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Larval Mosquito Fauna (Diptera: Culicidae) of Salikneta Farm

Billy Joel M. Almarinez[1,*] and Florencia G. Claveria[1,2]
[1] *Biology Department, College of Science, De La Salle University, Manila*
[2] *De La Salle AgriVet Sciences Institute, Salikneta Farm, SJDM, Bulacan*
**Corresponding Author: billy.almarinez@dlsu.edu.ph*

Abstract: A survey of mosquito fauna occurring in Salikneta Farm, San Jose Del Monte, Bulacan 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. Mosquito larvae were collected using improvised ovitraps placed in five selected sites in the farm during three periods covering November and December, 2012. With the aid of published and online identification keys, six species were found by examination of morphology and chaetotaxy of 546 third and fourth instar larvae that were collected and preserved in 10% formalin. 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 collection. With the exception of *Cx. mimeticus*, the species identified in the farm are recognized as medically important taxa with the potential to transmit arboviral and/or parasitic diseases. These findings imply the immediate need for proper 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*; larval morphology and chaetotaxy; vector surveillance

1. 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 viral and parasitic 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 percent of worldwide filariasis cases distributed over 15 countries, which includes 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 is the ever growing need for vector control through mosquito surveillance and vector ecology research. These tools are considered significant in reducing mortalities and morbidities (Santiago & 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 culicid fauna 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 & 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 culicid fauna in an area can afterwards be used to formulate strategies for vector monitoring and control. The present study represents a first attempt at uncovering 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.

2. METHODOLOGY

2.1 Study site

Salikneta Farm, located in Upper Ciudad Real, San Jose Del Monte, Bulacan, 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: (A) the mango tree outside the DLS-AGSI; (B) the mushroom culture substrate preparation area outside DLS-AGSI; (C) plantation area; (D) vermiculture facility area; and (E) large animal pen area. The sampling sites were selected based on presence of vegetation and shading, occurrence of humans or livestock as potential hosts for adults, and proximity to open bodies of fresh or stagnant water.

2.2 Collection of mosquito larvae

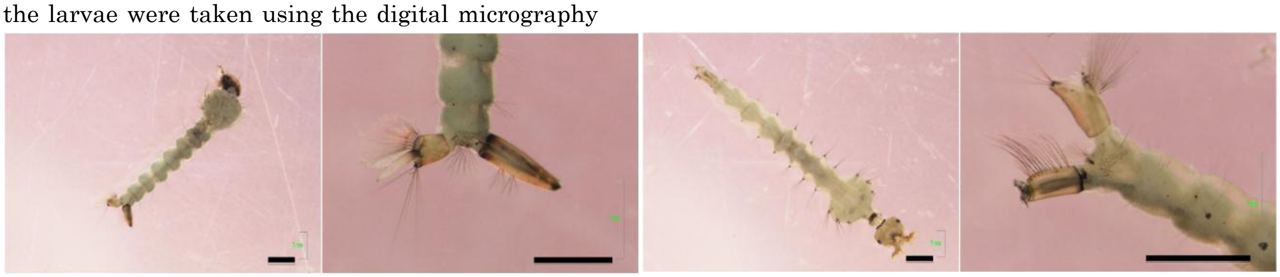
A modification of the method and improvised ovitrap design described by Deschamps (2005) was employed in the collection of larval mosquitoes. Instead of black plastic cups, black 500-ml round microwavable food containers with 5 inches brim diameter and about 4 inches bottom diameter were used as ovitrap 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. Cups were filled with approximately 200 ml of tap water or water from the lagoon situated in the middle of Salikneta Farm, and were allowed to season in the selected sites for 1 week without the ovipaddles. The ovipaddles were placed in the cups that remained untouched in the sites after 1 week, and were left for another week. Larvae present after 2 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 were previously collected on November 24, 2012 directly from troughs with stagnant water located in one of the selected sites. 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.

2.3 Identification and counting of mosquito larvae

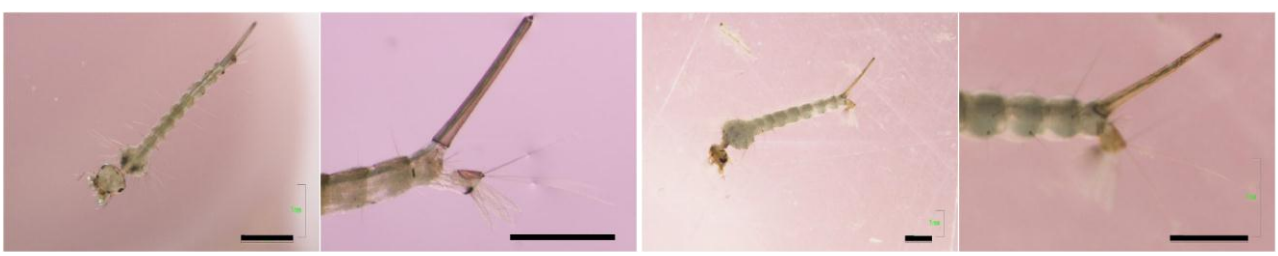
Species-level identification of mosquitoes was done through microscopic examination of the morphology and chaetotaxy of the head, thorax, and abdominal segments (particularly the terminal segments) of third or fourth instar larvae. The Lucid3-based online pictorial key of the Walter Reed Biosystematics 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.

3. RESULTS AND DISCUSSION

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 (Fig. 1). *Culex* represented 78.02% of



A. *Culex quinquefasciatus* B. *Cx. mimeticus*



C. *Cx. vishnui* D. *Cx. tritaeniorhynchus*

E. *Aedes aegypti* F. *Anopheles tessellates*

Figure 1. Larval mosquito fauna identified in Salikneta Farm. Left images: Whole larvae. Right images: Terminal abdominal segments showing chaetotaxy, saddle and siphon morphology. Arrows point to additional diagnostic features, such as the single row of denticulate comb scales of *Aedes aegypti* and the ventral palmate hairs of *Anopheles tessellates*. Scale bars = 1.0 mm.

Table 1. Distribution of collected mosquito larvae collected in selected potential breeding sites in Salikneta Farm

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

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* (Fig. 1A–D).

Only one species of *Aedes* was identified, *Aedes aegypti* (Fig. 1E), representing 20.33% (111) of the collected larvae. Likewise, only one anopheline species, *Anopheles tessellatus* (Fig. 3) was identified and represented the remaining 1.65% (9) of the larvae.

Of the 279 species of mosquitoes in the Philippines 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. However, the findings are worth noting since five out of the six species are of medical or veterinary importance.

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. This species' wide spread 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.

Ae. aegypti is well-known as the primary vector of dengue and yellow fever, and of the chikungungya virus. Additionally, it can also be a secondary vector of human filariasis. 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 hemorrhagic septicemia of buffaloes, fowl-pox, and enzootic hepatitis which can cause abortion in sheep and cattle (Christophers, 1960).

An. tessellatus has been reported to be a vector (primary or secondary) of *Wuchereria bancrofti* 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). *An. tessellatus* is recognized to be more 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 *An. 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 this, zoophilic anopheline species, like *An. flavirostris* as reported by Foley *et al.* (2003), can still pose a risk to humans. The same may possibly be true as well 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. Higher density of early and late *An. flavirostris* was found near human habitation in rural areas with low malaria endemicity in the Philippines, suggesting association of larvae with human habitation and a reinforced risk of of malaria in people situated within the area (Foley *et al.*, 2003).

Like the previous two species, the cosmopolitan *Cx. quinquefasciatus* is a known vector of Bancroftian filariasis in 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 Japanese B encephalitis, particularly in the Oriental region (Bram, 1967; Reuben *et al.*, 1994).

Cx. vishnui has been long in Tungkong Mangga as reported by Sirivanakarn (1976), a plausible explanation for this species' occurrence 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 Japanese encephalitis, particularly in the Southeast Asian region. Ingwavuma and Tembusu viruses have also been reported to be possibly be carried by this species (Sirivanakarn, 1976).

Cx. mimeticus is the only one among the six identified species as non-medically important. Identification of this species in plant matter-infused bodies of water in this study are similar to the observed simultaneous occurrence with *Cx. quinquefasciatus* in rice fields in Sukabumi, West Java, Indonesia (Stoops *et al.*, 2008) and with *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* in a rice paddy in Lian, Batangas (Santiago & 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 validates the first report of Santiago and Claveria (2012) of the existence of this species in the Philippines, at least in Luzon.

Muturi *et al.* (2008) noted how in Southeast Asia the bionomics, ecology, and epidemiological significance of culicid vectors, particularly *Anopheles*, remain poorly understood. Findings of this study imply the necessity of further studies on culicid vectors in the country, particularly in Salikneta Farm where baseline information is much needed. At least two cases of dengue fever in the farm were reported in the past months, and identification of *Ae. aegypti* as among the culicid fauna in the area confirms the need for proper vector control and prevention measures.

Vector surveillance and control requires sufficient understanding of the habitat preference and oviposition habits of culicid fauna. 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). In a choice test in Malaysia, storm-drain water has been found to be more attractive than seasoned tap water for oviposition of *Ae. 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 *Ae. aegypti* females were found to oviposit on a substrate that is readily available, including seasoned tap water (Chen *et al.*, 2007). 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 found in recent researches as aforementioned can easily be found in various areas of Salikneta Farm. This fact should be put into much consideration in coming up with vector control strategies.

One potential vector control strategy for the farm 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 arthropod parasites and vectors has been intensely studied. Among the numerous findings on *A. indica* are 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. These plants, as well as others that are found locally, may be considered as an alternative to the use of non-selective chemical insecticidal agents for vector control in Salikneta Farm.

4. CONCLUSIONS

In this study, six species were identified by examination of larval morphology and chaetotaxy. Five among them are generally recognized for their medical and veterinary importance: *Aedes aegypti*, the primary vector of dengue hemorrhagic fever and yellow fever in humans, and *Dirofilaria* in dogs; *Anopheles tessellatus*, a potential vector of malaria and Bancroftian filariasis; *Culex quinquefasciatus*, a cosmopolitan species that also has the potential to carry *Wuchereria bancrofti*; and *Cx. tritaeniorhynchus* and *Cx. vishnui*, both of which are recognized as important vectors of Japanese encephalitis. The sixth species, *Cx. mimeticus*, a non-medically important 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 span of time and more sampling sites spanning a greater extent of the 64-hectare area of Salikneta Farm. Finally, it is suggested that appropriate vector control measures be undertaken in the farm to prevent future cases of mosquito-borne arboviral and parasitic infections.

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