A novel negevirus isolated from *Aedes vexans* mosquitoes in Finland

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Abstract
Negeviruses are insect-specific enveloped RNA viruses that have been detected in mosquitoes and sandflies from various geographical locations. Here, we describe a new negevirus from Northern Europe, isolated from pool of *Aedes vexans* mosquitoes collected in Finland, designated as Mekrijärvi negevirus (MEJNV). MEJNV had a typical negevirus genome organization, is 9,740 nucleotides in length, and has a GC content of 47.53%. The MEJNV genome contains three ORFs, each containing the following identified conserved domains: ORF1 (7,068 nt) encodes a viral methyltransferase, an FtsJ-like methyltransferase, a viral RNA helicase, and an RNA-dependent RNA polymerase, ORF2 (1,242 nt) encodes a putative virion glycoprotein, and ORF3 (660 nt) encodes a putative virion membrane protein. A distinctive feature relative to other currently known negeviruses is a 7-nucleotide-long overlap between ORF1 and ORF2. MEJNV shares the highest sequence identity with Ying Kou virus from China, with 67.71% nucleotide and 75.19% and 59.00% amino acid sequence identity in ORF 1 and ORF 2, respectively. ORF3 had the highest amino acid sequence similarity to Daeseongdong virus 1 and negevirus Nona 1, both with 77.61% identity, and to Ying Kou virus, with 71.22% identity. MEJNV is currently the northernmost negevirus described. Our report supports the view that negeviruses are a globally distributed, diverse group of viruses that can be found from mosquitoes in a wide range of terrestrial biomes from tropical to boreal forests.
Kit (New England Biolabs), quantified using an NEBNext Library Quant Kit for Illumina (New England Biolabs), and sequenced on a MiSeq platform using a MiSeq Reagent Kit v2 with 150-bp paired-end reads. Raw sequence reads were trimmed, and low-quality (quality score < 30) and short (< 50 nt) sequences were removed using Trimmomatic [10]. Thereafter, de novo-assembly was conducted using MegaHit, followed by re-assembly against the de novo assembled consensus sequence using the BWA-MEM algorithm [11] implemented in SAMTools version 1.8 [12]. A total of 357,745 reads mapped to the largest contig, with mean coverage of 7.624 (range, 146-17,546).

A genomic sequence of 9,740 nt was identified as a negevirus using an NCBI BLASTx search, and the isolate was designated as “Mekrijärvi negevirus” (MEJNV) (GenBank accession number MT522375). Three ORFs were identified in the MEJNV genome using NCBI ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/) (Fig. 1), flanked by untranslated regions (unverified) of 509 nt (5’UTR) and 162 nt (3’UTR) in length. MEJNV was most similar to Ying Kou virus strain YK1714 (NC_040636.1) originating from Culex pipiens pallens mosquitoes in China, with 67.71% nucleotide sequence identity. Similar analysis done with amino acid sequences showed 75.19% identity between ORF1, 59.00% between ORF2, and 71.22% between ORF3 sequences. However, the closest matches to ORF3 of MEJNV were those of Daeseongdong virus 1 (NC_028487.1) and negevirus Nona 1 (AB972669.1), both with 77.61% identity. Notably, the GC% of MEJNV was similar to that of Ying Kou virus (GC% = 48.18%). Phylogenetic analysis suggested that MEJNV forms a monophyletic cluster (bootstrap value, 100) with Ying Kou virus. The closest relatives of MEJNV and Ying Kou viruses included viruses from Japan and South Korea (Daeseongdong virus 1 and negevirus Nona 1) and more distant relatives from South America, Australia, and the Philippines. Notably, the geographically closest negevirus sequence from Sweden in 2009 (Biggie virus, GenBank accession no. QGA70894) was not grouped with MEJNV. These results indicate that MEJNV is a distinct strain of negevirus related to negeviruses from distant geographical locations, and it is currently the northernmost negevirus strain to be isolated. Our results support the view that negeviruses are distributed globally in mosquitoes.

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**Fig. 1** Genome organization of the novel Mekrijärvi negevirus. The lengths (in nucleotides) of ORFs 1–3 and the intergenic region are shown in parentheses. The nucleotide positions of ORFs and their putative conserved protein domains within the genome are shown in square brackets.
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Compliance with ethical standards

Conflict of interest
The authors declare no conflict of interest.

Availability of data and material
The Mekrijärvi negevirus genome sequence has been deposited in the NCBI GenBank database under accession number MT522375.

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Fig. 2 Phylogenetic tree of negeviruses and related viruses (*n*=43) computed from protein sequences of ORF1 containing the RNA-dependent RNA polymerase (RdRp). The host, country of origin, and collection year are shown in square brackets. Unknown information is indicated by a hyphen. Sequences were aligned using MAFFT, and a maximum-likelihood tree with 1,000 bootstrap replicates was built using IQ-TREE. The tree is rooted at the midpoint.
References

1. Vasilakis N, Forrester NL, Palacios G, Nasar F, Savij N, Rossi SL, Guzman H, Wood TG, Popov V, Gorchakov R, Gonzalez AV, Haddow AD, Watts DM, da Rosa APAT, Weaver SC, Lippiak W, Tesh RB (2013) Negevirus: a proposed new taxon of insect-specific viruses with wide geographic distribution. J Virol 87(5):2475–2488. https://doi.org/10.1128/jvi.00776-12

2. O’Brien CA, McLean BJ, Colmant AMG, Harrison JJ, Hall-Mendelin S, van den Hurk AF, Johansen CA, Watterson D, Bielefeldt-Ohmann H, Newton ND, Schulz BL, Hall RA, Hobson-Peters J (2017) Discovery and characterisation of castlerea virus, a new species of Negevirus isolated in Australia. Evolut Bioinform 13:10–12. https://doi.org/10.1177/1176934317691269

3. Nunes MRT, Contreras-Gutierrez MA, Guzman H, Martins LC, Barbirato MF, Savit C, Balta V, Uribe S, Vivero R, Suaza JD, Oliveira H, Nunes Neto JP, Carvalho VL, da Silva SP, Cardoso JF, de Oliveira RS, da Silva Lemos P, Wood TG, Widen SG, Vasconcelos PFC, Fish D, Vasilakis N, Tesh RB (2017) Genetic characterization, molecular epidemiology, and phylogenetic relationships of insect-specific viruses in the taxon Negevirus. Virology 504:152–167. https://doi.org/10.1016/j.virol.2017.01.022

4. Carapeta S, do Bem B, McGuinness J, Esteves A, Abecasis A, Lopes A, de Matos AP, Piedade J, de Almeida APG, Parreira R (2015) Negeviruses found in multiple species of mosquitoes from southern Portugal: isolation, genetic diversity, and replication in insect cell culture. Virology 483:318–328. https://doi.org/10.1016/j.virol.2015.04.021

5. Kallies R, Kopp A, Zirkel F, Estrada A, Gillespie TR, Drosten C, Junglen S (2014) Genetic characterization of goutanap virus, a novel virus related to negeviruses, cileviruses and higreviruses. Viruses 6(11):4346–4357. https://doi.org/10.3390/v6114346

6. Zhao L, Mwaliko C, Atoni E, Wang Y, Zhang Y, Zhang J, Hu X, Yan Z (2019) Characterization of a novel tanay virus isolated from anopheles sinensis mosquitoes in Yunnan, China. Front Microbiol 10:1–11. https://doi.org/10.3389/fmicb.2019.01963

7. Charles J, Tangued C, Hurt SL, Tuneschif C, Firth AE, Garcia-Rejon JE, Machain-Williams C, Blitvich BJ (2018) Detection of novel and recognized RNA viruses in mosquitoes from the Yucatan Peninsula of Mexico using metagenomics and characterization of their in vitro host ranges. J Gen Virol 99(12):1729–1738. https://doi.org/10.1099/jgv.0.001165

8. Korhonen EM, Suvanto MT, Uusitalo R, Faolotto G, Smura T, Sane J, Vapalahti O, Huhtamo E (2020) Sindbis virus strains of divergent origin isolated from humans and mosquitoes during a recent outbreak in Finland. Vector-Borne Zoonotic Dis. https://doi.org/10.1089/vbz.2019.2562

9. Becker N, Petric D, Zgomba M, Boase C et al (2010) Mosquitoes and their control. Springer, Berlin, Heidelberg

10. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15):2114–2120

11. Li H (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv. 1303.3997v1

12. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Subgroup GPDP (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25(16):2078–2079

13. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30(4):772–780

14. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2014) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32(1):268–274

15. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermini LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14(6):587–589

16. Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2017) UBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol 35(2):518–522

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