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Identification of Blood Meals from Potential Arbovirus Mosquito Vectors in the Peruvian Amazon Basin

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Abstract. The transmission dynamics of many arboviruses in the Amazon Basin region have not been fully elucidated, including the vectors and natural reservoir hosts. Identification of blood meal sources in field-caught mosquitoes could yield information for identifying potential arbovirus vertebrate hosts. We identified blood meal sources in 131 mosquitoes collected from areas endemic for arboviruses in the Peruvian Department of Loreto by sequencing polymerase chain reaction amplicons of the *cytochrome b* gene. *Psorophora* (*Janthinosoma*) *albigenu*, *Psorophora* (*Grabhamia*) *cingulata*, *Mansonia humeralis*, *Anopheles oswaldoi* s.l., and *Anopheles benarrochi* s.l. had mainly anthropophilic feeding preferences; *Aedes* (*Ochlerotatus*) *serratus*, and *Aedes* (*Ochlerotatus*) *fulvus* had feeding preferences for peridomestic animals; and *Culex* (*Melanoconion*) spp. fed on a variety of vertebrates, mainly rodents (spiny rats), birds, and amphibians. On the basis of these feeding preferences, many mosquitoes could be considered as potential enzootic and bridge arbovirus vectors in the Amazon Basin of Peru.

Arboviruses are viruses transmitted by arthropods and constitute an important source of human disease,[1] especially in tropical and subtropical areas. The Amazon Basin of Peru has a wide variety of habitats for arthropods and hosts, providing an environment conducive for arbovirus transmission. Several arboviruses, including dengue virus, St. Louis encephalitis virus, Ilheus virus, Venezuelan equine encephalitis virus (VEEV), Mayaro virus (MAYV), Oropouche virus (OROV), Guaroa virus, and group C viruses, are endemic to the Peruvian Amazon Basin and have been associated with human disease.[2,3]

Field and laboratory studies have implicated some mosquito species as vectors of arboviruses in the Peruvian Amazon Basin, including *Culex* (*Melanoconion*) spp. and *Psorophora* spp.[4,5] However, the transmission cycle and vertebrate hosts of many endemic arboviruses are poorly understood.[6,7] This information is critical because understanding the vertebrate hosts involved in the transmission of arboviruses could help in the design and implementation of control strategies against arbovirus outbreaks. The purpose of this initial study was to use molecular techniques to identify blood meals in putative arbovirus vectors in an arboviral enzootic area of the Amazon Basin of Peru.

An entomological survey was carried out from January to March 2009 (rainy season), as part of a health assessment study in two villages in the Province Datem del Marañon (Saramiriza, Puerto America), located along the Marañon River, and two villages in the Province Alto Amazonas (Lagunas, Santa Cruz), located near the Huallaga River. This area consists of small rural communities with high rates of emerging and reemerging infectious diseases[8] (Figure 1).

Mosquitoes were collected using three different methods. Centers for Disease Control and Prevention (CDC) light traps (one trap/day) were set at 3 m above forest ground and more than 100 m from houses; these functioned over a 12-hour interval (6 PM–6 AM) over a period of 2–5 days/site. In addition, human landing catchers (one trap/day), who exposed their legs and aspirated mosquitoes as they landed, collected mosquitoes in peridomestic areas during the early evening (6 PM–9 PM) over a period of 2–4 days/site. Lastly, collections inside houses using backpack aspirators were performed (20 minutes per house) during daylight (8 AM–12:45 PM) over a period of 2–14 days/site. A total of four to nine night traps were set in each site. Backpack aspirations and human landing catcher collections were not performed on same dates as CDC light traps. The use of humans for collecting mosquitoes was approved by the Naval Medical Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects (protocol no. 2009.0002).

Captured mosquitoes were identified to species using dichotomous keys.[9] Female blood-engorged mosquitoes were placed individually and stored at −80°C. Mosquito abdomens were used for blood meal analysis (Supplemental Information). Also, to identify arboviruses from mosquito vectors with coincident host identification of blood meals, mosquito heads and thoraxes were tested on cell culture (C6/36 and Vero 76 cells), and by immunofluorescence assay.[3] In addition, RNA was extracted from the mosquito homogenate using the QIAamp viral RNA kit (Qiagen, Valencia, CA), and generic reverse transcription polymerase chain reaction (RT-PCR) was performed to detect nucleic acid from alphaviruses or flaviviruses.[10,11]

A total of 22,513 mosquitoes were collected during the study, belonging to 11 genera and 37 species (V. Zorrilla and others, unpublished data). The largest number of specimens (16,947 [75.3%] mosquitoes, 35 [94.6%] species) were collected with CDC light traps, followed by human landing catchers (3,888 [17.3%] mosquitoes, 22 [59.5%] species), and backpack aspirators (1,678 [7.4%] mosquitoes, 18 [48.6%] species).

One hundred and forty-six identified mosquitoes (0.6% of total) had evidence of blood in the abdomen and were tested for blood meal identification. Of them, 41, 10, and 95 mosquitoes were captured in a forest, domestic, and

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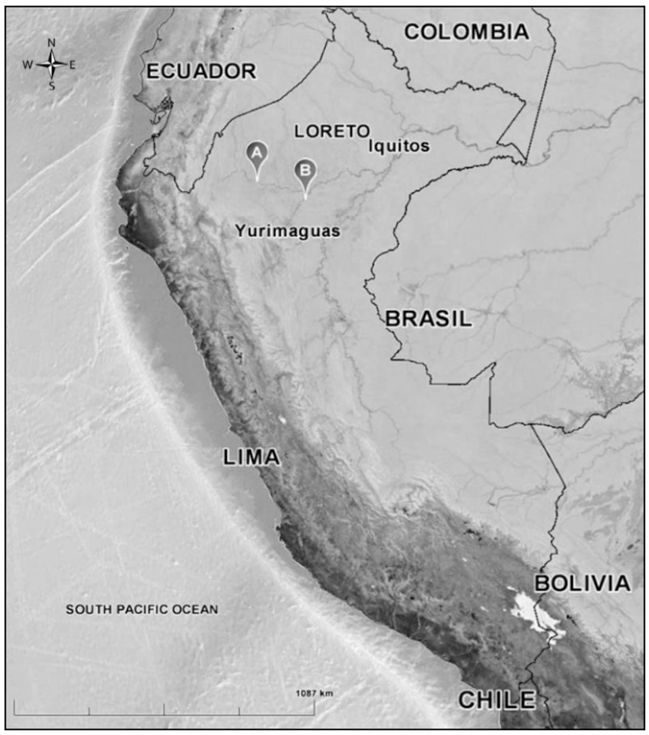

FIGURE 1. Map of Peru showing locations of mosquito collections in the Department of Loreto, Peru. (**A**) Saramiriza, Puerto America, and Nuevo Jerusalen. (**B**) Lagunas.

peridomestic setting, respectively. Specimens belonged to five of 11 (45.4%) genera and 12 of 37 (32.4%) different species: *Psorophora* (*Janthinosoma*) *albigenu* (Peryassu), *Psorophora* (*Grabhamia*) *cingulata* (Fabricius), *Aedes* (*Ochlerotatus*) *serratus* (Theobald), *Aedes* (*Ochlerotatus*) *fulvus* (Wiedemann), *Mansonia humeralis* Dyar and Knab, *Anopheles benarrochi* s.l. Gabaldon, Cova Garcia and Lopez, *Anopheles oswaldoi* s.l. (Peryassu), *Culex* (*Melanoconion*) *occosa* Dyar and Knab, *Culex* (*Melanoconion*) *dunni* Dyar, *Culex* (*Melanoconion*) *portesi* Senevet and Abonnenc, *Culex* (*Melanoconion*) *vomerifer* Komp, *Culex* (*Aedinus*) *amazonensis* (Lutz), and *Culex* (*Melanoconion*) spp. Of 146 samples assayed, blood meal sources were identified by DNA sequencing in 131 (89.7%) (Tables 1 and 2).

In the Province Datem del Marañon sites (Table 1), *Ps. albigenu* preferentially fed on humans ($N = 64$; 97.0% ± 4.2%), with one mosquito also feeding on dogs. *Aedes* spp. mainly fed on humans only, but a few *Ae. serratus* fed on dogs and pigs, and some *Ae. fulvus* fed on cows. The blood meal sources for the single *Cx. vomerifer* and *Cx.* (*Aedinus*) *amazonensis* captured were *Proechimys cuvieri* and *Proechimys brevicauda* spiny rats, respectively (Figure 2). The only source of blood of the single *Cx. portesi* specimen was a cat, whereas other *Culex* (*Mel.*) spp. fed on mammals and birds. Of the three *An. benarrochi* s.l., two fed on dogs and one on a pig. Humans were the only source of blood for *Ma. humeralis*, *Ps. cingulata*, and *An. oswaldoi* s.l., although only 3–5 specimens of each species were tested.

Only 11 specimens were tested from the Province of Alto Amazonas. Single specimens of *Ps. albigenu* and *Ae. serratus* fed on birds, whereas *Cx. vomerifer* and *Cx. ocossa* fed on humans and spiny rats (*Proechimys quadriplicatus*).

TABLE 1
Vertebrate blood meal sources identified in mosquitoes collected in sites located in the Province of Datem del Marañon, Loreto, 2009

Host (% Nt identity)	**Psorophora* (*Janthinosoma*) *albigenu*	*Psorophora* (*Grabhamia*) *cingulata*	*Aedes* (*Ochlerotatus*) *serratus*	*Aedes* (*Ochlerotatus*) *fulvus*	*Mansonia humeralis*	*Anopheles benarrochi* s.l.	*Anopheles oswaldoi* s.l.	*Culex* (*Melanoconion*) *portesi*	*Culex* (*Melanoconion*) *vomerifer*	*Culex* (*Aedinus*) *amazonensis*	*Culex* (*Melanoconion*) spp.
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Mammal											
Homo sapiens "human" (98%)	64 (97)	3 (100)	16 (88)	6 (75)	4 (100)	2 (67)	5 (100)				6 (60)
Sus scrofa "pig" (99%)			1 (6)			1 (33)					
Bos taurus "cow" (99%)	1 (1)			2 (25)							
Canis lupus "dog" (98%)	1 (1)		1 (6)								
Felis catus "cat" (98%)								1 (100)			
Dasyprocta fuliginosa "agouti" (99%)											1 (10)
Proechimys cuvieri "spiny rat" (99%)									1 (100)		1 (10)
Proechimys brevicauda "spiny rat" (99%)	1 (1)									1 (100)	1 (10)
Bird											
Butorides viriscens "green heron" (97%)											1 (10)
Total mosquitoes	66	3	18	8	4	3	5	1	1	1	10

Nt = nucleotide.
*Species with mixed blood meals.

TABLE 2
Vertebrate blood meal sources identified in mosquitoes collected in sites in the Province of Alto Amazonas, Loreto, 2009

Host (% Nt identity)	*Psorophora* (*Janthinosoma*) *albigenu* No. (%)	*Aedes* (*Ochlerotatus*) *serratus* No. (%)	**Culex* (*Melanoconion*) *dunni* No. (%)	*Culex* (*Melanoconion*) *ocossa* No. (%)	*Culex* (*Melanoconion*) *vomerifer* No. (%)	*Culex* (*Melanoconion*) spp. No. (%)
Mammal						
Homo sapiens "human" (98–99%)			1 (100)	1 (100)		1 (17)
Dasyprocta fuliginosa "black agouti" (99%)						1 (17)
Choloepus didactylus (92%)†¢ "sloth"			1 (100)			
Proechimys quadriplicatus "spiny rat" (99%)					1 (100)	
Bird						
Crypturellus undulatus (91%)† "undulated tinamou"						1 (17)
Sclateria naevia "silvered antbird" (98%)						1 (17)
Ixobrychus sinensis (91%)† "yellow bittern"						1 (16)
Crotophaga ani "smooth-billed ani" (98%)	1 (100)					
Gallinula chloropus (91%)† "common moorhen"		1 (100)				
Amphibian						
Bufo spp. "true toads" (99%)						1 (16)
Total mosquitoes	1	1	1	1	1	6

Nt = nucleotide.
*Species with mixed blood meals.
†Identity percentage (91–92%) in the GenBank database.

Culex (*Mel.*) spp. had a variety of blood meal sources from mammals, birds, and amphibians (Table 2). In addition, for one *Cx. dunni*, the sources of blood were from both a human and two-toed sloth (*Choloepus didactylus*). Cell culture and PCR failed to detect an arbovirus in any of the 146 mosquitoes.

This is the first study in the Peruvian Amazon Basin identifying blood meal sources from mosquitoes, like *Culex* (*Mel.*) spp., *Ps. albigenu*, *Ae. serratus*, and *Ae. fulvus*, all thought to be involved in the enzootic and epizootic cycle of arboviruses. *Psorophora albigenu* had mostly anthropophilic feeding preferences, which is consistent with studies conducted in Brazil[12] and in Peru using human landing catchers.[13] In addition, in a few specimens, we found DNA from multiple vertebrates (including a mosquito with two

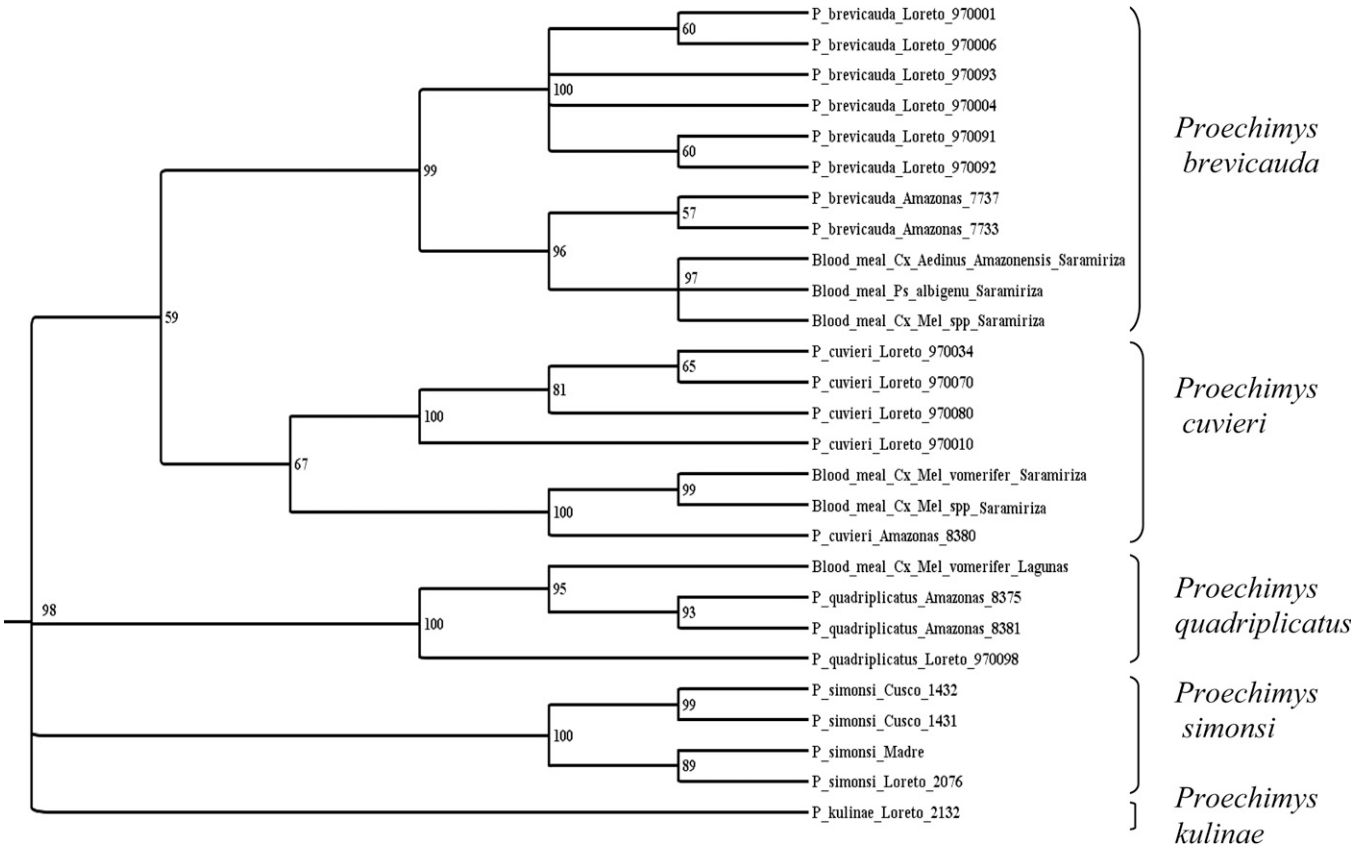

FIGURE 2. Maximum parsimony cladogram for the first 690 base pairs of the *cytochrome b* gene of Peruvian spiny rats *Proechimys*. Numbers at specific nodes are bootstrap values (1,000 replicates) above 50%. Sequences of *Echymis* and *Isothrix* were used as outgroups.

different blood meals) such as spiny rats, cows, dogs, and smooth-billed anis, the latter a suspected host of OROV and MAYV in Brazil.[14] Eclectic feeding behavior of *Ps. albigenu* has been reported in forest-protected areas of Brazil,[15,16] suggesting *Ps. albigenu* feeding preference was based on host availability. Our data suggests that *Ps. albigenu* could be a bridge vector in the transmission of alphaviruses between animals and humans. This observation is supported by the fact that *Ps. albigenu* is susceptible to VEEV and eastern equine encephalitis virus (EEEV) infection and is able to transmit these viruses.[4,5,7]

Aedes serratus and *Ae. fulvus* had anthropophilic and peridomestic feeding preferences in our study, findings consistent with previous reports from Brazil.[9,16] In the Amazon Basin, *Ae. serratus* and *Ae. fulvus* have yielded isolates of alphaviruses such as Trocara virus, Una virus, and EEEV.[7] Even though *Ae. fulvus* has been shown to be susceptible to VEEV and EEEV infection, its competence to transmit those alphaviruses was very limited.[4,5] Nevertheless, transmission studies with other alphaviruses circulating in the Amazon Basin are needed to evaluate the vector competence of *Aedes* (*Och.*) spp.

Culex vomerifer fed on spiny rats, including *P. quadriplicatus* and *P. cuvieri*. Phylogenetic analysis, using the *cyt b* sequences obtained from mosquito blood meals in this study and compared with the *cyt b Proechimys* database, identified separate species clades with monophyletic support (Figure 2). Spiny rats (Family Echimyidae) are considered to be enzootic hosts of VEEV that develop little or no disease after infection.[17] Our data demonstrated that *Culex* (*Mel.*) spp. fed on *Proechimys* spp. In addition, cases of VEEV subtype ID have been reported in Yurimaguas, a nearby location, suggesting possible enzootic VEEV (ID) transmission involving *Culex* (*Mel*) spp. and *Proechimys* spp.[18] *Culex dunni*, which has been implicated as a vector of VEEV and EEEV,[7] had blood meals of both sloth and human origin. *Culex portesi* and *Cx. occosa* had a peridomestic behavior also observed in studies from Brazil.[12] Both mosquito species have been identified as enzootic vectors for VEEV in Trinidad and Panamá.[19]

A limitation of our study was that morphological characterization alone prevented definitive species identification of some *Culex* (*Mel.*) spp. Sequences of internal transcribed spacer 2 (ITS2) of ribosomal DNA have been useful for solving taxonomy and phylogenetic relationships in *Culex* (*Mel.*) spp. mosquitoes.[20] Generation of an ITS2 sequence database from local mosquito specimens could improve the ability to properly identify mosquitoes in the Peruvian Amazon Basin. Also, the wide variety of blood meal sources, many with low GenBank identity (Table 2), in *Culex* (*Mel.*) spp. from Lagunas may be partially attributable to an absence of local host *cyt b* sequences in the database. Another limitation was that none of the mosquitoes tested yielded an arbovirus, which is not surprising due to the low infectivity rate normally found in field-collected mosquitoes.

In summary, our study has provided additional insight on the host-feeding patterns of some potential arboviral vectors in the Peruvian Amazon Basin. Our observations are consistent with previously reported data for *Culex* (*Mel.*) spp.[4,5] and, taken together, the feeding preference of this mosquito species supports a possible role in the enzootic cycle of alphavirus transmission in the Peruvian Amazon Basin. Severe and fatal VEEV (ID) cases have been reported in Lagunas and others localities in the Province of Alto Amazonas,[21] emphasizing the need for better clarification of its transmission dynamics. Future studies could evaluate the seroprevalence of VEEV (ID) and other arboviruses causing human disease in local animal populations and assess the susceptibility of these animals (e.g., spiny rats) to infection with these viruses.

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REFERENCES

1. Weaver SC, Reisen WK, 2010. Present and future arboviral threats. *Antiviral Res 85:* 328–345.
2. Morrison AC, Forshey BM, Notyce D, Astete H, Lopez V, Rocha C, Carrion R, Carey C, Eza D, Montgomery JM, Kochel TJ, 2008. Venezuelan equine encephalitis virus in Iquitos, Peru: urban transmission of a sylvatic strain. *PLoS Negl Trop Dis 2:* e349.
3. Forshey BM, Guevara C, Laguna-Torres VA, Cespedes M, Vargas J, Gianella A, Vallejo E, Madrid C, Aguayo N, Gotuzzo E, Suarez V, Morales AM, Beingolea L, Reyes N, Perez J, Negrete M, Rocha C, Morrison AC, Russell KL, Blair PJ, Olson JG, Kochel TJ; NMRCD Febrile Surveillance Working Group, 2010. Arboviral etiologies of acute febrile illnesses in western South America, 2000–2007. *PLoS Negl Trop Dis 4:* e787.
4. Turell MJ, O'Guinn ML, Dohm D, Zyzak M, Watts D, Fernandez R, Calampa C, Klein TA, Jones JW, 2008. Susceptibility of Peruvian mosquitoes to eastern equine encephalitis virus. *J Med Entomol 45:* 720–725.

5. Turell MJ, Jones JW, Sardelis MR, Dohm DJ, Coleman RE, Watts DM, Fernandez R, Calampa C, Klein TA, 2000. Vector competence of Peruvian mosquitoes (Diptera: Culicidae) for epizootic and enzootic strains of Venezuelan equine encephalomyelitis virus. *J Med Entomol 37:* 835–839.
6. Watts DM, Callahan J, Rossi C, Oberste MS, Roehrig JT, Wooster MT, Smith JF, Cropp CB, Gentrau EM, Karabatsos N, Gubler D, Hayes CG, 1998. Venezuelan equine encephalitis febrile cases among humans in the Peruvian Amazon River region. *Am J Trop Med Hyg 58:* 35–40.
7. Turell MJ, O'Guinn ML, Jones JW, Sardelis MR, Dohm DJ, Watts DM, Fernandez R, Travassos da Rosa A, Guzman H, Tesh R, Rossi CA, Ludwig V, Mangiafico JA, Kondig J, Wasieloski LP Jr, Pecor J, Zyzak M, Schoeler G, Mores CN, Calampa C, Lee JS, Klein TA, 2005. Isolation of viruses from mosquitoes (Diptera: Culicidae) collected in the Amazon Basin region of Peru. *J Med Entomol 42:* 891–898.
8. Sanchez JF, Halsey ES, Bayer AM, Beltran M, Razuri HR, Velasquez DE, Cama VA, Graf PC, Quispe AM, Maves RC, Montgomery JM, Sanders JW, Lescano AG, 2015. Needs, acceptability, and value of humanitarian medical assistance in remote Peruvian Amazon riverine communities. *Am J Trop Med Hyg 92:* 1090–1099.
9. Consoli RA, de Oliveira RL, 1994. *Principais mosquitos de importância sanitária no brasil*. Rio de Janeiro, Brazil: Editora FIOCRUZ.
10. Pfeffer M, Proebster B, Kinney RM, Kaaden OR, 1997. Genus-specific detection of alphaviruses by a semi-nested reverse transcription-polymerase chain reaction. *Am J Trop Med Hyg 57:* 709–718.
11. Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB, 1998. Phylogeny of the genus *Flavivirus*. *J Virol 72:* 73–83.
12. Forattini OP, Gomes Ad C, Natal D, Kakitani I, Marucci D, 1987. Preferências alimentares de mosquitos Culicidae no Vale do Ribeira, São Paulo, Brasil. *Rev Saude Publica 21:* 171–187.
13. Jones JW, Turell MJ, Sardelis MR, Watts DM, Coleman RE, Fernandez R, Carbajal F, Pecor JE, Calampa C, Klein TA, 2004. Seasonal distribution, biology, and human attraction patterns of culicine mosquitoes (Diptera: Culicidae) in a forest near Puerto Almendras, Iquitos, Peru. *J Med Entomol 41:* 349–360.
14. Dégallier N, Travassos da Rosa A, da Silva JC, Guerreiro Rodrigues S, Vasconcelos P, Travassos da Rosa J, Pereira da Silva G, Pereira da Silva R, 1992. As aves como hospedeiras de arbovírus na amazônia brasileira. *Bol Mus Para Emilio Goeldi ser Zoo 8:* 69–111.
15. dos Santos Silva J, Alencar J, Costa JM, Seixas-Lorosa E, Guimaraes AE, 2012. Feeding patterns of mosquitoes (Diptera: Culicidae) in six Brazilian environmental preservation areas. *J Vector Ecol 37:* 342–350.
16. Mucci LF, Junior RP, de Paula MB, Scandar SA, Pacchioni ML, Fernandes A, Consales CA, 2015. Feeding habits of mosquitoes (Diptera: Culicidae) in an area of sylvatic transmission of yellow fever in the state of Sao Paulo, Brazil. *J Venom Anim Toxins Incl Trop Dis 21:* 6.
17. Carrara AS, Gonzales G, Ferro C, Tamayo M, Aronson J, Paessler S, Anishchenko M, Boshell J, Weaver SC, 2005. Venezuelan equine encephalitis virus infection of spiny rats. *Emerg Infect Dis 11:* 663–669.
18. Aguilar PV, Greene IP, Coffey LL, Medina G, Moncayo AC, Anishchenko M, Ludwig GV, Turell MJ, O'Guinn ML, Lee J, Tesh RB, Watts DM, Russell KL, Hice C, Yanoviak S, Morrison AC, Klein TA, Dohm DJ, Guzman H, Travassos da Rosa AP, Guevara C, Kochel T, Olson J, Cabezas C, Weaver SC, 2004. Endemic Venezuelan equine encephalitis in northern Peru. *Emerg Infect Dis 10:* 880–888.
19. Seymour C, 1985. Sloths as hosts of arboviruses. Montgomery GG, ed. *The Evolution and Ecology of Armadillos, Sloths, and Vermilinguas*. Washington, DC: Smithsonian Institution Press, 269–278.
20. Navarro JC, Weaver SC, 2004. Molecular phylogeny of the Vomerifer and Pedroi groups in the Spissipes Section of the subgenus *Culex* (*Melanoconion*). *J Med Entomol 41:* 575–581.
21. Vilcarromero S, Aguilar PV, Halsey ES, Laguna-Torres VA, Razuri H, Perez J, Valderrama Y, Gotuzzo E, Suarez L, Cespedes M, Kochel TJ, 2010. Venezuelan equine encephalitis and 2 human deaths, Peru. *Emerg Infect Dis 16:* 553–556.