

Identification of a novel totivirus from *Culex tritaeniorhynchus* in China

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Abstract

Until the isolation of IMNV from penaeid shrimps, the first Totiviridae member to be isolated from an arthropod, no Totiviridae member had been isolated from an arthropod, and more toti-like virus was discovered from arthropod. In this paper, we effectively isolated a novel totivirus-like particle from *Culex tritaeniorhynchus* designated NODE2. Sequence and phylogenetic analysis shows that the NODE2 has the similar genome organization just as some other members of Totiviridae family, and it may represent an entirely novel genus within the Totiviridae family.

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Abstract

Until the isolation of IMNV from penaeid shrimps, the first Totiviridae member to be isolated from an arthropod, no Totiviridae member had been isolated from an arthropod, and more toti-like virus was discovered from arthropod. In this paper, we effectively isolated a novel totivirus-like particle from *Culex tritaeniorhynchus* designated NODE2. Sequence and phylogenetic analysis shows that the NODE2 has the similar genome organization just as some other members of Totiviridae family, and it may represent an entirely novel genus within the Totiviridae family.

Totiviridae viruses are nonsegmented, twofold RNA (dsRNA) viruses that attach, thus they are a family of non-enveloped, double-stranded RNA (dsRNA) viruses. Totiviridae genomes are 4.6 to 6.7 kb in size and contain two ORFs (RdRp)[1]. Totivirus, Victorivirus, Leishmanivirus, Giardiavirus, and Trichomonasvirus

are the five identified families. Totiviruses and Victoriviruses are fungus that attack yeast, smut fungi, and filamentous fungi, respectively, whereas Giardiaviruses and Leishmanniaviruses are parasitic protozoa[2]. Several totivirus-like genomes with comparable structures and morphology but limited similarity to members of other viruses have been discovered in recent times, for instance Totiviridae families have been found in non-fungal hosts such as shrimp, fish, and mosquitoes. [3-8].

Mosquitoes carry parasites and diseases that cause significant public health issues. Mosquito-borne viruses, in particular, are receiving increased focus and concern as a result of freshly appearing and re-emerging arboviral diseases, as well as recent global warming, which may assist in the spread of vector mosquitoes [9]. Deep sequencing from mosquitoes discovered some toti-like dsRNA viruses during an arbovirus study in China [10].

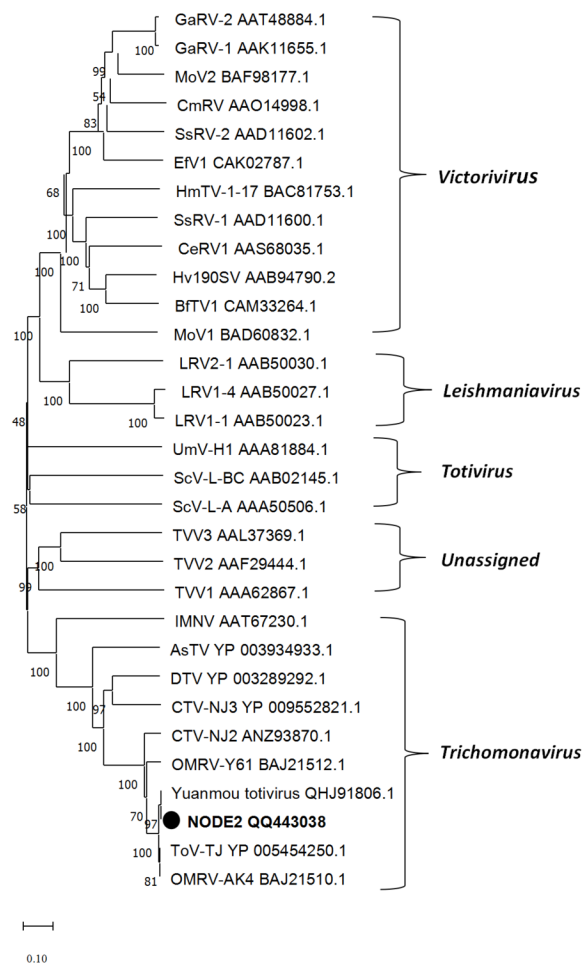
In this study, we isolate another novel virus from *Culex tritaeniorhynchus* in Chuxiong, Yunnan, China, which designated NODE2 tentatively belongs to the family Totiviridae. The cell lines used was C6/36 and inoculated with virus for 72h until a cytopathic effect was observed. Viral RNA was extracted and used to conduct a whole-genome sequencing on an Illumina HiSeq platform. The DNA sequence data was uploaded to GenBank with the entry code QQ443038. According to DNA research, the NODE2 genome is 7714bp long, with two ORFs and a 13-nt-long untranslated region (UTR) at the 5' end and a 161-nt-long UTR at the 3' end. ORF1 encodes a protein with 1689 amino acids ranging from nt 14 to 5083. (5070nt), which correlates to the capsid protein of other Totiviridae members. ORF2 spans nt 4789-7554 encoding 921 aa corresponds to the RNA dependent RNA polymerase and have a putative RaRp motif(RDRP-4) from aa position 408 to 480 .

Sequence analysis reveals an overlap area between ORF1 and ORF2, implying a possible ribosomal-1 in NODE2. A heptamer UUUUUUA was discovered in the overlap area at nt slippery position 4912, which corresponds to the earlier suggested XXXYYYZ (where X is A, C, G, or U, Y is A or U, and Z is A, C, or U)[11, 12]. This heptamer was followed by a pseudoknot at nt 4919-5053 (<http://bibiserv.techfak.uni-bielefeld.de/knotinframe/>). RNA pseudoknots are known to aid the ribosome in stopping translation, so an increase in frameshift and -1 frameshifting would have to occur within the ORF1-ORF2 overlap region[13, 14]. The NODE2 gene contained a ribosomal -1 translational frameshift. The discovery of a 'slippery heptamer' followed almost immediately by a pseudoknot lends credence to this hypothesis. As a result, a pseudosynaptic 'slippery heptamer' can be used to validate the existence or lack of ribosome-1 translational frameshift in the genome [8].

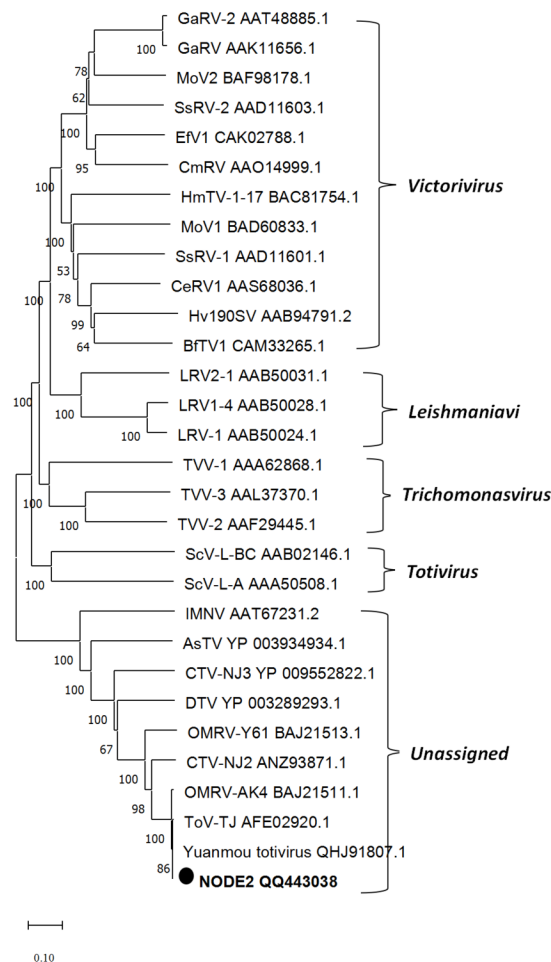
Some viruses with positive-stranded RNA and dsRNA have "2A" or "2A-like" nucleotide patterns, which induce ribosome "skipping" and apparent fragmentation of the viral polyprotein precursor [5]. Translation of the expected amino acid sequence from NODE2 ORF1, one 2A-like sequence motif (EGIEPNPGP) was discovered at aa location 504-512. The finding of such a structure in NODE2 suggests that the ORF1 product undergoes co-translational processing into two polypeptides. AsTV which isolated from mosquitoes is also observed one 2A-like sequence in ORF1 (GDVESNPGP, aa positions 313-321), and DTV has the same situation that observed one 2A-like sequence in ORF1 (EGVKPNPGP, aa positions 71-79). Other totiviruses like IMNV, OMRV, ToV-TJ, GSTV were found two conserved 2A-like sequence motifs in ORF1. IMNV are at aa position 86-94(GDVESNPGP) and 370-378(GDVEENPGP). OMRV-AK4 are at aa position 88-96(EGVEPNPGP) and 500-508(EGIEPNPGP), respectively. In the ToV-TJ genome sequence, at aa positions 88-96 and 500-508, there are two 2A-like sequence motifs(EGVEPNPGP), and the motifs for GSTV are EGVEKNPGP and GDIESNPGP at 305-313 and 466-474, respectively. Two conserved 2A-like sequence patterns are likely to split ORF1 polyprotein into three products[15].

The NODE2 CP was most similar to the strains Yuanmou totivirus and ToV-TJ (99% and 99.64%, respectively) based on amino acid sequence blastp analysis. NODE2 ORF2's amino acid sequence shared substantial parallels with RdRps from viruses in the Totiviridae family, especially ToV-TJ (99.73%) and OMRV-AK4 (98.4%). Genomic comparisons between mosquito strains (Yuanmou totivirus, OMRV-AK4, OMRV-Y61, CTV-NJ2 and CTV-NJ3) revealed that NODE2 shared the most similarities with the strain Yuanmou totivirus (98%) not the strain CTV-NJ2 or CTV-NJ3, while the stains of NODE2, CTV-NJ2, CTV-NJ3 isolated

from the same place.



(a)



(b)

Fig.1. MEGA 11 program produced neighbor-joining phylogenetic trees of Totiviridae members based on coat protein(a) and RaRp amino acid sequences (b). The tree was constructed with 500 bootstrap repeats of the neighbor-joining method, as well as the pairwise deletion and P-distance model parameters.

A phylogenetic analysis based on ORF1 CP and ORF2 RdRp sequences was performed to investigate NODE2's historical connection to other viruses in the Totiviridae family. NODE2, Yuanmou totivirus, TOV-TJ, OMRV-AK4, OMRV-Y61, DTV, IMNV are members of a distinct but tightly related cluster, and in both the CP and RaRp trees(Fig.1), showing the existence of a novel genus within the Totiviridae family. It suggests that these unidentified Totiviridae family members should be regarded members of a new species[8].

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