Isolation and full-length genome analysis of mosquito-borne Manzanilla virus from Yunnan Province, China

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Abstract

Background: There have been four strains on Manzanilla virus (MANV) identified to date. Here, we identify a novel MANV strain (DHL10M107) isolated from Culex tritaeniorhynchus Giles mosquitoes from Ruili city, Dehong prefecture, Yunnan Province, in the People’s Republic of China.

Results: The DHL10M107 L, M and S genes were sequenced at the nucleotide and deduced amino acid levels. The L, M and S gene sequences of DHL10M107 clustered with the MANV strains VN04-2108, TRVL3587, SA An 4165, and AV 782. DHL10M107 was most closely related to VN04-2108. Nucleotide homology ranged between 96 and 99% between DHL10M107 and VN04-2108. In terms of amino acid homology, all of the amino acid differences were in the L (96.3% homologous) and M (97.7% homologous) fragments.

Conclusions: DHL10M107 is likely a MANV isolated from mosquitos in the Yunnan Province. This is the first reported isolation of MANV in mainland China.

Keywords: Manzanilla virus, Oya virus, Orthobunyavirus, Phylogenetics

Findings

Manzanilla virus (MANV) belongs to the Simbu sero-group of the genus Orthobunyavirus of family Bunyaviridae [1–3]. MANV is a single-stranded negative sense RNA virus that contains three RNA fragments: large (L), medium (M) and small (S). Four strains of MNV have been isolated from to date. Anderson et al. were the first group to isolate MANV (TRVL3587) [1] from the blood of a Howler monkey (Alouatta seniculus insularis) in Trinidad in 1954. More recently, in 2004 Bryant et al. isolated a MANV strain identified as the Cat Que virus (VN04-2108) from mosquitoes (Culex sp.) in Vietnam [2]. In 2014, Ladner et al. [3] reclassified two viruses as MANV strains, the Ingwavuma virus (SA An 4165) isolated from a South African spectacled weaver (Hyphantaerus ocularis) in 1959 [4], and the Mermet virus (AV 782) isolated from a North American purple martin (Progne subis) in the United States in 1964 [5].

Yunnan Province is located in southwest China, adjacent to Myanmar, Laos and Vietnam. Ecologically, Yunnan Province supports the distribution of vector mosquitoes and the transmission of mosquito-borne viruses [6, 7]. The Japanese encephalitis virus (Genus Flavivirus, Family Flaviviridae), Dengue virus (Genus Flavivirus, Family Flaviviridae), Chikungunya virus (Genus Alphavirus, Family Togaviridae), Sindbis virus (Genus Alphavirus, Family Togaviridae), Getah virus (Genus Alphavirus, Family Togaviridae), Batai virus (BATV) (Genus Orthobunyavirus, Family Bunyaviridae), Banna virus (Genus Seadornavirus, Family Reoviridae), Yunnan orbivirus (Genus Obivirus, Family Reoviridae), and several other viruses have been isolated from human patients and from mosquitoes collected in Yunnan.
Province [8-16]. This study aimed to further investigate the distribution of mosquito-borne viruses in Yunnan Province.

In August 2010, mosquitoes were collected using the Kongfu Xiaoshuai trap (Wuhan Jixing Environmental Protection, Scientific, and Technological LLC) from cattle barns in the suburb of Ruili city, Dehong prefecture, Yunnan Province, People’s Republic of China. A total of 425 mosquitoes representing 10 species in four genera were collected. Of these, 318 (74.82%) were *Culex tritaeniorhynchus* Giles and the remaining 107 were from the other nine species (25.18%). The mosquito samples were sorted by species into 15 pools and ground together. The supernatant was used to inoculate Baby hamster kidney (BHK-21) cells to isolate any viruses present as previously described [15]. One of the pools containing *C. tritaeniorhynchus* (DHL10M107) caused significant cytopathic effects (CPE) in BHK-21 cells, characterized by the cells shrinking, rounding, and eventually floating.

The viral RNA was extracted from the culture supernatant of the DHL10M107 isolate, using the QIAamp viral RNA mini kit (Qiagen, Valencia, CA, USA) per the manufacturer’s instructions. cDNA was prepared with Ready-To-Go You-Prime First-Strand Beads” (American Amershams Pharmacia Biotech, Piscataway, NJ, USA), and amplified with MANV specific primers (see Additional file 1: Table s1). The reactions contained 1 µL cDNA template, 10 µL 2 × GCI Buffer, 0.5 µL 2.5 mM dNTP, 0.4 µL each of the upstream and downstream primers (10 mM), 0.4 µL ExTaq enzyme (5 U/µL), and 7.3 µL deionized water. The reaction conditions were: pre-denaturing at 95°C for 3 min, followed by 32 cycles of 95°C for 30 s, 58°C for 40 s, and 72°C for 1–2 min, and extension at 72°C for 10 min. The amplification was assessed using

![Figure 1](image-url)  
**Figure 1** Phylogenetic trees based on the nucleotide sequences of DHL10M107 strains isolated from Yunnan Province, China. **a** L sequence, **b** M sequence, **c** S sequence and **d** partial Oya virus S sequence (365 bp). The black triangle indicates DHL10M107.
2 µL of the product for 1% agarose gel electrophoresis. The whole genome sequence of the DHL10M107 strain was obtained (Beijing Liuhe Genomics Technology Co., Ltd. Shenzhen Branch). The L, M and S sequences of DHL10M107 strain were submitted to GenBank under accession nos. KP016012-KP016014. The sequence fragments were spliced, edited and corrected using SeqMan (DNAstar software package) and the phylogenetic analysis and nucleotide sequence analysis were performed using ClustalX (version 1.8), MEGA5.0, DNAStar alignment package, and MegAlign software.

Table 1 Nucleotide and amino acid homology in the sequences from DHL10M107 and other Orthobunya viruses

| Genome segment and strain | % Nucleotide and amino acid sequence identities | 1 | 2 | 3 | 4 | 5 | 6 |
|---------------------------|-----------------------------------------------|---|---|---|---|---|---|
| Small                     |                                               |   |   |   |   |   |   |
| DHL10M107                 |                                               |   |   |   |   |   |   |
| 1                         |                                               | 98.7| 98.9| 54.2| 51.4| 72.2|
| Manzanilla virus/VN04-2108|                                               | 100 | 99.6| 54.8| 50.8| 72 |
| 2                         |                                               | 100 | 100 | 54.9| 51.1| 72.2|
| Oya virus/SC0806           |                                               | 43  | 43  | 43  | 54.4| 52.8|
| 3                         |                                               | 39.1| 39.1| 39.1| 43.1| 51.1|
| Tahyna virus/XJ0708        |                                               | 74  | 74  | 74  | 44.3| 43.4|
| 4                         |                                               |     |     |     |     |     |     |
| Batai virus/MM2222         |                                               |     |     |     |     |     |     |
| 5                         |                                               |     |     |     |     |     |     |
| Oropouche virus/TRVL-9760  |                                               | 74  | 74  | 74  | 44.3| 43.4|
| Medium                    |                                               |   |   |   |   |   |   |
| DHL10M107                 |                                               |   |   |   |   |   |   |
| 1                         |                                               | 97.7| 97.2| 46.2| 48.9| 59 |
| Manzanilla virus/VN04-2108|                                               | 99.2| 97.5| 46.1| 48.9| 58.9|
| 2                         |                                               | 99.3| 99.2| 46.2| 49  | 58.9|
| Oya virus/SC0806           |                                               | 31.5| 31.5| 31.4| 51.2| 45.4|
| 3                         |                                               | 32.7| 32.8| 32.8| 43  | 48.5|
| Tahyna virus/XJ0708        |                                               | 52  | 52  | 52  | 30.5| 31.3|
| 4                         |                                               |     |     |     |     |     |     |
| Batai virus/MM2222         |                                               |     |     |     |     |     |     |
| 5                         |                                               |     |     |     |     |     |     |
| Oropouche virus/TRVL-9760  |                                               | 96.3| 92.6| 55.9| 56.1| 65 |
| Large                     |                                               |   |   |   |   |   |   |
| DHL10M107                 |                                               |   |   |   |   |   |   |
| 1                         |                                               | 99.5| 99  | 56  | 56.2| 65.1|
| Manzanilla virus/VN04-2108|                                               | 99  | 99.2| 55.9| 55.8| 64.9|
| 2                         |                                               | 50.8| 50.9| 51.2| 58.9| 56.2|
| Oya virus/SC0806           |                                               | 48  | 48.2| 48.3| 54.2| 55.3|
| 3                         |                                               | 31.7| 32.2| 31.7| 23.8| 23.3|

The percent nucleotide sequence identities are in the upper right half of the matrix and the percent amino acid sequence identities are presented in the lower left half. Virus strains in italics (homologous values) are members of Manzanilla virus complex of genus Orthobunyavirus.
Table 2 Sites of amino acid differences in L and M sequences of DHL10M107, VN04-2108 and SC0806

| Genome segment and strain | Sites of amino acid |
|---------------------------|---------------------|
| Large                     | 150 274 289 435 456 |
| DHL10M107                 | H E N D T C N T K T E G V |
| VN04-2108                 | H E N D N R S T N T D E V |
| SC0806                    | Q G E E T R S G K V D E A |
| Medium                    | 20 349 370 395 714 |
| DHL10M107                 | T D T Y S N T A |
| VN04-2108                 | A D T H S S T T |
| SC0806                    | T E I H P N A A |
| S                         | 922 1,202 1,634 1,660 2,055 |
| DHL10M107                 | T D T Y S N T A |
| VN04-2108                 | A D T H S S T T |
| SC0806                    | T E I H P N A A |

Abbreviations
- L: large RNA genome fragment; M: medium RNA genome fragment; S: small RNA genome fragment; MANV: Manzanilla virus; BATV: Batai virus; BHK-21: baby hamster kidney cells-21; CPE: cytopathic effects; OYV: Oya virus; ORF: open reading frame; ICTV: International Committee on Taxonomy of Virus.

Additional files

Additional file 1: Table s1. Specific primers for Manzanilla virus used in this study.
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