Journal of Medical Entomology, XX(X), 2021, 1–9
doi: 10.1093/jme/tjab113
Research

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Arthropod/Host Interaction, Immunity

Host-Feeding Patterns of the Mosquito Assemblage at Lomas Barbudal Biological Reserve, Guanacaste, Costa Rica

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Subject Editor: Maria Anice Sallum

Received 4 February 2021; Editorial decision 22 May 2021

Abstract

Mosquito-borne pathogens have spread throughout tropical regions of the Western Hemisphere causing increased burden of disease in the region. Outbreaks of dengue fever, yellow fever, chikungunya, West Nile, and Zika have occurred over the past several years. Mosquito blood-feeding patterns need to be assayed to assist in determining which vertebrates could act as hosts of these mosquito-borne pathogens and which mosquito species could act as vectors. We conducted bloodmeal analyses of mosquitoes collected at Lomas Barbudal Biological Reserve, a dry tropical forest reserve in Costa Rica. Mosquitoes were collected using backpack aspirators and light, gravid, and resting traps, and then identified morphologically. Blood-fed mosquitoes underwent DNA extraction, PCR amplification, and sequencing of the vertebrate cytochrome b and cytochrome c oxidase 1 genes to identify vertebrate bloodmeal hosts. Several mosquitoes known to vector pathogens were found including *Culex (Melanoconion) erraticus* Dyar & Knab (Diptera: Culicidae), *Cx. (Mel.) pedroi* Sirivanakarn & Belkin, *Aedes (Stegomyia) albopictus* Skuse, *Ae. (Ochlerotatus) scapularis* Rondani, *Ae. (Och.) serratus* Theobald, and *Ae. (Och.) taeniorhynchus* Wiedemann. The most common bloodmeal hosts were basilisk lizards (*Basiliscus vittatus*) Wiegmann (Squamata: Corytophanidae) in *Culex* (Linnaeus) species and white-tailed deer (*Odocoileus virginianus*) Zimmermann (Artiodactyla: Cervidae) in *Aedes* (Meigen) species. These results show the diversity of mosquito species in a tropical dry deciduous forest and identify associations between mosquito vectors and potential pathogen reservoir hosts. Our study highlights the importance of understanding interactions between vector species and their hosts that could serve as predictors for the potential emergence or resurgence of mosquito-borne pathogens in Costa Rica.

Key words: mosquito-borne disease, ecology, feeding-behavior, molecular biology, tropical entomology

Understanding mosquito-borne pathogen (MBP) transmission requires knowledge of interactions between vectors, pathogens, the environment, and bloodmeal hosts (Oliveira et al. 2018). Specifically, blood-feeding patterns are integral for understanding vectorial capacity of mosquito species (Kent 2009, Oliveira et al. 2018). Females take multiple bloodmeals during their lifetime which may facilitate the transmission and spread of MBPs between different host animal populations (Chaves et al. 2010). To determine the structure of ecological networks and the potential transmission dynamics of MBPs, there is a need to understand the natural interactions between mosquitoes and their bloodmeal hosts. These interactions can be characterized by using molecular and serological tools to help define the taxonomic origin of mosquito vertebrate host bloodmeals (Washino and Tempelis 1983, Kent, 2009, Reeves et al. 2018).

The circulation of several MBPs have been reported in Costa Rica, including Zika virus (ZIKV) (Sanchez et al. 2019), Chikungunya

virus (CHIKV) (Cauchemez et al. 2014), dengue virus (DENV) (Troyo et al. 2006), Venezuelan equine encephalitis virus (VEEV) (Martin et al. 1972), West Nile virus (WNV) (Hobson-Peters et al. 2011), Eastern equine encephalitis virus (EEEV), and *Plasmodium* spp. parasites (Chaves et al. 2020). However, DENV is the most prevalent MBP in the country, affecting mainly the North Pacific, Atlantic, and Central Pacific regions (Troyo et al. 2006). The largest dengue outbreak in Costa Rica occurred in 2013, with almost 50,000 cases suspected on the basis of laboratory testing and/or clinical symptoms (Soto-Garita et al. 2016). The role of nonhuman vertebrate hosts in the transmission of these MBPs is unclear in Costa Rica, however, recent studies have uncovered evidence of their potential involvement in pathogen transmission cycles. For instance, various studies have shown molecular and serological evidence of DENV and WNV in Costa Rican wildlife, including bats, nonhuman primates, and sloths (Vicente-Santos et al. 2017, Dolz et al. 2019). However, it is unclear from these studies which mosquito species could potentially function as enzootic vectors in natural habitats since *Ae. (Stg.) aegypti* Linnaeus (Diptera: Culicidae) is predominantly associated with urban settings. Moreover, which of these wild vertebrates maybe potential amplifying hosts or reservoirs for these pathogens and whether they could serve as disease sentinels for these pathogens have not been well-studied.

In order to understand the patterns of pathogen transmission in new geographical regions and how they increase global disease burden, it is important to study the ecological characteristics of known and potential mosquito vectors, including their blood-feeding patterns (Chaves et al. 2010). The goal of our study was to document the species diversity and blood-feeding hosts of mosquitoes collected in a dry tropical forest of northwestern Costa Rica. We conducted morphological identification of mosquito species and molecular identification of bloodmeal hosts. Our results reveal previously unreported blood-feeding relationships which may help to understand current or future patterns of transmission of MBPs.

Methods

Description of Study Site

Our study was conducted at the Lomas Barbudal Biological Reserve (LBBR), located in Guanacaste Province. This reserve has an area of 2,400 ha and is located approximately 15 km southwest of the town of Bagaces in the Pacific lowland watershed, with an elevation of 10–180 m (Frankie et al. 1988). It is primarily classified as a secondary growth dry deciduous and riverine forest and, though LBBR has been subjected to various disturbances (i.e., hunting and fires), it has remained largely intact. This region receives 1,000–2,200 mm of rain annually between the months of May and November. During the driest part of the year, between mid-December and May, most deciduous trees lose their leaves in this region (Frankie et al. 1988). The reserve is surrounded by small farms and residences, while a building located within the reserve serves as a park ranger residential station and visitor center.

The most common tree species found at LBBR include *Astronium graveolens*, *Spondias mombin*, *Luehea* spp., and *Tabebuia* spp. (Frankie et al. 1988). Vertebrate species reported at LBBR include white-faced capuchin monkeys (*Cebus capucinus*) and mantled howler monkeys (*Alouatta palliata*), jaguars (*Panthera onca*), jaguaroundis (*Herpailurus yagouaroundi),* armadillos (*Dasypus novemcinctus)*, opossums (*Didelphis marsupialis*), domestic dogs (*Canis familiaris*), caimans (*Caiman crocodilus*), coatis (*Nasua narica*), green iguana *(Iguana iguana*), raccoons (*Procyon lotor*), pigs (*Sus domesticus*), horses (*Equus caballus*), white-tailed deer (*Odocoileus virginianus*), cows (*Bos taurus indicus*), peccaries (*Tayassu tajacu*), agoutis (*Dasyprocta punctata*), as well as numerous species of small rodents, bats, birds, and amphibians (Personal communications with Susan Perry and Mahmood Sasa. For a more comprehensive list of vertebrate species found at LBBR, see Rose et al. 2003).

Collection Methods

Sampling was conducted at LBBR on two separate occasions. The first collection period was from 8th to 14th July in 2017. The second was from 3rd to 10th July in 2018. Mosquitoes were collected along a walking trail that extended from the park ranger residential station and visitor center, and the trail for the most part bordered Cabuyo River (Figure 1). In both 2017 and 2018, mosquitoes were collected daily using Prokopack aspirators (John W. Hock Company, Gainesville, FL) by three collectors. Aspiration is an effective method of capturing blood-fed mosquitoes because mosquitoes captured while resting tend to have a higher proportion of bloodmeals compared to other trapping methods, especially light traps (Williams et al. 2006, Burkett-Cadena et al. 2013, Brown et al. 2018). Mosquitoes were collected inside the reserve along the main walking trails, rocky riverbanks, and the park ranger station building. Specific resting habitats sampled included in and around vegetation, ground cover, tree cavities, woody debris and rocks, water-storing plants (i.e., bromeliads), building interior/exterior walls, and exterior human-made objects. In both years, aspiration sampling occurred only between the times of 07:00-12:15 h and 15:00–18:00 h. The exact amount of time each collector spent aspirating was recorded, yielding approximately 20 h of cumulative aspiration effort in 2017 and 26 h in 2018.

In order to enhance mosquito sampling, and to increase species diversity, collections were conducted using additional methods. During the 2017 sampling period, in addition to the aspiration methods described above, collections were conducted using three resting traps (BioQuip, Rancho Dominguez, CA) and three gravid traps (John H. Hock Company, Gainesville, FL). These traps were placed in three groups of two traps (one resting and one gravid) approximately 30 m apart, along the main preserve trail and near the park ranger building. They were set up for seven days and activated every evening at 16:00 h with specimens collected the next morning at 8:00 h, totaling 672 in hours of trapping collection effort. The gravid trap water bait solution consisted of a prepared mixture of tap water, baking yeast, and grass clippings and was “aged” for several days before use. During the 2018 sampling period, in addition to the aspiration methods described above, collections were conducted using two CDC mini light traps (BioQuip, Rancho Dominguez, CA) but no gravid or resting traps. These light traps were hung along the main preserve trail from tree branches about 1.2 m off the ground. Because of the unavailability of dry ice as complementary bait for these light traps, we used cotton balls with a few drops of octanol (BioQuip, Rancho Dominguez, CA) placed near the trap’s light bulb as an additional attractant. The light traps were set for three evenings at 16:00 h and specimens collected the following morning at 8:00 h totaling in 96 h of trapping collection effort.

All specimens collected into aspiration cups and traps were immediately placed in plastic bags containing cotton balls soaked in 90% acetone to kill the specimens. After discarding other non-mosquito arthropods and vegetation debris, mosquito specimens were then transferred to standard 100 mm petri dishes (Fisher Scientific, Pittsburgh, PA) and stored at –20 °C prior to identification. Each

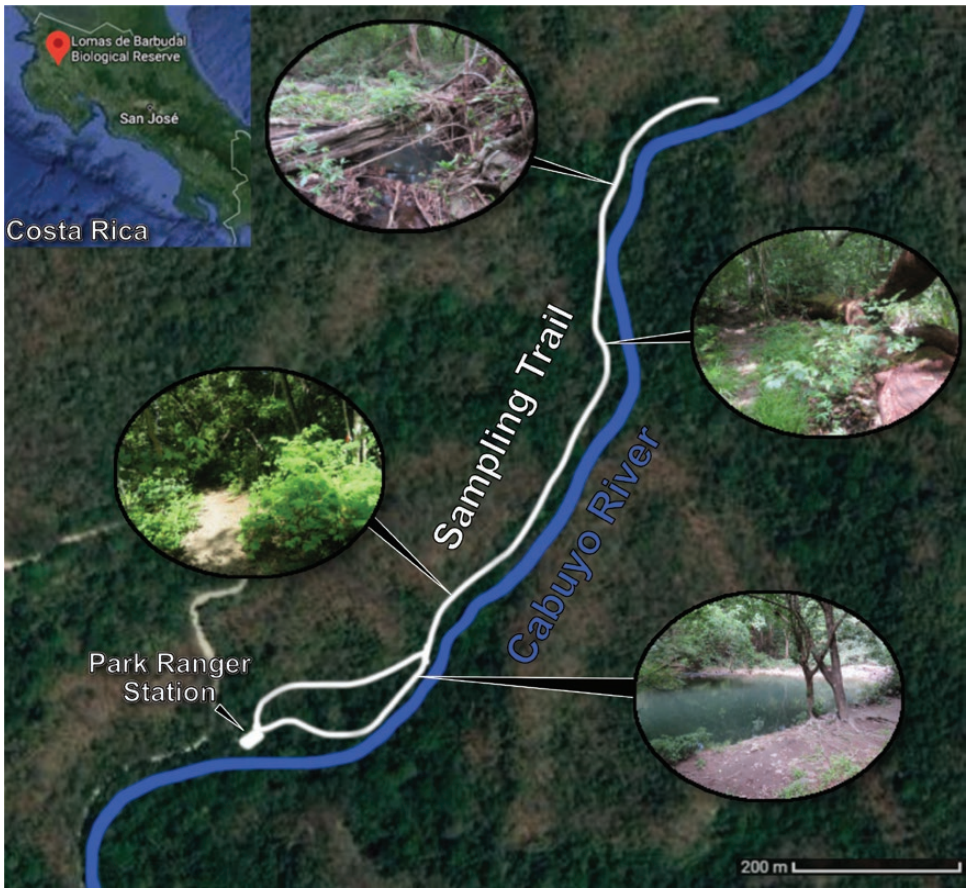

Fig. 1. Partial map of Lomas Barbudal Biological Reserve, Guanacaste, Costa Rica. All sampling occurred on the indicated trail. Pictures of select locations along the trail are shown to display different habitat types and features such as woody debris, foliage, and the margins of Cabuyo River. The satellite view was obtained from https://www.google.com/maps

plate was labeled with the collection method, researcher initials, location, time, and date.

This study was granted permits from Costa Rica's Ministry for the Environment and Energy and National Commission for the Management of Biodiversity (R-030-2017-OT-CONAGEBIO) for the collection and processing of mosquitoes and genetic material.

Mosquito Identification and Bloodmeal Scoring

All mosquitoes collected per sample site and method were sorted and placed in labeled microcentrifuge tubes. Both male and female mosquitoes were identified under a dissection microscope while maintained at a cool temperature using a petri dish on top of a portable chill table (BioQuip, Rancho Dominguez, CA). The taxonomic identification of specimens was based on morphological features following the keys of Berlin and Berkin (1980), Clark-Gil and Darsie (1983), Chaverri (1995), and Potter (2017, 2018). Specimens difficult to identify due to damage or lack of identifiable features were classified only to genera or subgenera. For the purposes of subgenera identification, *Ochlerotatus* Lynch Arribalzaga is recognized as a subgenus of *Aedes* Meigen (Walter Reed Biosystematics Unit 2021). The degree of bloodmeal digestion in blood-fed specimens was classified using the Sella scale (Darsie and Ramos 1969) from stage II (recently fed and fully engorged) to stage VI (bloodmeal almost completely digested). The Sella scale provides a simple and standardized visual method for determining the stage of bloodmeal digestion within a mosquito and is useful to assess the period over which a given molecular method will be effective for bloodmeal identification (Brugman et al. 2017). All blood-fed females were preserved in 95% ethanol (for 2017) and stored at –20°C (for both 2017 and 2018) until molecular bloodmeal analysis.

Bloodmeal Identification

Blood-fed mosquitoes were transported to the University of Costa Rica in San Jose (Centro de Investigación en Enfermedades Tropicales, CIET) and stored at –20°C until DNA extraction. In 2017, mosquitoes were selected for DNA extraction if they contained any visible traces of a bloodmeal (Sella stages II to VI). In 2018, in order to improve our rate of bloodmeal identification, mosquitoes were prioritized for DNA extraction if their bloodmeals corresponded to minimal stages of digestion (Sella stages II and III), although some mosquito specimens were still chosen that showed more advanced stages of digestion (Sella stages IV to VI). DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the protocol described by the manufacturer and stored at –20 °C until further analyses.

Polymerase chain reaction (PCR) assays were used to amplify regions of bloodmeal host DNA while trying to avoid amplification of mosquito DNA. Extracted DNA from engorged mosquitoes underwent different PCR protocols in 2017 and 2018, allowing us to improve our identification rate in 2018 compared to 2017. The PCR primers used in 2017 were designed to amplify the mammalian mitochondrial cytochrome b (*CYTB*) gene

(Kent and Norris, 2005). The PCR cycling conditions differ from what is published, as we modified them to optimize for our own use. The PCRs were performed in total reaction volumes of 25 µl per specimen: 12.5 µl of DreamTaq Master Mix (Thermo Fisher Scientific, Waltham, MA), 9.5 µl of deionized water, 1 µl each of the primers UNFOR403 and UNREV1025 with an initial concentration of 10 µM, and 1 µl of DNA template from extracted engorged mosquito samples. A negative control was also performed by replacing 1 µl of DNA template with 1 µL of deionized water. The PCR was carried out at 94°C for 2 min, followed by 45 cycles at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min, followed by a final extension step of 72°C for 10 min.

The DNA samples from engorged mosquitoes collected in 2018 underwent a nested PCR protocol designed to amplify the mitochondrial gene cytochrome c oxidase 1 (*CO1*) in all vertebrates (Alcaide et al. 2010). This new PCR protocol was implemented to improve the success rate of host DNA amplification and thereby increase the number of bloodmeal identifications, as the proportion of successful bloodmeal identifications was low in 2017. Additionally, some of the DNA samples extracted in 2017 that failed to amplify or coamplified off-target DNA were further analyzed using the Alcaide et al. (2010) nested PCR reaction. The PCR protocol was identical to what is published with one exception. In the second PCR reaction of the nested protocol, some samples underwent anywhere from 22 to 26 cycles as we modified the protocol for our own use.

All PCR products resulting from the aforementioned protocols (2017 and 2018) were separated by electrophoresis on 1% agarose gels at 100 volts for 45 min. DNA samples that yielded a single PCR product close to predicted amplicon sizes were chosen for sequencing. DNA samples that yielded no PCR products were not sequenced. Some samples amplified using the Alcaide et al. (2010) protocol yielded the predicted amplicons size in addition to at least one other amplicon of a different molecular weight which could pose problems to downstream sequencing reactions. To determine whether samples with multiple amplicons could be sequenced, a subset of samples yielding this pattern were also chosen for sequencing. PCR products selected for sequencing were treated with ExoSAP-IT Express PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA). After cleanup, these PCR products were sequenced using Sanger sequencing (Macrogen, Seoul, South Korea). To determine host identity of the bloodmeal, a Basic Local Alignment Search Tool (BLAST) was used to compare sequenced DNA samples to NCBI's nonredundant nucleotide database (Zhang et al. 2000), using default parameters. Sequences that matched ≥95% identity to a vertebrate sequence are reported as probable bloodmeal hosts, as this cutoff value has been previously used for host identification using molecular methods (Molaei et al. 2008, Mendenhall et al. 2012, Blosser et al. 2016).

Results

Mosquito Taxa Collected at LBBR

In 2017, 1,030 mosquitoes were collected (Table 1), mostly via aspiration (1,001 or 97.2%). Twenty-one (2%) were collected in gravid traps and 8 (0.8%) in resting traps. All mosquitoes were identified by sex with 350 (34%) females and 680 (66%) males. Among females, 76 (7.4%) had visible bloodmeals. Genera identified included *Aedes* Meigen, *Culex* Linnaeus, *Coquillettidia* Dyar, *Limatus* Theobald, *Uranotaenia* Lynch Arribalzaga, and *Wyeomyia* Theobald (Table 1).

In 2018, 3,628 mosquitoes were collected (Table 1), again mostly via aspiration (3,400 or 93.7%) while the remainder (228 or 6.3%) were collected via light traps. Of 3,510 mosquitoes identified by sex, 1,673 (47.7%) were females and 1,837 (52.3%) were males. Among females, 190 (11.4%) had visible bloodmeals. Genera identified in 2018 included all of those identified in 2017 except for *Coquillettidia,* and with the addition of *Haemagogus* Williston, *Psorophora* Robineau-Desoidy, and *Toxorhynchites* Theobald.

Combining 2017 and 2018 data, nine genera and 22 species of mosquitoes were identified at LBBR; 3,680 mosquitoes were identified to genera and 978 were left unidentified (Table 1). The most commonly collected genus was *Culex* which represented 84.4% (out of 3,680) of all identified mosquitoes, followed by *Aedes* which represented 14.2%. The other seven genera (*Coquillettidia*, *Haemagogus*, *Limatus*, *Psorophora, Toxorhynchites*, *Uranotaenia*, and *Wyeomia*) cumulatively represent the remaining 1.4% of identified mosquitoes. The three most commonly collected species identified were *Cx.* (*Anoedioporpa*) *restrictor* Dyar & Knab (5.5%), *Ae.* (*Och.*) *angustivittatus* Dyar & Knab (3.6%), and *Cx.* (*Mel.*) *erraticus* Dyar & Knab (2.9%).

Bloodmeal Analyses

From our 2017 collections, we extracted DNA of 60 blood-fed mosquitoes and then conducted the *CYTB* PCR protocol on each sample. Twelve PCR products out of the 60 total DNA extractions met the criteria to be chosen for sequencing. Five of the 12 sequenced PCR products matched ≥95% identity to known vertebrate *CYTB* sequences. Another five most closely matched *Culex*. sp. *CYTB* which indicates off-target amplification. The remaining two sequences matched <95% to a vertebrate sequence. Overall, five bloodmeal hosts were identified. All five of these host identifications were made from mosquitoes with bloodmeals categorized as Sella stage II.

From our 2018 collections, we extracted DNA of 89 blood-fed mosquitoes and then conducted the *COI* PCR protocol. We also attempted to amplify 18 DNA samples extracted during 2017 using this same protocol, totaling 107 samples. Forty-four PCR products out of the 107 total DNA extractions met the criteria to be chosen for sequencing including 15 PCR products with multiple amplicons. Twenty-two of the 44 sequenced PCR products matched ≥95% identity to known vertebrate *COI* sequences, six of which included PCR products with multiple amplicons None of the sequences matched mosquito DNA. The remaining 22 sequences matched <95% to a vertebrate sequence or had no known match to any known sequence. Overall, 22 bloodmeal hosts were identified. Seventeen of these 22 identifications were made from mosquitoes with bloodmeals categorized as Sella stage II and the remaining five were Sella stages III-V. The numbers of blood-fed mosquitoes, DNA extractions, PCR, sequencing reactions, and identifications for both 2017 and 2018 are summarized in Table 2.

Based on the findings from 2017 to 2018, there were 27 bloodmeal identifications and we identified bloodmeals for seven species or taxonomic groups of mosquitoes feeding on eight species of vertebrates (Table 3). Generally, *Culex* species fed upon reptiles and birds, and *Aedes* species upon mammals. The most common bloodmeal vertebrate hosts identified were *Basiliscus vittatus* Wiegmann (Squamata: Corytophanidae) (brown basilisk; 33%) and *Odocoileus virginianus* Zimmermann (Artiodactyla: Cervidae) (white-tailed deer; 33.3%).

Discussion

To our knowledge, this is the first report of species composition and identification of bloodmeal vertebrate hosts of mosquitoes collected

Table 1. Mosquito taxa collected with different collection methods at Lomas Barbudal Biological Reserve, Guanacaste, Costa Rica in 2017 and 2018 and identified to species or genus based on morphological characters

	2017 collection			2018 collection		Combined total
Mosquito taxa	Gravid trap	Resting trap	Aspiration	Light trap	Aspiration	
Aedes, unidentified	10	0	24	14	210	258
Ae. (*Och.*), unidentified	0	0	5	0	0	5
Ae. (*Och.*) *angustivittatus*	0	0	0	5	127	132
Ae. (*Och.*) *dupreii* Coquillett	0	0	2	0	0	2
Ae. (*Och.*) *euplocamus* Dyar & Knab	0	0	0	0	2	2
Ae. (*Och.*) *scapularis* Rondani	0	0	0	0	45	45
Ae. (*Och.*) *serratus* Theobald	0	0	0	0	16	16
Ae. (*Och.*) *stimulans* Walker	0	0	0	0	7	7
Ae. (*Och.*) *taeniorhynchus* Wiedemann	0	0	1	0	49	50
Ae. (*Stg.*), unidentified	0	0	1	0	0	1
Ae. (*Stg.*) *albopictus* Skuse	0	0	0	0	6	6
Coquillettidia, unidentified	0	0	1	0	0	1
Cq. (*Rhy.*) *arribalzagae* Theobald	0	0	1	0	0	1
Culex, unidentified	2	3	673	172	1,679	2,529
Cx. (*And.*), unidentified	0	0	0	0	31	31
Cx. (*And.*) *corrigani* Dyar & Knab	0	0	0	0	2	2
Cx. (*And.*) *restrictor*	3	1	167	0	32	203
Cx. (*Cux.*), unidentified	0	0	3	0	1	4
Cx. (*Cux.*) *nigripalpus* Theobald	0	0	0	0	3	3
Cx. (*Mel.*), unidentified	0	0	17	1	203	221
Cx. (*Mel.*) *erraticus*	0	4	97	0	6	107
Cx. (*Mel.*) *pedroi* Sirivanakarn & Belkin	0	0	0	0	3	3
Cx. (*Mel.*) *theobaldi* Lutz	0	0	1	0	0	1
Cx. (*Tin.*) *latisquama* Coquillett	0	0	0	0	1	1
Hg. (*Hag.*) *equinus* Theobald	0	0	0	0	2	2
Limatus durhamii Theobald	3	0	1	0	2	6
Psorophora, unidentified	0	0	0	0	1	1
Ps. (*Gra.*) *confinnis* Lynch Arribálzaga	0	0	0	0	1	1
Ps. (*Jan.*) *ferox* Humboldt	0	0	0	0	3	3
Toxorhynchites, unidentified	0	0	0	0	2	2
Uranotaenia, unidentified	0	0	4	3	8	15
Ur. (*Ura.*) *calosomata* Dyar & Knab	0	0	0	0	12	12
Ur. (*Ura.*) *hystera* Dyar & Knab	0	0	2	0	0	2
Wyeomyia, unidentified	0	0	4	0	1	5
Unidentified mosquitoes	0	0	0	35	943	978
Total	18	8	1,004	230	3,398	4,658

Table 2. Summary by year of numbers of blood-fed mosquitoes collected at Lomas Barbudal Biological Reserve, DNA extractions, PCR, sequencing reactions, and host identification

Year	Blood-fed mosquitoes collected	DNA extracted	PCR	Sequencing reactions	Host identification
2017	76	60	60	12	5
2018	190	89	89 + 18 from 2017	44	22

at LBBR. Several mosquito species identified in this study are known or suspected vectors of MBPs. The results of our study provide additional information regarding ecological interactions between mosquito species and their vertebrate hosts in northwestern Costa Rica, which also contributes to our knowledge on the potential spread of MBPs in this region of the country.

Bloodmeal Hosts

Uncovering the bloodmeal host range of mosquitoes is relevant to the study and control of MBPs, as some MBPs can change their amplifying host range which could result in novel disease emergence (Weaver and Barrett 2004). Some of the bloodmeal hosts identified in our study have been reported previously as hosts for different mosquito species, such as *Cx. (Cux.) pipiens* feeding on *Crax rubra* Linnaeus (Galliformes: Cracidae) in an urban zoo (Martínez-de la Puente et al. 2020), while we found *Cx. (And.) restrictor* feeding upon *C. rubra*. Additionally, our study was not comprehensive as only 27 individual bloodmeal hosts were identified and the associations between mosquitoes and hosts are therefore tenuous. However, some hosts reported are novel, for at the time of this study, we found no previous published reports of the following species as mosquito bloodmeal hosts: *Anolis cupreus* Hallowell (Squamata: Dactyloidae), *Basiliscus vittatus*, *Iguana iguana* Linnaeus (Squamata: Iguanidae), and *Nasua narica* Linnaeus (Carnivora: Procyonidae). These species should be considered in future studies as potential hosts of MBPs.

One-third of the bloodmeal hosts that we identified in mosquitoes from LBBR were from white-tailed deer (*O. virginianus*; Table 3). White-tailed deer is the most common mammalian source of

Table 3. Bloodmeal hosts of mosquitoes identified with PCR and sampled at Lomas Barbudal Biological Reserve, Guanacaste, Costa Rica

Mosquito species	Total identified	Bloodmeal host species
Cx. (*Mel.*) unidentified	1	*Anolis cupreus*
	8	*Basiliscus vittatus*
Cx. (*Mel.*) *erraticus*	1[a]	*Basiliscus vittatus*
	3[b]	*Iguana iguana*
	1[b]	*Homo sapiens* Linnaeus (Human) (Primates: Hominidae)
	1[b]	*Odocoileus virginianus*
Cx. (*And.*) *restrictor*	1[a]	*Crax rubra*
	1	*Nasua narica* Linnaeus
Ae. (*Och.*) *angustivittatus*	4	*Odocoileus virginianus*
	1	*Bos taurus* Linnaeus (Cow) (Artiodactyla: Bovidae)
Ae. (*Och.*) *scapularis*	1	*Nasua narica*
Ae. (*Och.*) *serratus*	2	*Odocoileus virginianus*
Ae. (*Och.*) *taeniorhynchus*	1	*Odocoileus virginianus*
Ae. (*Stg.*) *albopictus*	1	*Odocoileus virginianus*

All samples are from 2018 unless otherwise specified.
[a]Mosquito collected in 2017 and bloodmeal reanalyzed in 2018
[b]Mosquito collected and bloodmeal analyzed in 2017

blood for mosquitoes in rural North American sites (Apperson et al. 2004, Molaei et al. 2008) and has been identified as a probable amplifying host for Jamestown Canyon virus, Cache Valley virus, and Potosi virus (Issel et al. 1972, McLean et al. 1996, Blackmore and Grimstad 1998). Additionally, EEEV has been reported to be pathogenic to white-tail deer in the United States (Tate et al. 2005, Schmitt et al. 2007). In Costa Rica, white-tailed deer are common in dry-tropical forests, especially in Guanacaste, and are a protected species as well as the national symbol of wildlife in the country (Ortega et al. 2011). Their populations have been diminished by hunting and habitat destruction (ACG 2017, CCPC 2020). Since we found that they are also commonly bitten by potential pathogen vectors in Costa Rica, white-tailed deer is another species that should be monitored for MBPs in the region, both for conservation purposes and human health.

Another common bloodmeal host found in our study was *B. vittattus* (brown basilisk lizard, Table 3) fed on by *Cx.* (*Mel.*) species and one *Cx.* (*Mel.*) *erraticus*. *Cx.* (*Mel.*) *erraticus* is known to be an opportunistic feeder in the southeastern United States with bloodmeals predominantly from large mammals, but also birds, reptiles, and small rodents (Cupp et al. 2004, Savage et al. 2007, Cohen et al. 2009). However, a study conducted in Panama found *Cx.* (*Mel.*) *erraticus* feeding predominantly upon reptiles (Christensen et al. 1996). At LBBR, we documented *Cx.* (*Mel.*) *erraticus* feeding on two reptile species (*I. iguana* and *B. vittatus*) and humans (Table 3). Although *B. vittatus* is not known to be a host of MBPs, *I. iguana* has shown low levels of WNV viremia after experimental infection (Klenk and Komar 2003). Some reptilian species have been shown to be potential amplifying hosts for MBPs, including alligators (*Alligator mississippiensis*) for WNV, garter snakes (*Thamnophis sirtalis*) and green anoles (*Anolis carolinensis*) for EEEV and several species of turtles, frogs, toads, lizards, and snakes for CHIKV (Jacobson et al. 2005, White et al. 2011, Bosco-Lauth et al. 2018). Because basilisk lizards are major sources of bloodmeals for mosquitoes in this area, they should be monitored and assessed for their ability to act as amplifying hosts for MBPs in the region.

LBBR Mosquito Assemblage

Pooling together the 2017 and 2018 sampling, we identified a total of 3,680 out of the 4,658 mosquito specimens to genus or species, including nine genera and 22 mosquito species. However, we were not able to confidently identify 21% (978) of the mosquitoes. The mosquito assemblage identified in our study was similar to that found in study sampling resting sites of mosquitoes conducted at the Palo Verde Biological Station, about 20 km south of our study site (Burkett-Cadena et al. 2013). Several species have been reported in both Palo Verde and the LBBR, including *Ae.* (*Och.*) *euplocamus*, *Ae.* (*Och.*) *taeniorhynchus*, *Cx.* (*And.*) *restrictor*, *Cx.* (*Mel.*) *erraticus*, *Cx.* (*Cux.*) *nigripalpus*, and *Li. durhammi*. However, *Mansonia* and *Anopheles* were found in the Palo Verde study but not in our LBBR study. There were some genera found at LBBR but not at Palo Verde, including *Haemagogus*, *Psorophora*, *Wyeomyia*, and *Toxorhynchites*.

Given the close geographic proximity between the two sites, these differences in genera assemblages are unusual but could be explained by methodological and/or ecological differences. Methodologically, the Palo Verde study was conducted in August, while our LBBR study was conducted in mid-July, which could influence temporal differences in mosquito assemblages. Ecologically, the absence of *Mansonia* and *Anopheles* in our collections at LBBR may be due to a lack of suitable oviposition sites and larval habitats and/or lack of sampling in appropriate habitats. *Mansonia* species oviposit eggs on floating leaves of aquatic vegetation and larvae and pupae attach themselves to leaves and roots (Clements 1999). *Anopheles* larval habitats have been positively associated with low levels of aquatic vegetation (Tadei et al. 1998, Mwangangi et al. 2007, Chirebvu and Chimbari 2015). At Palo Verde, collections took place within 1 km of a freshwater marsh which may have been able to support local *Mansonia* and *Anopheles* populations. The Cabuyo River, near collections sites, may not provide adequate still water and aquatic vegetation to support *Mansonia* and *Anopheles* populations which could explain their absence at the LBBR at least in the specific areas we collected mosquitoes (Fig. 1).

The most relevant pathogen vector species found in our study were *Culex* and *Aedes* species. For example, *Cx.* (*Mel.*) *erraticus* and *Cx.* (*Mel.*) *pedroi* Sirivanakarn & Belkin are considered competent vectors of EEEV (Turell et al. 2008, Bingham et al. 2016) and VEEV (Ferro et al. 2003). *Ae.* (*Stg.*) *albopictus* is one of the most invasive and medically significant mosquito species in the world and a known vector of DENV, CHIKV, and ZIKV in some parts of its range (Powers and Logue 2007, Vasilakis et al. 2011, Leparc-Goffart et al. 2014, Musso and Gubler, 2016). Furthermore,

Ae. (*Och.*) *scapularis* and *Ae.* (*Och.*) *taeniorhynchus* are competent vectors of *Dirofilaria immitis* (Macêdo et al. 1998, Manrique-Saide et al. 2010). *Aedes* (*Och.*) *taeniorhynchus* is also an epizootic vector for VEEV (Navarro et al. 2017). Therefore, if cases of disease from these pathogens arise, the status of these mosquito species as potential vectors and their bloodmeal as potential reservoirs should be monitored.

Methodological Limitations

Modifications to our methods from 2017 to 2018 greatly improved our bloodmeal identification success (Table 2) and may be helpful for future researchers. As previously reported, bloodmeal DNA amplification success decreases with time since feeding (Oshaghi et al. 2006, Reeves et al. 2018). Furthermore, we observed that the Kent & Norris (2005) PCR protocol was not mammal-specific as it amplified both reptile and mosquito *CYTB*. In contrast, the Alcaide et al. (2010) PCR protocol showed no off-target (anything other than a vertebrate) amplification in our study. However, the Alcaide et al. (2010) was not ideal either because it sometimes produced multiple PCR products for a single sample that may have interfered with Sanger sequencing. There was no apparent pattern to which DNA templates produced multiple amplicons, as multiple amplicon samples were distributed across different species of mosquitoes and vertebrate hosts. Furthermore, some vertebrate host samples that produced the single expected amplicon in one sample produced multiple amplicons in a different sample of the same vertebrate host species. Additionally, the nested PCR protocol requires additional time, effort, and resources to perform, compared to a conventional PCR protocol. Future work will include the use of a protocol published after we completed the current study (Reeves et al. 2018), which requires only a single reaction and improved bloodmeal identification success (Sirot, unpublished data).

Conclusion

Our study focused on documenting interactions between potential mosquito vectors and their vertebrate bloodmeal sources in a northwestern Costa Rica dry tropical forest. The ecology of MBPs involves complex interactions between vectors, hosts, pathogens and environmental factors, such as climate, habitat, and geography. The ideal interface of vectors, hosts, pathogens, and the environment could potentially result in MBP transmission to humans and domestic animals (Oliveira et al. 2018). Our study addresses vector and host interactions, but not pathogens or the environment. Future studies should be extended to other regions of Costa Rica, other types of habitats, and at different times of the year to get a broader context of these ecological interactions. Such studies should also focus on screening both wild mosquitoes and their bloodmeal hosts for MBPs. Furthermore, the recent decision by the Costa Rican government to intentionally flood 113 hectares at LBBR (3.7% of the reserve), to increase the water supply to the neighboring Río Piedras Reservoir (Chaves 2018) could potentially alter the ecology of mosquito populations and their feeding hosts. Changes in land use and watershed structure could alter mosquito populations and their interactions with humans, domestic animals, wildlife, and any potential pathogen transmission (Norris 2004).

Acknowledgments

P. G. was supported by The College of Wooster Department of Biology and Henry J. Copeland Funds. T. K. and D. O. were supported by Westminster College's Drinko Center through the Student Travel Grant, Student Research Grant, and Summer Research Fellowship Program, and Westminster College's Dietz-Sullivan Research Experience. A. T. was supported in part by Universidade Costa Rica EcoVector Research Network (project B9-779). L. K. S. was supported by The College of Wooster's Henry Luce III Distinguished Scholarship Award, the Kendall-Rives Latin American Research Award, and the William H. Wilson Fund Award. We would like to thank Brian Carlson for guidance on bloodmeal analysis, Ivan Coronado for support of lab work, Rolando Moriera-Soto for help with trouble-shooting PCR, and Susan Perry and the staff of Lomas Barbudal Biological Reserve including Manrique Montes Obando, Seidy Rosales, and Orlando Ordóñez for their support during specimen collection. A. T. was supported in part by University of Costa Rica's EcoVector research network (project B9-779). We would like to thank Melania Muñoz and the staff of CONAGEBIO and MINAE for support and helpful guidance through the permitting process. We would like to thank Mick Smith for assistance with creating the sampling map of Lomas Barbudal Biological Reserve. This study was granted permits from Costa Rica's Ministry for the Environment and Energy and National Commission for the Management of Biodiversity (R-030-2017-OT-CONAGEBIO) for the collection and processing of mosquitoes and genetic material.

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