Journal of Entomological and Acarological Research

Original article, Entomology:

High mosquito diversity in an Amazonian village of Ecuador, surrounded by a Biological Reserve, using a rapid assessment method.

Paul-Leonardo Duque[1], Jonathan Liria[2], Sandra Enríquez[3], Elena Burgaleta[1,2], José Salazar[1], Jazzmin Arrivillaga-Henríquez[4], Juan-Carlos Navarro[1]

[1] Universidad Internacional SEK, Facultad de Ciencias Naturales y Ambientales, Center for Biodiversity, Emerging Diseases and Environmental Health, Quito, Ecuador.
[2] Universidad Regional Amazónica Ikiam, Grupo de Investigación en Población y Ambiente, Tena, Ecuador.
[3] Universidad Central del Ecuador, Instituto de Investigación en Salud Pública y Zoonosis-CIZ, Quito, Ecuador
[4] Universidad Central del Ecuador, Facultad de Comunicación, Carrera de Turismo Histórico, Ambiente y Territorio, Quito, Ecuador.

Acknowledgements: ASOKIL, Kichwa community of Limoncocha, Ana Ortega and students of Environmental and Biotechnology Engineering (UISEK 2016-2017) for field assistance. Jendry Moya and family for logistic at Scientific Station. MAE-RBL for permission. Collection permit by Ministerio del Ambiente No. MAE-DNB-CM-2015-0028-M001.

Correspondence: Juan-Carlos Navarro, Ph.D. Universidad Internacional SEK. Ecuador. Tel: +593-2-3970048, ext 166 / +593995479569.
Email: juancarlos.navarro@uisek.edu.ec

Keywords: Amazon, arboviruses, Culicidae, Natural Reserve, Kichwa.

Contributions: All authors contributed equally.

Conflict of interest: The authors declare that they have no conflicts of interest concerning this article.

Funding: This work was supported by SEK International University, Projects DII-P011617 and VCC-AMB217 (JCN) and UCE-CUP-91750000.0000.374044 (SE).

Abstract

This research represents a study in an Amazonian village that has similar structures to others Kichwa Amazonian villages of Ecuador. We evaluated the diversity, ecology, possibility of mosquitoes/pathogens translocation from forest to urban area, and the vulnerability by potential mosquito vectors of diseases through an intensive and fast method done January 2017. Our analyses registered a high diversity of mosquitos in Limoncocha village (33 spp, *H'* 2.76), which includes four new records of species for Ecuador. We propose the biological reserve and the lagoon are determinant environmental factors for the high mosquito diversity, plus the socio-economic characteristics related with a deficient water pipeline supply and lack of solid waste system. Furthermore, the high diversity of sylvan mosquitoes registered throughout the area, that include several potential vectors, suggest a moderate to high vulnerability for the transference of pathogens from the Biological Reserve to the urbanized area, which may increase the circulation of little-known arboviruses (Mayaro, Ilheus, St Louis encephalitis) across Ecuador.

Introduction

Limoncocha, an Amazonian small village, was founded in 1957 due to the growing interest of oil companies (Texaco and Occidental Oil Gas Corporation, OXY), the Ecuadorian government, and the Summer Institute of Linguistics (SIL) (Ortíz et al., 1995). The SIL mobilized a few young Kichwa people (over 50), from Tena city to borders of Limoncocha lagoon (Konecki et al., 2016). Presently, locality has increased to over 1,500 residents (Instituto Nacional de Estadísticas y Censos, 2010) with poor urban planning that causes deficient water supply, all the while lacking a solid waste disposition, although there are roads systems, dwellings, and educative and health centers with small agriculture lands near the urbanized center. Limoncocha population is influenced by western customs (religion, business, state), hence the lack of social and economic relationship with other ethnic groups in the surrounding area (Burgaleta et al., 2018). This social behavior has created a dependence with the biological resources from the Biological Reserve and Limoncocha Lagoon, which was declared as RAMSAR wetland in 1998 (Ramsar Convention on Wetlands, 1998).

The Biological Reserve presents tropical humid forest and flooded tropical lowland forest, thus, studies in mosquitoes have demonstrated high richness in similar areas (Pecor et al., 2000; Linton et al., 2013; Navarro et al., 2015). Nevertheless, the loss of primary forests during the last decades in Limoncocha because of human activities could be altering mosquito ecology cycles (Lyimo & Ferguson, 2009; Pongsiri et al., 2009). These changes could have destroyed or created new mosquito breeding sites in this small urban area, hence changing the species richness and abundance following demographic, environmental and social factors (Cardo et al., 2011; Ferraguti et al., 2016). Also, these ecological changes could increase the spread of arboviral disease from the forest to urban areas (Weaver, 2013; Wilder-Smith et al., 2017; Dexheimer Paploski et al., 2018).

In Ecuador, the vector-borne diseases are a big issue in public health. They are mainly transmitted by mosquitoes and cause major outbreaks in tropical and subtropical areas (Navarro et al., 2017). In the Amazonian towns, arboviral diseases as Dengue Virus (DENV), chikungunya Virus (CHIKV), Zika Virus (ZIKV), Yellow Fever Virus (YFV) occur frequently (reports of Dirección Nacional de Vigilancia Epidemiológica) and other probable circulation of viruses are unknown due to the absence of a health network with rapid virus isolation and serology capabilities in little-known arboviruses (Muñoz & Navarro, 2012). This research represents a study in an Amazonian village that has similar structures to other Kichwa Amazonian villages of Ecuador. We evaluated the diversity, ecology, possibility of mosquitoes/pathogens translocation from forest to urban areas, and the vulnerability of potential vector-borne diseases through an intensive and fast method that has been previously proved successful (Barrera et al., 1995; Navarro et al., 2007; Navarro et al., 2015). We hypothesized that Limoncocha, a small Kichwa village with little growth of urban infrastructures, poor pipeline water systems, a weak waste disposal system, and surrounded by a biological reserve with little social and commercial exchange with other communities (including other ethnic groups or localities with a mixed population), must have a diversity of mosquitoes strongly influenced by the mobility of species from the reserve towards the rural-urban population.

Materials and Methods

Study area

The population of Limoncocha is located in the Limoncocha Biological Reserve (RBL) in the Ecuadorian Amazon, Napo Biogeographic Province, and located in the political province of Sucumbíos, Canton Shushufindi (00 ° 24'25 "S; 76 ° 37' 14"W), parish of Limoncocha, at 203 m of altitude and with a total area of 59,853.32 ha. Its geographical limits are to the north with the parishes of Shushufindi and San Roque, to the south with the province of Orellana, to the east with the parish of Pañacocha, and to the west with the province of Orellana (Figure 1).

The RBL limits to the north with the Blanco River, to the East with the Itaya River, to the south with the Orellana province (Indillama River), to the Northwest with the Yasuní National Park, and to the West with the Jivino River and some populated centers like Playayacu, San Antonio, Limoncocha and Pompella (from north to south). The average annual temperature is 24.9 ° C, while the average annual rainfall is of 2,965 mm (INAMHi, 2016).

The total population of the parish of Limoncocha consists of approximately 6,700 residents, while in the town there are approximately 1,500 residents of the Kichwa ethnic group, being education, oil and agricultural the population's main activities. Other declared activities are fishing and hunting in the RBL (Konecki et al., 2016). The population consists of approximately 23 housing blocks and 120 buildings. Adjacent to the town is the Napo River (1-1.5 km away) and the Limoncocha Lagoon (abandoned meander of the Napo River, 1,230 years). The RBL is a RAMSAR area (wetland, declared in 1998) and is one of the smallest protected areas in Ecuador (4 613 ha.). It was declared as a Natural Reserve on September 23, 1985 (Ministerio del Ambiente, 2012).

Mosquito collection

We collected during three consecutive days in an intensive and fast sampling on January 2017 with different trap techniques in the populated zone (64 dwellings or 80% sampled), the ecotone near the lagoon and an intervened area based on a previously proved method (Barrera et al., 1995; Navarro et al., 2007; Navarro et al., 2015). Adult mosquitoes were collected through CDC light traps and human landing catches. The immature specimens were sampled using plastic bomb suckers in different breeding sites, both in natural (Phytotelmata) and artificial containers, and then reared in the laboratory to obtain adult mosquitoes with associated rearing (Belkin, 1965). The genera and species were identified from larvae, females and genitalia males based on *ad hoc* keys (Lane, 1953a; Lane, 1953b; Gorham et al., 1967; Valencia, 1973; Zavortink, 1979; Zavortink, 1981; Sallum & Forattini, 1996), as well as contrasting them with the National Collection Reference of Arthropods of Zoonotic importance (CoNRAZ-UCE) and the University collection reference (UISEK). Parallel to biological sampling, a survey about public service quality, socio-economic data, previous outbreaks and vector borne disease symptoms was carried out (Ortega et al., 2018).

Diversity analyses

We quantified the richness and its representativeness (Moreno, 2001) in two habitats (populated zone and ecotone) to obtain the estimated diversity. We evaluated the local diversity (α) using richness (species numbers or S), Shannon-Wiener index (diversity or $H' = - \sum p_i \ln p_i$), and Pielou index (evenness of $J' = H' / \ln S$). We used SHE analysis for studying the relative contribution for richness (S) and evenness (E) in mosquito community diversity (H'), following the relationship: $H' = \ln (S) + \ln (E)$. Finally, we performed a similarity analysis using Jaccard index ($I_J = c / a + b - c$, where: a = species presents in site A, b = species presents in site B, and c = common species in both sites) to show how mosquito composition is related between the two habitats. All calculations were made using PAST software version 2.17 (Hammer et al., 2017).

Results

Mosquito composition

A mosquito survey was carried out in several breeding sites in both urban and ecotone areas. It primarily focused on immature stages with dropper absorber (39% richness collected), as well as CDC light traps (67%) and manual aspirators (6%). Populated zones had the most species (88%) in contrast with ecotone areas (39% near the lagoon and 6% near intervened area). This study identified a total of 291 mosquitoes belonging to 33 species in seven tribes and 12 genera (Table 1). The Culicidae subfamily was the most diverse (94%) and abundant (82%) in relation to the diversity (6%) and abundance (18%) of the Anophelinae subfamily.

The Anophelinae subfamily was collected in adult stages with significant abundance in urbanized and ecotone areas beside the lagoon zone. Within the Culicidae subfamily, the Aedini tribe had three species: *Aedes aegypti* Linnaeus was collected in a barrel in larvae stage and restricted to the urbanized area, while the other two species were collected in adult stages in the ecotone zone. The Culicini tribe was the most diverse taxa with 14 species in three subgenera: *Culex* (*Carrollia*), *Cx.* (*Culex*) and *Cx.* (*Melanoconion*). The *Cx.* (*Carrollia*) was the only group to be collected in larvae stage and artificial containers, while the *Cx. Quinquefasciatus,* another typical urban species, was collected in urban and ecotone areas as well. Mansoniini tribe mosquitoes were collected in adult stages in urban and ecotones areas near the lagoon. Five species were identified in the Sabethini tribe: *Limatus* genus was collected in artificial containers in populated zones; *Wyeomyia melanocephala* Dyar & Knab was found in the leaf axils of phytotelmata plants belonging to the Araceae family (*Xanthosoma* sp and *Colocassia* sp); *Johnbelkinia longipes* Fabricius was registered in urban areas; and *Trichoprosopon compresum* Lutz was collected in broken bamboo internodes. One species of the Toxorhynchitini tribe and four species of the Uranotaeniini tribe were found in adult stages in urban zones, while *Uranotaenia briseis* Dyar was collected in ecotone areas close to the lagoon (Table 1).

Mosquito richness

The diversity of the whole zone sampled showed high values (*H* 2.76 in urban and ecotone areas). The richness (*S*) and equitability (*E*) were high in the urban zones (*S* 29 and *E* 0.82) and low in ecotone zones (*S* 14 and *E* 0.78). The diversity indices were complemented with SHE analysis (Figure 2) showing that accumulative values for richness and diversity increased, while evenness values decreased. The mosquito species composition between both sites (urban and ecotone) show a low level of similarity I_j *(30%)*.

Discussion

In tropical zones, vector-borne diseases are associated with social, demographic and environmental factors that primarily affect people who live in rural and urban areas (World Health Organization, 2014). In the Kichwa Amazonian village, we found a high mosquito diversity, including four new species records for Ecuador (Table 1). To understand the high diversity and the potential transmission risk of urban-like arboviruses, it is essential to identify the environmental and social factors that determine the spatial-temporal richness and abundance of the mosquito communities.

It has been reported that ecological changes influenced by human activities allow the expansion and establishment of vectors towards populated areas (Johnson et al., 2008; Pongsiri et al., 2009; Gleiser & Zalazar, 2010; Pires & Gleiser, 2010; Leisnham & Juliano, 2012; Weaver, 2013; Ferraguti et al., 2016; Romero-Alvarez & Escobar, 2017). Only ten sylvan species (30%) have been recorded using artificial/anthropogenic containers (Table 1) due to Kichwa inhabitants using different sources of water supply (wells, rivers, rain), which promotes the use of scarce water storage through large containers (like 200lt drums in the lowland and coastal areas of Ecuador and Latin America) and throughout long-term periods (due the small containers and continuous use). This peculiar storage behavior could explain the low abundance of the typical urban mosquito species reported as vectors with high invasive and vector capacities such as *Aedes aegypti* and *Culex quinquefasciatus* in the populated areas (Gleiser & Zalazar, 2010; Pires & Gleiser, 2010; Stein et al., 2011). The little abundance should be correlated with the Ecuadorian

Public Health Ministry epidemiological reports about the absence of recent outbreaks (Dirección Nacional de Vigilancia epidemiológica, 2018).

Mosquitoes exhibit several characteristics concerning the selection of breeding and feeding sites, which can vary spatially and temporally (Lyimo & Ferguson, 2009). However, in relatively stable environments, species find ideal oviposition sites with high survival rates such as floating vegetation in lagoons, as reported for *Mansonia* (Mulieri et al., 2005; Ghosh et al., 2006; Stein et al., 2011), *Uranotaenia, Anopheles,* and *Culex* (Lopes & Lozovei, 1995; Stein et al., 2011). We suggest that the Limoncocha lagoon, just behind the village, is the most significant contributing factor to the high richness of mosquitoes belonging to these genera. Although richness was high in urban areas (*H* 2.76) due to the collection of mosquitoes in adult phases that probably find additional blood-meal resources (humans and domestic animals), their preference for oviposition can be both natural environments and anthropogenic containers available in houses backyards since it has previously been demonstrated in other several mosquito species (Pires & Gleiser, 2010; Cardo et al., 2011; Schmidt et al., 2011; Confalonieri et al., 2012). Our data shows a low similarity of species between the ecotone and urban areas, which are characterized by four abundant species: *Anopheles (Anopheles) nr. mattogrossensis* Lutz & Neiva*, Cx. (Culex) spissipes* Theobald*, Cx. (Cux.) nigripalpus* Theobald and *Coquillettidia (Rhynchotaenia) albicosta* Peryassu. These findings suggest that in urbanized areas there is an increase of the vulnerability of the inhabitants towards sylvan transmission by mosquito vectors due to the heterogeneity of ecosystems that occur in the area surrounding the town (lagoon, forests, small crops).

Studies have shown a high diversity of mosquitoes (Culicidae and Anophelinae) in both longitudinal (ecological) and multi-localities sampling with high human population such as Iquitos with 48 spp in 12 months (Johnson et al., 2008), Peru and its vicinity forested areas (40 km around) (96 species in a longitudinal study) (Pecor et al., 2000), Manaus, Brazil with 50 spp in 12 months (Barbosa et al., 2008), Para, Brazil (55 spp, 10 months) (Confalonieri & Costa Neto, 2012), and the Venezuelan Amazonian (Alto Orinoco with 25 mosquito species in 18 Yanomami small and non-westernized villages (Shabonos) (Rubio-Palis et al., 2014). All this data was obtained through the collection of adult mosquitoes using different capture methods. In turn, we identified 33 spp using punctual sampling during three consecutive days of both adult and immature mosquitoes in different types of breeding sites. Such diversity in a punctual sampling like this highlights the need to sample both adult and immature mosquitoes, but also the effectiveness of our three-day sampling, which are ideal for a rapid assessment of biodiversity and epidemiological risks (Navarro et al., 2015; Ortega et al., 2018).

Concerning public service and epidemiological assessment, Schmidt et al., (2011) showed that human populations with high density and better water supply system (about 35,000 residents) have a lower transmission risk than rural and peri-urban area with low populations (3,000 to 7,000) due to poor infrastructure, and absence of water supply and waste storage. However, our case differs from Schmidt et al., (2011) since there is no information about the small Amazonian localities with a low number of inhabitants like Limoncocha. As a result, we are not able to properly evaluate the relationship between the potential vector-borne diversity and the potential risk and vulnerability of arboviral outbreaks.

Limoncocha village belongs to the Amazon, a region with high endemism and biological diversity. However, mosquito richness is still underestimated. This locality is an indigenous Kichwa community with a "Western" housing organization and infrastructure, thus we propose that the biological reserve and the lagoon are determinant environmental sources for the high mosquito diversity. Furthermore, the socio-economic characteristics of the village, including deficient water pipeline and solid waste system, as well as insufficient options to get consumable water due to high and long-term rainfall periods, have brought about a peculiar behavior of water storage and the use of waste containers by its inhabitants.

We suggest that Limoncocha have a low transmission risk of urban-like arbovirus as dengue, Zika and chikungunya due to its low *Aedes aegypti* occurrence. However, the high diversity of sylvan mosquitoes registered throughout the area, including several potential vectors, suggest a moderate to high vulnerability of the potential transference of pathogens from the Biological Reserve (including the lagoon) to the urbanized area, knowing the circulation of little-known arboviruses

(Mayaro, Ilheus, St Louis encephalitis) due to the presence of antibodies in soldiers who have lived in the Amazon (Calisher, et al., 1983; Horby, 2007; Manock et al., 2009; Izurieta et al., 2011).
This study will serve as a baseline for comparison with other locations of similar size in northern-south transects of the Ecuadorian Amazon, and also to compare the mosquito diversity with other ecosystems/land use surrounding the Limoncocha village, both researches in progress.

References

BARBOSA M.D.G.V., FÉ N.F., MARCIÃO A.H.R., DA SILVA A.P.T., Monteiro W.M., DE F. GUERRA M.V., et al., 2008 - Registro de Culicidae de importância epidemiológica na área rural de Manaus, Amazonas. - Rev Soc Bras Med Trop 41:658–663. doi: 10.1590/S0037-86822008000600019.
BARRERA R., NAVARRO J.C., MORA RODRIGUEZ J.D., DOMINGUEZ D., GONZALEZ J., 1995 - Public services deficiencies and breeding of *Aedes aegypti* in Venezuela. Bull Pan Am Health Organ 118:193–205.
BELKIN J., 1965 - Mosquito studies (Diptera, Culicidae) I: a project for a systematic study of the mosquitoes of Middle America. II. Methods for the collection, rearing and preservation of mosquitoes. - Contrib Am Entomol Inst 1:1–78.
BURGALETA E., RODRÍGUEZ N., MARTÍNEZ M., 2018 - Identidades amazónicas en conflicto: el indígena dócil frente al insurrecto. - Rev Mex Sociol 80:139–166.
CALISHER C.H., GUTIERREZ E., FRANCY D.B., ALAVA A., MUTH D.J., LAZUICK J.S., 1983 - Identification of hitherto unrecognized arboviruses from Ecuador: members of serogroups B, C, Bunyamwera, Patois, and Minatitlan. - Am J Trop Med Hyg 32:877–85.
CARDO M.V., DARÍO V., EDUARDO C.A., 2011 - Community structure of ground-water breeding mosquitoes driven by land use in a temperate wetland of Argentina. - Acta Trop 119:76–83. doi: 10.1016/j.actatropica.2011.04.011
CONFALONIERI U.E.C., COSTA NETO C., 2012 - Diversity of mosquito vectors (Diptera: Culicidae) in Caxiuanã, Pará, Brazil. - Interdiscip Perspect Infect Dis. 1-8. doi: 10.1155/2012/741273
DEXHEIMER PAPLOSKI I.A., SOUZA R.L., TAURO L.B., CARDOSO C.W., MUGABE V.A., PEREIRA SIMOES ALVEZ A.B., et al., 2018 - Epizootic Outbreak of Yellow Fever Virus and Risk for Human Disease in Salvador, Brazil. - Ann Intern Med 168:301. doi: 10.7326/M17-1949
DIRECCIÓN NACIONAL DE VIGILANCIA EPIDEMIOLÓGICA (2018) Gaceta epidemiológica Semanal No. 15 del 08 al 14 de abril. Ministerio de Salud Pública, República del Ecuador. Available on: https://www.salud.gob.ec/gacetas-vectoriales-2014-2015-2016-2017/ (Accessed 15 july 2018)
DIRECCIÓN NACIONAL DE VIGILANCIA EPIDEMIOLÓGICA. Anuario de Vigilancia epidemiológica 1994-2016. Ministerio de Salud Pública, República del Ecuador. Available on: https://www.salud.gob.ec/direccion-nacional-de-vigilancia-epidemiologica/ (Accessed 15 july 2018)
FERRAGUTI M., MARTÍNEZ-DE LA PUENTE J., ROIZ D., RUIZ S., SORINGUER R., et al., 2016 - Effects of landscape anthropization on mosquito community composition and abundance. - Sci Rep 6:1–9. doi: 10.1038/srep29002
GHOSH A., BISWAS D., CHATTERJEE S.N., BENGAL W., 2006 - Host Plant Preference of *Mansonia* Mosquitoes. - J Aquat Plant Manag 44:142–144.
GLEISER R.M., ZALAZAR L.P., 2010 - Distribution of mosquitoes in relation to urban landscape characteristics. - Bull Entomol Res 100:153–158. doi: 10.1017/S0007485309006919
GORHAM J., STOJANOVICH C., SCOTT H., 1967 - Clave ilustrada para los mosquitos anofelinos de Sudamerica Oriental / Illustrated key to the anopheline mosquitoes of Eastern South America. - 64. doi: 10.1215/00182168-2006-098
HAMMER Ø., HARPER D., RYAN P., 2017 - PAST: Paleontological Statistics Software Package for Education and Data Analysis. - Palaeontol Electron 4:9pp.
JOHNSON B.W., CRUZ C., FELICES V., ESPINOZA W.R., MANOCK S.R., GUEVARA C.,

et al., 2007 - Ilheus Virus Isolate from a Human, Ecuador. - Emerging Infectious Diseases, 13:956–958. http://doi.org/10.3201/eid1306.070118
INAMHI., 2016 - Boletin Climatologico Semestral 2016. - Instituto Nacional de Meteorología e Hidrología, República del Ecuador. quito, Ecuador. Available on: http://www.serviciometeorologico.gob.ec/meteorologia/boletines/bol_sem.pdf (Accessed 15 july 2018)
INSTITUTO NACIONAL DE ESTADÍSTICAS Y CENSOS 2010 - Población y Demografía | Instituto Nacional de Estadística y Censos. - In: INEC.
IZURIETA R.O., MACALUSO M., WATTS D.M., TESH R.B., GUERRA B., CRUZ L.M., et al., 2011 - Hunting in the rainforest and mayaro virus infection: An emerging alphavirus in Ecuador. - J Glob Infect Dis 3:317. doi: 10.4103/0974-777X.91049
JOHNSON M.F., GÓMEZ A., PINEDO-VASQUEZ M., 2008 - Land use and mosquito diversity in the Peruvian Amazon. - J Med Entomol 45:1023–30.
KONECKI K., KACPERCZYK A., CHOMCZYŃSKI P., ALBARRACIN M. ,2016 - The spirit of communitarianism and the cultural background of the Limoncocha community in the context of sustainable development and environmental protection. - SEK University Press, Quito, Ecuador.
LANE J., 1953a - Neotropical Culicidae - Volume II: Tribe Culicini, *Deinocerites, Uranotaenia, Mansonia, Orthopodomyia, Aedomyia, Aedes, Psorophora, Haemagogus*, Tribe Sabethini, *Trichoprosopon, Wyeomyia, Phoniomyia, Limatus*, and *Sabethes*. - Univ. of São Paulo, São Paulo, II:551–114.
LANE J., 1953b - Neotropical Culicidae - Volume I - Dixinae, Chaoborinae and Culicinae, tribes Anophelini, Toxorhynchitini and Culicini (Genus *Culex* only). - Univ. of São Paulo, São Paulo, I:1–549.
LEISNHAM P., STEVEN J., 2012 - Impacts os climate, land use, and biological invasion on the ecology of immature *Aedes* mosquitos: Implication for La Crosse emergence. - Ecohealth PMC 9:217–228. doi: 10.1007/s10393-012-0773-7
LINTON Y.M., PECOR J.E., PORTER C.H., MITCHELL LB., GARZÓN-MORENO A., FOLEY D.H., et al., 2013 - Mosquitoes of eastern Amazonian Ecuador: biodiversity, bionomics and barcodes. - Mem Inst Oswaldo Cruz 108 Suppl:100–9. doi: 10.1590/0074-0276130440
LOPES J., LOZOVEI A.L., 1995 - Ecología de mosquitos (Diptera: Culicidae em criadouros naturais e artificiais de área rural do Norte do Estado do Paraná, Brasil. I.-Coletas ao longo do leito de ribeirao. - Rev Saúde Pública 29:183–191. doi: 10.1590/S0034-89101995000300005
LYIMO I.N., FERGUSON H.M., 2009 - Ecological and evolutionary determinants of host species choice in mosquito vectors. - Trends Parasitol 25:189–196. doi: 10.1016/j.pt.2009.01.005
MANOCK S.R., JACOBSEN K.H., DE BRAVO N.B., RUSSELL K.L., NEGRETE M., OLSON J.G., et al., 2009 - Etiology of acute undifferentiated febrile illness in the Amazon basin of Ecuador. - Am J Trop Med Hyg 81:146–151.
MINISTERIO DEL AMBIENTE., 2012 - Sistema de Clasificación de los Ecosistemas del Ecuador Continental. - Ed. Subsecretaría de Patrimonio Natural, Quito
MORENO, C.E., 2001 - Métodos para medir la biodiversidad. - M&T - Manuales y Tesis SEA 1:84. doi: 10.1371/journal.pone.0103709
MULIERI P.R., TORRETTA J.P., SCHWEIGMANN N., 2005 - Host plant selection of two *Mansonia* Blanchard species (Diptera: Culicidae) in a heterogeneous habitat of Buenos Aires City, Argentina. - J Vector Ecol 30:201–5.
MUÑOZ M., NAVARRO J.C., 2012 - Mayaro: A re-emerging arbovirus in Venezuela and Latin America. - Biomédica 32:286–302.
NAVARRO J.C., BARRERA J.P., LIRIA J., AUGUSTE J., WEAVER S.C., 2017 - Alphaviruses in Latin America and the Introduction of Chikungunya Virus. - In: Human Virology in Latin America. 1st edn. Springer International Publishing, Cham
NAVARRO J.C., LIRIA J., PIÑANGO H., BARRERA R., 2007 - Biogeographic area relationships in Venezuela: A parsimony analysis of Culicidae - Phytotelmata distribution in National Parks. - Zootaxa 19:1–19.
NAVARRO J.C., ARRIVILLAGA J., MORALES D., PONCE P., CEVALLOS V., 2015 - Evaluación rápida de biodiversidad de mosquitos (Diptera: Culicidae) y riesgo en salud ambiental

en un área Montana del Chocó Ecuatoriano. - Entomotropica 30:160–173.
ORTEGA A., DUQUE P., LIRIA J., ARRIVILLAGA-HENRÍQUEZ J., SALAZAR J., BURGALETA E., NAVARRO JC. 2018. Mosquito Diversity and Public Services as Risk factors for Emerging Diseases in a Small Village, Ecuador Amazon. Entomology and Applied Science Letters. 5 (3):91-105.
ORTÍZ P., VAREA A., MARTÍNEZ E., BUSTAMANTE T., NAVARRO M., GARZÓN P., et al., 1995 - Marea negra en la Amazonia: conflictos socio ambientales vinculados a la actividad petrolera en el Ecuador. - UICN. Abya Yala, Quito
PECOR J.E., JONES J., TURELL M.J., FERNANDEZ R., CARBAJAL F., O'GUINN M., et al., 2000 - Annotated Checklist of The Mosquito Species Encountered During Arboviral Studies in Iquitos, Peru (Diptera: Culicidae). - J Am Mosq Control Assoc 16:210–218.
PIRES D.A., GLEISER R.M., 2010 - Mosquito fauna inhabiting water bodies in the urban environment of Córdoba city, Argentina, following a St. Louis encephalitis outbreak. - J Vector Ecol 35:401–409. doi: 10.1111/j.1948-7134.2010. 00099.x
PONGSIRI M.J., ROMAN J., EZENWA V.O., GOLBERG T., KOREN H., NEWBOLD S., et al., 2009 - Biodiversity Loss Affects Global Disease Ecology. - BioScience 59:945–954. doi: 10.1525/bio.2009.59.11.6
RAMSAR CONVENTION ON WETLANDS., 1998 - The Ramsar Bulletin Board, 3 August 1998. Available at: http://archive.ramsar.org/cda/es/ramsar-news-archives-1998-ramsar-bulletin-17633/main/ramsar/1-26-45-91%5E17633_4000_2__
ROMERO-ALVAREZ D., ESCOBAR L.E., 2017 - Vegetation loss and the 2016 Oropouche fever outbreak in Peru. - Mem Inst Oswaldo Cruz 112:292–298. doi: 10.1590/0074-02760160415
SALLUM M.A., FORATTINI O.P., 1996 - Revision of the Spissipes Section of *Culex* (*Melanoconion*) (Diptera:Culicidae). - J Am Mosq Control Assoc 12:517–600.
SCHMIDT W.P., SUZUKI M., DINH THIEM V., WHITE R., TSUZUKI A., YOSHIDA L., YANAI H., et al., 2011 - Population Density, Water Supply, and the Risk of Dengue Fever in Vietnam: Cohort Study and Spatial Analysis. - PLoS Med 8: e1001082. doi: 10.1371/journal.pmed.1001082
STEIN M., LUDUEÑA-ALMEIDA F., WILLENER J.A., ALMIRÓN W., 2011 - Classification of immature mosquito species according to characteristics of the larval habitat in the subtropical province of Chaco, Argentina. - Mem Inst Oswaldo Cruz 106:400–407. doi: 10.1590/S0074-02762011000400004
VALENCIA J.D., 1973 - A revision of the subgenus *Carollia* of *Culex*. - Contrib Am Entomol Inst 9:1–134.
WEAVER S.C., 2013 - Urbanization and geographic expansion of zoonotic arboviral diseases: mechanisms and potential strategies for prevention. - Trends Microbiol 21:360–363. doi: 10.1016/j.tim.2013.03.003
WILDER-SMITH A., GUBLER D.J., WEAVER S.C., MONATH T.P., HEYMANN D.L., SCOTT T.W., 2017 - Epidemic arboviral diseases: priorities for research and public health. - Lancet Infect Dis 17: e101–e106. doi: 10.1016/S1473-3099(16)30518-7
WORLD HEALTH ORGANIZATION, 2014 - A global brief on vector-borne diseases. - WHO DCO/WHD/2014.1. 56p.
ZAVORTINK T.J., 1979 - The new sabethine genus *Johnbelkinia* and a preliminary reclassification of the composite genus *Trichoprosopon*. - Contrib Am Entomol Inst 17: p1-61.
ZAVORTINK T.J., 1981 - Species complexes in the genus *Trichoprosopon*. - Mosq Syst 13:82–85.

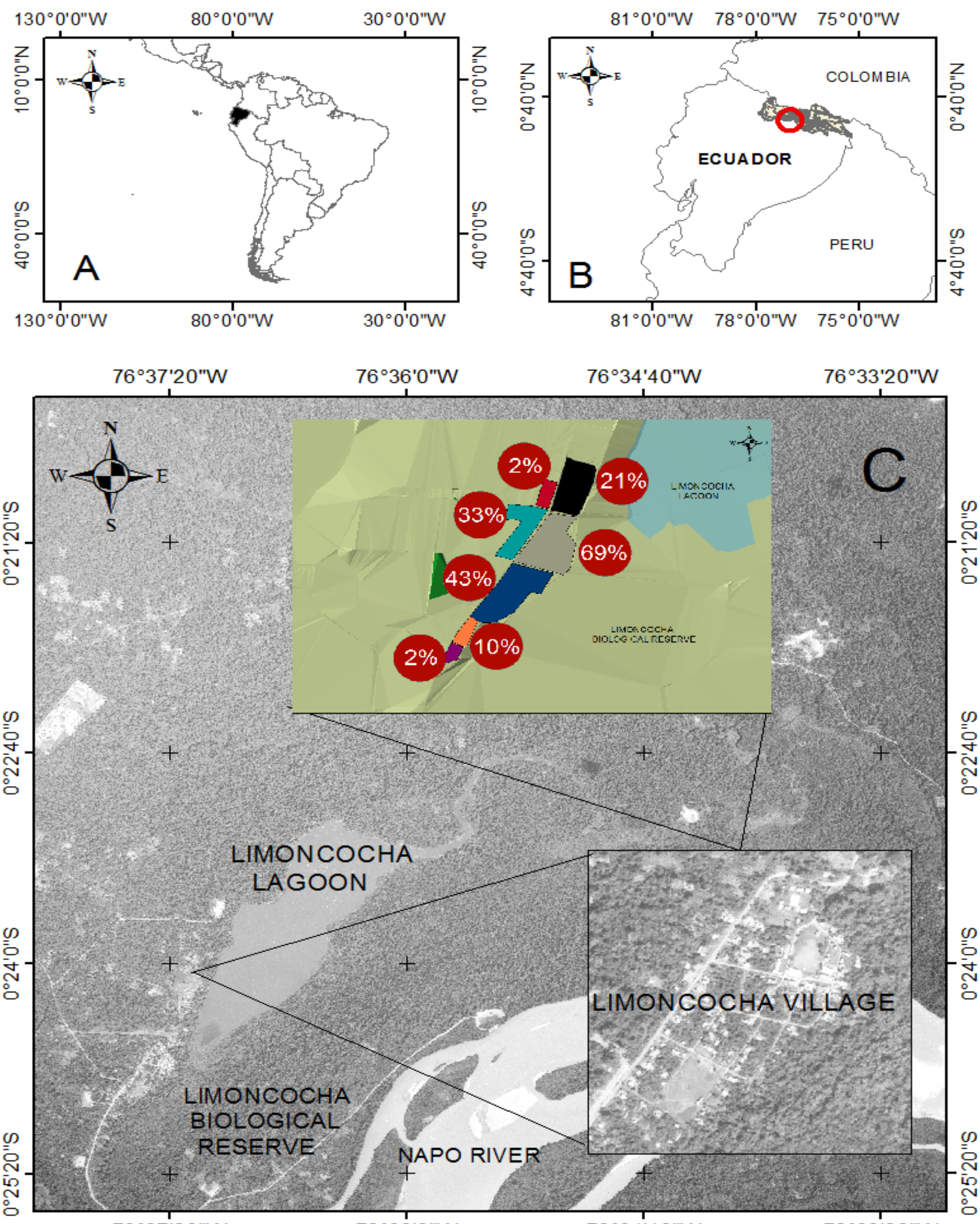

Figure 1. Map of locality. A. Ecuador location in South America, B. Sucumbíos Province location in Ecuador, C. Limoncocha Amazonian village with the percent of mosquito diversity by neighborhoods.

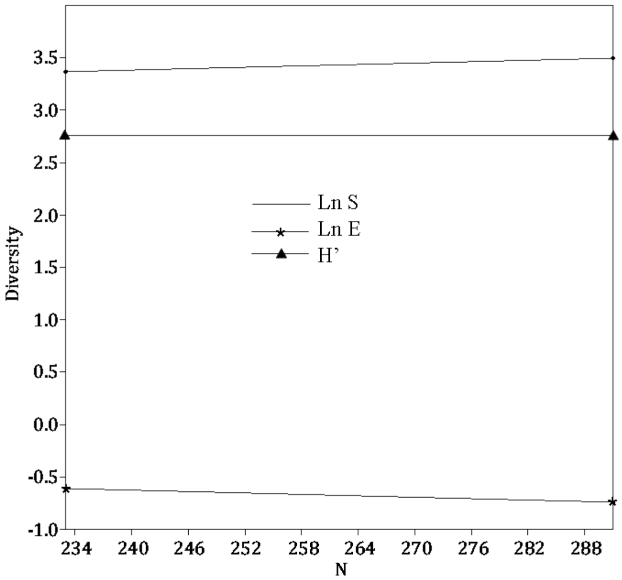

Figure 2. Graph of SHE analysis (S – species, H – Shannon-Wiener diversity index and E – Evenness index) for mosquito assemblages within the Limoncocha village.

Table 1. Species richness by collection site and sampling technique within Limoncocha village.

SPECIES	ENL	ENI	Urban Area		
	CDC	CDC	MAs	ADr	CDC
Aedes (Ochlerotatus) fulvus Wiedemann †		1			
Ae. (Stegomyia) aegypti Linnaeus †				1	
Anopheles (Anopheles) apicimacula Dyar & Knab †					2
An. (Ano.) nr. mattogrossensis Lutz & Neiva †	15		1		33
Coquillettidia (Rhynchotaenia) albicosta Peryassu **	3				18
Cq. (Rhy.) juxtamansonia Chagas	5				6
Culex (Carrollia) bonnei Dyar				15	
Cx. (Car.) infoliatus Bonne-Wepster & Bonne				5	
Cx. (Car.) secundus Bonne-Wepster & Bonne				11	
Cx. (Culex) sp1					1
Cx. (Cux.) sp2					5
Cx. (Cux.) declarator Dyar & Knab †				10	
Cx. (Cux.) nigripalpus Theobald †	1			3	17
Cx. (Melanoconion) nr. theobaldi Lutz 1904					1
Cx. (Cux.) quinquefasciatus Say †	1			1	1
Cx. (Mel.) nr. portesi Senevet & Abonnenc					4
Cx. (Mel.) sp1	1				
Cx. (Mel.) sp3	3				3
Cx. (Mel.) spissipes Theobald †	11	4			45
Cx. (Mel.) panocossa Dyar ** †	9				7
Johnbelkinia longipes Fabricius †			2	1	
Limatus asulleptus Theobald				2	
Li. durhami Theobald †				16	
Mansonia (Mansonia) humeralis Dyar & Knab	1				8
Ma. (Man.) wilsoni Barreto & Coutinho	1				
Psorophora (Grabhamia) dimidiata Cerqueira** †	1				
Toxorhynchites sp. 1				1	
Trichoprosopon compressum Lutz †				1	
Uranotaenia (Uranotaenia) briseis Dyar **	1				1
Ur. (Ura.) calosomata Dyar & Knab					1
Ur. (Ura.) geometrica Theobald					1
Ur. (Ura.) lowii Theobald					1
Wyeomyia melanocephala Dyar & Knab				8	
Abundance	53	5	3	75	155
Richness	13	2	2	13	18

ENL: Ecotone near to lagoon, ENI: Ecotone near to intervened area, MAs: Manual Aspirator, ADr: Absorber dropper, **New record for mosquito fauna from Ecuador, † species with vector disease role