Larval Mosquito Diversity and Its Public Health Implications in the Province of Cavite

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ABSTRACT

A study was conducted to determine the larval mosquito diversity and its implication to public health in the province of Cavite. The study was conducted across three altitude zones - upland, midland and lowland, covered by 13 municipalities and seven cities of the province. Mosquito larva collection employed simple random sampling on the possible and suitable biotopes in randomly selected areas using dippers, larval nets and pipettes/droppers. Identification of mosquito larvae was based on established morphological characteristics from larval identification keys. Results revealed that the province is inhabited by 10 mosquito species, five of which were considered medically important. They are *Aedes aegypti, Aedes albopictus, Culex quinquefasciatus, Culex tritaeniorhynchus* and *Anopheles* sp. which can transmit arboviral and/or other parasitic diseases. The first known record of two non-vector species, *Culex mimeticus* and *Toxorhynchites splendens,* with predatory behavior in their larval stages, were also collected in specific areas of the province. Occurrence, abundance and diversity analysis of the upland, midland and lowland areas present that the upland areas was the most diverse where *Ae. aegypti* and *Ae. albopictus* were the most abundant, dominant and constantly occurring species. The lowland area was the least diverse with *Cx. quinquefasciatus* as the most dominant species. Cluster analysis shows the similarity of community structure existing in the upland and midland areas of Cavite based on abundance of collected mosquito larva. These findings imply for an immediate and proper vector control programs for the medically important species to reduce public health risks.

Keywords: mosquito larva, diversity, arboviral diseases, public health in Cavite

INTRODUCTION

Valuable information on the occurrence, diversity and distribution of insects especially those with medical importance are significantly provided by entomological surveys. Mosquitoes are found in the different regions of the world, and even beyond the Arctic Circle where they exploit almost all types of aquatic habitat for possible oviposition and establishment of their population (Manguin & Boete, 2011). They are considered as the most important groups of insects due to their reliance on vertebrate blood for egg production thereby becoming parasitic and viral disease vectors (Santiago & Claveria, 2008; Almarinez & Claveria, 2015). The vectored diseases lead to epidemiological and public health concerns as they cause human morbidities and mortalities (Bernués - Bañares & Jiménez - Peydró, 2013; Sia Su et al., 2016). Vector borne diseases such as malaria, filariasis, Japanese encephalitis, dengue and chikungunya fever are the present public health burden of most countries like the Philippines which may even be classified as the highest contributors to disease burden in the future (Anandh & Sevarkodiyone, 2017).

In the Philippines, about 309 taxa of mosquitoes with 40 percent endemism is recorded where some species are known for their medical and veterinary importance (Cagampang - Ramos et al., 1985; Tsukamoto et al., 1985). Among the mosquito-vectored diseases, dengue was considered to be the most common arboviral disease showing an overall increase from year 2000-2011 (Bravo et al., 2014). Onuh et al. (2016) revealed that the average annual cost for a single case of dengue was PhP32,324.00, making it a major infectious disease comparable to tuberculosis suggesting its high disease burden and economic cost.

The recorded increase in dengue cases is attributed to vector biology and some anthropogenic factors. Mosquito species exhibit complex habit in feeding, oviposition, dispersal, high rate of fecundity and the capacity to exploit suitable environment (Anandh & Sevarkodiyone, 2017). These compliment with rapid urbanization and industrialization, increasing human population leading to decreased sanitation and limited vector control strategies (Collins & Blackwell, 2000; Suhasini & Sammaiah, 2014; Bravo et al., 2014). The ecological dynamics of changing rural to urban environment creates more mosquito larval habitats which support adult dispersal, diversity and species dominance most especially in transition zones between urban and rural communities (Juliano et al., 2010; Piovezan et al., 2017).

With the increasing threat posed by mosquito-vectored diseases, mosquito survey is of prime importance in setting the baseline information for mosquito related studies. It will widen the knowledge on existing mosquito species and their habitat thereby contributing in the development of an efficient vector control strategies for the spread of these arboviral diseases (Huang et al., 2017). Further understanding of mosquito diversity and their distribution in the province allows for the prediction of possible pathogens that may be transmitted by mosquitoes to their inhabitants.

This study generally aimed to determine the larval mosquito diversity and its implication to public health in the province of Cavite. Specifically, it aimed to identify the different mosquito species existing in the study sites using their larval forms; determine the possible mosquito-borne diseases in the province; and assess the similarity between and among the areas of study based on larval abundance.

METHODOLOGY

Study Area

The study was conducted across altitudinal zones covering the different cities and municipalities of the province of Cavite (Figure 1). The province has a total land area of 142,706 hectares bounded by the provinces of Rizal, Batangas and Laguna. Just like its neighboring provinces, Cavite has been described to have four physiographic areas characterized as lowest lowland area, low area, central hilly area and upland mountain area with a Type I climate season. Lowest lowland areas are located below the sea level and are prone to flooding. The central hilly or midland areas are generally flat with constant elevation and serve as transition zones from the mountainous and sloping upland areas. With a Type 1 climate system, dry season occurs from November to April while the wet season is observed in the remaining months of the year. The mean annual temperature ranges from 26.2 to 28.4ºC (PEMSEA & Provincial Gov't of Cavite, 2017).

Mosquito

Sampling and Collection

Simple random sampling on the possible and suitable biotopes of mosquito larvae was used in selected areas of the province from April 2015 to May 2016. Different methods of mosquito larval collection were applied depending on the specific habitat type. Dippers and larval nets were used to collect immature mosquitoes in open and wide sources while large-mouth pipettes were employed for tree holes and other artificial containers (Bernués - Bañares & Jiménez - Peydró, 2013; WHO, 2013 & 2016).

Collected mosquito larvae were placed in properly labeled containers and brought to the Department of Biological Sciences, Cavite State University for proper preservation and identification.

Identification of Mosquito Larvae

Collected mosquito larvae were properly prepared and preserved prior to microscopic examination following the procedure of Gaffigan and Pecor (1997). Species identification was based on established morphological characteristics from larval identification keys of Cutwa and Meara (2008), Littig and Stojanovish (2005), Rueda (2004), Reuben et al., (1994), Rattanarithikul (1982), Steffan and Evenhuis (1981), Christopher (1960) and online identification guide by the Water Reed Biosystematics Unit of the Smithsonian Institution.

Diversity and Analysis

Larval survey results were used to analyze species diversity indices using biodiversity estimators such as Margalef, Simpson, Shannon and Equitability. Ecological distance between and among the areas of study were determined using cluster and principal component analysis to validate the reliability of the systems of classification used. The Paleontological Statistics Software (PAST), a free software was used to perform all the calculations.

The occurrence pattern modified from Anandh and Sevarkodiyone (2017) of the mosquito distribution in the upland, midland and lowland areas was classified into five categories using the formula and the following conditions.

Formula: C = nN x 100

where, C = pattern of occurrence;
n = number of municipality/city positive for mosquito occurrence; and
N = total number of municipality/city in an area.

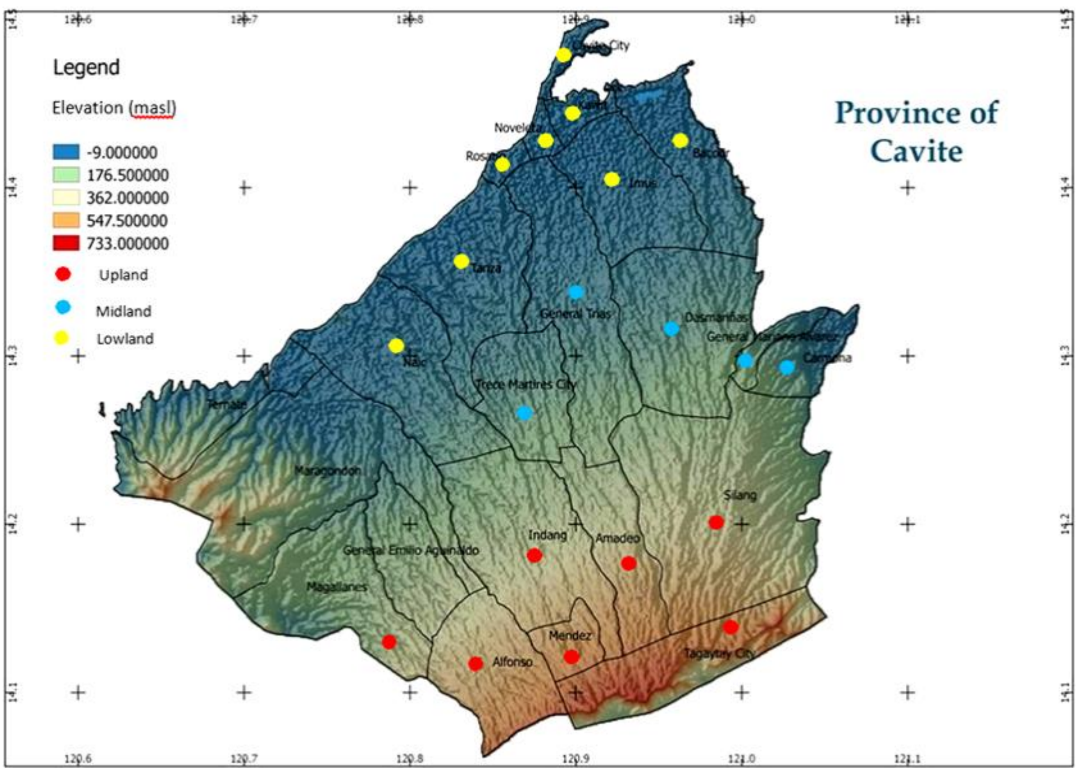

Figure 1. Map of Cavite province showing the upland, midland and low land areas

Interpretation: If the C value = 0.00 - 20%, sporadic; 20.1 - 40%, frequent; 40.1 - 60%, moderate; 60.1 - 80%, infrequent; 80.1 - 100%, constant.

RESULTS AND DISCUSSION

A total of 11,239 mosquito larvae were collected from 503 sampling sites across the 13 municipalities and seven cities surveyed for larval biotopes. Among the areas studied, the upland area had the highest number of collected mosquito larvae followed by the midland and lowland areas, respectively (Table 1).

Larval mosquito identification revealed that a total of 10 species belonging to four genera were recorded (Figures 2 and 3). Among the identified species, *Ae. aegypti* showed the highest percentage across the three areas of study. It was followed by *Ae. albopictus*, *Cx. quinquefasciatus* and *Tx. splendens* in upland Cavite and *Anopheles* sp. replaces *Tx. splendens* in midland areas, while *Cx. quinquefasciatus* had the second highest frequency in the lowland areas of Cavite (Table 2).

These data further suggest that five mosquito species were known to be of medical importance by acting as vectors for specific diseases. *Ae. aegypti*, a cosmo - tropical mosquito species is classified as the primary

Table 1. Mosquito larvae collected from different upland, midland and lowland municipalities and cities of Cavite from April 2015 to May 2016

MUNICIPALITY/CITY	NO. OF LARVAE COLLECTED	PERCENTAGE
UPLAND	**5,553**	**49.41**
Alfonso	982	8.74
Amadeo	1,097	9.76
Indang	586	5.21
Magallanes	97	0.86
Mendez	1,478	13.15
Silang	997	8.87
Tagaytay City	316	2.81
MIDLAND	**3,907**	**34.76**
Carmona	121	1.08
Dasmariñas City	1,365	12.15
Gen. Trias City	985	8.76
Gen. Mariano Alvarez	106	0.94
Trece Martires City	1,330	11.83
LOWLAND	**1,779**	**15.83**
Bacoor City	585	5.21
Cavite City	146	1.30
Imus City	107	0.95
Kawit	345	3.07
Naic	43	0.38
Noveleta	201	1.79
Rosario	198	1.76
Tanza	154	1.37
TOTAL	**11,239**	**100**

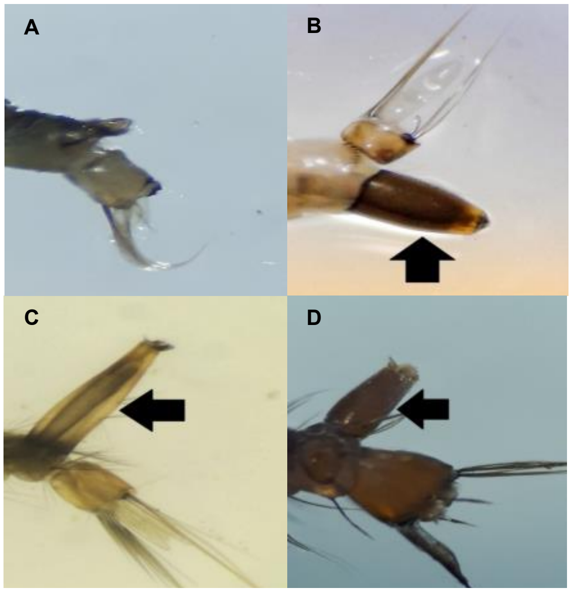

Figure 2. Anal segments of mosquito showing the distinct morphological characteristics (black arrow) and magnification at genus level: A. *Anopheles* with no siphon (32.5 X), B. *Aedes* with a siphon that is twice as long as the basal width (36.6 X), C. *Culex* with a siphon that is 5 times as long as the basal width (28.33 X), and D. *Toxorhynchites* possess a short siphon without a pointed tip (34 X)

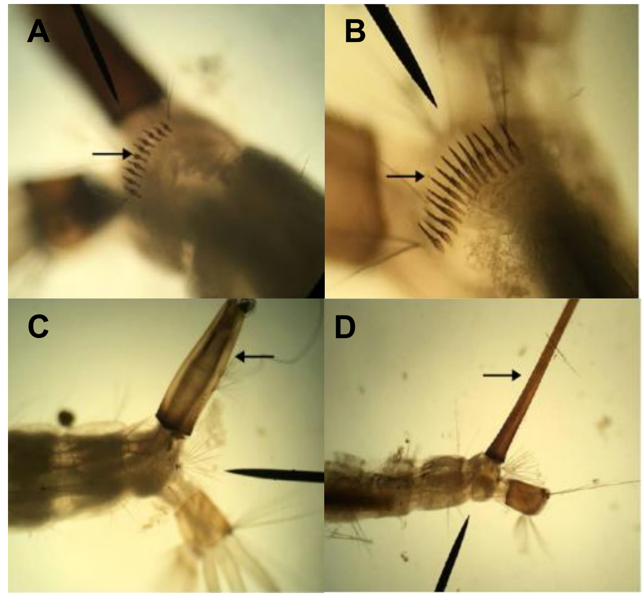

Figure 3. Anal segments of mosquito larvae showing their distinct morphological characteristics (black arrow) and magnification at the species level. A. *Aedes aegypti* with pitchfork - shaped comb scale (28.4 X), B. *Aedes albopictus* with thorn - like comb scale (29.4 X), C. *Culex quinquefasciatus* with air tube with 3 - 5 times as long as wide (27.5 X), D. *Culex tritaeniorhynchus* with weak siphonal tufts with 2 - 5 branches (26.6 X)

Table 2. Frequency of mosquito larvae collected and identified at the species level from the upland, midland and lowland areas of Cavite

MOSQUITO SPECIES	UPLAND		MIDLAND		LOWLAND	
	F	%	F	%	F	%
Aedes sp. 1	205	3.69	12	0.31	0	0.00
Aedes sp. 2	160	2.88	0	0.00	5	0.28
Ae. aegyti	2,365	42.59	1,974	50.52	728	40.92
Ae. albopictus	1,342	24.17	1,197	30.64	67	3.77
Anopheles sp.	0	0.00	119	3.05	0	0.00
Culex sp. 1	36	0.65	113	2.89	0	0.00
Cx. mimeticus	12	0.22	0	0.00	7	0.39
Cx. quinquefasciatus	872	15.70	424	10.85	967	54.36
Cx. tritaeniorhynchus	211	3.80	0	0.00	5	0.28
Tx. splendens	350	6.30	68	1.74	0	0.00
TOTAL	**5,553**	**100.00**	**3,907**	**100.00**	**1,779**	**100.00**

F – Frequency ; % - Percentage

vector of viruses that belongs to family Flaviviridae causing dengue, zika and yellow fever. Another virus that is primarily transmitted by *Ae. aegypti* is the Chikungunya virus which belongs to family Tongaviridae (CDC, 2012, 2014; Liu et al., 2017; Vezzani & Carbajo, 2008; WHO, 2016). At the same time, *Ae. aegypti* serves as a secondary vector for filarial worms affecting humans. It vectors *Dirofilaria* sp. in domestic animals like dogs and other carnivores (Christopher, 1960). Dirofilariasis is not only restricted to domestic and farm animals but studies and increasing cases suggest that it is an emerging zoonotic infection in the world although no known report of human dirofilariasis has been reported in the Philippines (Reddy, 2013; Simón et al., 2012). For the second most abundant species in the upland and midland areas of Cavite, *Ae. albopictus*, also serves as the secondary vector for dengue, chikungunya, dirofilariasis and possibly zika (ECDC, 2017; Liu et al., 2017; WHO, 2016).

The presence of *Cx. quinquefasciatus* across the study areas is suggestive of its possible threat to public health by acting as a vector of *Wuchereria brancofti* in Asia and other tropical countries (Almarinez & Claveriaa, 2015; Amarasinghe & Weerakkodi, 2014; Santiago & Claveria, 2012). However, Kron et al. (2000) revealed its poor vector competence in transmitting the filarial worm in the Philippines. This condition is not unique, considering that regional differences on its competence in transmitting pathogens like that of West Nile Virus has also been noted (Sardelis et al., 2001). Another disease that affects human population, most especially Filipino children below 15 years of age, is Japanese encephalitis (JE) which is primarily vectored by *Cx. tritaeniorhynchus*. Out of 497 cases tested from 2011 to 2014, 15% were laboratory confirmed JE cases with about 7% mortality (Lopez et al., 2015). In connection to this, findings suggest the competence of *Cx. quinquefasciatus* in acquiring and transmitting the JE virus 14 days after its infection. This acquired competence is a result of gradual displacement of genotype III with clade b thus causing the rapid dispersal pattern of JE virus in several regions of Asia posing additional threat to human health (Huang et al., 2015; Huang et al., 2016; Nitatpattana et al., 2005).

The presence of *Anopheles* sp. in the midland areas may lead to pathogen and disease dispersal considering their zoophilic and anthropophilic nature. *Anopheles* sp. is a known vector of some plasmodium species, and filariasis-causing *Wuchereria brancofti* in Asia (Almarinez & Claveria, 2015; Manguin et al., 2010). This condition poses greater human health risk as human-vector association increases with continuous development and progress in farms within communities (Foley et al., 2003).

This survey presents the first known record of *Cx. mimeticus* and *Tx. splendens* in the province of Cavite where both mosquito species were not medically important. Aside from being non-hematophagous and not medically important, *Tx. splendens* was known as a voracious feeder of other mosquito larvae and invertebrates in their aquatic habitat (Collins & Blackwell, 2000) while little is known about the observed predation of *Cx. mimeticus* against other mosquito larvae.

Observed occurrence pattern of the different mosquito species in the different areas of study showed that *Ae. aegypti* was the most constantly present species among all the survey sites followed by *Ae. albopictus* and *Cx. quinquefasciatus*. *Tx. splendens* moderately inhabited the upland areas with sporadic population in the midland communities. *Cx. tritaeniorhynchus* population was moderately inhabiting the upland communities while *Anopheles* sp. moderately inhabited the midland areas of the province (Table 3). Consequently, diversity analysis suggests that the upland communities are the most diverse areas while the lowland communities harbor the lowest mosquito diversity but with the highest dominance value (Table 4).

Altitude, environmental conditions, land use, available larval habitat and vertebrate blood source were contributory factors for the abundance, diversity and species richness of mosquitoes in the different areas (Manikandan &

Table 3. Occurrence and patterns of distribution of larval mosquito species in the upland, midland and lowland areas of Cavite

MOSQUITO SPECIES	UPLAND		MIDLAND		LOWLAND	
	% Occurrence	Pattern of Distribution	% Occurrence	Pattern of Distribution	% Occurrence	Pattern of Distribution
Aedes sp. 1	57.14	Moderate	20.00	Sporadic	0.00	Sporadic
Aedes sp. 2	57.14	Moderate	0.00	Sporadic	12.50	Sporadic
Ae. aegypti	100.00	Constant	100.00	Constant	100.00	Constant
Ae. albopictus	100.00	Constant	80.00	Frequent	50.00	Moderate
Anopheles sp.	0.00	Sporadic	60.00	Moderate	0.00	Sporadic
Culex sp. 1	14.29	Sporadic	40.00	Infrequent	0.00	Sporadic
Cx. mimeticus	14.29	Sporadic	0.00	Sporadic	25.00	Infrequent
Cx. quinquefasciatus	100.00	Constant	100.00	Constant	75.00	Frequent
Cx. tritaeniorhynchus	42.86	Moderate	0.00	Sporadic	12.50	Sporadic
Tx. splendens	85.71	Constant	20.00	Sporadic	0.00	Sporadic

Sevarkodiyone, 2014; Medeiros - Sousa et al., 2015). Similar findings in the upland areas were recorded by Manikandan and Sevarkodiyone (2014) where, high species richness was not only associated with the altitudinal modification on the environmental requirement of adult mosquitoes like humidity and temperature but the upland areas also support the establishment of a variety of larval habitats ranging from natural to man - made and artificial breeding sites. In the present study, the midland areas which act as the transition zone maintain natural breeding sites for some specific species which is minimal or absent in the lowland areas. This local habitat difference accounts for the richness and diversity of mosquito species (Tubaki et al., 2004).

Aside from the anthropophilic nature and artificial breeding site preference of *Ae. aegypti*, its constant presence in the entire province of Cavite reflects its plasticity in colonizing different habitats and in utilizing natural breeding sites for oviposition (Piovezan et al., 2017; Lounibos et al., 2010). Kaplan et al. (2010) further strengthened that the movement and exchange of common various containers most especially domestic trash like cups, cans, bottles and other plastic containers in developing areas provide new breeding habitat and new opportunities for *Ae. aegypti* dispersal. On the contrary, *Ae. albopictus*, was expected to be the most dominant species in the upland communities which project a rural setting and high vegetation cover with available artificial and natural breeding sites (ECDC, 2017; Lounibos et al.,

Table 4. Diversity indices of mosquito population in the upland, midland and lowland areas of Cavite

BIODIVERSITY ESTIMATOR	STUDY AREA		
	UPLAND	MIDLAND	LOWLAND
No. of Individuals	5,553	3,907	1,779
No. of Species	9	7	6
Dominance	0.2721	0.363	0.4644
Margalef index	0.9278	0.7255	0.6681
Simpsons index	0.7279	0.637	0.5356
Shannon index	1.566	1.245	0.8753
Equitability	0.7127	0.64	0.4885

2010). However, the said results may have been influenced by the availability of the breeding sites in community patches as surveys within forest and agricultural patches were limited.

The observed dominance of *Cx. quinquefasciatus* in the highly urbanized communities of lowland Cavite was not only dependent on high densities of human population but also on the availability of polluted water in canals and stagnant creeks (Medeiros - Sousa et al., 2015), while the existence of *Cx. tritaeniorhynchus* in the upland areas shows its association in ground waters found in canals, ditches and manmade ponds (Bashar et al., 2016; Amarasinghe & Weerakkodi, 2014). Additionally, *Tx. splendens* existence in the upland and midland areas does not only indicate the presence of suitable larval habitats that contain the prey mosquito larvae but also suggestive of the available vegetation and environment where plant-derived sugar sources and flower nectars are sufficient to support adult survival (Collins & Blackwell, 2000).

The low abundance of the other *Aedes*, *Culex* and *Anopheles* spp. may possibly indicate that these mosquito species are restricted only to specific sites and require specific conditions for survival, distribution and dispersal. However, low frequency of *Tx. splendens* in larval pools may be attributed to the cannibalistic behavior of this species most especially when prey density is low (Collins & Blackwell, 2000). Cohabitation and coexistence of mosquito species in the environment and in alternative breeding sites were noted. It is indicative of the various ecological relationships and the opportunistic adaptation response to the changing environment brought about by human activities (Medeiros - Sousa et al., 2015).

Cluster analysis using the Bray-Curtis coefficient represents the similarity of community structure existing in the upland and midland areas of Cavite based on abundance of collected mosquito larva (Figure 4). Based from the cophenetic correlation of 0.997, high level of correlation exists between the upland and midland areas as it supports specific species like *Anopheles* sp. and *Tx. splendens*.

These findings strengthen the need to review the existing vector control measures being implemented in the province of Cavite to reduce the threat and impact of possible epidemiological risks brought about by mosquito-vectored diseases. The changing environment caused by human activities reduces mosquito diversity which increases the chance of pathogen transmission. Thus, the necessity of maintaining vegetated area in the community which may support the survival and distribution of predatory species like *Tx. splendens* should be considered. Recrafting of the existing vector control measures in each community should also be done in relation to the specific species inhabiting their areas. However, these data are still limited, thus, a thorough ecological study on mosquito fauna and arboviral disease monitoring should strictly be done.

CONCLUSION

In this study, five out of the 10 mosquito species collected and identified in the province of Cavite are known for their medical and veterinary importance making it possible for enzootic cycles to occur between the vector and other vertebrate hosts including humans. This allows the inhabitants to be at high risk to pathogen infection and disease transmission. The first known record of the predatory species like *Cx. mimeticus* and *Tx. splendens* exist in specific areas of the province which may possibly support their larval and adult survival. Vector control program based on mosquito species in the different areas should not only consider mosquito vectors but beneficial species such as *Tx. splendens* as well.

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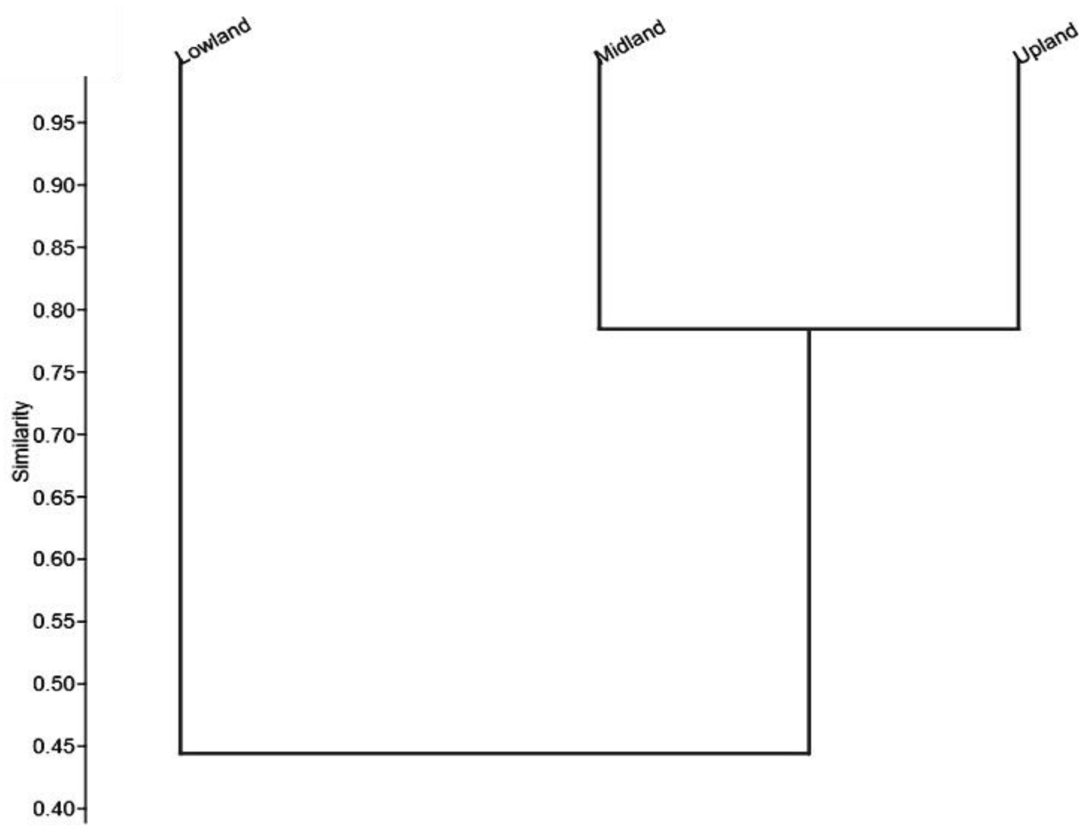

Figure 4. Cluster analysis of the study areas using the recorded mosquito larvae, based on the Bray - Curtis Coefficient with cophenetic correlation = 0.997

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