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RESEARCH NOTE

Larval Mosquito Fauna (Diptera: Culicidae) of Salikneta Farm, San Jose Del Monte, Bulacan, Philippines

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A survey of the mosquito fauna occurring in Salikneta Farm, San Jose Del Monte, Bulacan, Philippines was conducted with the primary aim of providing baseline data that may help in coming up with strategies for short-term and long-term vector control. Six species were identified by examination of larval morphology and chaetotaxy, and are reported herein. 340 (62.27%) *Culex quinquefasciatus*, 50 (9.16%) *Cx. mimeticus*, 28 (5.13%) *Cx. vishnui*, 8 (1.47%) *Cx. tritaeniorhynchus*, 111 (20.33%) *Aedes aegypti*, and 9 (1.65%) *Anopheles tessellatus* comprised the 546 third and fourth instar mosquito larvae collected from improvised ovitraps placed in five selected sites in the farm. With the exception of *Cx. mimeticus*, the species identified in the farm are recognized as medically important taxa with the potential to transmit agents of arboviral and/or parasitic diseases. These findings imply the importance of proper and sustainable vector control measures in Salikneta Farm where human activities and habitation have been gradually increasing as a consequence of ongoing development.

Key Words: *Aedes*, *Anopheles*, *Culex*, identification key, larval morphology and chaetotaxy, vector surveillance

INTRODUCTION

Mosquitoes (Order *Diptera*: Family *Culicidae*), classified into two subfamilies *Anophelinae* and *Culicinae*, are cosmopolitan insects. A number of members of this very diverse family are considered medically important as vectors of viruses and parasites associated with diseases that have been emerging as a threat in relation to global warming and environmental change (Harbach 2011). Among the diseases in which mosquitoes are implemented as vectors are dengue and yellow fever, Japanese encephalitis, malaria, and filariasis. Asia has been considered, by far, the most important region in terms of global number of active filarial infections, with about 59% of worldwide filariasis cases distributed over 15 countries, including the Philippines (Manguin et al. 2010). In the Philippines, 279 species, subspecies and varieties of mosquitoes, some of which are not only of medical but also of veterinary importance, have been reported (Cagampang-Ramos et al. 1985).

Rapid growth of human populations has been implicated in the increased presence of culicid vectors (Ravel et al. 2001). In light of the emerging threat posed by mosquito-borne diseases, there is an ever growing need for vector control through mosquito surveillance and vector ecology research. These tools are important in planning control strategies that can

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help significantly reduce mortalities and morbidities (Santiago and Claveria 2012), especially in cases such as that of dengue where prevention is entirely dependent on vector control due to the current lack of specific treatment and inavailability of vaccines (Ravel et al. 2001). In Southeast Asia, the bionomics, ecology and epidemiological significance of many mosquito vectors remain poorly understood (Muturi et al. 2008); hence, the greater need for more vector studies.

Mosquito surveillance requires identification of the culicid species that may serve as potential vectors, together with their breeding grounds, particularly in areas with human settlements. Larval morphology and chaetotaxy can be used in species identification and is an important part of larval surveillance (Harrison and Rattanarithikul 1973). With the aid of identification keys, species identification can be done rapidly through examination of the form of a mosquito larva together with the arrangement of setae on the different regions of its segmented body. Information on the diversity of the culicid fauna in an area can afterwards be used to formulate strategies for vector monitoring and control. The present study represents a first attempt to uncover the diversity of mosquitoes occurring in Salikneta Farm in San Jose Del Monte, Bulacan, Philippines through larvae collected from various sites and provides a preliminary peer at the richness and distribution of potential arboviral and parasitic disease vectors in the area.

MATERIALS AND METHODS

Study site

Salikneta Farm, located in Upper Ciudad Real, San Jose Del Monte, Bulacan at 14°48’14” N, 121°7’59” E (Figure 1), is the laboratory farm of De La Salle Araneta University, with a total land area of 64 hectares. Aside from the poultry, dairy, swine, and vegetation aspects of the farm, there are also available ecotourism facilities as well as an academic and research facility, the De La Salle AgriVet Sciences Institute (DLS-AGSI) that provides high school (Malayao 2011) and tertiary education (BS Agriculture) as of 2012. The farm served as the main study site, with five selected areas as sampling sites (Figure 2). The sampling sites were selected based on the presence of vegetation and shading, occurrence of humans or livestock as potential blood hosts for adult mosquitoes, and proximity to open bodies of fresh and stagnant water.

Collection of mosquito larvae

A modification of the method and improvised ovitrap design described by Deschamps (2005) was employed in the collection of larval mosquitoes. Instead of black plastic cups, black 500-ml round microwavable food containers with 5 inches brim diameter and about 4 inches bottom diameter were used as ovitrap cups. A hole of about 0.25 inch was drilled at about 1 to 1.5 inches below the brim to prevent overflow should rainwater fill the cup. Ovipaddles

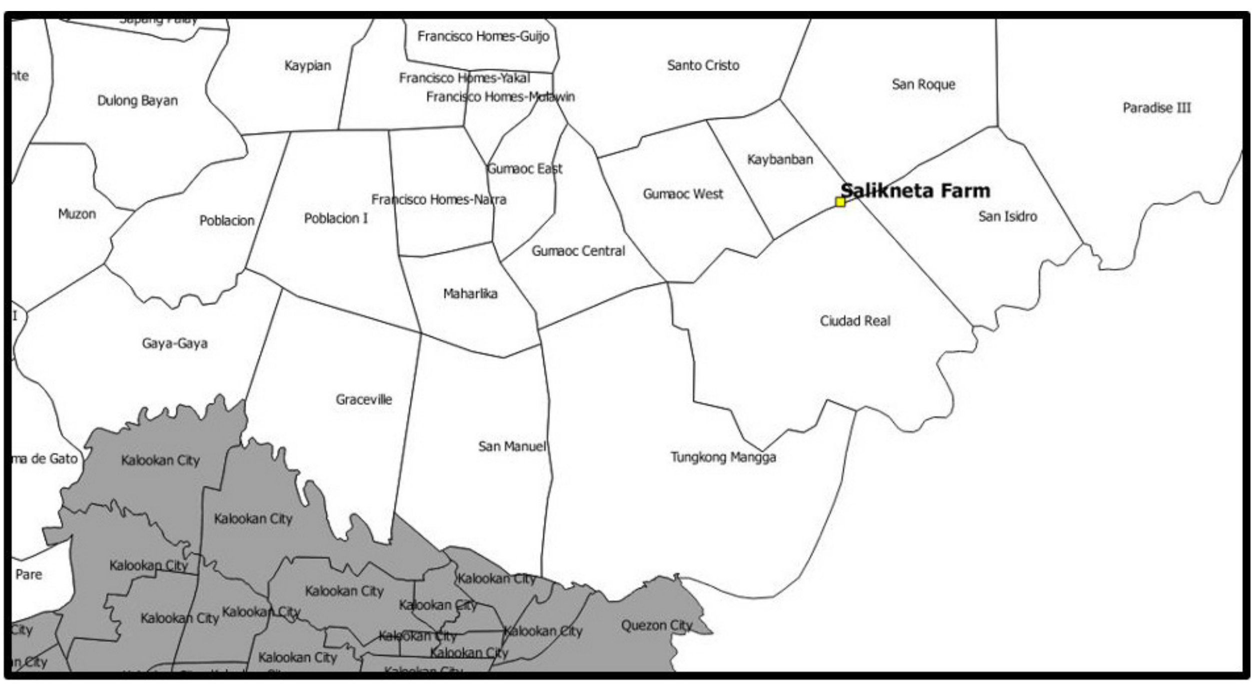

Figure 1. Location of Salikneta Farm (yellow marker) in San Jose Del Monte, Bulacan (white) and relative to Metropolitan Manila (grey) (generated via QGIS Desktop 2.0.1 using vector layers downloaded from the Philippine GIS Data Clearinghouse at http://www.philgis.org)

Figure 2. Selected sites for collection of mosquito larvae: (A) mango tree outside the De La Salle AgriVet Sciences Institute (DLS-AGSI); (B) mushroom culture substrate preparation area outside DLS-AGSI; (C) plantation area; (D) vermiculture facility area; (E) large animal pen area.

to promote oviposition by gravid females were constructed from standard 6-inch tongue depressors with unbleached paper towel wrapped around and secured at the top and bottom with staples. Each of the two cups placed in each of the sampling sites was filled with approximately 200 mL of tap water or water from the lagoon situated in the middle of Salikneta Farm, and was allowed to season in the selected sites for one week without the ovipaddle. The ovipaddles were placed in the cups that remained untouched in the sites after one week, and the cups were left for another week. Larvae present after two weeks were collected from the traps by sieving using a tea strainer. Prior to collection of larvae in the seasoned ovitraps, two samples of larvae had been previously collected on November 24, 2012 directly from troughs with stagnant water located in one of the selected sites (the vermiculture facility). Collection from the seasoned ovitraps was carried out on December 1 and 7. The collected larvae were placed immediately in 10% formalin for subsequent identification and counting. Ovipaddles with eggs were desiccated and discarded appropriately.

Identification and counting of mosquito larvae

Species-level identification of mosquitoes was done through microscopic examination of the morphology and chaetotaxy of the head, thorax, and abdominal segments (particularly the terminal segments) of third or fourth instar larvae. The Lucid3-based online pictorial key of the Walter Reed Biosystematics Unit (2010), together with the identification keys of Bram (1967), Sirivanakarn (1976), and Reuben et al. (1994) for *Culex*, of Gater (1935) and Reid (1968) for *Anopheles*, and of Christophers (1960) for *Aedes* were used. Images of the larvae were taken using the digital micrography function of the Nikon SMZ800 stereozoom microscope. First and second instar larvae were not included in the count.

RESULTS

In this survey, a total of 546 third and fourth instar larvae were collected. Three genera, namely *Culex*, *Aedes*, and *Anopheles*, were identified in the samples. *Culex* represented 78.02% of the larvae, with four species having been identified: 340 (62.27%) *Cx. quinquefasciatus*, 50 (9.16%) *Cx. mimeticus*, 28 (5.13%) *Cx. vishnui*, and 8 (1.47%) *Cx. tritaeniorhynchus* (Figures 3, 4, 5 and 6).

Only one species of *Aedes* was identified, *Aedes aegypti* (Figure 7), representing 20.33% (111) of the collected

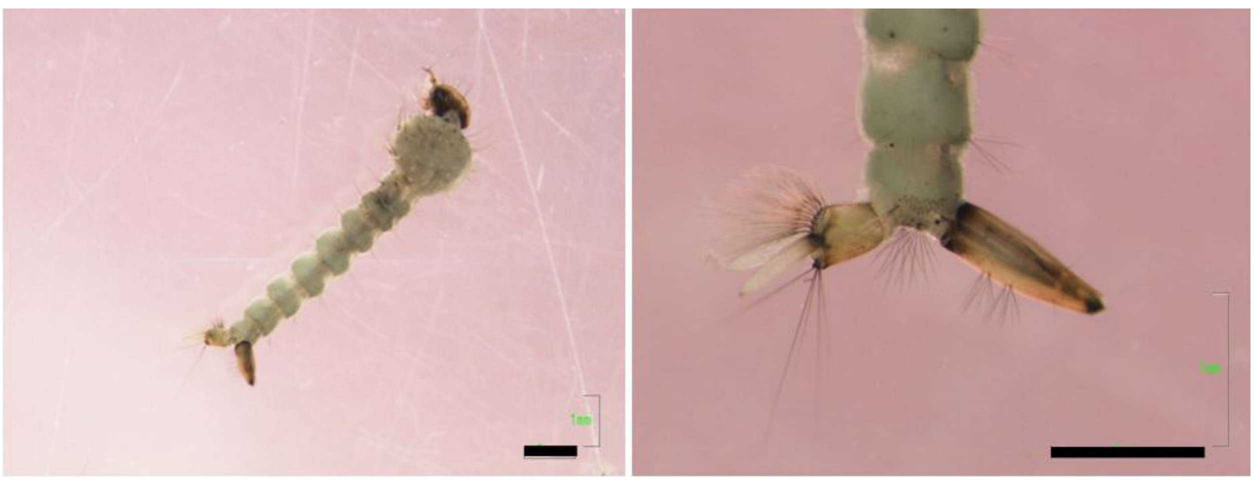

Figure 3. *Culex quinquefasciatus*. Left: Whole larva. Right: 6th, 7th, and 8th abdominal segments, showing chaetotaxy, saddle and siphon morphology. Scale bars = 1.0 mm.

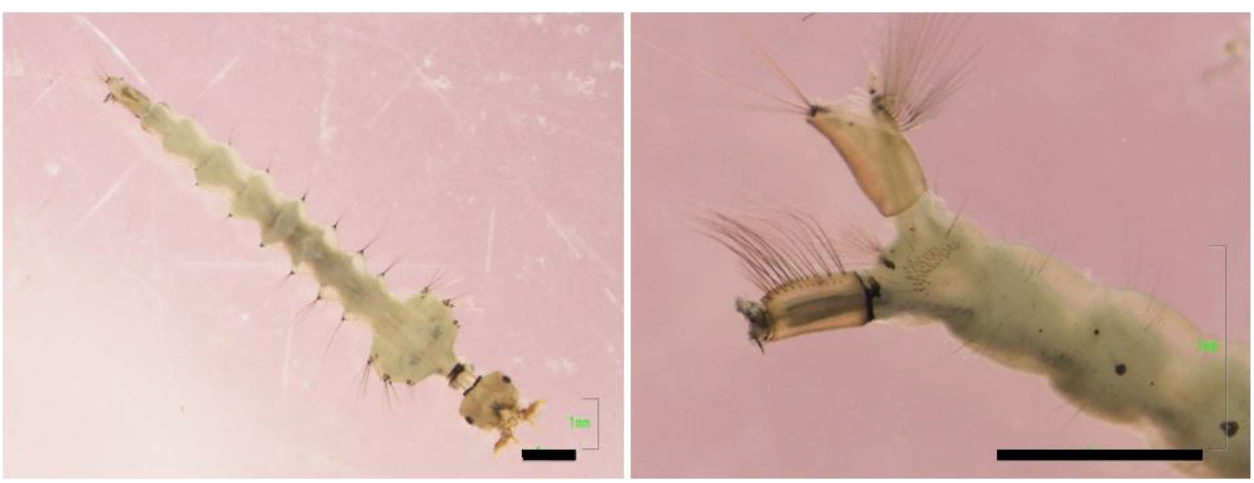

Figure 4. *Culex mimeticus*. Left: Whole larva. Right: 6th, 7th, and 8th abdominal segments, showing chaetotaxy, saddle and siphon morphology. Scale bars = 1.0 mm.

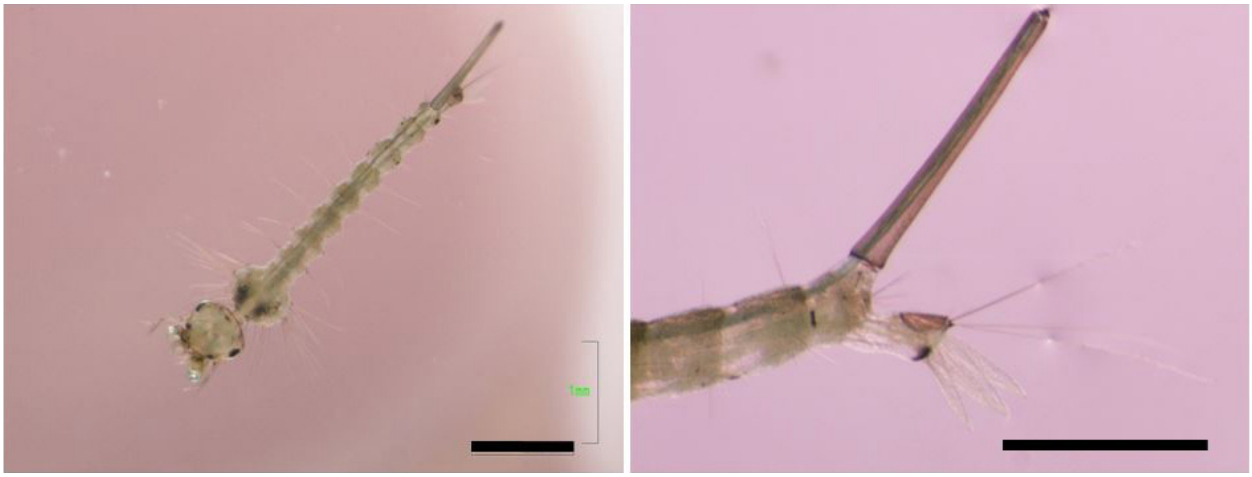

Figure 5. *Culex vishnui*. Left: Whole larva. Right: 6th, 7th, and 8th abdominal segments, showing chaetotaxy, saddle and siphon morphology. Scale bars = 1.0 mm.

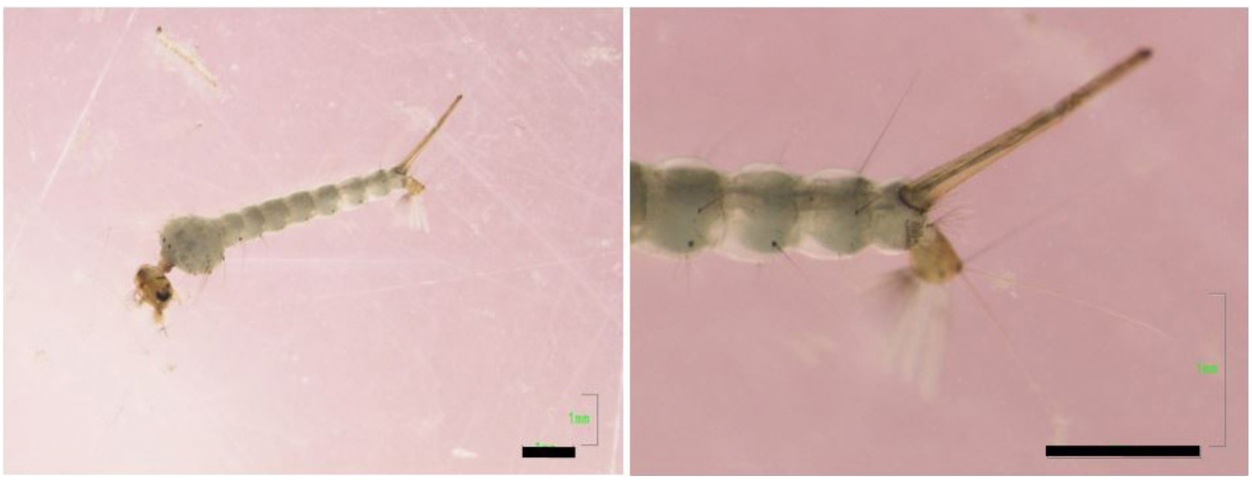

Figure 6. *Culex tritaeniorhynchus*. Left: Whole larva. Right: 5th, 6th, 7th, and 8th abdominal segments, showing chaetotaxy, saddle and siphon morphology. Scale bars = 1.0 mm.

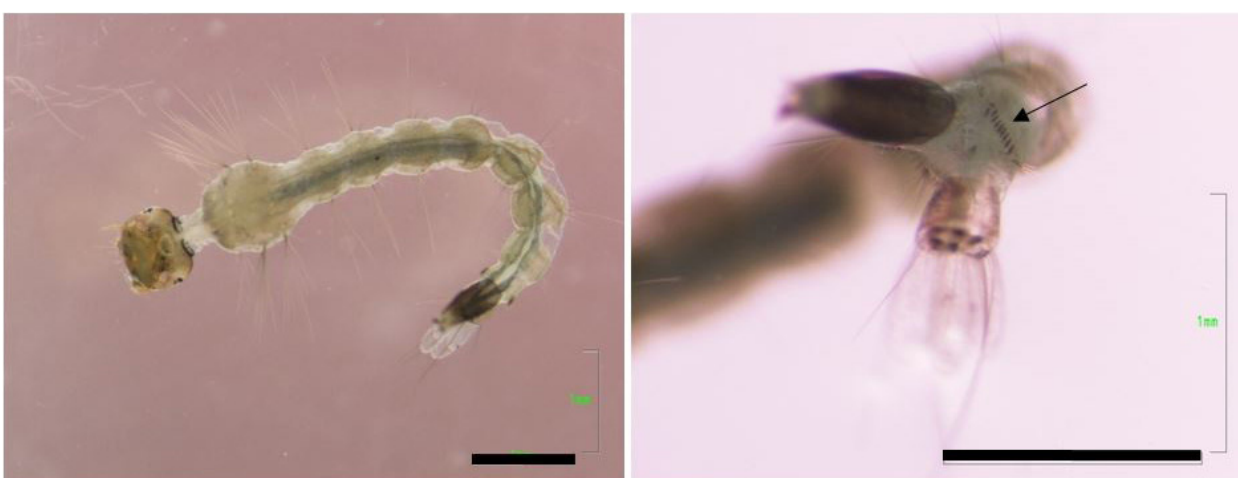

Figure 7. *Aedes aegypti*. Left: Whole larva. Right: 8th abdominal segment, showing single row of denticulate comb scales (arrow), chaetotaxy, saddle and siphon morphology. Scale bars = 1.0 mm.

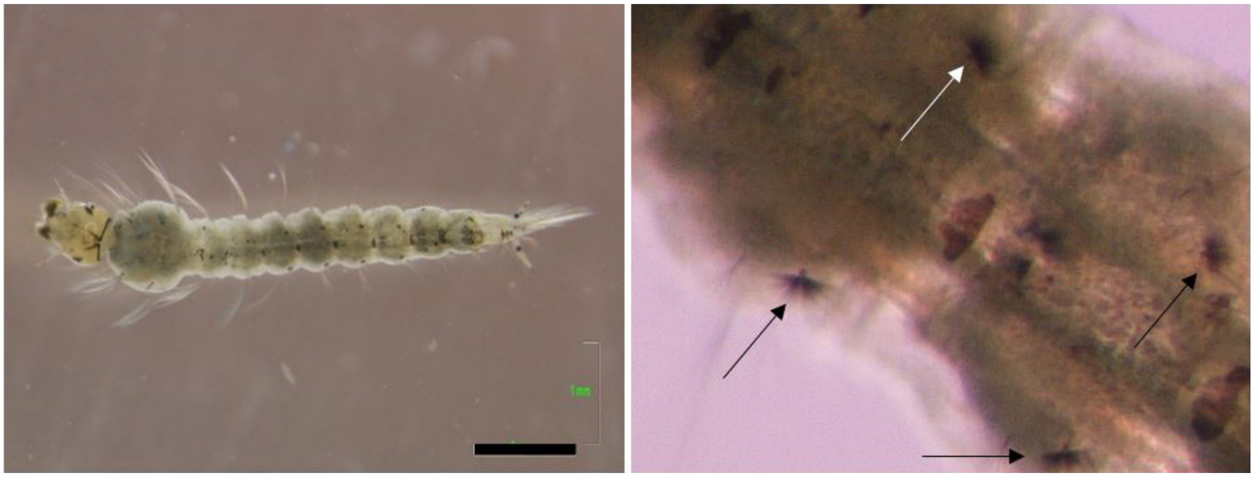

Figure 8. *Anopheles tessellatus*. Left: Whole larva. Right: Close-up of 6th and 7th abdominal segments, showing ventral palmate hairs (arrows). Scale bar = 1.0 mm.

Table 1. Distribution of collected mosquito larvae collected in selected potential breeding sites in Salikneta Farm, San Jose Del Monte, Bulacan, Philippines.

Species	Mango tree outside AGSI	MCSP*	Plantation area	Vermiculture facility	Large animal pen
Aedes aegypti	+	+	+		
Anopheles tessellatus					+
Culex mimeticus				+	
Cx. quinquefasciatus	+			+	
Cx. tritaeniorhynchus				+	
Cx. vishnui					+

*MCSP: Mushroom culture substrate preparation area

larvae. Likewise, only one anopheline species, *Anopheles tessellatus* (Figure 8) was identified and represented the remaining 1.65% (9) of the larvae.

Culex appears to be the dominant genus in this study, with more than three out of four sampled larvae belonging to this group. Results indicate that species-wise, *Cx. quinquefasciatus* is the most dominant species, with six out of ten collected larvae being of this taxon. *Cx. tritaeniorhynchus* appears to be the least dominant. *Ae. aegypti*, as can be seen in Table 1, appears to be the most widely distributed among species, occurring in three out of five sampling areas, namely the mango tree close to the AGSI building, the mushroom culture substrate preparation area (also right outside the AGSI building), and the plantation area. The wide spread of this species appears to be consistent with the fact that the plantation area is of a considerable distance from the AGSI areas. *An. tessellatus*, *Cx. triaeniorhynchus*, and *Cx. vishnui* were each identified in only one out of the five sampling sites, possibly indicating that these species may be limited only to certain areas in the farm.

Based on the morphology and chaetotaxy (particularly of the abdominal segments) of the collected and identified larvae, the following identification key may be used to discriminate among the taxa represented in this study:

1	A.	Siphon at 8th abdominal segment present; no ventral palmate hairs on abdominal segments	2
	B.	Siphon at 8th abdominal segment absent; with weak ventral palmate hairs on 1st and 2nd abdominal segments and strong ventral palmate hairs on 3rd to 7th abdominal segments	*Anopheles tessellatus*

2	A.	Siphon length/width ratio is < 5 (short siphon)	3
	B.	Siphon length/width ratio is >5 (long siphon)	4
3	A.	Siphon with several hair tufts and pecten; comb scales occur in more than 1 row	5
	B.	Siphon with single hair tuft posterior to row of pecten teeth; denticulate comb scales occur in a single row	*Aedes aegypti*
4	A.	Siphon more or less cylindrical, with weak hair tufts	*Culex vishnui*
	B.	Siphon apically tapered, with weak hair tufts	*Cx. tritaeniorhynchus*
5	A.	Siphon apically tapered, with branched siphonal hair tufts arranged randomly	*Cx. quinquefasciatus*
	B.	Siphon more or less cylindrical, with branched subventral siphonal tufts occurring in a row	*Cx. mimeticus*

DISCUSSION

Of the 279 species reported by Cagampang-Ramos et al. (1985), six species were identified in Salikneta Farm in this study. The survey was conducted in a span of less than a month, probably resulting in the identification of relatively few taxa only. However, the findings are worth noting since five out of the six species are of medical or veterinary importance.

Aedes aegypti is well-known as the primary vector of dengue and yellow fever viruses, and of the chikungungya virus. Additionally, it can be a secondary vector of human filarial worms. It is also of veterinary importance as it has been found to be a major vector of *Dirofilaria* in dogs, and has been implicated in transmission of *Pasteurella multocida* which can cause hemorrhagic septicemia in buffaloes, the fowlpox virus, and the enzootic hepatitis virus which can cause abortion in sheep and cattle (Christophers 1960).

In 2011, Bulacan was reported to have the most cases of dengue in Central Luzon (Wallis et al. 2011), with the city of San Jose Del Monte identified as having the highest number of cases among all the province's cities and towns (Provincial Public Awareness Office 2011a). Although a decline in the number of dengue cases in Central Luzon was reported in 2013, Bulacan still remains at the top of the list of provinces with the highest incidence of dengue in the region (Pavia 2013). Two cases of dengue in the farm were reported in the past months, and identification of *A. aegypti* as among the culicid fauna in the area confirms the urgent need for proper vector control and prevention measures not only in the farm but in the greater San Jose Del Monte area.

Anopheles tessellatus has been reported to be a vector (primary or secondary) of *Wuchereria bancrofti*, which causes filariasis, in Asia, including the Philippines. It has also been recognized as a species that can co-transmit *Plasmodium* together with the filarial worm (Manguin et al 2010). *A. tessellatus* is recognized to be rather zoophilic than anthrophophilic, as it has been reported that a single calf can attract about 20 times more than two men, and two goats about twice as many (Reid 1968). This zoophilic nature may account for its occurrence in the vicinity of the large animal pen where the some of the carabaos, sheep, and goats are kept. The very low abundance of larvae in the single site where *A. tessellatus* was identified may possibly be due to the tendency of anopheline species to usually scatter individual eggs while hovering around an area for more preferred oviposition sites, much unlike *Culex* species that lay eggs in rafts.

Despite zoophily, anopheline species like *A. flavirostris* as reported by Foley et al (2003), can still pose a risk to humans. The same may possibly be true for *An. tessellatus*, considering that the increased human activity in the farm as brought about by its continuing development can also lead to greater association with this anopheline vector. In 2011, the Bulacan Provincial Public Health Office reported cases of malaria in six barangays in Norzagaray, which is immediately north of San Jose Del Monte (Provincial Public Awareness Office 2011b). Athough it has been reported that malaria incidence in the province has declined between 2011 and 2012 (Philippine Information Agency 2013), the occurrence of this anopheline species necessitates caution by the people inhabiting the farm. Higher densities of *A. flavirostris* were found near human habitations in rural areas with low malaria endemicity in the Philippines, suggesting association of larvae with human habitation and a reinforced risk of malaria within the area (Foley et al 2003).

Like the previous two species, the cosmopolitan *Culex quinquefasciatus* is a known vector of *W. bancrofti* in the tropical Americas, in tropical Africa, and in Asia (Manguin et al. 2010). Vector competence studies have shown, however, that this species is a poor vector for *W. bancrofti* in endemic areas in the Philippines (Kron et al 2000).

Similar to *An. tessellatus*, *Cx. tritaeniorhynchus* females are primarily zoophilic to cattle and swine, only occasionally feeding on avians and humans. Larvae have been noted to prefer sunlit freshwater bodies with vegetation (Bram 1967). This probably explains its occurrence in the water troughs with soaked plant matter kept in the vermiculture facility, which is not very far from the piggery. Despite its primary zoophilic nature, *Cx. tritaeniorhynchus* has been long regarded as the principal vector of the Japanese encephalitis virus, particularly in the Oriental region (Bram 1967; Reuben et al. 1994).

Cx. vishnui has been occurring for a long time in Tungkong Mangga as reported by Sirivanakarn (1976), a plausible explanation for the occurrence of this species in Salikneta Farm. Females are known to oviposit in ground pool habitats that include (but are not limited to) those with emergent and aquatic vegetation. Larvae are reported to have been found alongside those of *Anopheles* species, as was observed in this study particularly among the samples collected from the large animal pen area. Together with *Cx. tritaeniorhynchus*, *Cx. vishnui* has been implicated as possibly among the important vectors of the Japanese encephalitis virus, particularly in the Southeast Asian region. Ingwavuma and Tembusu viruses have also been reported to be possibly transmitted by this species (Sirivanakarn 1976).

Cx. mimeticus is the only one among the six identified species without medical importance. Identification of this species in plant matter-infused bodies of water in this study is similar to the observed simultaneous occurrence with *Cx. quinquefasciatus* in rice fields in Sukabumi, West Java, Indonesia (Stoops et al. 2008). Simultaneous occurrence of *Cx. mimeticus* with *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* in a rice paddy has likewise been reported in Lian, Batangas (Santiago and Claveria 2012). The larvae of *Cx. mimeticus* collected in this study were found to be very large compared to those of the other five species, consistent with the description by Wise (1912) of *Cx. mimeticus* larvae being notably large and very voracious. Its occurrence and identification in Salikneta Farm confirms the first report of Santiago and Claveria (2012) of the existence of this species in the Philippines, at least in Luzon.

Vector surveillance and control requires sufficient understanding of the habitat preference and oviposition habits of culicids. In South Korea, anopheline species were found to be associated with flooded rice paddies (Sithiprasasna et al. 2005). This was likewise reported by Santiago and Claveria (2012) for the Philippines. Despite the reported association with rice paddies, anopheline species can still breed in other habitats, as observed in Salikneta Farm where rice paddies were not yet existent during the time the survey was conducted.

In a choice test in Malaysia by Chen et al. (2007), storm-drain water has been found to be more attractive than seasoned tap water for oviposition of *A. aegypti*, indicating how clear stagnant water in a concrete drainage system can provide a suitable medium for the colonization of the primary dengue vector. Nevertheless, gravid *A. aegypti* females were found in that study to additionally oviposit on a substrate that is readily available, including seasoned tap water, as was also observed in the current study.

In Guilan Province in Iran, *Culex* larvae have been found to prefer transient, standing water with or without vegetation in a variety of natural and man-made habitats (Azari-Hamidian 2007). While rice paddies may be absent, the other preferred habitats and oviposition substrates reported in previous studies can easily be found in various parts of Salikneta Farm. This fact should be taken into account in planning for sustainable vector control strategies.

One potential vector control strategy could be the use of plants and plant derivatives that have been found to possess properties and biological activities detrimental to culicid vectors. In recent years, the insecticidal property of neem, *Azadirachta indica*, and its derivatives against medically important arthropods and vectors, has been intensely studied. Among the numerous findings on *A. indica* were a putative dose-dependent impairment of blood intake and oviposition, hormonal control of oogenesis, and a potential cytotoxicity against oocytes in *An. stephensi* (Lucantoni et al. 2006). The larvicidal activity of *baleteng-baging*, *Ficus benghalensis* (=*indicus*), against the same culicid species and additionally against *Cx. quienquefasciatus* and *Ae. aegypti* has been reported (Govindarajan 2010). Crude extracts of *Ervatamia coronaria* (=*Tabernaemontana divaricata*) and *Caesalpinia pulcherrima* have been found to possess ovicidal and repellent properties against the three aforementioned culicid species (Govindarajan et al. 2011). These two plants are locally known in the country as *pandakaking-tsina* and *bulaklak ng paraiso*, respectively.

Muturi et al. (2008) noted that the bionomics, ecology, and epidemiological significance of Southeast Asian culicid vectors, particularly *Anopheles*, remain poorly understood. Findings of the current study imply the necessity of further studies on culicid vectors in the country, particularly in remote areas like Salikneta Farm where baseline information is much needed. The results and findings generated by this preliminary work has guided the management of Salikneta Farm in the implementation of sustainable vector control strategies which include (but are not limited to) planting of *A. indica* trees within areas regularly occupied by people, particularly the students of the AgriVet Sciences Institute, De La Salle Araneta University.

CONCLUSION

In this study, six culicid species were identified by examination of larval morphology and chaetotaxy. Among them, five are generally recognized for their medical and veterinary importance: *Aedes aegypti*, the primary vector of viruses that cause dengue hemorrhagic fever and yellow fever in humans, and of the parasitic *Dirofilaria* in dogs; *Anopheles tessellatus*, a potential vector of malaria-causing *Plasmodium* and *Wuchereria bancrofti*; *Culex quinquefasciatus*, a cosmopolitan species that also has the potential to transmit *W. bancrofti*; and *Cx. tritaeniorhynchus* and *Cx. vishnui*, both of which are recognized as important vectors of the Japanese encephalitis virus. The sixth species, *Cx. mimeticus*, a medically unimportant culicid, was also found together with other *Culex* species. In light of these findings, it is recommended that the baseline information provided by this study be used as a starting point for further vector studies that will cover a longer period of time and more sampling sites spanning a greater extent of Salikneta Farm. Finally, it is suggested that appropriate vector control measures be continuously undertaken in the farm to prevent future cases of arboviral and parasitic diseases caused by pathogens transmitted by these culicid vectors.

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