An Acad Bras Cienc (2024) 96(2): e20230452 DOI 10.1590/0001-3765202420230452
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

BIOMEDICAL SCIENCES

Diversity and molecular characterization of insect-specific flaviviruses in mosquitoes (Diptera: Culicidae) collected in central and northern Argentina.

ADRIÁN A. FARÍAS, VICTORIA LABERDOLIVE, MARINA STEIN, MARÍA JULIA DANTUR JURI, ANDRÉS VISINTIN, WALTER R. ALMIRÓN, MARTA S. CONTIGIANI, VIVIANA E. RE & ADRIÁN DIAZ

Abstract: The genus Flavivirus comprises approximately 80 different viruses. Phylogenetic relationships among its members indicate a clear ecological separation between those viruses transmitted by mosquitoes, ticks, with no known vector, and insect-specific Flaviviruses. The diversity and phylogenetic relationships among insect-specific flaviviruses circulating in the central and northern regions of Argentina were studied by performing molecular detection and characterization of the NS5 protein gene in mosquitoes collected in Córdoba, Chaco and Tucumán provinces. Overall, 68 out of 1776 pools were positive. CxFV, KRV and CFAV circulate in the 3 studied provinces. Several mosquito species *(Aedes aegypti, Culex bidens, Cx. dolosus, Cx. interfor, Cx. quinquefasciatus, Cx. saltanensis, Haemagogus spegazzini)* were found infected. A wide circulation of CxFV was observed in the central-northern region of Argentina. CxFV strains detected in our study clustered with strains circulating in Santa Fe and Buenos Aires provinces (Argentina), and other countries such as Indonesia, Mexico, Uganda and Taiwan. The presence of these viruses in mosquitoes could play an important role from the public health perspective, because it has been shown that previous CxFV infection can increase or block the infection of the mosquito by other pathogenic flaviviruses.

Key words: ISF, Flavivirus, Culicidae, PCR, molecular characterization.

INTRODUCTION

Members of the genus Flavivirus, family Flaviviridae are enveloped viruses that have a positive-sense single-stranded RNA genome. During recent years cell culture virus isolation and molecular screening of mosquitoes for flaviviral pathogens has resulted in the isolation and detection of numerous novel flaviviruses from various locations that seem to be specific for mosquito hosts. These viruses were previously defined as mosquito-only flaviviruses or insect-specific flaviviruses (ISFs), and have been widely detected in mosquitoes in different countries in the last decades (Blitvich et al. 2015, Calisher & Higgs 2018, Sang et al. 2003, Crabtree et al. 2003). The first virus characterized within the ISF group was cell fusing agent virus (CFAV), which was isolated from an *Aedes aegypti* cell line in 1974 (Stollar et al. 1975, Crabtree et al. 2003). Besides CFAV, many other ISFs, including Culex flavivirus (CxFV), Kamiti River virus (KRV) and Aedes flavivirus (AeFV), have been isolated and characterized (Blitvich et al. 2015, Calisher & Higgs 2018). CxFV is the most frequently reported ISF to date (Fernandes et al. 2018). Based on their phylogenetic and antigenic

relationships, ISFs can be separated into two distinct groups. The largest group consists of the classical insect-specific flaviviruses (cISFs), such as CFAV, CxFV, and KRV. cISFs form a separate clade from the vertebrate pathogenic flaviviruses. The other ISFV group consists of the arbovirus-related or dual host affiliated insect-specific flaviviruses (dISFs) (Blitvich et al. 2015). dISFs are phylogenetically more similar to flavivirus vertebrate pathogens than to cISFs. These similarities raise the possibility that some of the dISFs might modulate arbovirus infection and transmission in a dually infected mosquito host; in addition, they could be useful in the development of potential flavivirus vaccines or reagents (Blitvich et al. 2015, Guzman et al. 2018). However, evidence is scarce, unclear, and in some cases contradictory (Bolling et al. 2011, Huanyu et al. 2012). Despite the search and detection of ISFs in different regions of the world, knowledge for South American countries is scarce. Like studies carried out in Brazil, in which CxFV was isolated from *Culex* sp. (Machado et al. 2012), CFAV in mosquitoes from the Amazon Region (Fernandes et al. 2018), newly Alfavirus (Tscha et al. 2021) and DNA similar to flavivirus from Ae. in Argentina (Bonica et al. 2021). The aim of the present study was to analyze the natural diversity and phylogenetic relationships among ISFs circulating in mosquitoes collected in the central and northern region of Argentina.

MATERIALS AND METHODS

Mosquitoes were sampled using CDC light traps baited with CO_2. Sampling was performed in the provinces of Chaco (Resistencia, Pampa del Indio and Monte Alto), Córdoba (Córdoba, Mar Chiquita) and Tucumán (San Miguel de Tucumán) from 2001 to 2012 (Table I). Between December 8, 2009 and March 18, 2010, 177 ovitraps were placed in different neighborhoods of Córdoba city. Collected adult mosquitoes and larvae were transported under refrigerated conditions to the Laboratory of Arbovirus (Institute of Virology "Dr. JM Vanella", Faculty of Medical Sciences, National University of Córdoba), where they were taxonomically identified on top of a cold plate and stored at -20 °C (Darsie et al. 1985). Mosquitoes were sorted by species, sex, collection site, and date, and gathered in pools of a maximum of 50 individuals each. Each pool was homogenized with MEM in sterile mortars

Table I. Mosquito collection carried out in central and northern area of Argentina during 2001 and 2012.

Province	City of collection	Time period/Year	Pools Collected (n)	Total (n)
Córdoba	Córdoba	2001-2004	331	1259
		2006	36	
		2008-2013	696	
	Altos de Chipión	2008-2009	112	
		2004-2006	84	
Chaco	Resistencia	2001-2003	188	388
	Monte Alto	2001-2003	129	
	Pampa del Indio	2009	71	
Tucumán	San Miguel de Tucumán	2005	129	129

and pestles, centrifuged and stored at -80 ºC until processing for virological studies.

Molecular screening was performed using a generic flavivirus RT-Nested PCR that amplifies a 143-base pair (bp) fragment of the flavivirus NS5 region (Sánchez-Seco et al. 2005). To test whether the positive pools were the results of genomic RNA amplification or DNA forms, nucleic acid extracts were treated with RNAsa (Promega) before amplification. Then, positive pools were analyzed using a generic RT-Nested PCR technique for the amplification of an 860-bp fragment of the NS5 region of the *Flavivirus* genome for phylogenetic analysis (Vázquez et al. 2012). This region of the genome was chosen because it provides useful information for phylogenetic studies, allowing a clear molecular differentiation of the members of the genus *Flavivirus*; it is also one of the most representative regions in GenBank (Cook et al. 2012). All the amplified and purified fragments were sequenced in both directions through automatic sequencers (MACROGEN, Korea).

The genomic sequences obtained were initially identified by comparing them with all the sequences available in GenBank using BLASTn software (http://www.ncbi.nlm.nih.gov/BLAST/). The blast coverage percentages are greater than 98%. Multiple sequence alignments were generated with the relevant ISFV genomes available in GenBank. The final dataset contained 824 positions. Consequently, the construction of the maximum likelihood (ML) was completed under the GTR + I + G model for the NS5 gene. The ML tree was performed using MEGA XI software, with 1000 bootstrap replications (Kumar et al. 2018).

RESULTS

A total of 1776 mosquito and larva pools were analyzed, which were collected in the city of Córdoba (center of the province) between 2001 and 2012 (except for 2005) (n = 800); in the town of La Para (northeast of Córdoba province) in 2008, 2009 and 2010 (n = 129), in Resistencia city (Chaco province) in 2003, 2004 and 2009 (n = 143), in the town of Pampa del Indio (Chaco province) in 2009, 2010 and 2011 (n = 71); and in San Miguel de Tucumán city (Tucumán province) in 2005 and 2006 (n = 129). CxFV was detected in 2 of the 3 provinces. In Córdoba city, the 3 ISF species were detected: CxFV, CFAV and KRV, with CFAV being the predominant one, whereas in Chaco CFAV and CxFV were detected (Figure 1). Only one virus was detected in La Para (CxFV) as well as in Tucumán (KRV). The presence of CxFV, KRV and CFAV was not constant during the collection period (2001 - 2012) in the three provinces (Figure 1).

We found that 68 of the 1776 pools were positive by generic RT Nested PCR using the short fragment (Sánchez Seco PCR). According to the BLASTn analysis, 14 pools were classified as CFAV, 4 pools as KRV and 19 pools as CxFV. Only 27% of the pools (10/37) were amplified by PCR using the long fragment of NS5 (Vázquez PCR) and then sequenced. The identity of all (100%) of these sequences of the viral species agreed between both fragments. CFAV was found mainly (85.71%) in *Ae. aegypti* (12/14); it was also found in a pool of *Ochlerotatus scapularis* and in another pool of *Mansonia titillans,* whereas KRV was found in a 3:1 ratio in *Ae. aegypti* and *Culex interfor*. On the other hand, 94.73% (18/19) of CxFV was found in mosquitoes of the genus *Culex*, except for one positive pool of *Haemagogus spegazzini* collected in Chaco province. It should be noted that ISFs were found in both adult male and female mosquitoes as well as in larvae. Detailed information on species distribution, geographic origin, collection year, sex and stage of development of positive pools are shown in Table II.

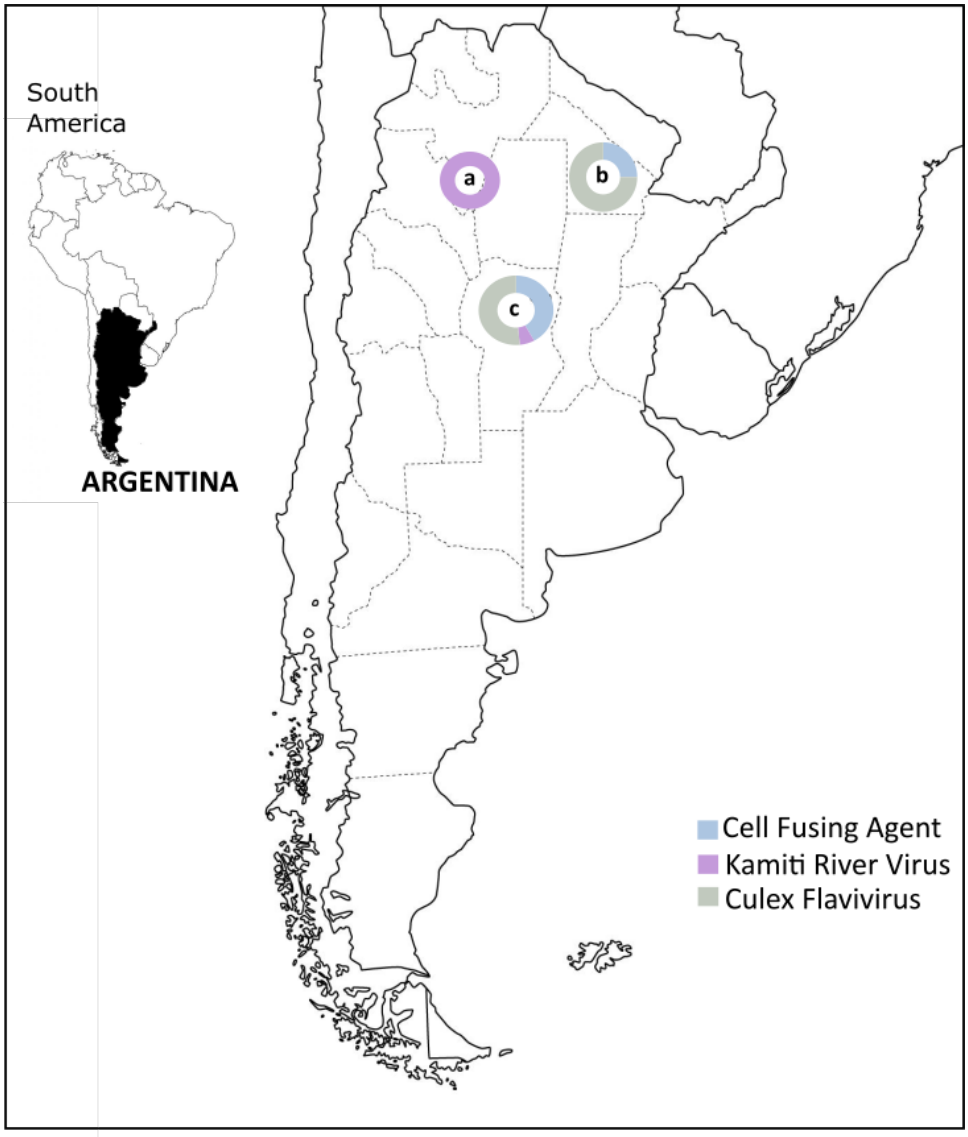

Figure 1. Geographical distribution and proportion of insect-specific flavivirus positive pools detected in 3 provinces of Argentina. a- Tucumán, b- Chaco, c- Córdoba.

To analyze the phylogenetic relationships between CxFV and other ISFs, we used the long NS5 Flavivirus fragment. The analysis revealed two well-differentiated groups, one including viruses associated with *Aedes* spp. and *Ochlerotatus* spp., and the other including viruses associated with *Culex* species. The latter group includes Quang binh (QBV), Calbertado (CLBV), Spanish Culex flavivirus (SCxFV), Nakiwogo (NKV) and CxFV. The group associated with CxFV is the most heterogeneous and diverse one, and shows a clear separation into two subgroups at the host species level: one subgroup that is not clearly defined due to the scarcity of samples and the lack of details of the identified species (subgroup 1) and the other composed of *Culex pipiens, Culex quinquefasciatus* and *Culex tritaeniorhynchus* (subgroup 2) (Figure 2). CxFV strains detected in our study clustered with strains circulating in Santa Fe and Buenos Aires provinces (Argentina), and other countries such as Indonesia, Mexico, Uganda and Taiwan.

Table II. Summary of the insect-specific flaviviruses detected in different mosquito pools captured in Central and Northern Region of Argentina, during 2001 to 2012.

Pool	Host	Collection date	Stage	Province	City of collection	Virus	GenBank ID	Sex
LP2_045	*Culex saltanensis*	04/2009	Adult	Córdoba	La Para	CxFV[2]	KF316946	Female
LP2_047	*Culex dolosus*	04/2009	Adult	Córdoba	La Para	CxFV[2]	KF316947	Male
CHPI 79	*Haemagogus spegazzini*	12/2009	Adult	Chaco	Pampa del Indio	CxFV[2]	KF316948	Female
819	*Culex interfor*	12/2009	Adult	Córdoba	Córdoba	CxFV[2]	KF316939	Female
CA13	*Culex sp.*	12/2009	Adult	Córdoba	La Para	CxFV[2]	KF316941	Female
CA18	*Culex sp.*	12/2009	Adult	Córdoba	La Para	CxFV[2]	KF316942	Female
CA19	*Culex sp.*	12/2009	Adult	Córdoba	La Para	CxFV[2]	KF316943	Female
CA26	*Culex sp.*	12/2009	Adult	Córdoba	La Para	CxFV[2]	KF316944	Female
CA28	*Culex sp.*	12/2009	Adult	Córdoba	La Para	CxFV[2]	KF316945	Female
925	*Culex interfor*	01/2010	Adult	Córdoba	Córdoba	CxFV[2]	KF316940	Female
3_95	*Aedes aegypti*	02/2004	Adult	Córdoba	Córdoba	CFA[1]		Female
3_86	*Culex interfor*	02/2004	Adult	Córdoba	Córdoba	KRV[1]		Female
3_76	*Culex quinquefasciatus*	03/2004	Adult	Córdoba	Córdoba	CxFV[1]		Female
3_75	*Ochlerotatus scapularis*	03/2004	Adult	Córdoba	Córdoba	CFA[1]		Female
AR374	*Aedes aegypti*	03/2004	Adult	Córdoba	Córdoba	CFA[1]	DQ335466	Male
AR336	*Aedes aegypti*	03/2004	Adult	Córdoba	Córdoba	KRV[1]	DQ335465	Female
AR349	*Mansonia titillans*	04/2004	Adult	Córdoba	Córdoba	CFA[1]	DQ335467	Female
AR310	*Aedes aegypti*	04/2004	Adult	Córdoba	Córdoba	CFA[1]	DQ431718	Female
ChRS7	*Culex quinquefasciatus*	03/2009	Adult	Chaco	Resistencia	CxFV[1]		Female
ChRS20	*Aedes aegypti*	03/2009	Adult	Chaco	Resistencia	CFA[1]		Female
LP2_046	*Culex bidens*	04/2009	Adult	Córdoba	La Para	CxFV[1]		Female
CA21	*Culex spp.*	12/2009	Adult	Córdoba	La Para	CxFV[1]		Female
CA23	*Culex sp.*	12/2009	Adult	Córdoba	La Para	CxFV		Female
CHPI 158	*Culex sp.*	05/2011	Adult	Chaco	Pampa del Indio	CxFV[1]		Female
SMT105	*Aedes aegypti*	12/2005	Adult	Tucumán	San Miguel de Tucumán	KRV[1]		Female
SMT114	*Aedes aegypti*	11/2005	Adult	Tucumán	San Miguel de Tucumán	KRV[1]		Female
933	*Culex interfor*	01/2010	Adult	Córdoba	Córdoba	CxFV[1]		Female
942	*Culex interfor*	01/2010	Adult	Córdoba	Córdoba	CxFV[1]		Female
cba_52	*Aedes aegypti*	01/2011	Adult	Córdoba	Córdoba	CFA[1]		Female
cba_43	*Aedes aegypti*	01/2011	Adult	Córdoba	Córdoba	CFA[1]		Female
cba_103	*Aedes aegypti*	02/2011	Adult	Córdoba	Córdoba	CFA[1]		Female
cba_104	*Aedes aegypti*	02/2011	Adult	Córdoba	Córdoba	CFA[1]		Male
cba_86	*Aedes aegypti*	02/2011	Adult	Córdoba	Córdoba	CFA[1]		Female
cba_87	*Aedes aegypti*	02/2011	Adult	Córdoba	Córdoba	CFA[1]		Male
cba_132	*Culex saltanensis*	03/2011	Adult	Córdoba	Córdoba	CxFV[1]		Female
L897	*Aedes aegypti*	01/2010	Larvae	Córdoba	Córdoba	CFA[1]		Female
L497	*Aedes aegypti*	01/2010	Larvae	Córdoba	Córdoba	CFA[1]		Female

[1]Small segment sequenced is showed in Supplementary Material – Appendix 1, [2]Large Fragment sequenced, CFAV = Cell Fusing Agent virus, CxFV = Culex Flavivirus virus, KRV = Kamiti River virus.

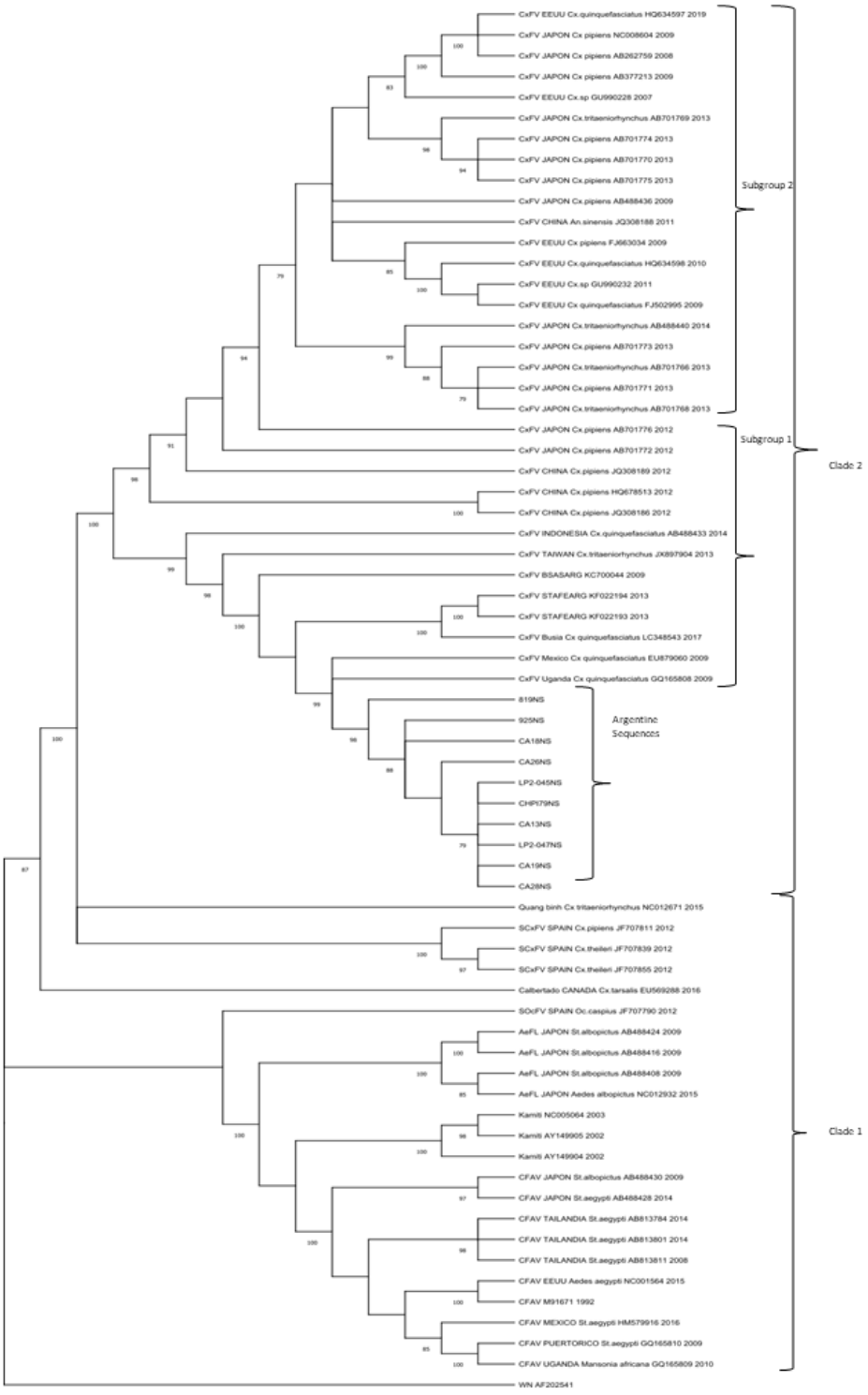

Figure 2. **Phylogenetic tree estimated using the Maximum Likelihood method and GTR +I+G model. This analysis involved 68 nucleotide sequences. The final dataset contained 824 positions. Evolutionary analyses were conducted in MEGA XI. The tree was constructed based on the NS5 target gene of mosquito samples positive for Culex flavivirus and ISF sequences from GenBank. This figure represents the Maximum Likelihood cladogram; bootstrap values are superimposed to indicate nodal support**

DISCUSSION

Recently, several ISFs have been sequenced, characterized, and identified in arthropods (Bonica et al. 2021, Guarido et al. 2021, Tscha et al. 2021). In this study, we aimed to investigate the presence of ISFs in mosquitoes and to analyze the natural diversity and phylogenetic relationships among them in the central-northern region of Argentina.

The temporal analysis of CxFV, KRV and CFA in Córdoba city provides evidence of an erratic maintenance, with the occurrence of presence-absence events and with a permanence of the genetic identity of the circulating CxFV over time. This is consistent with the seasonal fluctuations detected in the *Culex* mosquito populations in the temperate regions of Argentina. The absence of this virus during certain periods could be explained by the low number of mosquitoes collected and analyzed.

The screening performed using Sánchez Seco PCR for Flaviviruses (Sánchez-Seco et al. 2005) allowed us to identify the activity of three ISF species, CFAV, CxFV and KRV, in central-northern Argentina. Although only 10 pools belonging to CxFV were amplified using Vázquez PCR technique (Vázquez et al. 2012), our results indicate 100% of coincidence between the short and long fragments of the NS5 gene. This result shows that this PCR screening technique is an appropriate tool for surveillance and identification of circulating flaviviruses. The NS5 gene is widely used as target for molecular characterization and identification of flaviviruses, providing useful information about phylogenetic relationships (Sánchez-Seco et al. 2005, Vázquez et al. 2012, Fang et al. 2018, Talavera et al. 2018). The obtained sequences strongly suggest the presence of other still not isolated putative viruses; this was the case of OcFV, which was isolated after the detection of field sequences (Calzolari et al. 2012, 2016).

The group of ISFs is highly divergent within the genus Flavivirus and shares a common ancestor with all the other members, including those capable of infecting mammalian hosts. A possible explanation for the origin of ISFs is the existence of a co-divergence between vectors and viruses; this hypothesis is supported by the virus-vector association pattern observed in this work (Figure 2). In agreement with other studies (Huanyu et al. 2012, Bittar et al. 2016), there is a clear divergence between Clade 1: ISFs associated with mosquitoes of the genus Aedes / Stegomyia (CFAV, KRV, AeFV and Spanish Ochlerotatus flavivirus (SOcFV)) and Clade 2: ISFs associated with Culex (CLBV, QBV, CxFV, SCxFV). ISFs would have undergone multiple introductions with frequent vector changes and occasional genetic reassortments, particularly in CFAV. However, there are no studies to support that CxFV was generated by codivergence between these viruses and their vectors (Cook et al. 2009, Bittar et al. 2016, Chatterjee et al. 2021). As expected, based on previous evidence, CFAV (Clade 1) was found in mosquitoes of the genera Mansonia and Ochlerotatus which, according to the phylogeny of mosquitoes, are more related to the genus Aedes / Stegomyia than to Culex (Cook et al. 2009).

CxFV primarily infects globally distributed mosquito species of the genus Culex, which are vectors for pathogenic flaviviruses like WNV, SLEV, and Japanese encephalitis virus (JEV) (Bittar et al. 2016). CxFV has a greater diversity of reservoirs, which might influence its phylogeny (Fernandes et al. 2018, Miranda et al. 2019). This is the first record of CxFV in the mosquito species *Haemagogus spegazinii*; the incorporation of this new record increases the diversity of reservoirs in this viral species. This CxFV strain was grouped in subgroup 1 (Fig.

2). The phylogenetic tree shows two clades: the first one is associated with Asian and USA strains, where *Cx. pipiens* is the main reservoir. However, other mosquito species, such as *Culex tritaeniorhynchus* and *Anopheles sinensis* (Liang et al. 2015), have been reported infected with CxFV belonging to the first clade (Asian/USA; Fig. 2). The second phylogenetic group of CxFV has been reported mostly in the Americas and Africa, with *Culex quinquefasciatus* being the main reservoir (Goenaga et al. 2014, Bittar et al. 2016, Liang et al. 2015). The sequences detected in this study are more phylogenetically related to this group (Africa/Caribbean/Latin America; Fig. 2) than to the first group. Our findings agree with this previously reported phylogeographic separation (Goenaga et al. 2014, Liang et al. 2015). Our sequences clustered with previous detections reported for Argentina (Buenos Aires, Córdoba, Chaco and Santa Fe provinces) (Goenaga et al. 2014). Moreover, all these Argentine sequences are close to that detected in Mexico and Africa. A wide circulation of CxFV was observed in the central-northern region of Argentina (Figure 1). CxFV seems to have been introduced several times in the New World in association with Culex species, which could explain the distribution of the viruses detected in this work (Bolling et al. 2011, Cook et al. 2012, Fernandes et al. 2018).

Although viruses of the genus Flavivirus share complex antigenic relationships, they can be divided into four phylogenetic/ecological groups. These divisions largely reflect the selective constraints imposed on the viruses by vertebrate hosts, invertebrate vectors, and the associated ecologies. Some authors described the evolution and possible origins of individual flaviviruses, showing a correlation of ecological and epidemiological characteristics with their phylogenies and geographic dispersal (Gould et al. 2003, Calzolari et al. 2016, de Oliveira Ribeiro et al. 2021). Many of the phylogenetic lineages that define viral species diverged relatively recently; therefore, the subsequent dispersal and epidemiology of these viruses have been significantly influenced by increasing human population densities and activities, such as recreation, urbanization, land reclamation, transportation, and deforestation (Guzman et al. 2018). In the region, DNA sequences similar to flaviviruses have been found, but in years different from this study (Bonica et al. 2021).

It has been suggested that, since these viruses do not replicate in vertebrate cells, the main route of transmission and maintenance would be the vertical and venereal routes (Bolling et al. 2011, Cook et al. 2006, Lutomiah et al. 2007, Bonica et al. 2021). However, in this study this virus was detected in another species (*Haemagogus spegazzini*) for the first time, suggesting other possible modes of transmission that have still not been discovered.

In conclusion, this work has gathered most of the existing information on insect-specific Flaviviruses circulating in central/northern Argentina, their diversity, phylogenetic relationships, and maintenance dynamics. More specifically, CxFV, KRV and CFAV circulate in several mosquito species in central Argentina. The presence of these viruses in mosquitoes could play an important role from the public health perspective, because it has been shown that previous CxFV infection can increase or block the infection of the mosquito by other pathogenic flaviviruses.

Acknowledgments

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This study was supported by PICT 2013, PICT 2016-3283 and PICT 2018-01172 grants from Agencia Nacional de Promoción Científica y Técnica (Ministerio de Ciencia y Tecnología de Argentina) and Secyt-CONSOLIDAR – Universidad Nacional de Córdoba. This article does not

contain any studies with human participants or animals performed by any of the authors.

REFERENCES

BITTAR C, MACHADO DC, VEDOVELLO D, ULLMANN LS, RAHAL P, ARAUJO JUNIOR JP & NOGUEIRA ML. 2016. Genome sequencing and genetic characterization of Culex Flavirirus (CxFV) provides new information about its genotypes. Virol J 13(1): 158.

BLITVICH BJ & FIRTH AE. 2015. Insect-specific flaviviruses: A systematic review of their discovery, host range, mode of transmission, superinfection exclusion potential and genomic organization. Viruses 7: 1927-1959.

BOLLING BG, EISEN L, MOORE CG & BlLAIR CD 2011. Insect-specific flaviviruses from Culex mosquitoes in Colorado, with evidence of vertical transmission. Am J Trop Med Hyg 85: 169-177.

BONICA MB, BALCAZAR DE, CHUCHUY A, BARNECHE JA, TORRES C & MICIELI MV. 2021. Detection of Flaviviral-Like DNA Sequences in Aedes aegypti (Diptera: Culicidae) Collected From Argentina. J Med Entomol 9;58(6): 2406-2411. doi: 10.1093/jme/tjab073. PMID: 33939805.

CALISHER CH & HIGGS S. 2018. The Discovery of Arthropod-Specific Viruses in Hematophagous Arthropods: An Open Door to Understanding the Mechanisms of Arbovirus and Arthropod Evolution? Annu Rev Entomol 7: 87-103.

CALZOLARI M ET AL. 2012. Detection of mosquito-only flaviviruses in Europe. J Gen Virol 93 (6): 1215-1225.

CALZOLARI M, ZE-ZE L, VAZQUEZ A, SANCHEZ SECO MP, AMARO F & DOTTORI M. 2016. Insect-specific flaviviruses, a worldwide widespread group of viruses only detected in insects. Infect Genet Evol 40: 381-388. doi: 10.1016/j.meegid.2015.07.032.

CHATTERJEE S, KIM CM, YUN NR, KIM DM, SONG HJ & CHUNG KA. 2021. Molecular detection and identification of Culex flavivirus in mosquito species from Jeju, Republic of Korea. Virol J 19;18(1): 150. doi: 10.1186/s12985-021-01618-9. Erratum in: Virol J 20;18(1): 172.

COOK S, BENNETT SN, HOLMES EC DE CHESSE R, MOUREAU G & DE LAMBALLERIE X. 2006. Isolation of a new strain of the flavivirus cell fusing agent virus in a natural mosquito population from Puerto Rico. J Gen Virol 87: 735-748.

COOK S, MOUREAU G, HARBACH RE, MUKWAYA L, GOODGER K, SSENFUKA F, GOULD E, HOLMES EC & DE LAMBALLERIE X. 2009. Isolation of a novel species of flavivirus and a new strain of Culex flavivirus (Flaviviridae) from a natural mosquito population in Uganda. J Gen Virol 90(Pt 11): 2669-2678. doi: 10.1099/vir.0.014183-0.

COOK S, MOUREAU G, KITCHEN A, GOULD EA, LAMBALLERIE X, HOLMES EC & HARBACH RE. 2012. Molecular evolution of the insect-specific Flaviviruses. J Gen Virol 93: 223-234.

CRABTREE MB, SANG RC, STOLLAR V, DUNSTER LM & MILLER BR. 2003. Genetic and phenotypic characterization of the newly described insect flavivirus, Kamiti River virus. Arch. Virol 148: 1095-1118.

DARSIE RF. 1985. Mosquitoes of Argentina. Part I. Keys for identification of adult females and fourth stages larvae in English and Spanish (Diptera, Culicidae). Mosquito Systematics 17: 153-253.

DE OLIVEIRA RIBEIRO G ET AL. 2021. Guapiaçu virus, a new insect-specific flavivirus isolated from two species of Aedes mosquitoes from Brazil. Sci Rep 25;11(1): 4674. doi: 10.1038/s41598-021-83879-6.

FANG Y, ZHANG Y, ZHOU ZB, SHI WQ, XIA S, LI YY, WU JT, LIU Q & LIN GY. 2018. Co-circulation of Aedes flavivirus, Culex flavivirus, and Quang Binh virus in Shanghai, China. Infect Dis Poverty 16;7(1): 75. doi: 10.1186/s40249-018-0457-9.

FERNANDES LN ET AL. 2018. A Novel Highly Divergent Strain of Cell Fusing Agent Virus (CFAV) in Mosquitoes from the Brazilian Amazon Region. Viruses 24;10(12): 666. doi: 10.3390/v10120666.

GOENAGA S, FABBRI CM, GARCIA JB, RONDAN JC, GARDENAL N, CALDERON GE, ENRIA DA & LEVIS SM. 2014. New strains of Culex flavivirus isolated in Argentina. J Med Entomol 51: 900-906.

GOULD EA, DE LAMBALLERIE X, ZANOTTO PM & HOLMES EC. 2003. Origins, evolution, and vector/host coadaptations within the genus Flavivirus. Adv Virus Res 59: 277-314. doi: 10.1016/s0065-3527(03)59008-x.

GUARIDO MM, GOVENDER K, RIDDIN MA, SSHRAMA M, GORSICH EE, BROOKE BD, ALMEIDA APG & VENTER M. 2021. Detection of Insect-Specific Flaviviruses in Mosquitoes (Diptera: Culicidae) in Northeastern Regions of South Africa. Viruses 25;13(11): 2148. doi: 10.3390/v13112148.

GUZMAN H ET AL. 2018. Characterization of Three New Insect-Specific Flaviviruses: Their Relationship to the Mosquito-Borne Flavivirus Pathogens. Am J Trop Med Hyg 98(2): 410-419. doi: 10.4269/ajtmh.17-0350.

HUANYU W, HAIYAN W, SHIHONG F, GUIFANG L, HONG L, XIAOYAN G, LIZHI S, RAYNER S, AIQIANG X & GUODONG L. 2012. Isolation and identification of a distinct strain of Culex Flavivirus from mosquitoes collected in Mainland China. Virol J 27;9: 73. doi: 10.1186/1743-422X-9-73.

KUMAR S, STECHER G, LI M, KNYAZ C & TAMURA K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547-1549.

LIANG W ET AL. 2015. Distribution and phylogenetic analysis of Culex flavivirus in mosquitoes in China. Arch Virol 160(9): 2259-2268.

LUTOMIAH JJ, MWANDAWIRO C, MAGAMBO J & SANG RC. 2007. Infection and vertical transmission of Kamiti river virus in laboratory bred Aedes aegypti mosquitoes. J Insect Sci 7: 1-7. doi: 10.1673/031.007.5501.

MACHADO DC, MONDINI A, SANTANA VS, YONAMINE PT, CHIARAVALLOTI NETO F, ZANOTTO PM & NOGUEIRA ML. 2012. First identification of Culex flavivirus (Flaviviridae) in Brazil. Intervirology 55: 475-483.

MIRANDA J, MATTAR S, GONZALEZ M, HOYOS-LOPEZ R, ALEMAN A & APONTE J. 2019. First report of Culex flavivirus infection from Culex coronator (Diptera: Culicidae), Colombia. Virol J 3;16(1): 1. doi: 10.1186/s12985-018-1108-2.

SANCHEZ-SECO M, ROSARIO D, DOMINGO C, HERNANDEZ L, VALDES K, GUZMAN M & TENORIO A. 2005. Generic RT-nested-PCR for detection of Flaviviruses using degenerated primers and internal control followed by sequencing for specific identification. J Virol Methods 126: 101-109.

SANG RC, GICHOGO A, GACHOYA J, DUNSTER MD, OFULA V, HUNT AR, CRABTREE MB, MILLER BR & DUNSTER LM. 2003. Isolation of a new flavivirus related to cell fusing agent virus (CFAV) from field-collected flood-water Aedes mosquitoes sampled from a dambo in central Kenya. Arch Virol 148: 1085-1093.

STOLLAR V & THOMAS VL. 1975. An agent in the Aedes aegypti cell line (Peleg) which causes fusion of Aedes albopictus cells. Virology 64: 367-377.

TALAVERA S, BIRNBERG L, NUÑEZ AI, MUÑOZ-MUÑOZ F, VAZQUEZ A & BUSQUETS N. 2018. Culex flavivirus infection in a Culex pipiens mosquito colony and its effects on vector competence for Rift Valley fever phlebovirus. Parasit Vectors 23;11(1): 310. doi: 10.1186/s13071-018-2887-4.

TSCHA MK ET AL. 2021. Pirahy virus: Identification of a new and potential emerging arbovirus in South Brazil. Virus Evol 17;7(2): veab105. doi: 10.1093/ve/veab105. PMID: 35310294; PMCID: PMC8928568.

VAZQUEZ A ET AL. 2012. Novel Flaviviruses detected in different species of mosquitoes in Spain. Vector Borne Zoonotic Dis 12: 223-229.

SUPPLEMENTARY MATERIAL

Appendix S1.

How to cite

FARÍAS AA, LABERDOLIVE V, STEIN M, JURI MJD, VISINTIN A, ALMIRÓN WR, CONTIGIANI MS, RE VE & DIAZ A. 2024. Diversity and molecular characterization of insect-specific flaviviruses in mosquitoes (Diptera: Culicidae) collected in central and northern Argentina. An Acad Bras Cienc 96: e20230452. DOI 10.1590/0001-3765202420230452.

Manuscript received on April 18, 2023;
accepted for publication on February 28, 2024

ADRIÁN A. FARÍAS[1]
https://orcid.org/0000-0003-0497-0226

VICTORIA LABERDOLIVE[1]
https://orcid.org/0009-0006-0884-2634

MARINA STEIN[2,3]
https://orcid.org/0000-0001-7102-9474

MARÍA JULIA DANTUR JURI[3,4]
https://orcid.org/0000-0002-9219-2577

ANDRÉS VISINTIN[5]
https://orcid.org/0000-0003-2153-7512

WALTER R. ALMIRÓN[3,6]
https://orcid.org/0000-0003-0559-2407

MARTA S. CONTIGIANI[1]
https://orcid.org/0000-0002-7171-0013

VIVIANA E. RE[1,3]
https://orcid.org/0000-0002-4106-6642

ADRIÁN DIAZ[1,3]
https://orcid.org/0000-0001-5953-2907

[1]Universidad Nacional de Córdoba, Laboratorio de Arbovirus, Instituto de Virología "Dr. J. M. Vanella", Facultad de Ciencias Médicas, Enfermera Gordillo Gomez s/n, Ciudad Universitaria, CP 5016, Córdoba, Córdoba, Argentina

[2]Universidad Nacional del Nordeste, Departamento de Entomología, Instituto de Medicina Regional, Avenida Las Heras 727, CP 3500, Resistencia, Chaco, Argentina

[3]Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET), Godoy Cruz 2290, CABA, Argentina

[4]Unidad Ejecutora Lillo (CONICET–Fundación Miguel Lillo), Miguel Lillo 251, CP 4000, San Miguel de Tucumán, Tucumán, Argentina

[5]Universidad Nacional de la Rioja, Instituto de Biología de la Conservación y Paleobiología (IBiCoPa), Centro de Investigación e Innovación Tecnológica (CENIIT), Avenida Luis Vernet y Apóstol Felipe s/n, F5200, La Rioja, Argentina

[6]Universidad Nacional de Córdoba, Centro de Investigaciones Entomológicas de Córdoba, Instituto de Investigaciones Biológicas y Tecnológicas (IIBYT), CONICET-Av. Vélez Sarsfield 1611, X5016, Córdoba, Argentina

Correspondence to: **Adrián Diaz**
E-mail: adrian.diaz@conicet.gov.ar / adrian.diaz@fcm.unc.edu.ar

Author contributions

Adrian A. Farías: Writing – original draft - Reviewing-Data analyses Victoria Laberdolive: Data curation, Writing- Original draft preparation Marina Stein: Investigation, Writing - review & editing. María Julia Dantur Juri: Investigation, Writing - review & editing. Andres Visintin: Investigation, Writing - review & editing. Walter Almirón: Investigation, Funding acquisition, Writing - review & editing. Marta S. Contigiani: Conceptualization, Methodology, Funding acquisition, Writing - review & editing. Viviana E. Re: Conceptualization, Methodology, Writing - review & editing. Adrián Diaz: Writing – original draft, Supervision and Editing Conceptualization, Methodology, Writing - review & editing.