





Genome Sequence of a Ranavirus Isolated from a Red-Eared Slider (*Trachemys scripta elegans*) in Poland

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ABSTRACT The red-eared slider (RES) ranavirus (RESRV) was isolated from a free-ranging RES turtle that died with evidence of respiratory disease. The RESRV genome sequence (106,878 bp) was determined, and phylogenetic analysis revealed that it is a common midwife toad virus (CMTV) strain. This study is the first report of CMTV in RES.

Red-eared sliders (RES; *Trachemys scripta elegans*) are semiaquatic turtles with a native range extending from the southeastern United States to northern Mexico. They are listed as an invasive alien species (IAS) by the European Union legislation (1). A RES was captured from a shallow retention reservoir in Majdan Zahorodynski in eastern Poland (51°13'44"N, 23°08'26"E) and soon after died with signs of respiratory disease. A necropsy revealed congestion and edema with petechial hemorrhages in the tracheal mucosa and liver. Splenomegaly as well as hepatic and tracheal necrosis strongly suggested a viral infection. Homogenates prepared from the animal's pooled tissues (e.g., liver, kidney, spleen) were used for virus isolation attempts in *Terrapene carolina* heart (TH-1) cells (CCLV-RIE 1131), as previously described (2). Cytopathic effects, including cell rounding, detachment, and lysis, were observed in the first cell passage. DNA was extracted from the cell culture supernatant using a Qiagen DNeasy blood and tissue kit, and the sample tested positive for ranavirus by quantitative PCR (qPCR) (3).

Viral DNA served as the template for constructing a DNA library using a TruSeq Dual Index high-throughput (HT) DNA PCR-free library preparation kit (Illumina), followed by sequencing on an Illumina MiSeq sequencer using a v3 chemistry 600-cycle kit. *De novo* assembly of the paired-end reads was performed in SPAdes v3.13.0 with default parameters (4). BLASTN analysis of the resulting three contigs was performed against the National Center for Biotechnology Information (NCBI) nonredundant nucleotide database. The largest contig was 106,878 bp, with a G+C content of 56% and an average coverage of 11,055 reads/nucleotide, and showed the highest nucleotide identity (98.85%) to a common midwife toad virus (CMTV) strain (*Pelophylax esculentus* virus [PEV]; GenBank accession no. [MF538627](https://www.ncbi.nlm.nih.gov/nuclseq/MT000000)).

The genome of the red-eared slider ranavirus (RESRV) was annotated using the Genome Annotation Transfer Utility with default parameters, and the CMTV strain PEV was used as the reference genome. Additional putative open reading frames (ORFs) were identified using GeneMarkS, and gene functions were predicted based on BLASTP searches against the NCBI GenBank nonredundant protein sequence database. A total of 101 putative ORFs were predicted in RESRV, compared to strains of CMTV

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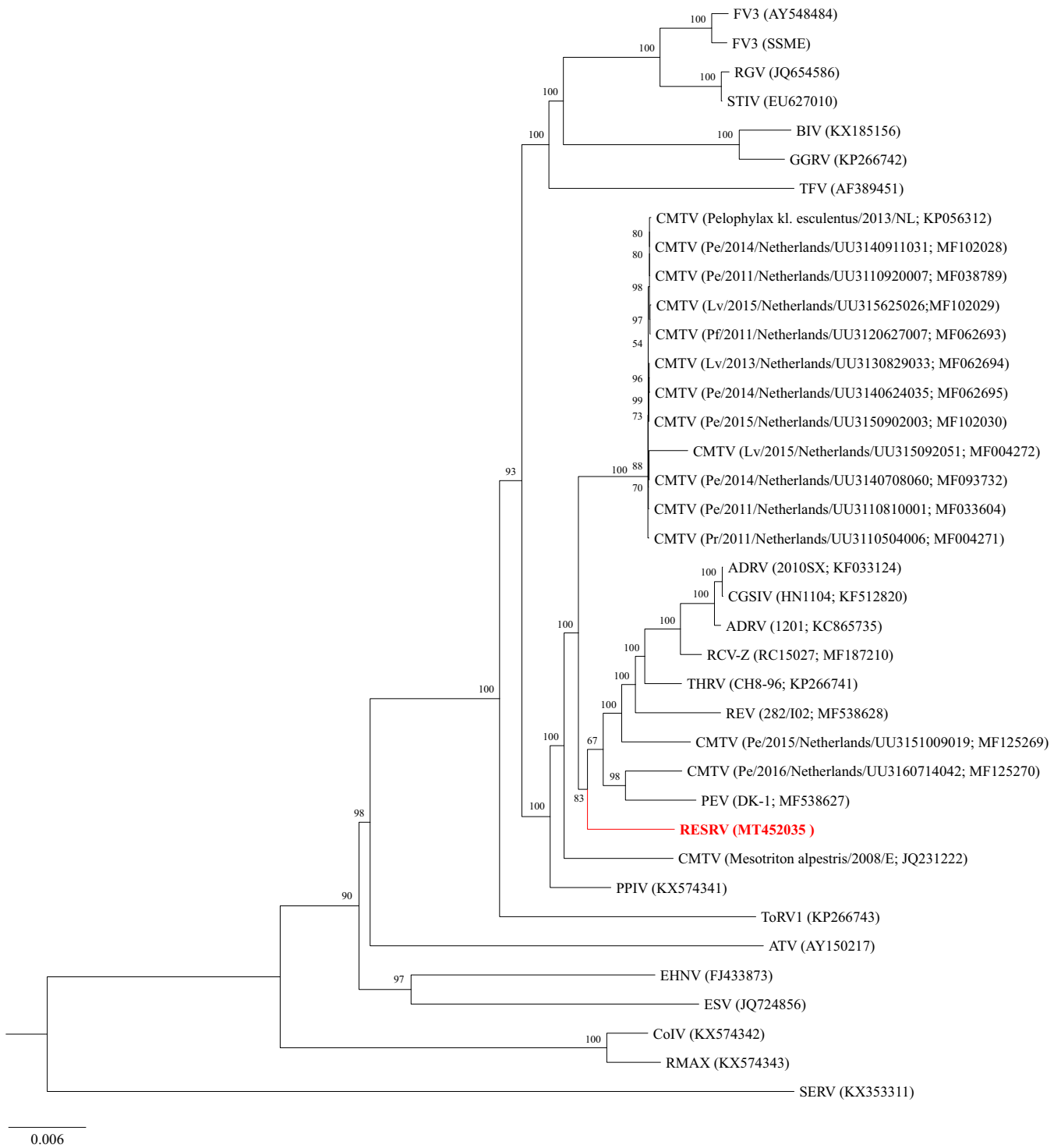


FIG 1 Maximum-likelihood phylogram depicting the relationship of RESRV (in red) to 37 ranaviruses based on the concatenated genome-wide locally colinear block alignments. Isolate/strain identification (where available) and GenBank accession numbers are listed in parentheses. The bootstrap values are provided at each node.

predicted to encode between 98 and 112 ORFs (5–12). An analysis of locally colinear blocks (LCB) in Mauve, with default parameters, revealed that the RESRV displays the same genome arrangement as other strains of CMTV (data not shown). The genome-wide LCB alignments were concatenated in Geneious v10.2.6 (13) and used in a maximum-likelihood (ML) analysis in IQ-Tree (<http://iqtree.cibiv.univie.ac.at>) with default

parameters and 1,000 bootstrap replicates. The resulting ML tree supported the Polish RESRV as a strain of CMTV (Fig. 1).

Members of the genus *Ranavirus* (family *Iridoviridae*) are globally emerging viruses that have been reported in wild and captive populations of ectothermic vertebrates (14). Although previous experimental challenge studies demonstrated that red-eared sliders are susceptible to frog virus 3 (15, 16), our study confirms the susceptibility of a free-ranging RES to CMTV. Thus, this invasive chelonian represents a new host for CMTV and may potentially spread ranaviruses into native populations of fish, amphibians, and reptiles. Ethical approval for turtle trapping and euthanasia was not required according to the local bioethical committee at the University of Life Sciences in Lublin, Poland (statement of 14 October 2014).

Data availability. The genome sequence and raw sequence data for RESRV have been deposited in the NCBI GenBank and Sequence Read Archive (SRA) databases under accession no. [MT452035](#) and [SRX8622342](#), respectively.

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