Journal of Medical Entomology, 55(3), 2018, 646–653
doi: 10.1093/jme/tjx254
Advance Access Publication Date: 30 January 2018
Research Article

Mosquito Species (Diptera: Culicidae) Diversity from Ovitraps in a Mesoamerican Tropical Rainforest

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Subject Editor: Scott Ritchie

Received 6 September 2017; Editorial decision 13 December 2017

Abstract

Mosquito sampling using efficient traps that can assess species diversity and/or presence of dominant vectors is important for understanding the entomological risk of mosquito-borne disease transmission. Here, we present results from a survey of mosquito species sampled with ovitraps in a neotropical rainforest of Costa Rica. We found the method to be an efficient sampling tool. With a total sampling effort of 29 traps, we collected 157 fourth-instar larvae and three pupae belonging to eight mosquito taxonomic units (seven species and individuals from a homogenous taxonomic unit identified to the genus level). In our samples, we found two medically important species, *Sabethes chloropterus* (Humboldt) and *Trichoprosopon digitatum* (Rondani). The former is a proven vector of Yellow Fever in sylvatic environments and the later has been found infected with several arboviruses. We also found that mosquito species abundance and diversity increased with canopy cover and in environments where leaf litter dominated the ground cover. Finally, our results suggest that ovitraps have a great potential for systematic sampling in longitudinal and cross-sectional ecological "semi-field" studies in neotropical settings.

Key words: biodiversity, canopy cover, disease vector, mosquito sampling, regression tree

Mosquito species (Diptera: Culicidae) abundance and diversity are key parameters to understand the entomological risk of mosquito-borne disease transmission (Brown et al. 2008) and, more generally, the landscape eco-epidemiology of vector-borne diseases (Reisen 2010). For example, increased mosquito diversity has been associated with a decrease in the infection rate of dominant vector species (Chaves et al. 2011). Moreover, the study of abundance and diversity patterns in mosquitoes has the potential to uncover inter-specific interactions between mosquitoes that might be useful to "naturally" control major vector species, especially in the aquatic stages (Miyagi and Toma 1980).

Mosquito species diversity also seems to play a role in current global invasions by major mosquito vector species (Lounibos 2002). For example, in Costa Rica, the Asian Tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), has been reported as occurring in microenvironments where its co-occurrence with other mosquito species is very limited (Calderón Arguedas et al. 2012). In Costa Rica, environments where *Ae. albopictus* has been found are agricultural areas whose overall diversity is reduced by the monoculture nature of commodity crops for export (Chaves et al. 2015b, Futami et al. 2015). More specifically, persistent infestations by *Ae. albopictus* have been observed in the Sarapiqui county, Heredia Province, North Eastern Costa Rica. This area is dominated by the production of pineapples for export (Chaves et al. 2015b), but sizeable patches of natural tropical rainforest remain (Vargas 2006). In Sarapiqui, *Ae. albopictus* females have been found infected with dengue virus serotypes DENV-1, DENV-2, and DENV-4 (Calderón-Arguedas et al. 2015). Then a relevant question is the evaluation of mosquito diversity patterns associated with the different environmental gradients that emerge in heterogeneous landscapes like the one of Sarapiqui. For example, gradients in canopy or ground cover are known to be associated with overall mosquito species diversity and abundance (Chaves et al. 2015a, Chaves 2016), but also with the abundance, or presence, of dominant vector species like *Ae. albopictus* (Tsuda et al. 2003).

Ovitraps have been frequently used to study mosquito species diversity and abundance in temperate East Asia, where *Ae. albopictus* is a widespread common species (Moriya 1974). Ovitraps

have also been used in Africa (Dunn 1927, Service 1965), Panamá (Yanoviak 1999, Yanoviak 2001), Australia (Ritchie et al. 2004), and South America (Navarro and Machado-Allison 1995). An evaluation of ovitraps as a suitable tool for mosquito species diversity sampling (Zea Iriarte et al. 1991) revealed that ovitraps were successful to sample mosquito species found in treeholes of similar volume across an urban altitudinal gradient in Nagasaki, Japan (Tsuda et al. 1994). Despite this encouraging result, to the best of our knowledge, similar studies have not been made in Mesoamerican neotropical settings, thus warranting the test of ovitraps as a tool for the systematic sampling of mosquito aquatic stages under "semi-field" conditions, that is, controlling some aspects of the observations by introducing, for example, ovitraps which are homogeneous sampling units that can be placed at fixed heights or controlling some of their characteristics unlike most natural phytotelmata. Here, it is also important to highlight that container mosquitoes are generally studied by directly inspecting artificial water containers (Schneider et al. 2004) or phytotelmata (Tsuda et al. 1994), but also by using adult traps (Maciel-de-Freitas et al. 2006, Brown et al. 2008, Torres et al. 2017), resting boxes (Edman et al. 1997, Barata et al. 2007), and by the aspiration of resting habitats (Clark et al. 1994, Scott et al. 2000, Vazquez-Prokopec et al. 2009). These methods are costly by being labor intensive and/or by relying on traps whose price is orders of magnitude above that of an ovitrap (Ritchie et al. 2003). For example, while any commercially available adult trap is above 100 US$ per unit, after externalizing the cost of cans which can be obtained for free from recycling material, an ovitrap costs around 0.25 US$, making these traps exceptionally cheap, which eases their large-scale deployment (Ritchie et al. 2003, Ritchie et al. 2004).

In this study, we evaluate ovitraps as a method for mosquito sampling in a Mesoamerican rainforest environment. We performed this evaluation at La Selva Biological Station (LSBS), which is located in Sarapiqui county where persistent infestations by *Ae. albopictus* have been observed (Chaves et al. 2015b). LSBS is an ideal study site to perform biodiversity studies given its heterogeneous land use, where primary and secondary forests are mixed with residential areas, thus featuring gradients of canopy and ground cover useful to study mosquito biodiversity patterns (Hoshi et al. 2014). Furthermore, LSBS has had extensive surveys about its larval mosquito fauna (Heinemann and Belkin 1977) setting an advantage for results comparison, especially about the habitats of previously collected mosquito species with data from ovitraps, which have never been deployed at LSBS. For the evaluation of ovitraps as a method for mosquito sampling in sylvatic environments, we describe mosquito species diversity and abundance in ovitraps set across heterogeneous land use areas in LSBS. We also compare mosquito species diversity with observations made at LSBS by Heinemann and Belkin (1977) and records in the Diptera collection at Museo Nacional de Costa Rica.

Materials and Methods

Study Site

Mosquitoes were collected using ovitraps set in LSBS, Puerto Viejo de Sarapiquí, Heredia Province, Costa Rica (10.430644°N, -84.007003°W). LSBS is within a lowland tropical rainforest, with a long rainy season from April to November and a short dry season from December to March (Tosi 1969, Vargas 2006). Nowadays, most of the land surrounding LSBS has been converted into pesticide intensive commercial pineapple plantations for export (Chaves et al. 2015b, Futami et al. 2015). LSBS currently has different land use types, including primary and secondary tropical lowland forests, but also residential areas with high human activity. For this study, we placed ovitraps in a total of 15 trees, whose locations were randomly chosen across walking paths in three areas: (i) a not forested residential area (NF), (ii) a primary forest (PF), and (iii) an old growth, around 20 yr, secondary forest (SF). Tree locations, five by area (Fig. 1), were recorded using a GPS. Around each tree, we measured the ground cover by dividing the ground into 12 30° sections with a radius of 3 m (Chaves et al. 2015a, Chaves 2016) and assigned one of the following categories (leaf litter, bare earth ground, root, understory, tree cover, concrete, bamboo). We also measured canopy cover using a standard densiometer, where four measurements were taken 90° apart in a 2 m radius from the tree stem (Chaves et al. 2015a). In each tree, with the exception of one that did not reach 150 cm of height, we then fixed two ovitraps at 75 and 150 cm of height. Each trap consisted of a 350 ml, 54mm diameter, metallic

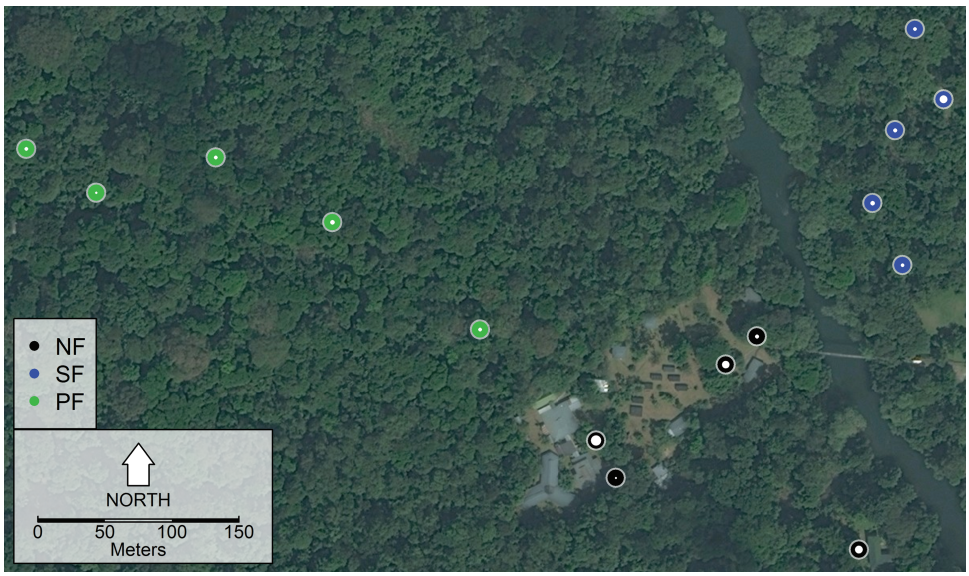

Fig. 1. Study site. Ovitraps were set at "La Selva" biological station, Sarapiqui, Heredia Province, Costa Rica. In the map location color indicates the study area (NF = Not Forested, SF = Secondary Forest, PF = Primary Forest). The white spot at the center of each sampling location is proportional to the canopy openness, where a larger white dot indicates less canopy cover.

can, open in the upper end, painted with black acrylic paint inside and outside, following the standard description of ovitraps used to study mosquito larvae (Moriya 1974). Since the observations were made during the rainy season, we did not add water to the traps but let them fill naturally with rainwater. Traps were set on 9 September 2016. Traps were left in the field for an average of 17 d.

Mosquito Collection

Mosquitoes were collected between 25 and 27 September 2016 from the 29 traps we set. During mosquito collection, we emptied the content of each trap, measured its water volume, and then moved the contents into a transparent tray. Any trap containing small larvae (<5 mm length) was considered as positive for first to third instar larvae, but no further identification of mosquito species was done due to the lack of reliable morphological characters for identification. Larger, that is, fourth instar, larvae (over 5 mm length) and pupae were collected alive and brought to the lab for morphological identification. Fourth-instar larvae were killed using 100% ethanol and later identified, while pupae were allowed to emerge into adults for identification.

For larval mosquito identification, we mounted specimens on microscope slides with euparal medium, while adults emerged from pupae were flash frozen. Larvae and adults were identified using a taxonomic key for mosquitoes of Costa Rica (Darsie Jr 1993). Vouchers for all the identified species were deposited in the Diptera collection at Museo Nacional de Costa Rica.

Statistical Analysis

We summarized information about the ground cover using a principal component analysis (Venables and Ripley 2002), where the first component, which accounted for 47% of the variability in the data, was used as a metric for ground coverage (Chaves et al. 2015a). In this ground cover index, positive values were associated with leaf litter and tree cover, while negative values with concrete and bare earth grounds.

We analyzed the quality of our species diversity sampling by analyzing the total number of fourth-instar larvae species per trap using a species accumulation curve built through the rarefaction method (Colwell and Coddington 1994). Briefly, with the rarefaction method, the expected number of species, that is, species richness, and its standard deviation are obtained by sampling individuals based on their abundance per sampling effort, which in the specific case of this study is an ovitrap. We also examined the patterns of clustering of the Bray distances between sampling locations that had fourth-instar larvae, and studied the association between those Bray distances and geographical distance between the traps. To assess the significance of the association between the Bray and geographic distances, we employed a Mantel test based on the Pearson correlation, whose inference was based on 1000 permutations. Briefly, the Bray distance is a metric for the compositional dissimilarities between two sites. This metric has a range between 0 and 1 where a value of 0 indicates that species composition is the same for two sites and 1 that there is no single common species between the two studied sites (Bray and Curtis 1957). The Mantel test is a correlation test for two distance matrices, which unlike a single correlation coefficient considers that observations are distances between objects (Legendre et al. 2015). For the cluster analysis, we employed an agglomerative nesting, a method for hierarchical clustering, where ovitraps are clustered based on their species similarity (Venables and Ripley 2002).

We also studied species richness and larval abundance, as function of the ground cover, canopy openness, water volume collected in the ovitraps, study area, and trap height using regression trees. Regression trees are a set of rules that can predict a response variable when a series of nested conditions are met (Olden et al. 2008). Regression trees is a powerful analysis technique that can capture non-linear, as well as linear, relationships between a response variable and a set of predictors (Faraway 2006), and without strict assumptions about independence in the samples (Chaves 2010). Moreover, the fitting method is by itself useful for variable selection, since covariates not informative for the generation of predictive rules are discarded during the iterative process of model fitting (Olden et al. 2008).

All statistical analyses and maps were made using the language R version 3.4.0. For the maps, we used the GISTools, rgdal, and OpenStreetMap libraries. For the cluster analysis, species accumulation curve, and to estimate and analyze the Bray distances, we employed the libraries vegan and cluster, while the tree analysis was made with the library rpart.

Results

Of the 29 traps set, a total of 26 had water, 25 had larvae (any instar), 21 had small larvae, 16 had large larvae, and two had pupae (Table 1). We found eight species, which are listed in Table 1 which also includes the total number of fourth-instar mosquito larvae collected by area and trap height. Interestingly, the ovitraps with *Toxorhynchites hypoptes* (Knab) had no small larvae or other mosquito species. We collected a total of 157 fourth-instar mosquito larvae. The most common species were *Trichoprosopon digitatum* and *Culex secundus* Bonne-Wepster & Bonne, with 49 (present in NF, PF, and SF ovitraps, mainly at 150 cm) and 43 (present in NF and PF ovitraps at 75 cm) individuals, respectively. On the other extreme, the least common species was *Sabethes chloropterus* with two individuals, which were found in NF ovitraps at 150 cm (Table 1). We also found three individuals belonging to *Wyeomyia* spp. Theobald, which although not identifiable at the species level, formed a unique taxonomic unit. We only collected three mosquito pupae, two belonging to *Tr. digitatum* and one to *Limatus durhamii* Theobald. The total number of larvae was similar between traps at 75 cm (a total of 82 fourth-instar larvae) and 150 cm (a total of 75 fourth-instar larvae), but pupae were only found at 75 cm. In general, most of the species we found naturally colonize treeholes, bamboo stumps, and artificial containers (Table 2).

Figure 2 shows the species accumulation curve for our ovitraps, where the three dry traps were not considered nor data from the pupae. The flattening of the curve suggests that our sampling effort was large enough so that most species that could be sampled with ovitraps were collected, during the season and conditions when our traps were deployed, that is, that our species sampling was comprehensive for our collection method.

In general, most of the Bray distances in species composition among ovitraps were close to one, signifying a large dissimilarity between traps (Fig. 3A). However, in some instances, a few traps had the same species, and in general, the clustering pattern suggests that there were no major differences in the mosquito fauna between the primary and secondary forest, as also suggested by the raw data in Table 1. Bray distance in species composition and geographical distance between ovitraps did not show any clear association (Fig. 3B). The Mantel correlation between the Bray and geographic distances was $\hat{r} = -0.123$ ($P > 0.918$). The regression tree for the number of species in an ovitrap as function of the environmental variables (Fig. 3C) explained a high proportion of the variability in the data we collected ($R^2 = 0.84$). In regression trees values at the end of the branches are predictions for

Table 1. Mosquito species abundance by study area and ovitrap height

Study area	Not forested (NF)		Secondary forest (SF)		Primary forest (PF)		
Ovitrap height	150 cm	75 cm	150 cm	75 cm	150 cm	75 cm	Total
Aedes podographicus Dyar & Knab	0	4	0	0	0	0	4
Culex corrigani Dyar & Knab	0	3	21	0	0	0	24
Culex secundus Bonne-Wepster & Bonne	0	10	0	0	0	33	43
Limatus asulleptus Theobald	0	0	0	0	0	3	3
Limatus durhamii Theobald	0	0	0	9	3	13	25
Sabethes chloropterus (Humboldt)	2	0	0	0	0	0	2
Toxorhynchites hypoptes (Knab)	0	0	1	1	1	1	4
Trichoprosopon digitatum (Rondani)	12	0	0	2	35	0	49
Wyeomyia spp Theobald	0	0	0	3	0	0	3
Small Larvae	3	3	3	4	4	4	21
Large Larvae	2	2	2	3	3	4	16
Pupae	0	1 (2 Tp[a])	0	0	0	1 (1 Ld[b])	2 (3)
No. traps	5	5	4	5	5	5	29

In the table rows, Small Larvae (first-third instar), Large Larvae (fourth instar), and Pupae indicate the number of ovitraps (where the total number of traps set is indicated in row No. Traps) that had mosquitoes in that ontogenetic stage. For the containers that had pupae, the species, and number of collected individuals, are indicated within parentheses.

[a]Tp = *Trichoprosopon digitatum*.

[b]Ld = *Limatus durhamii*.

Table 2. Habitats of the collected mosquito

Species/Habitat	AC	BS	GP	TH	T	RP	FPS	FH	FLW	HFB
Aedes podographicus	X	X		X	X					
Culex corrigani		X		X						
Culex secundus	X	X	X			X	X	X	X	
Limatus asulleptus	X	X		X			X	X	X	
Limatus durhamii	X	X		X	X		X	X	X	X
Sabethes chloropterus				X						
Toxorhynchites hypoptes		X		X				X		
Trichoprosopon digitatum	X	X			X		X	X		X
Wyeomyia spp	X	X								X

In the Table an "X" denotes presence. This table is based on observations from Heinemann and Belkin (1977) and records at Museo Nacional de Costa Rica. Habitats include: AC (artificial container), BS (bamboo stump), GP (ground pool), T (tire), TH (tree hole), RP (rock pools), FPS (fallen palm fronds), FH (fallen fruit husks holding water), FLW (fallen leaves holding water), HFB (heliconia flower bracts).

the set of rules leading to them. In Fig. 3C, the most basal node is the canopy openness, meaning this is the most important variable associated with species richness patterns, where values below 0.27 (i.e., high canopy cover) were associated with more species per trap. Similarly, the number of species was largest in traps at 75 cm, where ground cover was dominated by leaf litter and trees. On the opposite extreme, no species were found when the canopy cover was low (i.e., openness high) and grounds were mainly formed by concrete. The regression tree for the number of collected larvae had a lower fit (R^2 = 0.44) than the richness tree (R^2 = 0.84), and it shows similar patterns to the species richness tree (Fig. 3D). Nevertheless, the most important variable (basal branching node) was ground coverage, where a ground coverage dominated by leaf litter and trees had more fourth-instar mosquito larvae. Canopy cover and water volume were also important to explain mosquito abundance. High canopy cover (i.e., low canopy openness) was associated with an increased mosquito abundance, while the relationship with water volume was non-linear, with more mosquitoes being observed at certain, bounded, water volumes (below 134 ml).

Discussion

Our survey revealed new species records for Sarapiqui county, as *Aedes podographicus* Dyar & Knab and *Sa. chloropterus*, both of which were found in the NF area, were not reported by Heinemann and Belkin (1977) or more recently by Calderón Arguedas et al. (2012). Here, it is worth highlighting that *Sa. chloropterus* is a Yellow Fever vector (de Rodaniche et al. 1956, Trapido and Galindo 1957) which often bites humans, and its finding in a residential area (NF) deserves further inquire for its potential to transmit pathogenic arboviruses to humans in Sarapiqui county. The use of ovitraps for mosquito larval sampling in a mesoamerican neotropical rainforest was successful, as we were able to sample eight taxonomic units out of 68 taxonomic units (32 identified at the species level) recorded at LSBS, using several sampling techniques not including ovitraps, by Heinemann and Belkin (1977). We are confident our sampling was comprehensive for the conditions where and when traps were set, given the flattening of the species accumulation curve (Colwell and Coddington 1994). Our data also showed that, as observed elsewhere (Chaves et al. 2015a, Chaves 2016), mosquito species richness and abundance increased with forest canopy and in places were the ground was dominated by standing vegetation and/or leaf litter. Thus, in the specific setting of our study mosquitoes were more abundant and diverse in the forested areas. Nevertheless, species similarity patterns as function of geographical distance does not suggest that the areas have differentiated faunas as product of distance (Chaves et al. 2011), while the cluster analysis suggests that the two

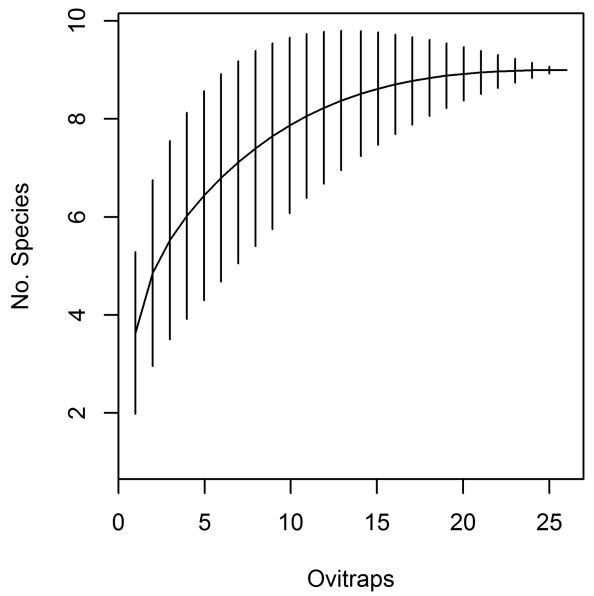

Fig. 2. Species accumulation curve built by rarefaction, and as function of the cumulative number of ovitraps that contained water.

forested areas were more likely to be similar, something also supported by the raw data in Table 1.

Almost all the species we found normally colonize treeholes, bamboo stumps, and artificial containers, which might indicate that ovitraps for larval sampling (Moriya 1974) might be good to sample mosquitoes that occur in artificial containers, treeholes, and bamboo stumps holding similar water volumes, as observed elsewhere (Zea Iriarte et al. 1991, Tsuda et al. 1994). Indeed, Heinemann and Belkin (1977) found six taxonomic units, with only three units identified to the species level, developing in bamboo stumps, and 10 species in treeholes, of which only *T. hypoptes* and *Culex corrigani* Dyar & Knab were among the species we found. There were eight species that we did not find despite their recording by Heinemann and Belkin (1977) in bamboo stumps and threeholes, three species were from bamboo stumps: *Sabethes cyaneus* (Fabricius), *Sabethes identicus* Dyar & Knab, and *Culex bahahoyensis* Levi-Castillo, and five from treeholes: *Culex bonneae* Dyar & Knab, *Culex coronator* Dyar & Knab, *Culex declarator* Dyar & Knab, *Culex mollis* Dyar & Knab, and *Lutzia allostigma* Howard, Dyar & Knab. These mosquito species were likely undetected because of the limited sampling period, but their absence from our samples might also reflect the metacommunity dynamics of mosquitoes, where some species do not co-occur by having different phenologies (Tsuda et al. 1994).

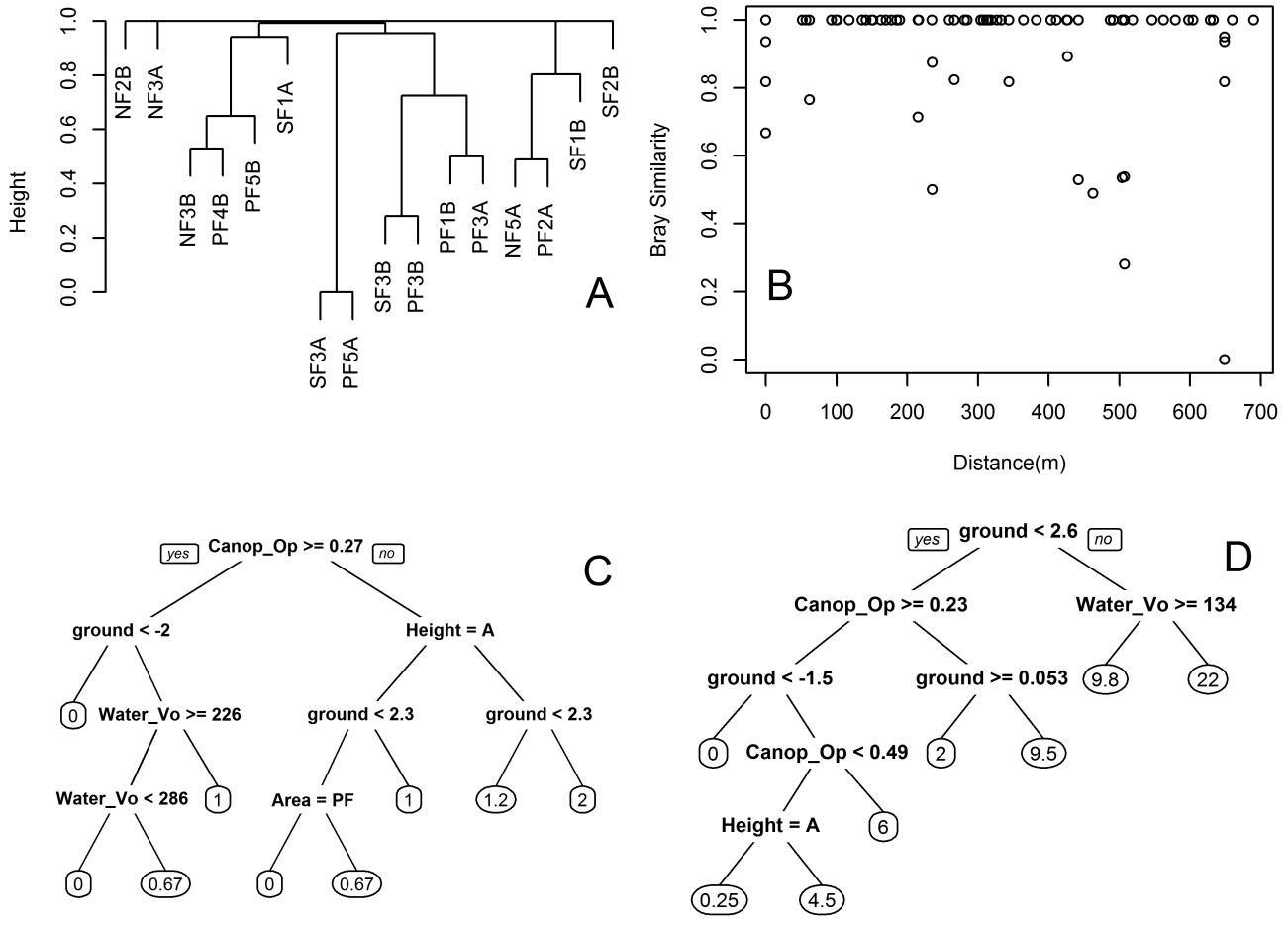

Fig. 3. Diversity and mosquito abundance patterns. (A) Cluster Analysis of Bray distances between ovitraps where fourth-instar larvae were found. The agglomerative coefficient of the cluster was 0.44 ($P < 0.05$). Codes indicate the study area (NF = Not Forested, SF = Secondary Forest, PF = Primary Forest), tree inside the area (from one to five), and trap height (A = 150 cm and B = 75 cm). (B) Bray distance between ovitraps as function of their geographical distance. (C) Regression Tree for the number of species found in ovitraps. (D) Regression tree for the number of larvae found in ovitraps. In the regression trees: Canop_Op = Canopy Openness, Height = trap height (A = 150 cm, B = 75 cm), Water_Vo = Water Volume (in ml), ground = ground cover index, Area = study area (PF = Primary Forest).

In addition, some species might only occasionally colonize ovitraps, for example, *Cx. secundus* is a species associated with ground pools, yet we found it in our ovitraps. Our ovitraps also caught species previously found at artificial containers in Sarapiqui (Heinemann and Belkin 1977, Calderón Arguedas et al. 2012): *Tr. digitatum*, *Limatus asulleptus* Theobald, and *L. durhamii*. We did not find any species belonging to the subfamily Anophelinae, even though mosquitoes from this subfamily have been recorded at LSBS (Heinemann and Belkin 1977). However, this lack of Anophelinae species could be expected, given that, to the best of our knowledge and based on records at Museo Nacional de Costa Rica, only *Anopheles eiseni* Coquillet has been found in bamboo stumps and only *An. eiseni, Anopheles neivai* Howard, Dyar & Knab, and *Anopheles powderi* Zavortink have been found in treeholes in Costa Rica. We could also have expected more species in the ovitraps if the sampling was extended to the forest canopy (Galindo et al. 1956, Yanoviak 1999). Although our samples were collected during the rainy season, which normally has the largest mosquito species richness in the study site (Heinemann and Belkin 1977), an open question is whether some species, whose phenology might be restricted to the dry season, could also be sampled with ovitraps during the dry season.

The taxonomic units we found belong to four Culicinae tribes, two species belong to the Culicini tribe, one species is from the Aedini tribe, and one species is from the Toxorhynchitini tribe. Most mosquito species belong to the Sabethini tribe, with five taxonomic units (four identified at the species level and a homogenous taxonomic unit identified to the genus level).The two Culicini species we found were *Cx. corrigani* and *Cx. secundus*, were sampled in all areas and do not have any known medical importance (Arnett 1948, Heinemann and Belkin 1977). The only Aedini species we found was *Ae. podographicus*, a species we found in the NF area, has no known medical importance, but it co-occurs with *Aedes aegypti* (L.), the most important dengue vector worldwide, in Mexico (Baak-Baak et al. 2016). The Toxorhynchitini was *Toxorhynchites hypoptes*, a species belonging to a genus without medical importance given the reliance of adults on carbohydrate rich materials, not blood-sucking like most non-autogenous mosquito species (Chaves et al. 2010). Nevertheless, its patterns of co-occurrence are suggestive of a major predatory role (Arnett 1950, Zavortink and Chaverri 2009), since larvae were found alone in the four traps where we found this mosquito species, our sampling during the rainy season coincides with previous observations about population peaks in this species (Carpenter and Peyton 1952). About the Sabethini we found, we are confident all the *Wyeomyia* spp. individuals we collected belonged to a single taxonomic unit, but we were unable to identify them to the species level, given the lack of good taxonomic keys for *Wyeomyia* spp. identification as larvae (Campos 2016). *L. durhamii* and *L. asulleptus*, are species that are commonly attracted to humans and other animals during daytime (Carpenter and Peyton 1952, Salas et al. 2001). *L. asulleptus* has been found infected with viruses of the Bunyamwera group (Family *Bunyaviridae,* Genus *Orthobunyavirus*) (Mores et al. 2009). *Tr. digitatum* is one of the most interesting mosquitoes in the neotropics, with diurnal biting as adults, parental care of recently layed egg rafts, and predacious and cannibalistic behaviors as larvae, which might explain why this species was alone in the three ovitraps we found it (Zavortink et al. 1983). Pixuna virus and Wyeomyia virus have been isolated from *Tr. digitatum*, while Bussuquara virus, Ilheus encephalitis virus, St. Louis encephalitis virus, and Triniti virus have been isolated from pools of mixed species containing *Tr. digitatum* (Zavortink et al. 1983). *Sa. chloropterus* is an important Yellow Fever vector, whose adults are diurnal and concentrate their biting activity in the afternoons, and are non-autogenous, requiring blood feeding before oviposition (Galindo et al. 1950, Galindo et al. 1951). The larvae exhibit negatively phototropic characteristics and are cannibalistic (Galindo 1958), which might be related with the low abundance we found and the fact this species was also alone in the ovitrap where it was sampled.

Finally, our results are very encouraging about the potential use of ovitraps for larval mosquito biodiversity sampling in neotropical settings, since these traps are cheap, roughly at least 400 ovitraps can be deployed by the price of the least expensive adult trap in the market, and because ovitraps might serve as reliable sampling units for longitudinal "semi-field" studies, those were sampling is systematically done in the field while controlling a few aspects of the subjects/objects under observation, aiming at better understanding the aquatic ecology of mosquitoes, beyond cross-sectional diversity surveys like the one presented here. Moreover, ovitraps for larval mosquito sampling are useful to collect medically important species, as was the case with *Tr. digitatum* and *Sa. chloropterus*. Although we did not find *Ae. albopictus*, we think LSBS and nearby farms with patches of primary tropical rainforest are ideal to monitor for infestations of this invasive species of medical importance, given *Ae. albopictus* year-round presence in nearby pineapple farms (Calderón Arguedas et al. 2012, Calderón-Arguedas et al. 2015, Chaves et al. 2015b, Futami et al. 2015), especially considering whether native species only present in patches of primary tropical lowland rainforest are able to keep *Ae. albopictus* in check.

Acknowledgments

We thank all the students enrolled in the fall 2016 "Tropical Disease" study abroad semester from the Organization of Tropical Studies (OTS), who helped with data collection as part of a faculty led project in their research methods class. We also thank Dr. Carlos de La Rosa for his help with the logistics associated with the implementation of this study. C.D., D.L., and C.R. were students in the fall 2016 "Tropical Disease" semester from OTS. Finally, Museo Nacional de Costa Rica kindly provided materials, equipment, and space for the identification of mosquito larvae and emerged adults from pupae.

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