










Complete Genome Coding Sequences of Artashat, Burana, Caspiy, Chim, Geran, Tamdy, and Uzun-Agach Viruses (*Bunyavirales: Nairoviridae: Orthonairovirus*)

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ABSTRACT The bunyaviral monogeneric family *Nairoviridae* currently includes 12 species for 35 distinct viruses. Here, we present the complete genome coding sequences of an additional seven nairoviruses. Five of them can be assigned to established species, whereas two of them (Artashat and Chim viruses) ought to be assigned to two novel species.

In 2016, the family *Bunyaviridae* was thoroughly revised into an order (*Bunyavirales*) including nine families. In parallel, the bunyaviral genus *Nairovirus* (*Nairoviridae: Orthonairovirus*) has been expanded by the creation of 5 novel species and now includes 12 species for 35 distinct viruses (1, 2). Partial genomic sequences have been published for numerous tentative orthonairoviruses (2), but their classification requires the determination of complete genome coding sequences. Here, we report the complete coding sequences of seven putative orthonairoviruses.

We obtained Artashat, Burana, Caspiy, Chim, Geran, Tamdy, and Uzun-Agach viruses (3–9) from the Russian State Collection of Viruses in the form of a lyophilized 10% (wt/vol) suspension of infected suckling mouse brain tissue. The viruses were recovered by intracerebral infection of 1- to 2-day-old mice. We isolated total RNA from the brains of infected mice with 1 ml of TRI reagent (Molecular Research Center, Cincinnati, OH, USA), purified RNA with the RNeasy MinElute cleanup kit (Qiagen, Hilden, Germany), and depleted rRNA with the GeneRead rRNA depletion kit (Qiagen). Depleted RNA was fragmented at 85°C for 5 min in 2× reverse transcriptase buffer (Thermo Fisher Scientific, Grand Island, NY, USA). Reverse transcription was performed with hexameric random primers (Promega, Madison, WI, USA) and RevertAid reverse transcriptase (Thermo Fisher Scientific). We used the NEBNext second-strand synthesis module (New England BioLabs, Ipswich, MA, USA) to convert first-strand cDNA to double-stranded cDNA, which was used to prepare next-generation sequencing libraries with the TruSeq DNA LT library prep kit (Illumina, San Diego, CA, USA). Sequencing of indexed libraries was done on an Illumina MiSeq instrument with a paired-end 250-bp protocol, followed by *de novo* genome assembly with CLC Genomics Workbench 7.0 (CLC bio, Waltham, MA, USA).

Genetic and phylogenetic analyses were performed as outlined by Kuhn et al. (2). Briefly, genomes were aligned using the CLUSTAL algorithm at the amino acid level

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using MEGA6 (10). Neighbor-joining (NJ) analysis at the amino acid level was performed due to the observed high variability of the underlying nucleotide sequences. The statistical significance of the tree topology was evaluated by bootstrap resampling of the sequences 1,000 times. Phylogenetic analyses were performed using MEGA6 (2). These analyses indicate that Artashat virus and Chim virus ought to be assigned to novel species (proposed to be named “*Artashat orthonairovirus*” and “*Chim orthonairovirus*,” respectively). Furthermore, the analysis suggests that Burana virus and Tamdy virus are both members of the species *Burana orthonairovirus* (proposed to be renamed “*Tamdy orthonairovirus*”), Caspiy virus is a member of the species *Hughes orthonairovirus*, Uzun-Agach virus is a member of the species *Keterah orthonairovirus*, and Geran virus is a member of the species *Qalyub orthonairovirus*. An official taxonomic proposal to this effect was submitted on 8 June 2017 to the International Committee on Taxonomy of Viruses (ICTV). This proposal (2017.008 M) has been accepted by the ICTV Executive Committee and is now awaiting ratification.

Accession number(s). The GenBank accession numbers for the determined complete genome coding sequences of S, M, and L segments are [KP792746](#), [KP792745](#), and [KP792744](#) (Artashat virus), [KP792707](#), [KP792706](#), and [KP792705](#) (Burana virus), [KP792710](#), [KP792709](#), and [KP792708](#) (Caspiy virus), [KP792713](#), [KP792712](#), and [KP792711](#) (Chim virus), [KP792716](#), [KP792715](#), and [KP792714](#) (Geran virus), [KP792728](#), [KP792727](#), and [KP792726](#) (Tamdy virus), and [KP792743](#), [KP792742](#), and [KP792741](#) (Uzun-Agach virus), respectively.

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