EcoHealth 16, 726–733, 2019
https://doi.org/10.1007/s10393-019-01442-2

EcoHealth

Original Contribution

Insights into the Host Specificity of Mosquito-Borne Flaviviruses Infecting Wild Mammals

Jesús Sotomayor-Bonilla,[1,2] María José Tolsá-García,[1,2] Gabriel E. García-Peña,[1,2,3] Diego Santiago-Alarcon,[4] Hugo Mendoza,[1,2] Paulina Alvarez-Mendizabal,[1,2] Oscar Rico-Chávez,[1,2] Rosa Elena Sarmiento-Silva,[5] and Gerardo Suzán[1,2]

[1]*Laboratorio de Ecología de Enfermedades y Una Salud, Departamento de Etología, Fauna Silvestre y Animales de Laboratorio, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Circuito Interior S/N, Ciudad Universitaria, Coyoacán, 04520 Ciudad de México, Mexico*

[2]*Asociación Mexicana de Medicina de la Conservación Kalaan Kab AC, Ciclistas 63 Col. Country Club, Coyoacán, Ciudad de Mexico, Mexico*

[3]*Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Circuito Interior S/N, Ciudad Universitaria, Coyoacán, Ciudad de México, Mexico*

[4]*Red de Biología y Conservación de Vertebrados, Instituto de Ecología AC, Carretera Antigua a Coatepec 351, Xalapa, Veracruz, Mexico*

[5]*Departamento de Microbiología e Inmunología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Circuito Interior S/N, Ciudad Universitaria, Coyoacán, Ciudad de México, Mexico*

Abstract: Mosquito-borne flaviviruses (MBFVs) are of public and animal health concern because they cause millions of human deaths annually and impact domestic animals and wildlife globally. MBFVs are phylogenetically divided into two clades, one is transmitted by *Aedes* mosquitoes (*Ae*-MBFVs) associated with mammals and the other by *Culex* mosquitoes (*Cx*-MBFVs) associated with birds. However, this assumption has not been evaluated. Here, we synthesized 79 published reports of MBFVs from wild mammals, estimating their host. Then, we tested whether the host specificity was biased to sampling and investigation efforts or to phylogenetic relationships using a viral phylogenetic tree drawn from analyzing whole flavivirus genomes obtained in GenBank. We found in total 18 flaviviruses, nine related to *Aedes* spp. and nine to *Culex* spp. infecting 129 mammal species. Thus, this supports that vectors are transmitting MBFV across available host clades and that ornithophilic mosquitoes are readily infecting mammals. Although most of the mosquito species are generalists in their host-feeding preferences, we also found a certain degree of MBFV's specificity, as most of them infect closely related mammal species. The present study integrates knowledge regarding MBFVs, and it may help to understand their transmission dynamics between viruses, vectors, and mammal hosts.

Keywords: Mammals, *Flaviviridae*, Mosquitoes, Virus–host association, West Nile virus, Dengue

Background

Mosquito-borne flaviviruses (MBFVs; genus Flavivirus, family *Flaviviridae*) produce dengue fever, Zika virus disease, and West Nile encephalitis among other diseases

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s10393-019-01442-2) contains supplementary material, which is available to authorized users.

Published online: October 29, 2019

Correspondence to: María José Tolsá-García, e-mail: marijotols@hotmail.com

affecting millions of people in more than 120 countries (Gould et al. 2017). Some MBFVs can be fatal in domestic animals, *e.g*,. West Nile virus (WNV) in horses (Calistri et al. 2010). Other MBFVs seriously threaten wild populations, like Yellow fever virus (YFV) and WNV, which caused mortality events of *Alouatta* primates and birds, respectively (Holzmann et al. 2010; Kilpatrick et al. 2013).

Interactions of viruses and their vertebrate and invertebrate hosts are influenced by multiple factors including the ecology, biology, and evolution of these three (Lobo et al. 2009, Grubaugh and Ebel 2016). For example, MBFVs are transmitted by several mosquito genera with great plasticity of their feeding behavior that depends on multiple intrinsic (e.g., genetic) and extrinsic (e.g., presence of available warm-blooded hosts) factors (Takken and Verhulst 2013). Therefore, these factors determine strongly their transmission dynamics in the multi-host, multi-pathogen systems.

Phylogenetically, this viral group is divided into two vector clades, one is commonly linked with mosquitoes of the genus *Aedes* (*Ae*-MBFVs) and the other is frequently associated with mosquitoes of the genus *Culex* (*Cx*-MBFVs) (Gupta et al. 2014). Nonetheless, these two MBFV groups have already been reported infecting a wide range of wild mammal species. In addition, there are certain generalizations about MBFVs–host associations. Similarly, generalizations occur in the mosquito feeding preferences, for example, *Aedes* spp. are primatophilic and research regarding dengue viruses (DENV), YFV, and Zika virus (ZIKV) is biased toward nonhuman primates.

Furthermore, *Culex* spp. are ornitophilic and research regarding WNV and St. Louis encephalitis virus (SLEV) is biased toward birds, which could underestimate the potential relevance of some mammal groups within viral transmission cycles. Because experiments on most host–parasite associations are not feasible to test, results from widespread epidemiological surveys can be used to measure host specificity of MBFVs and to redirect viral research efforts as needed.

We investigated whether MBFVs are specific to some wild mammal lineages, based on the published records and consensus mammal phylogenies. Particularly, our objectives were (1) to quantify how often wild mammal hosts are reported positive for MBFVs transmitted by *Culex* (ornithophilic) mosquitoes and (2) to estimate specificity of MBFVs to mammal hosts. This study integrates multidisciplinary knowledge and improves understanding of MBFVs' transmission dynamic suggesting new directions for research efforts.

Methods

Database

We searched for reports in the scientific literature in the ISI Web of Knowledge (Thompson-Reuters 2017) using MBFVs' names and mammal as keywords. All studies reporting serology, molecular, and isolation tests were included in our analyses. We did not include review articles, experimental studies, zoo records, and reports that did not specify the MBFV or the host species.

We considered as positive all reports identifying antibodies, viral RNA, viral proteins, or viral isolation. All records suggest microbiological contact between viruses in a susceptible mammal. In this case, antibodies reports recognize an immune response in the host that limits infection. Molecular analyses including the identification of viral proteins and viral isolation show that the virus entered the host cell and replicated in an active infection (Valiakos et al. 2013; Tolsá et al. 2018). Data are available as Supplementary Material 1 (SM1).

Statistical Analyses

We completed a systematic research on the published reports of MBFVs in mammals, and we first performed a two-sided Pearson's Chi-square test to determine whether wild mammals are more likely to be infected with *Ae*-MBFV or *Cx*-MBFV. The null hypothesis was that the number of reports of *Ae*-MBFVs and *Cx*-MBFVs is similar. However, based on mosquito host-feeding preferences (*Culex* and *Aedes* mosquitoes are ornithophilic and primatophilic, respectively), we expected to find a significantly higher number of MBFVs reports of the *Ae*-MBFV clade.

Host Range Estimation

We tested only mammal species reported as positive for MBFVs for the host range estimation. The nomenclature and phylogenies of Bininda-Emonds et al. (2007) were followed to examine whether MBFVs infect specific mammal phylogenetic orders as a function of sampling (number of individuals tested) and investigation efforts (number of studies).

Host phylospecificity index (mpd.z) was used to measure whether these viruses were reported in more closely related hosts than expected by chance (Kembel et al. 2010). This index is built on the mean phylogenetic distance (mpd) between all the possible pairs of hosts infected (with at least one positive report) by MBFVs (mpd.obs). We used the formula: mpd.z = ((mpd.obs − mpd.n)/s(mpd.n)), where mpd.n is the mpd expected for n hosts drawn after randomizing the data for 999 iterations in the mammal phylogeny (Bininda-Emonds et al. 2007) and s(mpd.n) is the standard deviation of mpd.

We estimated mpd.z values for each MBFV that infected at least two mammal species using the R package picante (Kembel et al. 2010). Negative values of mpd.z, significantly different from mpd.n, indicate that viruses are host specialists or infect certain clades/groups of phylogenetically related wild mammals (Kembel et al. 2010). Positive values indicate that viruses are generalists. This index is the inverse value of the net relatedness index (NRI) used by Cooper and colleagues (2012). To ensure that our results were not sensitive to the inference on the branch lengths of the mammal phylogeny, we repeated our analysis by using the three phylogenetic hypotheses reported by Bininda-Emonds and colleagues (2007). We performed analyses in three ways using data of all diagnostic tests, only serological data, and only molecular data (Table 1).

Finally, we tested whether the observed host phylospecificity was not sensitive to bias in the sampling and investigation efforts, or to the phylogenetic relationships between the viruses, by performing a phylogenetic generalized linear model (pglm). In this model, we considered host phylospecificity index as a response variable and as predictors: (1) the number of studies (investigation effort) and (2) the number of individuals tested (sampling effort).

Then, to control for phylogenetic relatedness between viruses, we weighted the analysis with the maximum likelihood of lambda (Pagel 1999; Freckleton et al. 2002). The lambda parameter maximizes the fit of the data to the covariance matrix expected by the relationships between viruses. When lambda is close to 1, this indicates that the observed data are explained by the viral phylogeny. When lambda is close to 0, there is no effect of the phylogeny on the data (Pagel 1999). This measure is robust to incomplete phylogenetic information (Freckleton et al. 2002).

As a surrogate of the phylogeny of flaviviruses, we used a phylogenetic tree drawn from analyzing whole genomes obtained in GenBank (SM3). We aligned sequences using MAFFT and FFT-NS-i strategies (Katoh and Standley 2013). Phylogenetic reconstructions were performed in the MrBayes software (Huelsenbeck and Ronquist 2001), with a GTR model with inverse gamma-distributed rate variation across sites. To obtain a consensus tree, we implemented 20 Markov chains of 20,000,000 generations each, which were sampled every 1000 generations to obtain independent samples of the posterior distribution of the parameters, discarding the first 25% as burn-in. The outcome was a phylogenetic tree supported by 98% of the posterior probabilities (SM3).

Results

General Findings

We found records of nine MBFVs transmitted by *Aedes* mosquito species and nine transmitted by *Culex* mosquito species, reported from 129 wild mammal species out of 218 mammal species tested (Table 1). These species belong to 45 families and ten different orders (Fig. 1, SM4). Additionally, we observed significantly more positive records of MBFVs transmitted by *Culex* mosquitoes ($n = 352$) than those transmitted by *Aedes* mosquitoes ($n = 159$) in wild mammals ($\chi^2 = 61.088$, $df = 1$, $P < 0.0001$, SM1).

Difference in Host Specificity of MBFVs

A total of 61 wild mammal species with positive reports of *Ae*-MBFVs were documented, of which, 42, 19, and 11 of them were positive to Dengue virus (DENV), YFV, and Zika virus (ZIKV), respectively. We found a total of 94 mammal species with positive reports of *Cx*-MBFV, of which, 57, 33, and 24 different mammal species were positive for WNV, St. Louis encephalitis virus (SLEV), and Japanese encephalitis virus (JEV), respectively (Fig. 1).

Host phylospecificity values varied from positive values observed in DENV (mpd.z 1.40) to the most negative values observed for NTAV (mpd.z − 5.27) (Table 1). We found that ten MBFVs phylogenetically clustered with specific mammal groups (i.e., negative index values). For example, WNV is a specialist virus with an mpd.z value of − 2.29, because it is reported in 26 rodent species out of 56 different species (Table 1, Fig. 1).

Quality of these results did not change across the three trees reported by Bininda-Emonds et al. (2007). When we analyzed serological data, only DENV host specificity index changed from positive to negative values, because 19 out of the 42 positive species are bats (Table 1). Using only

Table 1. Host Specificity Indexes of Mosquito-Borne Flaviviruses Reported in Wild Mammals, Including All Diagnostic Tests, Serologic and Molecular Studies, Separately, Positive Mammal Orders, Number of Tested and Positive Mammal Species, Number of Studies, Positive Mosquito Genera, and Reference Identification Number.

Virus	Vector	# studies	# mammal species tested	# taxa/all	mpd.z/all	*P*	# taxa/serol	mpd.z/serol	*P*	# taxa/molec	mpd.z/molec	*P*	References
Banzi virus	*Culex*	1	1	1									(1)
Bussuquara virus	*Culex*	4	3	1									(2, 3)
Cacipacore virus	*Culex*	1	3	0									4
Dengue virus	*Aedes*	15	84	42	1.39	0.92	28	− 1.67	0.03	24	1.37	0.91	(2, 5–18)
Ilheus virus	*Culex*	5	6	4	− 1.15	0.09	4	− 1.06	0.12				(2, 8, 18–20)
Japanese encephalitis virus	*Culex*	10	33	24	− 2.67	0	23	− 2.33	0	2	− 0.8	0.16	(13, 14, 17, 21–28)
Ntaya virus	*Culex*	1	17	6	− 5.25	0	6	− 6.38	0				(29)
Potiskum virus	*Aedes*	1	1	1			1						(1)
Rocio virus	*Culex*	2	4	1			1						(4, 20)
Saboya virus	*Aedes*	1	5										(30)
Spowendi virus	*Aedes*	1	17	4	− 1.94	0.02	4	− 2.05	0.01				(2–5,8–10, 15, 18, 20, 28, 31–47)
St. Louis encephalitis virus	*Culex*	27	84	33	0.48	0.68	33	1.85	0.97				(29)
Tembusu virus	*Culex*	1	1	1			1						(17)
Uganda S virus	*Aedes*	1	9	2	− 1.64	0.05	2	− 1.8	0.05				(13)
Usutu virus	*Culex*	3	4	3	− 0.68	0.2	2	− 0.92	0.13	1			(48–50)
Wesselsbron virus	*Aedes*	3	22	8	− 1.4	0.02	8	− 1.27	0.06				(3, 5, 8, 13, 15, 18, 29, 31, 32, 35–38, 41, 42, 49–76)
West Nile virus	*Culex*	44	113	57	− 2.21	0.01	56	− 1.36	0.1	5	− 0.63	0.25	(1, 29, 69)
Yellow fever virus	*Aedes*	9	31	19	− 1.87	0.02	19	− 1.55	0.04				(1, 2, 78, 4, 5, 8, 10, 20, 39, 69, 77)
Zika virus	*Aedes*	5	31	11	− 2.45	0	9	− 2.23	0.01	2	− 2.55	0.02	(13, 17, 29, 69, 79)

taxa/all = # positive mammals including all diagnostic tests
mpd.z/all = host specificity index including all diagnostic tests.
taxa/serol = # positive host taxa including serological tests.
mpd.z/serol = host specificity index serological tests.
taxa/molec tests = # positive host taxa including molecular tests.
mpd.z/molec = host specificity index including molecular tests.
Refs. References within Table 1 are listed in SM2.
P = *P* value.

molecular data, host specificity indexes were calculated only for DENV, JEV, WNV, and ZIKV. DENV was the only generalist, and it has been reported in 13, 6, and 5 species belonging to the Chiroptera, Didelphimorphia, and Rodentia orders, respectively (Fig. 1, SM1, SM4). Considering the scarce molecular data, JEV, WNV, and ZIKV are specialists to bats, rodents, and primates, respectively.

We did not find consistent or significant associations between host specificity, investigation, and sampling efforts when these variables were controlled for MBFV's phylogeny (F (2, 7) = 1.426, P = 0.3024; CI 95%; Table 2). The maximum likelihood of lambda analyses showed that the host specificity indexes did not have phylogenetic dependence (test of lambda = 0, P = 1; test of lambda = 1, P = 0.25). Inference of the model parameters was adequate as the residual values were normally distributed (Shapiro–Wilk = 0.96, P = 0.7847).

Discussion and Conclusions

We synthesized the published information about MBFVs from wild mammals. We quantified all positive records of MBFVs transmitted by *Culex* mosquitoes in wild mammal hosts and estimated the specificity of MBFVs to mammal hosts (Table 1). In both cases, we found interesting results that should be considered in future field and laboratory studies to understand the MBFV transmission dynamics.

We documented more positive records of MBFVs in wild mammals transmitted by *Culex* than *Aedes* mosquitoes. Firstly, these results suggest that generalizations of mosquito host-feeding preferences should be carefully analyzed, especially if we want to describe MBFVs diversification and viral emergence, since feeding preferences depend mostly on what is available in the local vertebrate community (Takken and Verhulst 2013).

A large array of potential mosquito vectors suggests that there are current interaction possibilities between different viruses and hosts that we have not detected by premises or assumptions. Although it is known that *Aedes* and *Culex* mosquitoes feed preferentially on nonhuman primates and birds, respectively (Takken and Verhulst 2013), we found different potential patterns of feeding behavior. We found more positive reports of MBFVs that are transmitted by ornithophilic *Culex* mosquitoes, suggesting mosquito generalist feeding patterns (e.g., Jansen et al. 2009, Abella-Medrano et al. 2018). *Ae*-MBFVs have been reported in eight mammal orders; all of them, except DENV, are specialized on primates and rodents (Table 1; SM1).

Detailed analysis of vertebrate and mosquito communities that co-occur in time and space should be implemented for MBFV studies, and that means multi-host–multi-vector–multi-pathogens studies should be considered on these viral infections (Stephens et al. 2009). Likewise, the analysis of habitat type and species assemblages in human-dominated landscapes should be contemplated, because *Culex* spp. are mainly distributed by human settlements where other synanthropic species co-occur including rodents and other invasive mammals (Esser et al. 2019).

Similarly, climatic variability on interannual fluctuations of mosquito abundance, hydrological factors, soils, and socioeconomic variables should be considered, especially in those infections that commonly occur in urban and suburban settings like DENV (Kyle and Harris 2008). DENV is a generalist virus that has been reported in five mammal orders, but if we only consider serological data DENV is a specialist, because 13 (out of 28) species are bats (Aguilar-Setién et al. 2008; Machain-Williams et al. 2013; Vicente-Santos et al. 2017).

SLEV was the only generalist virus when we considered all diagnostic tests and serological tests separately. SLEV is distributed across all over the America suggesting unidentified interactions with a wide diversity of vectors and hosts (Reisen 2003).

Our results are consistent with the previous studies suggesting that rodents and bats have ecological traits and life histories that make them good reservoirs of zoonotic pathogens including MBFVs (Plourde et al. 2017; Guy et al. 2019). Bats, rodents, and marsupials deserve special attention given the conclusive evidence of MBFVs infecting them. Similarly, nine out of ten MBFVs are specialists to rodents (WNV, Ntaya virus), bats (JEV), artiodactyls (Usutu virus), or primates/sloths (Ilheus virus).

Thus, if we want to establish effective programs to prevent MBFVs zoonoses, we must expand our research scope to include those insect vectors and vertebrate host species that might be thought unlikely to be involved in transmission cycles. Although, we are still far to identify the true role of each species in the transmission dynamics, multi-host, multi-vector, and multi-pathogen approaches should be applied to pave the way for further studies. Simultaneously, systematic surveillance efforts on mammal communities and MBFVs will help to establish baseline data and then we can further identify potential competent

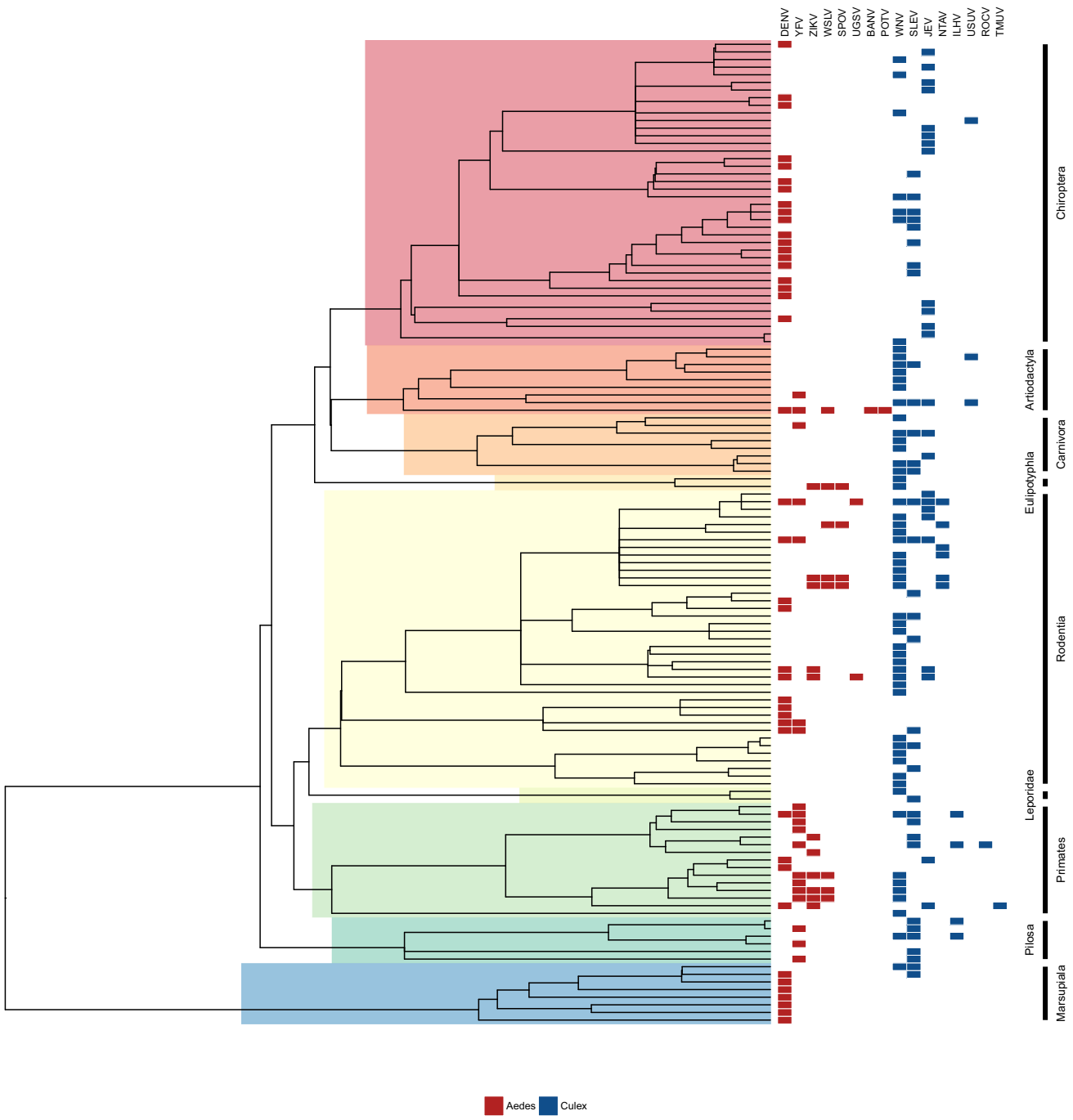

Figure 1. Host phylospecificity of mosquito-borne flaviviruses. Red and blue boxes include MBFVs transmitted by Aedes and Culex mosquitoes, respectively.

hosts for MBFV and implement integrated prevention and management programs with ecosystemic approach.

Although it is difficult to know, both empirically and experimentally, the role of each mosquito vector and mammal host in MBFV transmission cycles is crucial to understand how the system works. We must enhance MBFVs surveillance in as many taxa as possible and should not underestimate the role of other species that were not included in this study. For example, armadillos are not included in our database, but they are a potential host of ZIKV, as a recent experiment showed (Ragan et al. 2017).

It must be recognized that most of the reports found in the literature do not demonstrate the role of a given species in transmitting MBFVs, and many of these species are

Table 2. Estimated Results of the Phylogenetic Generalized Linear Model (pgls).

Predictor	Estimate	Standard error	*t* Value	*P*
Intercept	− 2.91	0.95	− 3.06	0.018
Investigation effort	− 0.09	0.11	− 0.83	0.430
Sampling effort	0.06	0.04	1.42	0.199

incidental hosts. Furthermore, serological tests are prone to classification errors due to crossreactivity by closely related viral species, and they do not necessarily demonstrate viral circulation and it is not possible to identify reservoir competence. Thus, further research on reservoir and vector competence is needed to corroborate our findings and improve our understanding of MBFV dynamics within enzootic and epizootic cycles.

According to our results and the generalized blood-feeding patterns of mosquitoes (Takken and Verhulst 2013), it is necessary to extend MBFVs to other taxa and no mammal species should be neglected in future ecological, epidemiological, and evolutionary studies of MBFVs. Although mosquito feeding preferences seem not to drive the phylogenetic division between MBFV clades, our findings support a degree of mammal host specificity in most (10 out of 12) of the MBFVs, suggesting that either mosquito species feed on phylogenetically related mammal species or that MBFVs are only feasible in specific mammal lineages.

Determining the role of wild mammal hosts and mosquitoes in MBFVs sylvatic cycles is far from being established, but synthesis and analysis of known information, measuring pathogen and host associations (e.g., host specificity), will aid to determine the complexity of MBFVs' cycles including future research agendas, surveillance programs, and public policies. This type of study is necessary because it integrates knowledge of different disciplines, tools, and scales of analysis. The present study may reveal emerging patterns that can not be observed at local scales; thus, macroecological and macroevolutionary approaches are useful to understand the infection dynamics of MBFVs which are important for public and animal health, as well as for wildlife conservation.

Acknowledgements

We acknowledge the Posgrado en Ciencias de la Producción y Salud Animal, UNAM, PAPIIT Project IN221715, and CONACYT PhD grant for JSB.

Funding

This study was funded by the CONACYT PhD grant for JSB.

Compliance with Ethical Standards

Conflict of interest The authors have no competing interests.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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