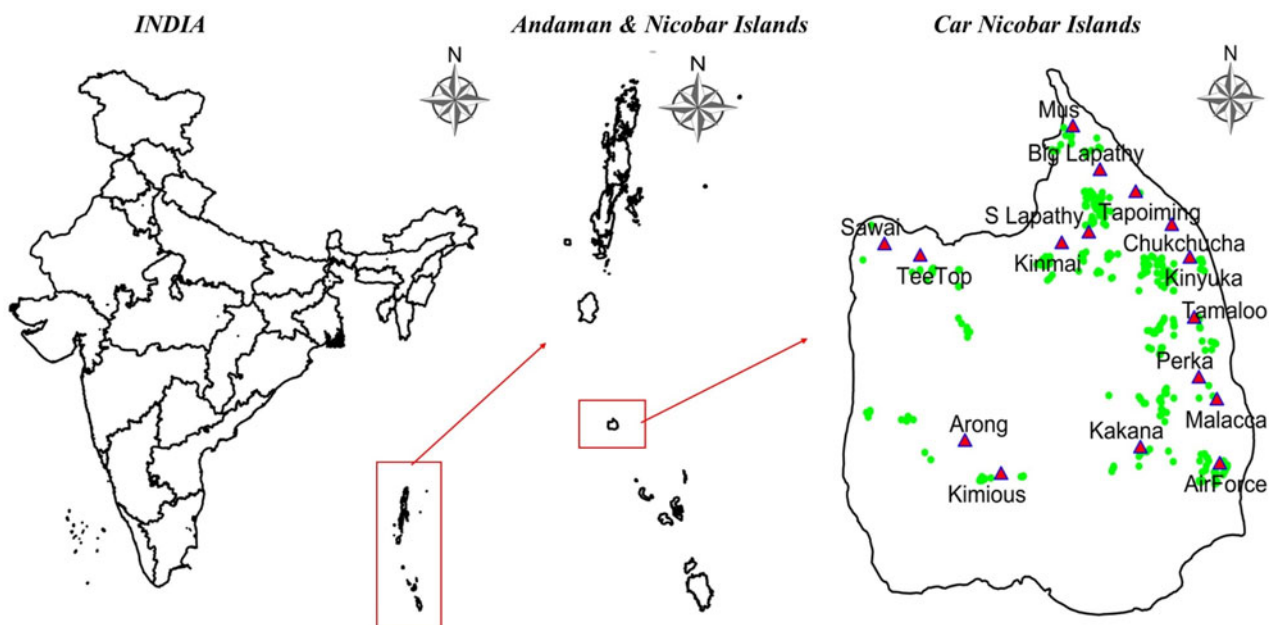


Figure 1. Map of study sites at Car Nicobar in the Andaman and Nicobar Islands.

The image depicts the study area, specifically the Car Nicobar Islands, within the Andaman and Nicobar Islands. Triangle indicates the study villages and the green dots indicate the sample collection coordinates.

constructed elevated dwellings on supports 10 feet above the ground for every family on the island. Car Nicobar consists of fifteen villages, all of which are inhabited by the indigenous ‘*Nicobari*’ tribe, who are of Mongoloid descent. Coconuts, followed by Pandanus pulp, salmon, and rice, are the primary mainstay of the population’s diet. The weekly ship service is the primary mode of conveyance to and from Car Nicobar, dependent to weather conditions. Forests encompass roughly sixty per cent of the island, with coconut plantations dominating the areas surrounding the villages and natural vegetation flourishing in the interior regions. Numerous live waterways enable seawater to inundate the island, but some streams that once transported monsoon water to the sea have become stagnant as a result of the tsunami’s altered geology. As a result, numerous water bodies and marshy regions formed during the monsoon season can persist for as long as nine months after rainfall.

Larval collection and identification

Between April and September 2019, mosquito larvae were collected from 16 areas (includes 15 villages and one Air Force station) on Car Nicobar Island. Leaf axils on all plants at ground level were examined, along with tree holes and bamboo stumps, up to 50 m in height. Pipettes and plastic tubes (with siphon) were employed to draw out the water along with the mosquito immatures from each phytotelmata. After pipetting out the water along with mosquito immatures, the water was returned to the respective phytotelmata at least twice, to flush out the organic matter and mosquito immatures from the bottom. As suggested by Jocque *et al.*, in 2010, pipetting may not be the most effective method for investigating the entire faunal community, but it proved effective for capturing mosquitoes. The volume of water extracted from each plant or tree hole was recorded. With a limited quantity of habitat water, the larvae and pupae were transported to the laboratory in a plastic container, and reared until they emerged into adults. The larvae were reared by providing larval food, viz, a mixture of dog biscuit and yeast powder in a ratio of 3:1. The newly emerged adults were identified by using dichotomous keys of Barraud in 1934 (for culicines) and Nagpal and Sharma in 1995 (for anophelines). Specimens with unknown characteristics were identified by dissecting their terminalia. The trees and plants surveyed (with phytotelmata) were identified with the help of experts from the Botanical Survey of India (BSI) at Port Blair. The samples of leaves, blossoms, and other plant elements were sent to BSI for identification.

Data analysis

The structure of each village and habitat was characterised by several attributes, including the percentage of larval positive habitat, species richness, species composition, abundance and density of immatures, and diversity and evenness measures. The Shannon-H index was used to assess species diversity, taking into account both abundance and evenness of the mosquito species (Shannon and Weaver, 1949). Simpson’s index was used to evaluate the degree of dominance (Shannon, 1948), while Margalef’s index measured evenness (Death, 2008). Chao’s index was used to estimate the species richness (Chao and Chiu, 2016) where Chao 2 estimates the incidence of individual samples belonging to specific sub-groups (Hoshi *et al.*, 2014). Species accumulation curve was also estimated to compare the number of species by phytotelmata type. Alpha diversity

indices were calculated using PAleontological STatistics (PAST) version 3.21. The container index (CI) was estimated for each habitat at village level. To test the significant difference in larvae positive observations among subgroups (villages, habitats, and trees), Dunn’s test was applied, followed by multiple testing correction using Holm’s test after the Kruskal–Wallis test. Karl Pearson coefficient of correlation was used to estimate the relationship between the species count and the water volume and height from where the samples were collected. Correspondence analysis (PCA) was performed using the R programming language version 3.5.2 (R Core Team, 2018) to assess the relationship between mosquito communities among villages and among the four phytotelmata types. For statistical computing, the R Foundation for Statistical Computing, Vienna, Austria, was utilised (URL: <https://www.R-project.org/>) along with the packages such as stats, FSA, corrr, FactoMineR, MASS, sf, and ggplot2.

Results

To investigate mosquito immatures, a comprehensive survey of 750 phytotelmata was conducted in 16 areas (15 villages and one Air Force station) of Car Nicobar Island. Kinyuka had the greatest number of phytotelmata examined, totalling 85 (11.3%), followed by Malacca, where 60 (8.0%) were examined. These phytotelmata belonged to 87 distinct species of trees and plants, which were further classified into 43 families for analytical convenience. The Arecaceae family contained the greatest number of tree types, with 144 (19.2%) phytotelmata found across all the 16 areas. Both the Myrtaceae and Moraceae families were observed in 16 areas with 88 (11.7%) and 56 (7.5%) tree species, respectively. The maximum number of trees examined was coconut trees, with 131 (17.5%), followed by 77 (10.3%) Java apple trees and 53 (7.1%) bamboo. Phytotelmata were grouped into four types: bamboo stumps (53; 7.1%), tree holes (609; 81.2%), root holes (33; 4.4%), and leaf axils (55; 7.1%). Almost 291 (38.8%) of the 750 phytotelmata examined were positive for the presence of mosquito larvae. From these 291 habitats, 5434 mosquito larvae were collected, from which 2587 mosquitoes were identified. There were 16 species of mosquitoes belonging to seven genera. *Aedes edwardsi* species was collected from more phytotelmata (124; 42.6%), followed by *Aedes malayensis* (64; 22%) and *Ae. albopictus* (49; 16.4%). Other significant mosquito vector species, including *An. sundaicus* and *Cx. quinquefasciatus*, were found in 4 (0.5%) and 26 (8.9%) phytotelmata respectively (table 1).

Among the 291 positive phytotelmata, 185 (63.6%) habitats had one mosquito species, while 75 (25.8%), 26 (8.9%), and 5 (1.7%) habitats had a combination of two, three, and four mosquito species co-existing together, respectively (fig. 2). The co-existence of four species was found mainly in tree holes. The highest proportion of habitats with more than two species co-existing was tree holes (31%), but comparatively low for the other habitats, with 2.7% for bamboo stumps and 2.4% for root holes. On the other hand, leaf axils did not have any co-existing species. Among the four mosquito vector species collected, one phytotelmata (tree hole – *Macaranga* species) was found with both *Ae. albopictus* and *Cx. quinquefasciatus* to exist together. The predatory mosquito species, viz, *Tx. splendens*, was found to co-exist with *Ae. aegypti* (3 times) and with *Ae. albopictus* (once).

These phytotelmata were observed at various heights ranging from ground level to 51 m. There was a statistically significant

Table 1. Mosquito species recorded in the Phytotelmata of Car Nicobar Island

Genus	Mosquito species	n_1	%	n_2	%	Ratio
Aedes 265 (35.3%)	<i>Aedes cancricomus</i>	7	0.9	30	1.2	1 : 4.3
	<i>Aedes aegypti</i>	21	2.8	137	5.3	1 : 6.5
	<i>Aedes albopictus</i>	49	6.5	283	10.9	1 : 5.8
	<i>Aedes edwardsi</i>	124	16.5	767	29.6	1 : 6.2
	<i>Aedes malayensis</i>	64	8.5	392	15.2	1 : 6.1
Anopheles 8 (1.1%)	<i>Anopheles insulaeflorum</i>	4	0.5	42	1.6	1 : 10.5
	<i>Anopheles sondaicus</i>	4	0.5	29	1.1	1 : 7.3
Culex 81 (10.8%)	<i>Culex minor</i>	11	1.5	52	2.0	1 : 4.7
	<i>Culex brevipalpis</i>	25	3.3	128	4.9	1 : 5.1
	<i>Culex fragilis</i>	13	1.7	52	2.0	1 : 4
	<i>Culex pallidothorax</i>	6	0.8	39	1.5	1 : 6.5
	<i>Culex quinquefasciatus</i>	26	3.5	183	7.1	1 : 7
Others 79 (10.5%)	<i>Armigeres subalbatus</i>	13	1.7	77	3.0	1 : 5.9
	<i>Orthopodomyia anopheloides</i>	47	6.3	288	11.1	1 : 6.1
	<i>Toxorhynchites splendens</i>	13	1.7	50	1.9	1 : 3.8
	<i>Tripteroides indicus</i>	6	0.8	38	1.5	1 : 6.3

n_1 indicates the number of positive phytotelmata among 750 examined; n_2 indicates the number of mosquito identified; Ratio indicates the presence of larvae per positive drawn [$n_1:n_2$].

difference ($P < 0.001$) in the abundance of mosquito species based on their heights. Only one species, *Ae. malayensis* was collected at a height of above 20 m, while six species above 10 m. Eight species seemed to be limited to heights below 10 m. *Aedes malayensis* was

observed precisely at a height of 51 m on a coconut tree (*Cocos nucifera*). *Culex (Lop) minor* and *Orthopodomyia anopheloides* were collected at a height of 13.9 m, while the disease vectors, viz, *An. sondaicus*, *Cx. quinquefasciatus*, *Ae. aegypti*, and

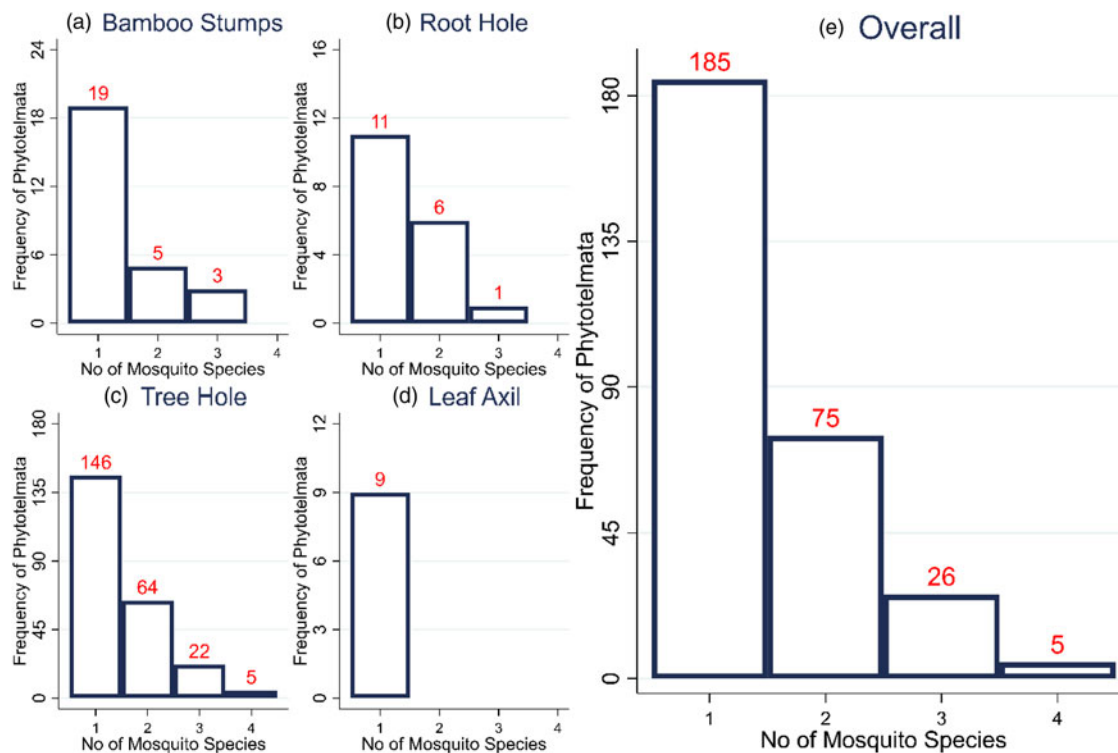


Figure 2. Frequency distribution of the number of mosquito species in the four habitat types. The data represents the distribution and combination of mosquito species across four different habitats in the study area.

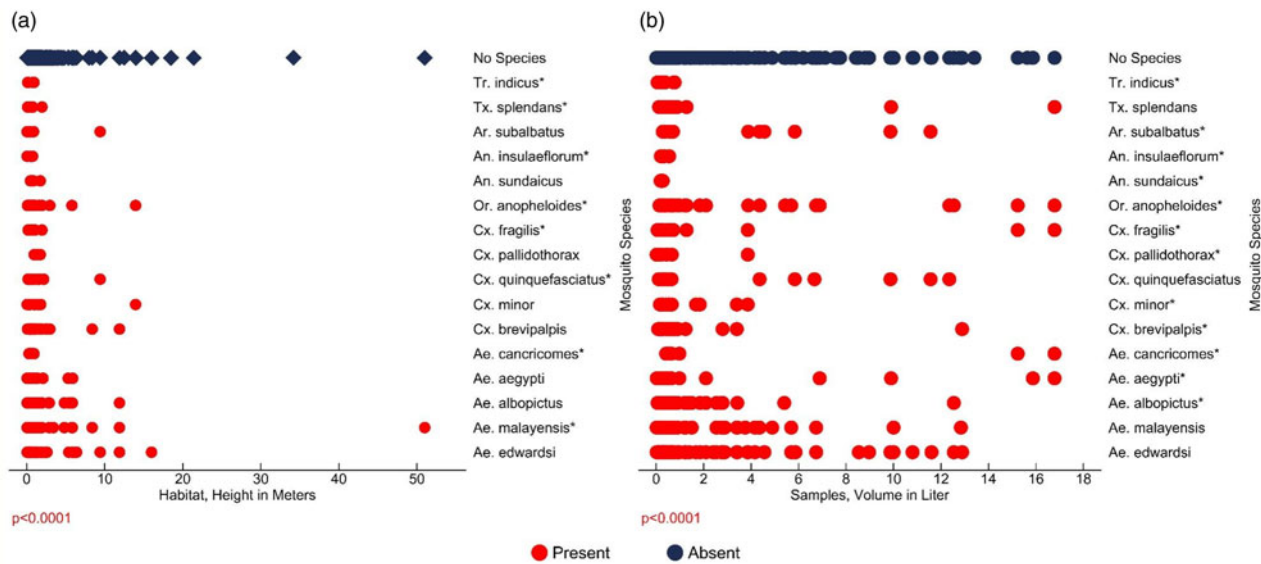


Figure 3. Mosquito species collected in Phytotelmata at varying heights (in metres) and volume (in litres). The data represents the height as well as the volume of water in the phytotelmata site classified by the different mosquito species. $P < 0.0001$ indicates there is a significant difference observed between the height and volume with respect to mosquito species; *indicates the occurrence of the particular species is significantly different from other species.

Ae. albopictus were observed at heights lower than 12 m (fig. 3a). Correlation analysis was performed between abundance and height of phytotelmata. Two mosquito species, viz, *Ae. edwardsi* and *Cx. pallidothorax* were significantly associated with height negatively.

The water content in the examined phytotelmata spanned a wide spectrum, from 0.002 to 16 litres. The number of larvae per 10 ml of water ranged from 0.003 to 3.833 on average. In root holes, the lowest larval density was observed, ranging from 0.10 to 1.07 larvae per 10 ml of water. Correlation analysis was performed between abundance and water volume. Two mosquito species, viz, *Ae. cancricomes* and *Cx. brevipalpis*, were significantly associated with water volume. As water quantity increased the abundance of larvae increased.

Among the four habitat categories, the CI of mosquito larvae infestation was estimated (fig. 4). The CI for *Anopheles* mosquitoes was highest in root holes (6.1%), whereas *Aedes* and *Culex* mosquitoes were more prevalent in bamboo stumps (39.6 and 15.0%, respectively). Several tree families exhibited a CI greater than 50%, including Cycadaceae (3 containers; 100%), Primulaceae (1 container; 100%), Rhizophoraceae (3 containers; 100%), Araucariaceae (2 containers; 66.7%), and Clusiaceae (2 containers; 66.7%) (fig. 4b). *Anopheles* mosquitoes were more prevalent in Primulaceae (1 container; 100%) and Rhizophoraceae (3 containers; 100%), whereas *Culex* mosquitoes were more prevalent in Araucariaceae (2 containers; 66.7%) and *Aedes* mosquitoes were present in Cycadaceae (3 containers; 100%). There was a significant disparity in the abundance of mosquitoes between habitats. The average number of mosquitoes in root holes was 10.7 ± 5.6 , which was substantially greater than the average of 3.2 ± 2.8 in leaf axils ($P < 0.001$). Perka (35 containers; 63.6%), Chukchucha (25 containers; 50.0%), Malacca (27 containers; 45.0%), Air Force (15 containers; 42.9%), Kakana (23 containers; 42.6%), Mus (18 containers; 41.9%), TeeTop (15 containers; 41.7%), and Small Lapathy (16 containers; 40.0%) had the highest percentage of containers infested with larvae (fig. 4c).

Aedes edwardsi was the most prevalent of the 16 identified mosquito species, accounting for 767 specimens (29.6%) and being found in 13 out of 16 areas (81.3%). *Ae. malayensis* and *Or. anopheloides* were collected from 9 (56.3%) and 10 (62.5%), respectively) areas, respectively. The Kakana village had the greatest number of mosquito species, with ten (62.5%) species collected, followed by Sawai village with eight (50.0%) species. *An. sundaicus*, the malaria vector, was found in two (12.5%) villages, namely Kimiuos and Sawai. *Aedes aegypti* and *Ae. albopictus*, the arboviral vectors, were collected from three and six areas, respectively (table 2).

The average number of mosquitoes in each village varied significantly ($P < 0.001$). In Small Lapathy, *Culex* species were significantly more prevalent ($P = 0.002$), with an average of 11.3 mosquitoes per collection (standard deviation: 7.6). Significantly more *Aedes* mosquitoes were found ($P < 0.001$) in Mus, with an average of 12.5 per collection (standard deviation: 9.0). *Aedes edwardsi* was more prevalent in Sawai and Tapoiming villages ($P = 0.020$), whereas *Ae. albopictus* was prevalent in Kakana, Mus, and Malacca villages ($P = 0.049$).

On Car Nicobar Island, species diversity was calculated for four distinct habitats and sixteen areas. The alpha biodiversity indices revealed that the island's environment exhibited a high level of mosquito diversity, with 16 distinct species identified. The results showed a high species richness, evenness, dominance, and infinite diversity. The Shannon-H index was 2.260 (95% CI; 2.220–2.293), and the Margalef index was 1.909 (table 3). The overall *Chao2* species richness estimate was found to be 15.99 (95% CI; 15.72–16.26). Habitat wise analysis shows that species richness also estimated by *Chao 2*, was high in tree holes. These patterns of diversity were shared by all the mosquito species, with the exception of *Aedes*, which exhibited significantly greater diversity than *Culex*. All 16 species were found in tree holes, the most diverse habitat, followed by bamboo stumps and root holes.

Species accumulation curve was prepared for the number of species with respect to phytotelmata type (fig. 5). Flattening of the curve suggest that the sampling effort in the present study

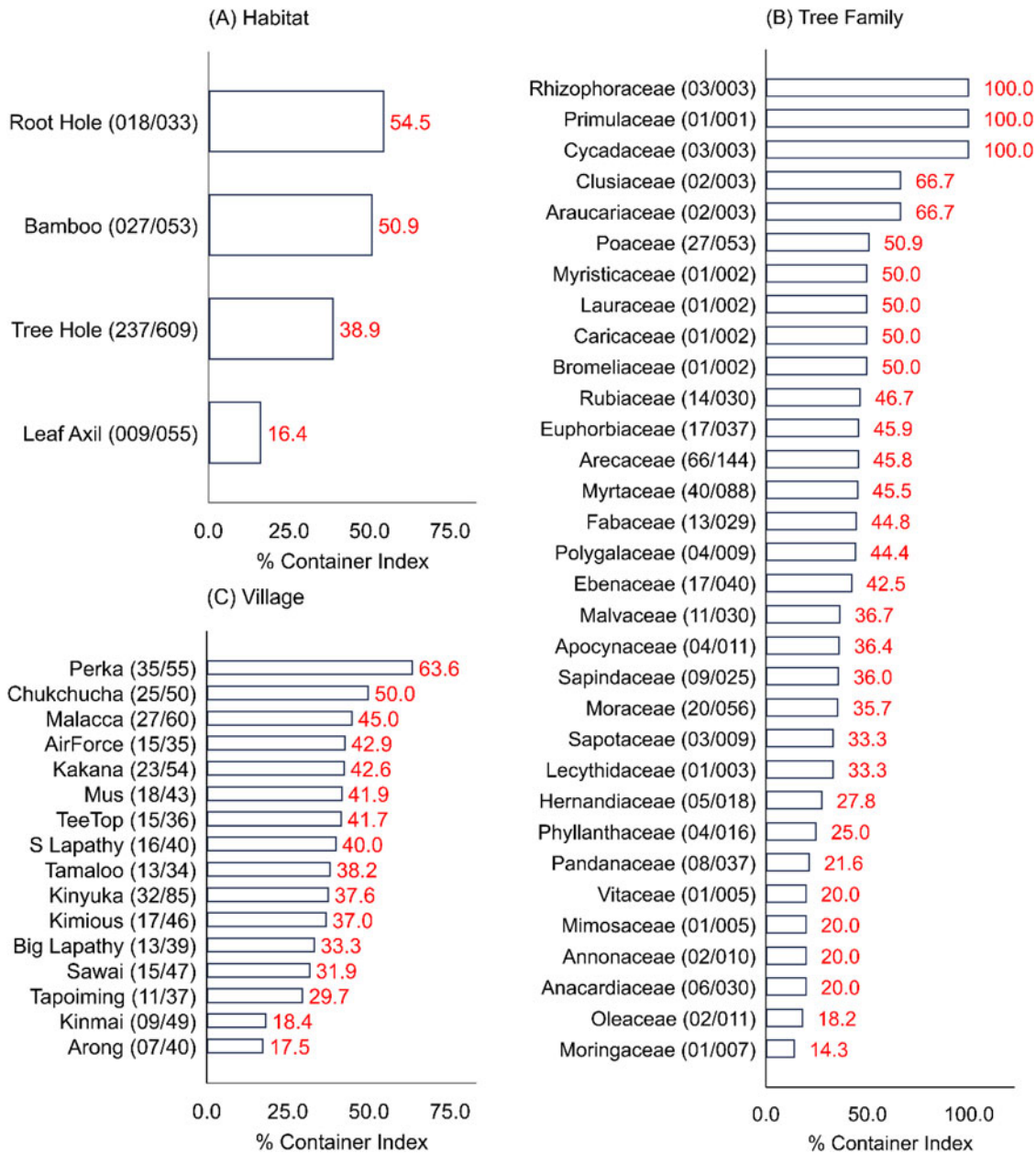


Figure 4. Container index variation in habitat, tree families, and village. The data represents the container index, ranked based on its percentage and shown by classification of their habitat, tree family type, and village.

was large enough that most species that could be sampled in the phytotelmata were collected. In terms of rank and abundance of different species collected, *Ae. edwardsi* was found to be most abundant followed by *Ae. malayensis*, and the least abundant was *An. sundaicus* in the different phytotelmata examined (fig. 6). Habitat wise analysis revealed that bamboo stumps had nine species, with *Ae. malayensis* more abundant. In leaf axil also, this species dominated. In tree holes and root holes, *Ae. edwardsi* was more abundant. Analysis of correspondence revealed associations between habitat and mosquito species, and it accounted for approximately 88% of the variance. *An. insulaeflorum* and *Ar. subalbatus* was overrepresented in root-hole habitats, while in leaf axils and bamboo stumps *Ae. malayensis* and *Cx. brevipalpis* were overrepresented, and other mosquito species were found in tree-hole habitats (fig. 7a). Analysing mosquito

species in relation to tree families revealed that the two anopheline species were more represented with Rhizophoraceae and Primulaceae (fig. 7b). *An. sundaicus* was over-represented in Rhizophoraceae, and *Tx. splendans* was over-represented in Alauraciaceae and Phyllanthaceae phytotelmata. For the villages, Kinyuka was predominantly associated with four mosquito species, including *Ae. aegypti*, a vector of dengue (fig. 7c). Small Lapathy village was found associated more with *Cx. quinquefasciatus*, *An. insulaeflorum*, and *An. sundaicus*.

Discussion

The Nicobarese tribe inhabits the level terrain of Nicobar Island, which is dominated by tropical evergreen forests. The Nicobarese extensively cultivate coconut trees (*C. nucifera*) as a source of

Table 2. Cross tabulation by villages and mosquito species

Mosquito / Village	Air Force	Arong	Big Lapthi	Chukchucha	Kakana	Kimious	Kinmai	Kinyuka	Malacca	Mus	Perka	S Lapathy	Swau	Tamalo	Tampimim	Tee Top	N	n ¹	%
<i>Aedes edwardsi</i>	21	28	-	62	73	57	-	-	32	83	50	63	114	83	61	40	767	13	81.3%
<i>Aedes malayensis</i>	48	-	90	-	48	-	-	-	74	76	26	-	10	-	20	-	392	9	56.3%
<i>Aedes albopictus</i>	36	-	-	-	84	-	-	-	74	41	48	-	-	-	-	-	283	6	37.5%
<i>Aedes aegypti</i>	-	-	-	-	29	-	-	89	-	-	19	-	-	-	-	-	137	3	18.8%
<i>Aedes cancricomes</i>	-	-	-	-	5	-	-	25	-	-	-	-	-	-	-	-	30	2	12.5%
<i>Culex brevipalpis</i>	-	-	-	34	-	-	-	-	31	23	-	-	-	-	40	-	128	4	25.0%
<i>Culex minor</i>	18	10	-	-	8	-	-	-	-	6	-	-	10	-	-	-	52	5	31.3%
<i>Culex quinquefasciatus</i>	-	-	-	34	6	-	-	-	-	-	-	90	1	40	-	12	183	6	37.5%
<i>Culex pallidothorax</i>	-	23	-	-	3	-	-	-	-	-	-	-	13	-	-	-	39	3	18.8%
<i>Culex fragilis</i>	-	-	-	-	-	-	-	47	-	-	-	-	5	-	-	-	52	2	12.5%
<i>Orthopodomyia anopheloides</i>	31	1	-	53	6	33	48	82	1	31	-	-	2	-	-	-	288	10	62.5%
<i>Anopheles sundaicus</i>	-	-	-	-	-	21	-	-	-	-	-	-	8	-	-	-	29	2	12.5%
<i>Anopheles insulaeflorum</i>	-	-	-	-	-	42	-	-	-	-	-	-	-	-	-	-	42	1	6.3%
<i>Armigeres subalbatus</i>	-	-	16	-	-	-	18	-	-	-	-	19	-	24	-	-	77	4	25.0%
<i>Toxorhynchites splendans</i>	-	-	-	-	-	-	-	28	-	-	8	-	-	-	-	14	50	3	18.8%
<i>Tripteroides indicus</i>	-	17	-	-	5	-	-	-	16	-	-	-	-	-	-	-	38	3	18.8%
N	154	79	106	183	267	153	66	271	228	260	151	172	163	147	121	66	2587		
n ²	5	7	2	4	10	4	2	5	6	6	5	3	8	3	3	3			
%	31.3%	43.8%	12.5%	25.0%	62.5%	25.0%	12.5%	31.3%	37.5%	37.5%	31.3%	18.8%	50.0%	18.8%	18.8%	18.8%			

Green cells indicates the lower side (minimum specimens collected), yellow indicates middle (median) and red indicates the higher side (maximum); N denotes the number of mosquito identified. n¹ denotes the number of villages with each mosquito species; n² denotes the number of the mosquito species in each village.

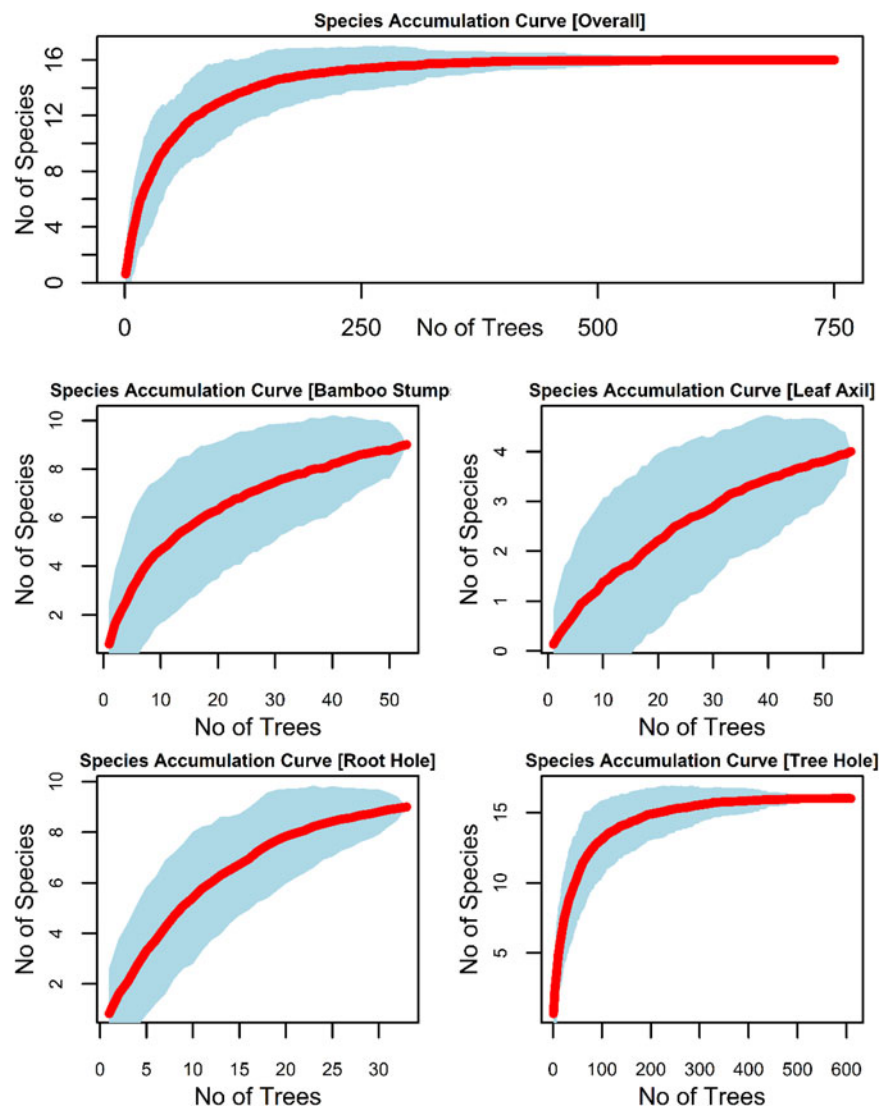
Table 3. Alpha (α) biodiversity estimates for mosquitoes by habitats

	Overall	Bamboo stumps	Leaf axil	Root hole	Tree hole
Specific richness (S)	16	9	4	9	16
Individuals	2587	166	29	193	2199
Simpson index (λ)	0.852 (0.843, 0.860)	0.796 (0.750, 0.824)	0.589 (0.406, 0.692)	0.853 (0.826, 0.866)	0.848 (0.838, 0.856)
Shannon–Weiner index (H')	2.260 (2.220, 2.293)	1.810 (1.676, 1.900)	1.071 (0.741, 1.265)	2.036 (1.928, 2.090)	2.252 (2.209, 2.288)
Margalef index (DMg)	1.909 (1.909, 1.909)	1.565 (1.565, 1.565)	0.891 (0.594, 0.891)	1.520 (1.520, 1.520)	1.949 (1.949, 1.949)
Chao-1	16.00 (0.00, 32.00)	9.50 (0.50, 18.50)	4.00 (1.00, 7.00)	9.00 (0.00, 18.00)	16.00 (0.00, 32.00)
Chao 2	15.99 (15.72, 16.26)	8.60 (6.32, 10.88)	3.47 (2.53, 4.40)	8.55 (6.78, 10.32)	15.88 (15.43, 16.34)

sustenance, so they compose the primary canopy. Remarkably, these coconut trees provide an abundance of tree holes for mosquito larvae to inhabit. In fact, mosquito larvae inhabiting nearly 31 tree holes were found in these coconut trees. The Java apple tree (*Syzygium samarangense*) was found to be the second most prevalent tree species among these larval habitats. This observation highlights the ecological significance of these tree species on the island, where the interdependent relationship between

the Nicobarese and their environment plays a crucial role in determining the population dynamics of mosquitoes.

A remarkable spectrum of mosquito behaviour has been observed among phytotelmata dwelling organisms. Some insects exhibit a marked preference for a single plant species, whereas others occupied a variety of plants within the same phytotelmata class. In the present study, *An. sundaicus* was collected from mangrove trees during all the four occasions. Similarly, *Tr. indicus* was

**Figure 5.** Species accumulation curve for all phytotelmata and by habitat types.

The data represents the number of species by phytotelmata and also by habitat types.

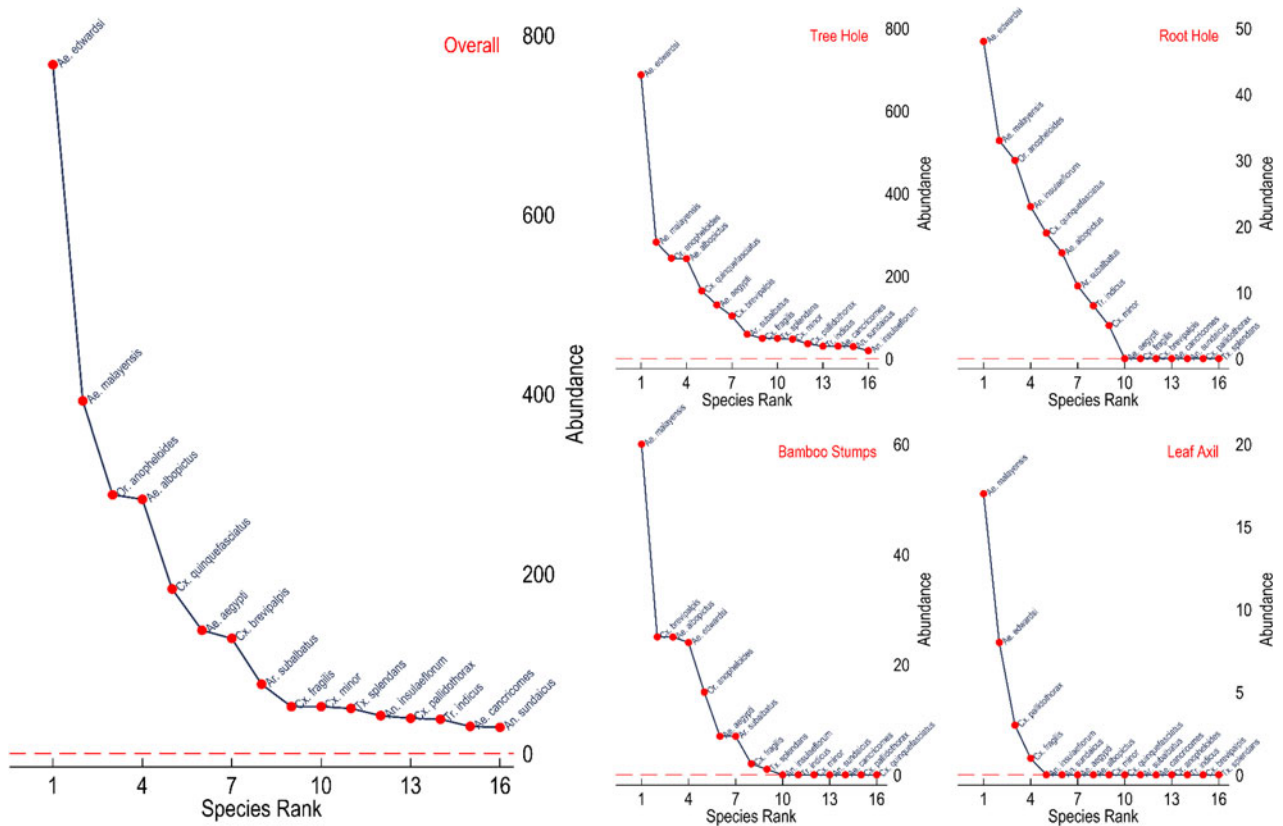


Figure 6. Abundance and ranking of mosquito species in phytotelmata examined. The data represents the abundance and ranking of mosquito species in phytotelmata, including the four habitat types.

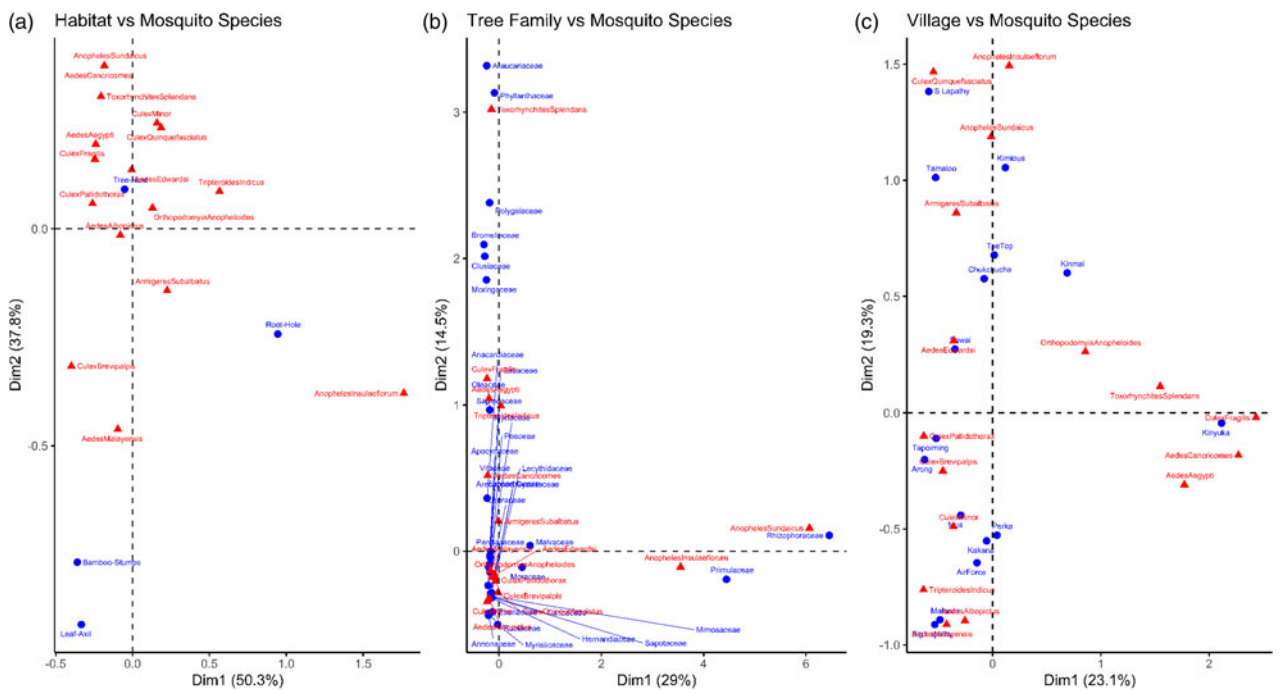


Figure 7. Correspondence analysis of mosquito species, habitat, tree family, and village associations. The data represents the association between sixteen mosquito species and their respective habitats, tree families, and villages. This information aims to enhance our understanding of the diversity dynamics within the mosquito species.

collected from java apple tree on 4 out of 6 occasions. A few were more diverse in their larval habitat preferences. In this study, larvae of *Aedes* species; *Ae. malayensis*, *Ae. edwardsi*, and *Ae. cantans* were collected more frequently from coconut tree. *Aedes aegypti*, the vector of dengue virus, was also present in these tree holes, albeit in small numbers. In contrast, *Ae. albopictus*, another important vector of dengue and chikungunya viruses, did not appear to favor this tree species. The two Anopheline species, on the other hand, primarily inhabited oriental mangrove trees. Notably, the main phytotelmata dweller, *Tx. splendens* (Kitching, 2001), which is also a known predator of mosquito larvae, was observed more frequently in cook pine (*Araucaria columnaris*) and coconut tree holes. It is noteworthy that Sota *et al.* (1994) found a similar diversity of mosquito species in tree cavities on a temperate Japanese Island. This shows that species diversity is not inherently related to latitude gradients. Multiple factors, such as the complexity of microhabitats and the presence of predators may influence the number of species inhabiting particular type of phytotelmata.

The present investigation discovered nearly 109 phytotelmata (39%) harbouring multiple mosquito species. The range of mosquito species across holes in a single plant species was analysed. One mosquito species was observed from 185 phytotelmata. Up to four species were found co-existing in five tree holes. In coconut trees, which is the most common tree type examined, up to four mosquito species were collected from two trees. Notable is the fact that mosquito richness in tropical phytotelmata can attain values equal to or greater than 10 for certain plant species (Delgado and Machado-Allison, 2006). In the present study the number of species found in holes was typically less than the number of species found across that tree species, due to the fact that, competition among species or predation risk by predatory species deters adults from laying eggs where other larvae already occur. This complex interaction between mosquito preference and habitat dynamics highlights the complexity of mosquito ecology in this tropical ecosystem. The only mosquito-infested habitats in Argentina's primary temperate wetland were water-filled leaf axils and tree holes (Albicocco *et al.*, 2011). Particularly, leaf axils have evolved as an important phytotelmata for mosquito larvae. In South America, *Culex castroi*, *Cx. hepperi*, *Cx. renatoi*, *Is. paranensis*, and *Wy. leucostigma* have been collected exclusively from water retained in leaf axils (Marti *et al.*, 2007). Leaf axils of *C. hastatum* were discovered to be the most prevalent phytotelmata habitat in New Zealand. The majority of *Cx. asteliae* larvae, with a lesser presence of *Ae. notoscriptus*, inhabited majority of the 470 plants surveyed, of which 50% tested positive for mosquito larvae (Derraik, 1990). In the present investigation, 55 leaf axils were examined, and 16% contained mosquito larvae. Four distinct species were identified, however, these species were also collected from other habitat types, and no mosquito species specific to leaf axils were observed. Co-existence of species was not observed in any of the leaf axils examined. Height of phytotelmata also has a role to play in the abundance of mosquito species. In the present survey, there was a statistically significant difference ($P < 0.001$) in the abundance of mosquito species based on their heights. Only one species, *Ae. malayensis* was collected at a height of above 20 m, while six species above 10 m. Eight species (mainly the discarded container breeding *Aedes* mosquitoes) seemed to be limited to heights below 10 m. In Cost Rica (Romero *et al.*, 2019), *Cx. nigripalpus* was only found at 75 cm, while *Cx. erethyzonpher* was common at 2.25 m, and is known for having a preference for arboreal epiphytes. These findings contribute to comprehend the

diverse mosquito reproductive habitats in various geographical regions and ecologies.

According to Sota and Mogi (1996), bamboo stumps are a prominent mosquito breeding habitat in numerous parts of the world. In the current study, surveys were conducted on 53 bamboo stumps, and the results were noteworthy, with nearly 51% of them harbouring mosquito larvae. This habitat supported nine mosquito species, with *Ae. malayensis* being the most abundant and *Cx. brevipalpis* following closely behind. Root holes, on the other hand, are frequently neglected but have emerged as essential habitats for certain mosquito species, a finding that has been infrequently reported in previous studies. In the present investigation, 33 root holes were meticulously examined, and 55% of them contained mosquito larvae. Notably, this habitat had the highest CI for anopheline mosquitoes. This emphasises the significance of contemplating root holes as significant anopheline habitats in addition to other surface water bodies when conducting anopheline surveys. These findings enhance our knowledge of the various mosquito productive sites and their implications for mosquito population dynamics and control initiatives.

The amount of water within phytotelmata stands out as a significant factor influencing the number of mosquito larvae. In temperate deciduous forests in North America, Europe, and Japan, tree holes frequently have capacities greater than 10 l, with some in Florida reaching 23 l (Bradshaw and Holzapfel, 1983). In Japan, it was once possible to collect 490 l of water from a single tree hole (Arakawa *et al.*, 1990). In the present study, the maximum water volume observed in tree holes was 16 l, in bamboo stumps it was 3 l, in root holes it was 2 l, and 580 ml in leaf axils. Larval mortality due to habitat overflow caused by rainfall has been documented for a number of mosquito species developing in both natural and artificial containers (Koenraad and Harrington, 2008). This phenomenon is especially pertinent in the context of tiny, ephemeral water collections. Due to the limited resources available in these confined habitats, container-breeding mosquitoes exhibit pronounced competitive interactions (Washburn, 1995; Juliano, 1998). In natural containers with extremely low water volumes, such as 5 ml, the selection of plants previously inhabited by immature mosquitoes of other species appears improbable. Alternately, it is plausible to hypothesise that certain plants provide more favourable conditions – possibly a combination of pH, shade, and detritus for the oviposition of various mosquito species, thereby fostering their coexistence. In addition, Walker and Merritt (1988) observed that tree holes with larger water volumes tend to harbour greater mosquito densities and lower nitrate concentrations. In the present study, two mosquito species (*Ae. cancricomes* and *Cx. brevipalpis*) were found to have a significant positive relationship with volume of water in phytotelmata. In Japan, *open-type* and drought-resisting bigger tree holes, in the beginning of breeding season was found to be preferable for *Ae. albopictus* (Tsuda *et al.*, 1994). Nutrients play a crucial role in the growth and survival of tree hole mosquitoes. In New Jersey, *Ae. albopictus* were common in containers with leaf litter, which is an important nutrient source for bacteria, which in turn are the primary food source for mosquito larvae (Bartlett-Healy *et al.*, 2012). Gut microbiota of these larval stages has important implication in the species oviposition specificity. Numerous studies have shown diverse microbiota colonized by many insects and varies across the life stages (Ali *et al.*, 2018, 2019). However, this aspect was not investigated in the present study, leaving scope for future research into these ecological dynamics.

Nagpal and Sharma (1985) in Orissa documented various mosquito species reproducing and resting in tree holes, including *An. barbirostris*, *An. culicifacies*, *An. fluviatilis*, *An. jeyporiensis*, *An. nigerrimus*, *Cx. tritaeniorhynchus*, *Cx. vishnui* and *Ae. aegypti*. Clearly, additional research is necessary to thoroughly comprehend the ecology of mosquitoes in Car Nicobar, specifically their use of phytotelmata in their native habitats. Environmental factors play a significant role in determining the distribution of both adult and larval mosquito populations (Smith *et al.*, 2004), a factor that was not investigated in this study and thus represents a limitation.

Our investigation included exhaustive sampling and analysis of phytotelmata, yielding valuable larval records. The species accumulation curve showed that the species sampling was comprehensive for our collection method in phytotelmata, as observed for ovitrap sampling carried out by Chaverri *et al.* (2018). The alpha biodiversity indices revealed that the island's environment exhibited a high level of mosquito diversity, with 16 distinct species identified. Habitat wise analysis shows that *Chao2* value was highest in tree-holes denoting more species richness and more number of immatures collected. Given the paucity of information on the reproductive habitats of phytotelmata-dwelling mosquitoes in the Car Nicobar Islands, this contribution is significant. This is the first systematic update on the inventory and distribution of mosquitoes in the Phytotelmata of Nicobarese tribal territory on Car Nicobar Island. Tree holes were the most diverse habitat for mosquitoes, harbouring sixteen species, followed by bamboo stumps and root holes. The mosquito species collected included the vectors of disease causing pathogens, viz, *An. sundaicus* (malaria vector), *Ae. Aegypti*, and *Ae. albopictus* (vectors of dengue and chikungunya viruses), *Cx. quinquefasciatus* (vector of lymphatic filarial parasite). *Anopheles sundaicus* is the incriminated vector of malaria parasites in this island, and they were found breeding both in fresh and brackish water. This mosquito is predominantly endophagic and zoophagic (Sunish *et al.*, 2015). *Aedes* species have been found in man-made container habitats such as tanks, barrels, and abandoned containers (Sunish *et al.*, 2014). Hence, during the vector control operation, the phytotelmata habitats also need to be considered for devising any control operation. Further, the predatory mosquito, *Tx. splendens* was observed from 30 phytotelmata and can be considered as an option for use to control tree hole dwelling mosquito vectors and, consequently, mosquito-borne maladies in these tribal islands. The survey did have few limitations, as it was not carried out for different seasons of the year, to determine the mosquito species occurring at various time periods. This will facilitate in collection of more number of mosquito species, which were not observed in the present survey, as the study was a cross sectional and the period was restricted to April – September.

In conclusion, our research provides a comprehensive description of larval mosquito communities in phytotelmata within a tropical wetland, thereby filling a significant knowledge gap. This data provides a basis for evaluating the biotic and abiotic factors that shaped the community structures observed in this investigation. Due to the presence of important disease-transmitting mosquito vectors in phytotelmata, these habitats should not be ignored during control operations to effectively combat mosquito-borne diseases.

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Authors' contributions. IPS and ANS designed the study, collated the data, carried out preliminary analyses and prepared the draft. APK carried out the fieldwork and collected the data as per the study design. TK carried out detailed data management and statistical analysis. All authors contributed equally to the preparation of the manuscript and the preparation of all figures and tables.

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