ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/224867405

First record of a mosquito iridescent virus in Culex pipiens L. (Diptera: Culicidae)

Article *in* Archives of Virology · April 2012

DOI: 10.1007/s00705-012-1302-2 · Source: PubMed

CITATIONS
4

READS
49

7 authors, including:

Evangelina Muttis
National Scientific and Technical Research Council
5 PUBLICATIONS **32** CITATIONS
SEE PROFILE

Solange Ana Belén Miele
National University of Quilmes
8 PUBLICATIONS **142** CITATIONS
SEE PROFILE

Mariano Belaich
National University of Quilmes
31 PUBLICATIONS **196** CITATIONS
SEE PROFILE

María V Micieli
National University of La Plata
45 PUBLICATIONS **402** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Encapsulation of mosquito repellents for textile application View project

Development of an innocuous viral nanovector for the transport of therapeutic genes in mammals View project

All content following this page was uploaded by Pablo Ghiringhelli on 22 December 2014.

The user has requested enhancement of the downloaded file.

First record of a mosquito iridescent virus in *Culex pipiens* L. (Diptera: Culicidae)

Evangelina Muttis[1*], Solange A. B. Miele[2], Mariano N. Belaich[2], Maria V. Micieli[1], James J. Becnel[3], Pablo D. Ghiringhelli[2] and Juan J. García[1]

[1]Centro de Estudios Parasitológicos y Vectores (CEPAVE-CONICET-CCT-La Plata-UNLP); Calle 2 # 584, (1900) La Plata, Bs As, Argentina; Tel: 54-0221-4233471 Fax: 54-0221-4232327.

[2]LIGBCM-AVI (Laboratorio de Ingeniería Genética y Biología Celular y Molecular - Area Virosis de Insectos), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes (UNQ); Roque Saenz Peña 352, (1876) Bernal, Bs. As., Argentina; Tel: 54-11-43657100 (ext. 4152), Fax: 54-11-43657132

[3]United Status Department of Agriculture, Agricultural Reserch Service. Center for Medical, Agricultural, and Veterinary Entomology, 1600/1700 SW 23rd Drive, Gainsville, FL 32604, USA

**Corresponding author: Muttis Evangelina*

Centro de Estudios Parasitológicos y de Vectores, CEPAVE (CONICET- CCT -La Plata - UNLP), Calle 2 Nº 584, (1900) La Plata, Buenos Aires, Argentina. Fax: +54-0221-4232327. Tel: +54-0221-4233471. E-mail: evangelinamuttis@hotmail.com

Abstract

The mosquito iridescent viruses (MIVs) are large icosahedral DNA viruses that replicate and assemble in the cytoplasm of the host. Paracrystalline arrangements of virions that accumulate in the cytoplasm produce an iridescent color that is symptomatic of acute infections. In August 2010 we found larvae of *Culex pipiens* with these symptoms in suburban ditches around La Plata city, Argentina. Electron microscope studies, PCR amplification of the Protein (MCP) gene arrangement, DNA sequencing and phylogenetic analysis were carried out.

Key words: Iridescent Virus, Culicidae, *Culex pipiens*, Argentina.

Iridoviruses are pathogens of both vertebrates and invertebrates. Among invertebrates the most common host are the aquatic stages of Diptera, particularly mosquitoes [10]. The viruses characterized from invertebrates have been assigned to two genera, both belong to the family *Iridoviridae*; the genus *Iridovirus* which contains the majority of iridescent viruses and the genus *Chloriridovirus* which contains the single species type IV3 from *Aedes taeniorhynchus* (Wiedemann) [5, 2]. The presence of an iridescent virus in mosquitoes was first reported in *Aedes taeniorhynchus* from Florida [5]. In subsequent years these viruses have been detected in several other genera, such as *Ochlerotatus* Lynch Arribalzaga, *Psorophora* Robineau-Desvoidy and *Culiseta* Felt, but they have not been detected in *Anopheles* Meigen and there are two reports of an iridescent virus infecting *Culex territans* Walker from Russia and Ukraine [10]. The mosquito iridescent viruses (MIVs) are large icosahedral viruses that replicate and

61 assemble in the cytoplasm of the host, primarily in fat body cells. Paracrystalline
62 arrangements of virions that accumulate in the cytoplasm produce an iridescent color
63 when exposed to light on a dark background [1]. MIVs have been reported from US,
64 Europe and Russia [10] while no data are available for the Neotropical Region.

65 *Culex pipiens* (Linnaeus) is a vector of pathogens that cause disease and an
66 important pest world-wide. Immature stages develop in different habitats, including
67 temporary and permanent pools. In Argentina the most common breeding sites for this
68 mosquito are man-made drainage ditches in suburban areas of the cities from which a high
69 number of mosquito adults emerge throughout the year [4].

70 In August 2010, during a survey for natural enemies of *C. pipiens* we found fourth
71 instar larvae with infections symptomatic of MIV. These larvae were collected from
72 suburban ditches around La Plata city Argentina (34°52'12.34"S, 57°52'8.59"W) that
73 contained permanent water. The habitat was polluted with greasy substances and abundant
74 organic matter from domiciliary drainage. Larvae were examined under a dissecting
75 microscope using a black background. From the 151 larvae collected 16 (10.6 %) showed
76 turquoise iridescence similar than that described as typical for the family Iridoviridae.

77 Small pieces of infected larvae were prepared for electron microscopic studies.
78 These were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.3) for 3 h,
79 postfixed in 1% osmium tetroxide for 2 h, dehydrated through ascending concentrations of
80 ethanol and embedded in Epon-Araldite resin. Ultrathin sections (60 nm), stained in 2%
81 uranyl acetate and lead citrate, were examined with an electron microscope at 75 kv [3].
82 The virus particle exhibits a hexagonal shape and has a dense central core surrounded by a
83 lighter staining outer shell (**Fig 1**).The virus particles have an average diameter of 158 nm
84 (n= 30).

Two infected larvae were semi-purified for DNA extraction. The larvae were homogenized with distilled water and filtered through a syringe with cotton, then centrifuged twice in order to remove cellular debris (1000 rpm for 10 min. and 1500 rpm for 10 min.). The supernatant was centrifuged at 15000 rpm for 30 min. Finally, the pellet was resuspended in 500 µl of distilled water. One hundred microliters of this suspension containing MIV was digested with proteinase K (0.1 mg/ml) in lysis buffer plus SDS (10 mM Tris-HCl pH 8.0; 200 mM NaCl; 1 mM EDTA pH 8.0; 0.5% SDS). The mixture was incubated at 37 °C for 3 hr and then centrifuged at 16000*g* for 15 min. Viral DNA was purified by standard phenol/chloroform extraction and ethanol precipitation [6]. The final pellet was dissolved in 20 µl of double-distilled water.

Using the MCP primers described by Webby and Kalmakoff [9] and 1 µl of MIV DNA (1/100 dilution) as template, a PCR amplification with Taq DNA polymerase (Invitrogen) was carried out under standard conditions [1 X standard buffer, 0.25 U Taq DNA polymerase, 1.5 mM $MgCl_2$, 1 mg/ml BSA, 0.2 mM of each dNTP and 1 µM of each primer]. The amplification profile used was: one initial denaturing step of 1 min at 94 ºC, 35 cycles of 10 sec at 94 ºC, 10 sec at 56 ºC and 20 sec at 72 ºC, followed by a final extension step of 3 min at 72 ºC. The PCR samples were electrophoresed in 1% agarose gels and visualized by ethidium bromide staining.

An expected fragment of ~300 bp was obtained. This fragment was cut off and recovered using silica matrix adsorption (Gene Clean II kit, BIO101). Purified fragment was ligated to pGemT™ vector (Promega) and electroporated into *E. coli* Top10 competent cells. After selection in LB agar plates containing 100 µg/ml Ampicillin and 20 µg/ml X-Gal, the recombinant clones were grown in liquid LB plus Ampicillin. Plasmid DNAs were purified using the alkaline lysis method [6] and sequenced in the Macrogen Center (Korea), employing T7 and SP6 universal primers. In silico translated protein and

type species proteins corresponding to each *Iridoviridae* genus were analyzed using multiple and pairwise alignments obtained with ClustalX software [8] and phylogeny inference using MEGA4 software [7]. *Culex pipiens* MIV MCP protein sequence fragment cluster more nearly with *Chloriridovirus* genus than the others (**Fig 2**).

Based on these results, we were able to confirm the presence of an iridescent virus infecting *C. pipiens* larvae based on symptoms, morphology, and molecular methods. This report constitutes the first documentation of an iridescent virus in *C. pipiens* larvae and also the first time that an iridescent virus has been described infecting mosquito species in the Neotropical Region.

Acknowledgments

This work was supported in part by grant PICT 2008-0653 from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT). MNB, PDG and MVM are members of the Research Career of CONICET (Consejo Nacional de Ciencia y Tecnología). JJG is a member of the Research Career of CIC (Comisión de Investigaciones Científicas de la provincia de Buenos Aires), EM holds a fellowship from CIC. SABM holds a fellowship from CONICET.

Conflict of interest

The authors declare that they have no conflict of interest.

Figure legends

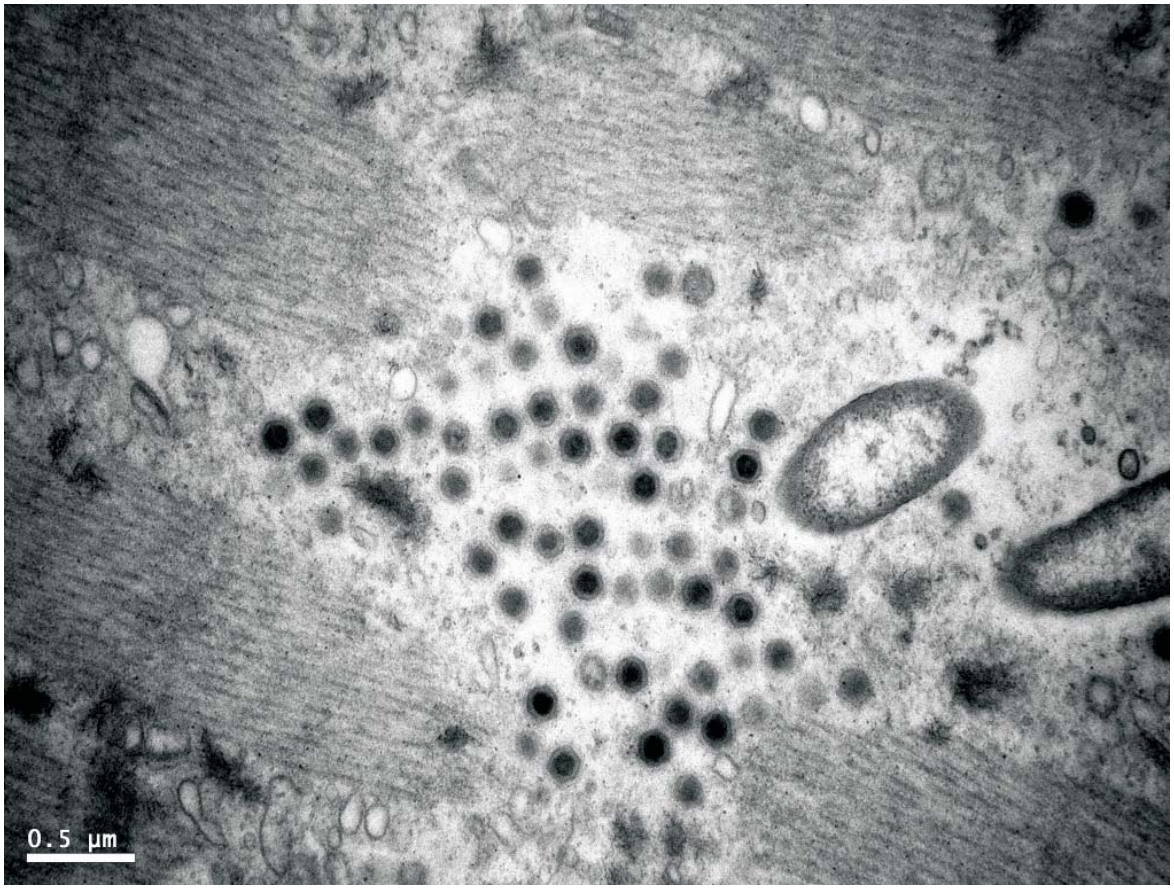
Fig 1 Transmission electron micrograph. Ultrathin sections of infected *Culex pipiens* larvae showing particles of Mosquito Iridescent Virus

Fig 2 Sequence analysis. **A.** Multiple alignment of *Culex pipiens* MIV MCP deduced protein sequence and corresponding protein fragments of type species from different genera in the family *Iridoviridae.* **B.** Pairwise identity (upper right triangle) and homology (lower left triangle) between all analysed protein sequences. **C.** Evolutionary relationships of 6 taxa, inferred using the minimum evolution method . Node consistency (bootstrap test, 1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the following parameters: Model [amino: Poisson correction], Substitutions to include [All], Pattern among Lineages [Same (homogeneous)], Rates among Sites [Different (Gamma distributed)] and Gamma parameter [2.25]. Between parenthesis are shown the GenBank accesion codes

References

1. Anthony DW, Comps M (1991) Iridoviridae. In: Adams JR, Bonami JR (eds) Atlas of Invertebrate Viruses, CRC Press, Inc. Boca Raton, Florida, pp 56-86
2. Becnel JJ (2002) The Chloriridovirus. In: Tidona CA, Darai G (eds) The Springe Index of Viruses. Springer-Verlag, Heidelberg, Germany, pp 472-474
3. Becnel JJ (1997) Complementary Techniques: Preparations of Entomopathogens and Diseased Specimens for more Detailed Study using Microscopy. In: Lacey LA (ed) Manual of Techniques in Insect Pathology. Academic Press, San Diego, pp 337-353

4. Campos RE, Maciá A, García JJ (1993) Fluctuaciones estacionales de culícidos (Díptera) y sus enemigos naturales en zonas urbanas de los alrededores de La Plata, provincia de Buenos Aires. Neotrópica 39: 55-66
5. Clark TB, Kellen WR, Lum PTM. (1965) A mosquito iridescent virus (MIV) from *Aedes taeniorhynchus* (Wiedemann). J. Invertebr. Pathol. 7: 519-521
6. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. 2. ed., 3. vol., Cold Spring Harbor Laboratory Press, New York
7. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 Molecular Biology and Evolution 10.1093/molbev/msm092
8. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22(22): 4673-4680
9. Webby R, Kalmakoff J (1998) Sequence comparison of the major capsid protein gene from 18 diverse iridoviruses. Arch. Virol. 143: 1949–1966
10. Williams T (2008) Natural invertebrate host of iridoviruses (Iridoviridae). Neotrop. Entomol. 37(6): 615-632



178

179

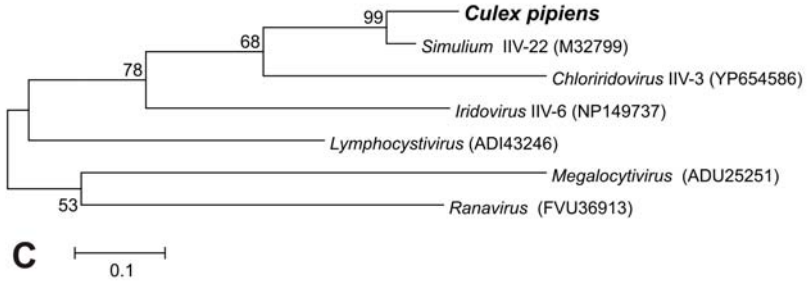
```
Megalocytivirus (ADU25251)      QSGYNKMIGMRSDLV-----AGITNGQTMPAVYLNLPIPLFFTRDTGLALPTVSLPYNEV
Ranavirus (FVU36913)            RTGYYNMIGNTSDLINPAPATGQDGARVLPAKNLVLPLPFFFSRDSGLALPVVSLPYNEI
Culex pipiens                   RIGYDNMIGNVSSLINP--VAPGGTLGNAGGINLNLPLPFLFSRDTGVALPTAALPYNEM
Chloriridoviridae IIV-3 (YP654586) RNGYDNMIGNISTLTDP--VGPGGSLGHTGGTVLNLPISLFFTRDTGVSLPTAALPYNEI
Simulium IIV-22 (M32799)        RNGYDNMIGNVSSLINP--VAPGGTLGSVGGINLNLPLPFFFSRDTGVALPTAALPYNEM
Iridovirus IIV-6 (NP149737)     AVGYDNMIGNVSALIQPQPVPVAPATVSLPEADLNLPLPFFFSRDSGVALPTAALPYNEM
Lymphocystivirus (ADI43246)     RTGYDNMIGNTIDMTQP-----VGPEGRLPGKVLVLPLPYFFSRDSGVALPSAALPYNEI
                                  ** :***    :                * **:. :*:**:*::** .:*****:

Megalocytivirus (ADU25251)      RIHFKLRRWEDLLISQSNQADM---AISTVTLANIGN-VAPALTNVSVMG-----
Ranavirus (FVU36913)            RITVKLRAIHDLLILQHN-TTG---AISPIVASDLAG-GLPDTVEANVYMTVALI
Culex pipiens                   QINFNFRDWTELLILTDSSVAPPASPYVPIVVGTHIA-SAPVLGPVQVWANYAI-
Chloriridoviridae IIV-3 (YP654586) QLNFSMRDWKDLLILTDTAITT-GNPYQTIDVSKHLGGVAPSLSNVQVWANYAI-
Simulium IIV-22 (M32799)        QINFNFRDWHELLILTNSALVPPASPYVPIVVGTHIS-AAPVLGPVQVWANYAI-
Iridovirus IIV-6 (NP149737)     RINFQFHDWQRLLILDNIAAVA-SQTVVPVVGATSDIATAPVLHHGTVWGNYAI-
Lymphocystivirus (ADI43246)     RLTFHLRDWTELLIFQNKQDST----IMPLTAADLMW-GKPDLKDVQVWI-----
                                :: . ::    ***             .: .       *     *
```

A

B

	Culex pipiens	Simulium IIV-22 (M32799)	Chloriridovirus IIV-3 (YP654586)	Iridovirus IIV-6 (NP149737)	Lymphocystivirus (ADI43246)	Megalocytivirus (ADU25251)	Ranavirus (FVU36913)
Culex pipiens		90.1	61.6	60.7	47.7	37.8	41.2
Simulium IIV-22 (M32799)	98.2		63.4	60.5	48.6	39.6	43.0
Chloriridovirus IIV-3 (YP654586)	90.2	91.1		44.2	49.5	40.5	36.8
Iridovirus IIV-6 (NP149737)	78.6	79.8	73.5		46.9	36.3	42.6
Lymphocystivirus (ADI43246)	71.2	70.3	71.2	69.9		41.6	50.0
Megalocytivirus (ADU25251)	69.4	70.3	73.0	65.5	70.3		42.3
Ranavirus (FVU36913)	71.9	74.6	71.9	72.2	74.5	70.3	



180

View publication stats