RESEARCH ARTICLE

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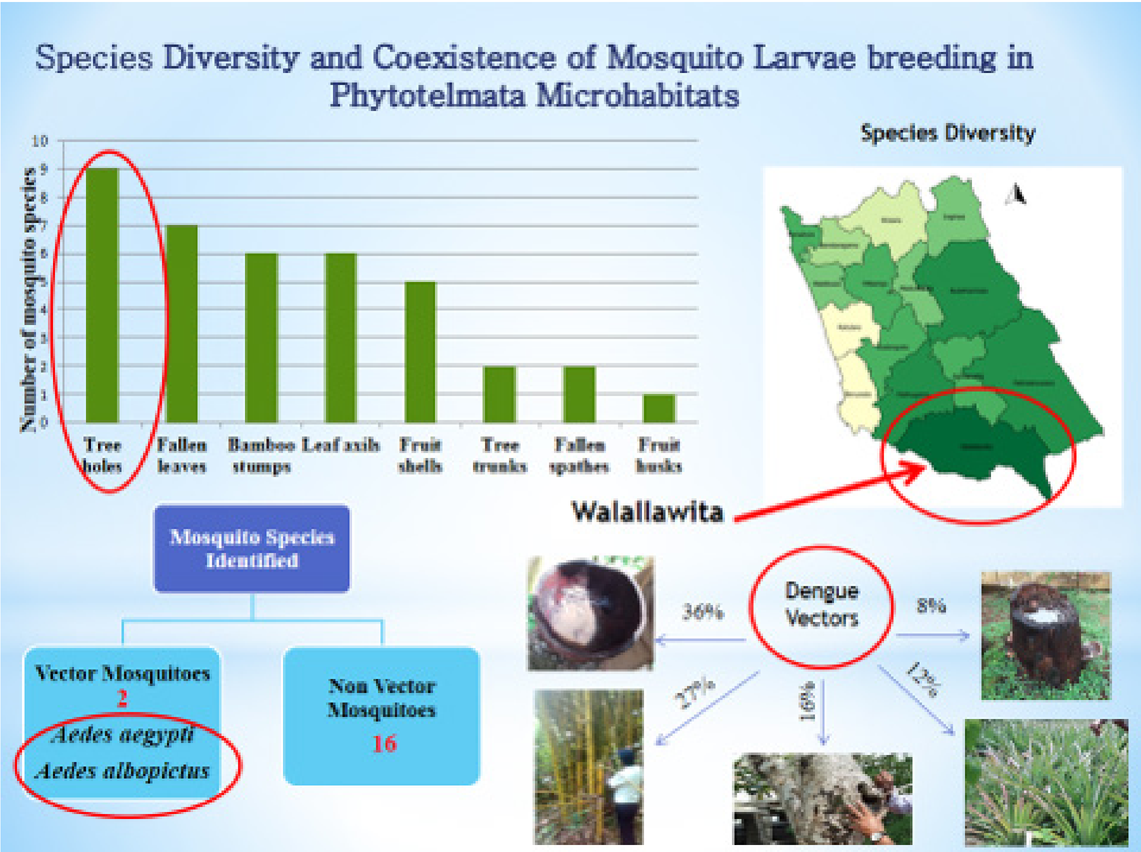
C.S. Kariyawasam* and H.C.E. Wegiriya



Highlights

- A total of 18 mosquito species were identified in eight types of phytotelmata habitats in Kalutara, Sri Lanka.
- Mosquito species diversity was highest in fallen leaves.
- A total of 17 types of coexistence were observed among mosquito larvae.
- Species richness and species diversity were highest in the Walallawita area.
- *Aedes aegypti* and *Aedes albopictus* were the only disease vector mosquitoes identified.

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Species diversity and coexistence of mosquito larvae breeding in phytotelmata microhabitats; a cross-sectional study from Kalutara district, Western Province, Sri Lanka

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Abstract: Phytotelmata are used by many insects for breeding. This study was designed to identify mosquito species breed in phytotelmata available in the Kalutara district. A larval survey was carried out once every two months from January 2019 to April 2021 in thirteen Medical Officer of Health (MOH) areas. For each survey, 20 premises including houses, institutions, public places, roadsides, open areas, and plantations were examined in one randomly selected Grama Niladari (GN) division in each MOH area. According to the study, 18 mosquito species belonging to 6 genera were identified in 8 types of phytotelmata namely**;** tree holes, bamboo stumps, leaf axils, tree trunks, fruit husks, fruit shells, fallen leaves, and fallen spathes. Species richness was highest in tree holes and species diversity was highest in fallen leaves. Similarly, 17 types of coexistence could be observed. The coexistence of four species *Aedes chrysolineatus*, *Ae. downsiomyia*, *Ae. w-albus*, and *Ae. krombeini* were observed in *Dillenia suffruticosa* fallen leaves. Species richness and species diversity of mosquitoes that breed in phytotelmata were highest in Walallawita. The correlation between the volume of water in phytotelmata and the number of larvae in the phytotelmata habitat was statistically insignificant. Although phytotelma is a hidden aquatic habitat, this study indicated that it is an important breeding place for a variety of mosquitoes including vector mosquitoes such as *Ae. aegypti* and *Ae. albopictus*.

***Keywords*:** Mosquito breeding; phytotelmata; diversity; coexistence; Kalutara district.

INTRODUCTION

Phytotelma is a water body held by living or dead terrestrial plants (Motoyoshi, 2012). The source of water may be rain, plant secretions, sap from wounds, or their mixtures. The plants may be ornamental plants, wild plants, or crop plants and those are found especially in humid places such as in tropical areas (Emantis, 2017a). These freshwater aquatic habitats are characterized by small size, discreteness, and ephemerality. Classification of phytotelmata is mainly based on their position in the plant and on the nature of the liquid they contain (Albicocco *et al*., 2011). Tree holes, bamboo stumps and internodes, fallen leaves and spathes, fruit husks and shells, leaf axils, flower bracts, and pitchers are the structures of the terrestrial plants which create phytotelmata (Motoyoshi, 2012; Munirathnam, 2014). These aquatic habitats held by soft parts of plants are generally short-lived and constantly replaced by new ones and phytotelmata held by hard parts of plants are potentially more durable but water persistence is subject to rainfall (Motoyoshi, 2012).

These microenvironments are important in the conservation of biodiversity. The water accumulated within these plants may serve as the habitat for fauna and flora. These aquatic habitats are used in a variety of ways by a wide range of organisms including insects, mites, entomostracods, microorganisms, annelids, crabs, and anurans (Harold, 2001). Those aquatic microcosms are used by many insects for breeding and foraging. Odonates, water bugs, beetles, and dipterans are the most common phytotelmata species.

Among Diptera, one of the most commonly found groups in various phytotelmata is the larvae of mosquitoes (Culicidae). Mosquito larvae and pupae develop in a wide range of aquatic habitats and they cannot withstand desiccation. The only absolute requirement of all development sites is that they maintain at least a film of water for the duration of the larval and pupal periods (Mike, 2012). However, individual species tend to oviposit and develop in sites with specific structural and chemical properties (Gary and Lance, 2019). For example, larvae of *Mansonia* and *Coquillettidia* mosquitoes are restricted to water bodies with aquatic vegetation such as *Pistia stratiotes*, *Salvinia*, or *Eichhornia*, and pH, salt concentration, suspended particulate matter, biological oxygen demand, chemical oxygen demand, and coliform level act as the limiting factors which determine the presence and abundance of those mosquito larvae in aquatic habitats (Serandour *et al*., 2010; Pratiwi *et al*., 2018). In addition to those structural and chemical properties Emantis (2017b) has shown that humidity and rainfall factors have a positive correlation and temperature has a negative correlation with the number of individual Dipteran larvae inhabiting in Phytotelmata.

There are three types of phytotelmata inhabitant species. Some species completely inhabit phytotelmata. Some are common in phytotelmata but also utilize other

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types of aquatic habitats even artificial containers. Another group of species occur in phytotelmata only accidentally but commonly utilize other types of aquatic habitats (Motoyoshi, 2012). *Tripteroides aranoides*, *Tr. bambusa*, *Malaya genurostris*, and *Ma. jacobsoni* are some of the mosquito species identified as completely inhabiting phytotelmata (Lein, 1962; Lang and Ramos, 1981). *Culex breviplapis* has been most frequently identified from tree holes, bamboo stumps, and bamboo internodes but also found from various artificial containers, rock holes, ditches, and ponds (Ralph, 1967). *Culex pseudovishnui* vector of Japanese encephalitis has been found in a wide range of groundwater habitats including ditches, rice fields, ponds, streams, and sumps (Ralph, 1967). Although it is not common in phytotelmata, it has been found in tree holes in India (Munirathnam, 2014).

Phytotelmata ecosystems are often ignored by humans because of their small size and concealment. Some of the species that develop in phytotelmata have public health significance as vectors of diseases such as dengue - *Ae. albopictus* (Emantis, 2017b), malaria - *Anopheles stephensi*, *An. subpictus*, *An. culicifacies* (Selvan and Jebanesan, 2014), and filariasis - *Cx. quinquefasciatus* (Selvan and Jebanesan, 2014). Also, those ecosystems are atypical and understudied freshwater ecosystems. Knowledge of mosquito breeding site preference is important for planning effective mosquito control strategies. The most practical way to reduce the local population of vector mosquitoes is to eliminate their habitats as much as possible (Leopoldo, 2007). Lack of knowledge on mosquito breeding places can adversely affect the ecological balance and biodiversity. Excessive use of insecticides without proper knowledge on vector breeding places kills not only vector mosquitoes but also the predators and harmless mosquito species which do not act as the vectors of diseases. In addition, insecticide resistance can be developed in mosquitoes especially due to excessive and repetitive exposure to insecticides such as pyrethroids, organophosphates and carbamates over the years (Chaudhry *et al.*, 2019). By eliminating more sensitive, harmless mosquito species from an environment more resistant and aggressive vector species can easily expand their niche. So that unplanned vector control interventions can harmfully affect the ecological balance and biodiversity. According to the global invasive species database, the Asian tiger mosquito (*Ae. albopictus*) and the common malaria mosquito (*An. quadrimaculatus*) are listed among the world's worst invasive alien species (Lowe *et al.*, 2000). When local populations in the native range invade into a human-altered environment some populations are quickly established by adapting to this new habitat. This scenario is known as Anthropogenically Induced Adaptation to Invade (AIAI) and it results in speciation (Hufbauer *et al.*, 2011). AIAI has been postulated to be associated with speciation in the most important afro-tropical malaria mosquito *An. gambiae* and this speciation have created problems in vector control interventions and consequences upon malaria transmission (Kamdem *et al.*, 2012).

The present study was designed to investigate mosquito species that breed in phytotelmata since it is an overlooked ecosystem due to its concealment. The study concentrates on assessing species richness, species diversity, and coexistence of phytotelmata breeding mosquitoes in different locations of Kalutara district, which is an endemic area for dengue and urban filariasis and with heavy routine vector control activities. Studies on phytotelmata have not been done expansively compared to other mosquito breeding places in the Kalutara district. This study aimed to identify the physicochemical parameters *i.e.* pH, turbidity, light intensity, and volume of water which determine the selection of phytotelmata as the breeding site by female mosquitoes and to investigate the significance of phytotelmata as vector mosquito breeding sites. Therefore, the findings will contribute towards a complete understanding of the significance of phytotelmata as one of the vector mosquito breeding places and will be useful for health authorities in planning vector control activities.

MATERIALS AND METHODS

Study area

The study was conducted in 13 MOH areas in Kalutara District namely; Panadura, Wadduwa, Bandaragama, Horana, Mathugama, Dodangoda, Agalawatta, Madurawala, Millaniya, Walallawita, Palindanuwara, Bulathsinhala, and Ingiriya. Kalutara district is situated in the western province of Sri Lanka and has an area of 1598 km^2. It is bounded by Colombo district from North, Rathnapura district from East, Galle district from South, and the Indian Ocean from West. In the Kalutara district, climatic conditions differ geographically and periodically, and there are different ecosystems, such as fresh water and marine ecosystems, forests and mountain ecosystems, and diverse croplands.

Mosquito sampling

The larval survey was carried out once every two months in each MOH area during the study period from January 2019 to April 2021. Different GN divisions were randomly selected from each MOH area throughout the study period. In each survey, twenty premises including houses, institutions, public places, roadsides, open areas, and plantations *i.e.* pineapple plantations, rubber plantations were examined from the selected GN divisions. All available phytotelmata habitats were examined. Pipetting method (10 ml pipette) was used to collect all mosquito larvae and pupae and the total volume of water was collected from each phytotelma habitat.

Identification of mosquitoes

All larvae were observed under the compound microscope and identified up to the genera (Amarasinghe, 1995a) and then to the species level using published taxonomic keys (Ralph, 1967; Kenneth, 1968; Amarasinghe, 1995b; Rattanarithikul *et al.*, 2010). All collected pupae were kept separately in labeled cups filled with some water and covered with net cloths until adults emerged. Adults were identified using published taxonomic keys (Amarasinghe, 1995a; Rattanarithikul *et al.*, 2010).

Identification of physicochemical parameters

Turbidity, light intensity, pH, and volume of water were the parameters used to analyze the breeding site preference of female mosquitoes. The pH of breeding places was measured using pH indicator papers (pH 1 - 14 universal indicator). Turbidity of the water in phytotelmata was recorded as clear, slightly turbid, and turbid (Salit *et al*., 1996) by looking at the transparency and suspended solids of water after adding it into a glass test tube. Light intensity at the phytotelmata habitats was classified relatively as sunny, semi-shaded, and deeply shaded (Salit *et al*., 1996). Throughout the study period, turbidity and light intensity were recorded by one person. In addition, the volume of water was measured by collecting water into a measuring cylinder.

Data analysis

Data were analyzed mainly using descriptive statistical techniques. Species diversity was calculated by Shanon Weiner Diversity Index (H) using Microsoft Excel 2013. It was calculated as $H= -\sum pi \ln pi$. Where *pi* was the proportion of the total sample represented by species i. Correlation between the volume of water in phytotelmata habitats and the number of mosquito larvae and pupae in phytotelmata habitats were analyzed by bivariate Pearson product-moment correlation coefficient using IBM SPSS statistical software package. Q GIS 2.18.13 software was used to present the analyzed spatial data using maps. Abundance, species richness, and species diversity were used as the layers, and graduated colour maps were prepared.

RESULTS

Mosquito species breeding in phytotelmata microhabitats

A total of 2262 mosquito larvae and pupae were collected from 174 phytotelmata during the study period. Those phytotelmata belong to 8 types namely; tree holes, bamboo stumps, leaf axils, tree trunks, fruit husks, fruit shells, fallen leaves, and fallen spathes (Figure 1). Fruit husk and shells were categorized separately depending on their hardness and durability. Among that 52% (n = 90) of the phytotelmata were water bodies held by living terrestrial plants and 48% (n = 84) of phytotelmata were water held by dead terrestrial plant structures. Collected mosquito larvae and pupae belonged to 18 mosquito species (Table 1).

Table 1 shows that 8 out of 18 species have more than one type of phytotelmata habitats selection for oviposition. *Armigeres subalbatus* was found in all types of phytotelmata habitats found during the study.

Species richness was highest in tree holes. According to the study, 213 mosquito larvae and pupae belonging to 9 species were found in 18 tree holes. *Aedes albopictus* (n = 114, 54%), *Ar. subalbatus* (n = 39, 18%), and *Ae. krombeini* (n = 37, 17%) were the mosquito species mainly found in tree hole habitats.

According to the Shanon Weiner diversity index species diversity was highest in fallen leaves (Figure 2) and 45 mosquito larvae belonging to 7 species were found in fallen leaves (Table 1). Fallen leaves of *Dillenia suffruticosa* (Rata Diyapara) (Figure 1 - f) and *Musa* sp. (Banana) were the phytotelmata habitats positive for the immature stages of the mosquitoes. In *Dillenia suffruticosa* fallen leaves 56% (n = 14) of larvae were *Ae. chrysolineatus*. In *Musa* sp. 60% (n = 12) of mosquito larvae found were *Ar. subalbatus*.

Ananas comosus (Pineapple), *Colocasia esculenta* (Elepahant Ear Trees), *Alocasia macrorrhizos* (Giant Tora Trees), *Neoregelia* sp. (wild and ornamental Bromeliad plants), and *Pandanus amaryllifolius* (Pandan) were the phytotelmata leaf axils positive for mosquitoes. Pineapple cultivation could be seen mainly in Horana, Palindanuwara, Dodangoda, and Madurawala area. Pineapple leaf axils were mainly utilized by *Malaya genurostris* (n = 23, 38%), *Tripteroides* sp. (n = 21, 35%), and 57% (n = 12) of *Colocasia* sp. and *Alocasia* sp. were utilized by *Ma. genurostris.* However, *Neoregelia* sp. Bromeliad leaf axils were primarily consumed by *Ae. albopictus* (n = 65, 54%) and *Tripteroides* sp. (n = 46, 38%). *Malaya genurostris* was the only mosquito species identified in Pandan leaf axils.

Out of the 174 phytotelmata habitats positive for mosquito larvae in this study, 64 (37%) phytotelmata habitats were bamboo stumps. So bamboo stumps were the main phytotelma habitats positive for mosquito immature found in Kalutara district. *Armigeres subalbatus* (n = 456, 53%), *Ae. albopictus* (n = 195, 23%), and *Tripteroides* sp. (n = 192, 23%) were the main mosquito species identified from bamboo stumps.

Discarded coconut shells were the only fruit shell positive for mosquito immature stages. Five mosquito species were identified from those breeding places and 59% (n = 389) of mosquito species breeding in discarded coconut shells were *Ar. subalbatus* (Table 1).

Banana and coconut trunks were the tree trunk phytotelmata habitats positive for mosquito immature stages. Utterly of Banana tree trunks were positive for *Ar. subalbatus* and 100% of coconut tree trunks were positive for *Ae. albopictus.*

Coconut and king coconut husks were the fruit husks positive for mosquitoes. *Armigeres subalbatus* was the only mosquito species found in fruit husk phytotelmata habitats. Discarded areca nut and coconut spathes were the fallen spathes positive for mosquitoes. *Armigeres subalbatus* (n = 87, 81%) and *Cx. fragilis* (n = 20, 19%) species were found in those breeding places.

Coexistence of mosquito larvae breeding in phytotelmata microhabitats

According to the results, 17 types of coexistence were observed among mosquito larvae in phytotelmata (Table 2). Coexistence of four species (*Ae. chrysolineatus*, *Ae. downsiomyia*, *Ae. w-albus*, and *Ae. krombeini*) was observed in *Dillenia suffruticosa* fallen leaves. Two species

Table 1: Mosquito species identified from phytotelmata habitats in Kalutara district.

Mosquito species	Number and percentage of immature stages of mosquitoes collected from different types of phytotelmata							
	Tree holes	Bamboo stumps	Leaf axils	Tree trunks	Fruit husks	Fruit shells	Fallen leaves	Fallen spathes
Aedes aegypti		1, (0.12%)						
Aedes albolateralis	1, (0.47%)							
Aedes albopictus	114, (53.52%)	195, (22.86%)	84, (40.58%)	54, (35.06%)		254, (38.66%)	5, (11.11%)	
Aedes chrysolineatus						1, (0.15%)	14, (31.11%)	
Aedes (downsiomyia)							4, (8.89%)	
Aedes krombeini	37, (17.37%)	5, (0.59%)	1, (0.48%)			11, (1.67%)	3, (6.67%)	
Aedes niveus			3, (1.45%)					
Aedes (phagomyia)	3, (1.41)							
Aedes pseudotaeniatus	10, (4.69%)							
Aedes vittatus						2, (0.30%)		
Aedes w-albus							4, (8.89%)	
Armigeres subalbatus	39, (18.31%)	456, (53.46%)	7, (3.38%)	100, (64.94%)	26, (100%)	389, (59.21%)	12, (26.67%)	87, (81.31%)
Culex brevipalpis	4, (1.88%)	4, (0.47%)						
Culex fragilis								20, (18.69%)
Culex nigropunctatus							3, (6.67%)	
***Heizmannia* sp.**	1, (0.47%)							
Malaya genurostris			40, (19.32%)					
***Tripteroides* sp.**	4, (1.88%)	192, (22.51%)	72, (34.78%)					

Ae. *albopictus* and *Tripteroides* sp. were the frequently found coexisting species in phytotelmata habitats and 53% (n = 8) of their coexistence was observed in bamboo stumps.

Abundance and distribution of phytotelmata breeding mosquitoes in Kalutara district

Leaf axils, bamboo stumps, and fruit shells were the most abundant and widely distributed phytotelmata habitats found in the Kalutara district and they were principally identified from rural areas like Dodangoda, Palindanuwara, and Agalawatta which are away from the Colombo district boundary (Figure 3). Phytotelmata habitats positive for mosquito immature stages were very rarely found in urban areas like Bandaragama, Wadduwa, and Panadura which are close to the Colombo district border in the North.

Species richness of the mosquitoes which breed in phytotelmata habitats was highest in Walallawita MOH (Figure 4a) area and eight mosquito species were identified in different phytotelmata habitats. The species diversity of phytotelma breeding mosquitoes were also highest in Walallawita area (Figure 4b) and Shanon Weiner diversity index was 1.5404.

Physico-chemical parameters that determine the selection of sites by females for oviposition

According to the linear regression analysis (Figure 5) and Pearson product-moment correlation analysis, there was no significant correlation between the volume of water in phytotelmata habitat and the number of larvae and pupae in each phytotelmata habitat ($R^2 = 0.007$, $r = 0.08$, $p = 0.328$). Moreover, 18%, 52%, 21%, and 9% of mosquito larvae and pupae were found in phytotelmata habitats respectively with < 50ml, 50 < 250 ml, 250 < 500 ml and 500 < 1000 ml of water.

Figure 1: Phytotelmata habitats identified in Kalutara district: (a) Tree hole, *Plumeria* sp.; (b) Tree trunk, *Cocos nucifera*; (c) Fruit shell, *Cocos nucifera*; (d), (e) Bamboo stump; (f) Fallen leaf, *Dillenia suffruticosa*; (g) Leaf axil, *Ananas comosus*; (h) Leaf axil, *Neoregelia* sp. and (i) Fallen spathe, *Cocos nucifera*.

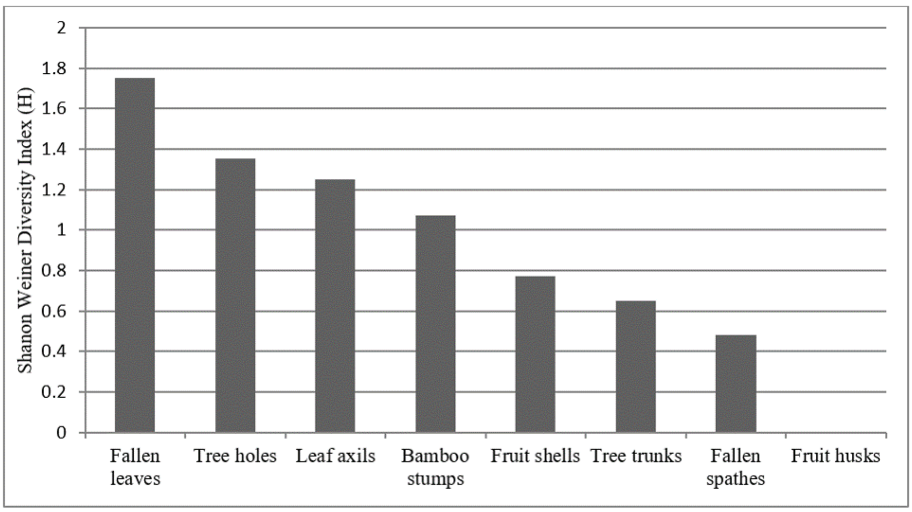

Figure 2: Shanon Weiner Diversity index for mosquito species identified from phytotelmata habitats in Kalutara District.

Table 2: Coexistence identified among mosquito larvae inhabited in phytotelmata of the present study.

	Coexisting species in phytotelmata				Number of Times Coexist
1	*Aedes aegypti*	*Aedes albopictus*			1
2	*Aedes albopictus*	*Aedes chrysolineatus*			1
3	*Aedes albopictus*	*Aedes krombeini*			4
4	*Aedes albopictus*	*Aedes krombeini*	*Armigeres subalbatus*		1
5	*Aedes albopictus*	*Aedes (Phagomyia)*			1
6	*Aedes albopictus*	*Armigeres subalbatus*			7
7	*Aedes albopictus*	*Armigeres subalbatus*	*Tripteroides* sp.		2
8	*Aedes albopictus*	*Tripteroides* sp.			15
9	*Aedes chrysolineatus*	*Aedes w-albus*			1
10	*Aedes chrysolineatus*	*Aedes (downsiomyia)*	*Aedes w-albus*	*Aedes krombeini*	1
11	*Aedes krombeini*	*Malaya genurostris*			1
12	*Aedes krombeini*	*Malaya genurostris*	*Tripteroides* sp.		1
13	*Armigeres subalbatus*	*Culex brevipalpis*			1
14	*Armigeres subalbatus*	*Culex fragilis*			1
15	*Armigeres subalbatus*	*Tripteroides* sp.			3
16	*Armigeres subalbatus*	*Culex nigropunctatus*			1
17	*Malaya genurostris*	*Tripteroides* sp.			2

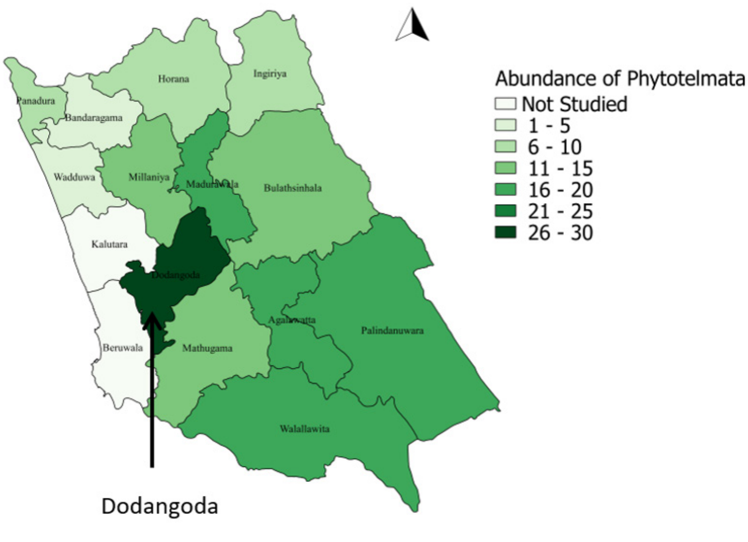

Figure 3: Abundance of phytotelmata habitats positive for mosquito immature stages in Kalutara district.

The pH of water in the phytotelmata habitats where mosquito immature stages were found varied from 7 to 9. According to the results, 98% (n = 2237) of mosquito larvae were found in pH 7 water in phytotelmata. Fifty-two percent (n = 3) of *Ae. krombeini* were found in tree holes with pH 8 water, 50% (n = 4) of *Cx. brevipalpis* and 0.5% (n = 6) of *Ar. subalbatus* were found in tree holes with pH 8 and 9.

According to figure 6, *Ae. aegypti*, *Ae. chrysolineatus*, *Ae.* (*downsiomyia*), *Ae. niveus*, *Ae. w-albus*, and *Cx. fragilis* were found only in clear water and *Ae. albolateralis*, *Ae.* (*phagomyia*), and *Heizmannia* sp. were found only in turbid water. Therefore, the level of turbidity is a limiting factor for the selection of phytotelmata as a breeding site by the adult female mosquitoes of those species. *Aedes pseudotaeniatus*, *Ae. Vittatus*, and *Cx. nigropunctatus* were found entirely in slightly turbid water (Figure 6). *Aedes albopictus*, *Ar. subalbatus*, and *Tripteroides* sp. were found in all three types of turbidity levels - clear, slightly turbid, and turbid water. Forty-nine percent (n = 1125) of mosquito immature stages were found in slightly turbid water, 28% (n = 634) and 23% (n = 503) were found separately in clear and turbid water in phytotelmata habitats.

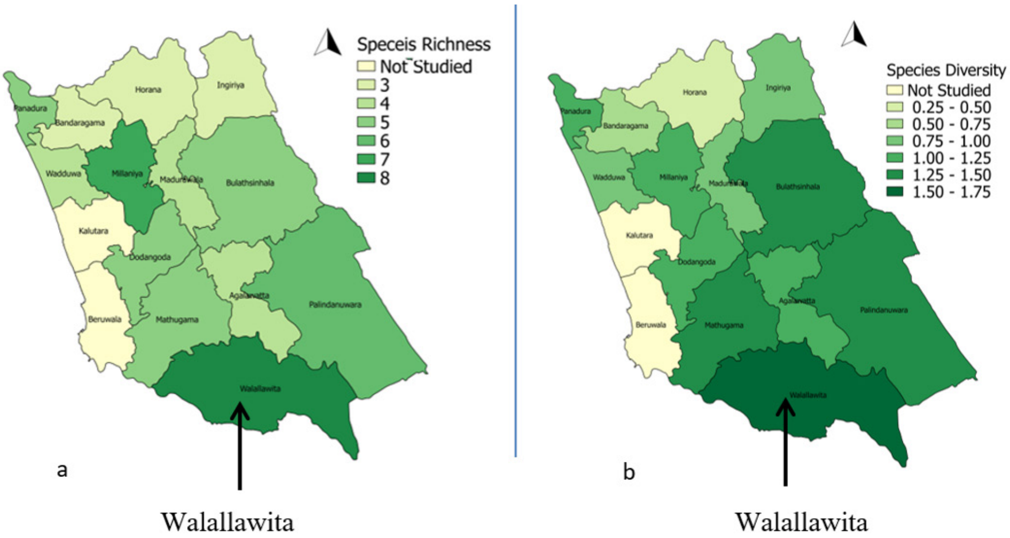

Figure 4: (a) Species richness and (b) species diversity of mosquito species breed in phytotelmata habitats in Kalutara district.

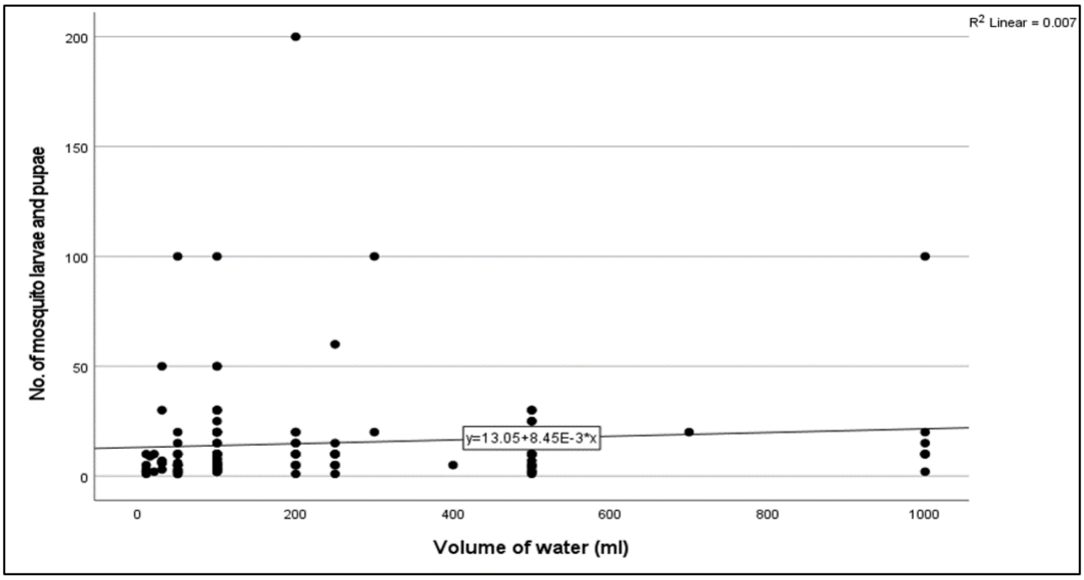

Figure 5: Correlation in-between volume of water in phytotelmata habitats and number of mosquito larvae and pupae in phytotelmata habitats.

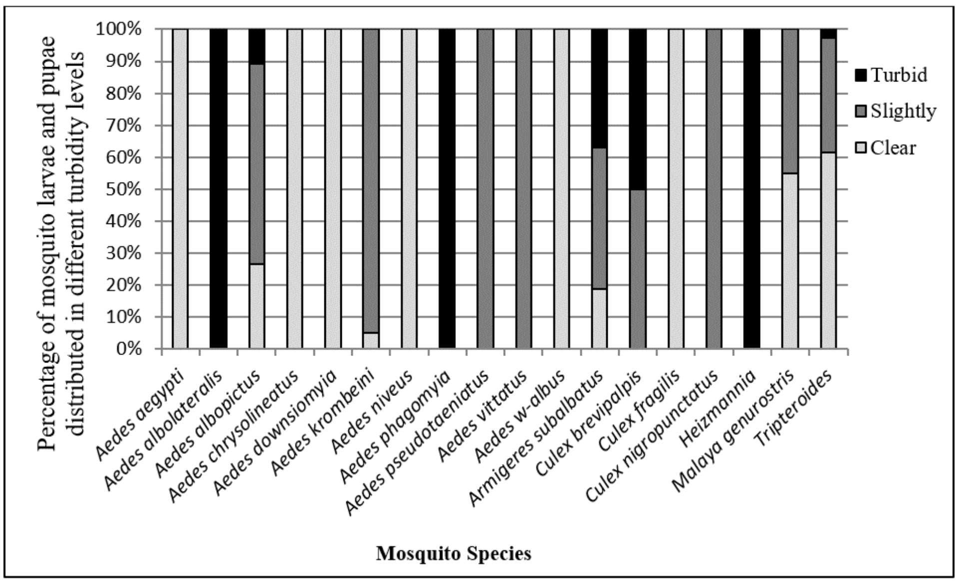

Figure 6: Percentage stacked column chart showing the distribution of mosquito species in different levels of turbidity

According to the study, 30% of mosquito immature stages were found in phytotelmata habitats in sunny environments. Among them were 100% (n = 1) of *Ae. aegypti*, 28% (n = 198) of *Ae. albopictus*, 39% (n = 435) of *Ar. subalbatus*, 29% (n = 12) of *Ma. genurostris*, and 10% (n = 27) of *Triptroides* sp.. Other 70% of mosquitoes were found in semi-shaded environments.

DISCUSSION

According to the present study, 18 mosquito species belonging to 6 genera were identified in 8 types of phytotelmata habitats. Among these species *Ae. aegypti* and *Ae. albopictus* were the only two mosquito species that act as vectors of human diseases. *Aedes aegypti* mosquito is the primary vector of dengue, chikungunya, yellow fever, and zeka. *Aedes aegypti* is highly associated with human dwellings and most often breeds in man-made containers (David *et al*., 2009). Emantis *et al*. (2017) reported that *Ae. aegypti* was absent in phytotelmata habitats in Indonesia. Munirathinam *et al*. (2014) stated that in India two dengue/ chikungunya vectors *i.e. Stegomyia aegypti* and *St. albopicta*, were recorded from tree holes, bamboo stumps, reed stumps, and log holes. In this study *Ae. aegypti* was found in one bamboo stump in Nalluruwa area in Panadura. It is an urban area with high *Ae. aegypti* density and frequent outbreaks of Dengue. *Aedes albopictus* is the secondary vector of dengue in South East Asia. It is considered a sylvatic species and breeds mainly in natural containers. According to this study *Ae. albopictus* was found in fruit shells, bamboo stumps, tree holes, leaf axils, tree trunks, and fallen leaves. Consequently, those breeding places need to be focused on during dengue control activities. According to a study done by Munirathinam *et al*. (2014) in India, not only the dengue vector mosquitoes but one malaria vector (*An. culicifacies*) and two Japanese encephalitis vectors (*Cx. pseudovishnui*, *Cx. whitmorei*) have also been recorded from phytotelmata habitats indicating these habitats as important breeding sites for vector mosquitoes of human diseases. However, before implementing vector control activities focusing on phytotelmata habitats, it is essential to study the pupal productivity of vector mosquito species and other phytotelmata breeding species as there may be predators of the mosquito larvae breeding in phytotelmata habitats. Tadpoles of *Phyllidytes luteolus* living in bromeliad axils have been identified as potential predators of mosquito larvae (Aila *et al*., 2018). According to them, *Phyllidytes luteolus* tadpoles of any size were able to prey on mosquito larvae and large tadpoles preyed a larger number of mosquito larvae than small size tadpoles. Further, larvae of dragonflies and damselflies are predators of mosquito larvae. Frank *et al*. (2009) cited that dragonflies and damselflies breed in bromeliad leaf axils and their nymphs can climb out of one leaf axil to another with the assistance of their well-developed legs.

Armigeres subalbatus was the most abundant and widely dispersed mosquito species breed in phytotelmata habitats. According to the present study they were found in all types of phytotelmata habitats indicating that they could tolerate a wide range of pH, turbidity, and light intensity than other species. Comparatively, *Ar. subalbatus* larvae were found in high density in each phytotelmata habitat and the average density was 24 (± 37) larvae per breeding site. So they may be less specific of their requirements and show substantial flexibility in their breeding place selection. Rajavel (1992) has found ammonia nitrogen as the only factor significantly correlated with the immature density of *Ar. subalbatus*. In this study the coexistence of *Ar. subalbatus* could be observed with *Ae. albopictus*, *Cx. fragilis*, *Cx. brevipalpis*, *Cx. nigropunctatus*, and *Tripteroides* species. Also different larval stages of *Ar. subalbatus* were found in the same breeding places. However, under laboratory conditions without alternative food supplements and by using *Ae. albopictus* and *Cx. uniformis* larvae as prey organisms, Chathuranga *et al*. (2019) have found predatory and cannibalistic behavior of *Ar. subalbatus*.

Three *Culex* species were identified from phytotelmata. Those were *Cx. brevipalpis*, *Cx. fragilis*, and *Cx. nigropunctatus*. According to Ralph (1967) in Thailand *Cx. brevipalpis* larvae were most frequently found from tree holes and from bamboo stumps and internodes. Also in this study *Cx. brevipalpis* immature stages were found in tree holes and bamboo stumps. During this study, *Tripteroides* species were found in bamboo stumps, leaf axils, and tree holes. According to Gunathilaka (2018) in Sri Lanka, there are three *Tripteroides* species and they belong to the subgenus *Rachinotomyia*. The immature stages of *Rachinotomyia* found in tree holes, hollow logs, bamboo, and tree stumps, root holes, split bamboo, plant axils (including banana, *Allocasia*, *Colocasia*, Nipa, Pandanus, pineapple, and others), *Nepenthes* pitchers, ginger florescence, fallen leaves, coconut shells and husks, rock holes, and artificial containers (Harbach, 2014). Chathuranga *et al*. (2017) have found *Tr. affinis* in tree holes in Kandy district, Sri Lanka.

Except for the genera *Anopheles* and *Culex*, there are no proper published morphological identification keys available for local/ Sri Lankan mosquito species. Therefore, collected *Heizmannia* and *Tripteroides* species were identified only up to the genus level. Seven *Aedes* larvae were identified only up to the subgenera level as *Ae*. (*downsiomyia*) and *Ae*. (*phagomyia*). According to the available records, two species under the subgenera *downsiomyia* (namely; *Ae. albolateralis* and *Ae. mohani*) and one species under the subgenera *phagomyia* (namely; *Ae. gubernatoris*) have been identified in Sri Lanka (Gunathilaka, 2018).

A large accumulation of water in a phytotelmata habitat would provide prolonged breeding habitat for mosquitoes even though other small water bodies have dried up. So, the volume of water in phytotelmata is a key factor to the colonization of the phytotelmata as in the dry season habitat dryness would prevent their availability to mosquito breeding. In this study volume of water in phytotelmata habitats varied from 10 - 4000 ml and 99% of the phytotelmata habitats positive for mosquitoes were with less than 1L of water. This study shows that there is no significant correlation between the volume of water in the phytotelmata habitat and the number of larvae and pupae in each phytotelmata habitat. Likewise, 70% (n = 1651) of mosquito immature stages were found in less than 250 ml volume of water. A study done by Adebote *et al*. (2008) shows that *Aedes* genus of mosquitoes breeds in phytotelmata at significantly smaller water volumes than *Culex* genus and the abundance of all species of mosquitoes correlated positively and significantly with water volumes in the phytotelmata. Adebote *et al*. (2008) also show that not only the water volume but also the surface area and depth variously affect mosquito species occurrence and abundance.

Species diversity was highest in fallen leaves and the coexistence of four species was observed in *Dillenia suffruticosa* fallen leaves. It is an invasive plant and it is widely distributed in wastelands and stream banks especially in the Walallawita area. Those leaves are thick, 15 - 30 cm long, and 6 - 15 cm broad. Fallen dried-up leaves do not degrade easily and 100 - 250 ml of rainwater could be held for more than five days. In past years, these plants have been highly emphasized and criticized by villagers because of the mosquito nuisance and the dengue outbreaks arising in the Walallawita area. However, according to this study, dengue vector mosquito larvae were not found in *Dillenia suffruticosa* fallen leaves. *Aedes chrysolineatus* were the main mosquito species found in those breeding places. Nevertheless, *Ae*. *albopictus* was positive in *Musa* sp. fallen leaves and it is a very common fruit plant cultivated in home gardens, plantations, and any other places.

CONCLUSION

Although phytotelmata are neglected aquatic habitats because of its size and concealment, this study shows that they are important breeding places for mosquito species considering species richness, species diversity, and their coexistence. According to this study volume of water in phytotelmata is not a limiting factor for the abundance of mosquito larvae. Most of the phytotelmata inhabited by mosquito larvae had neutral pH, slight turbidity, and less than 250 ml water volume, and were in semi-shaded environments.

Presence of *Ae*. *aegypti* and *Ae*. *albopictus* in different types of phytotelmata shows the significance of phytotelmata in dengue transmission. Therefore, this study on mosquito species breeds in phytotelmata is particularly important to both biologists and epidemiologists.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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