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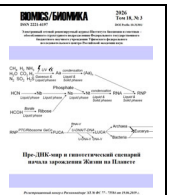

Phylogenetic analysis of amino acid sequences of DNA polymerases of *Chordopoxvirinae* poxviruses

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Resume

The family *Poxviridae* is subdivided into two subfamilies: *Chordopoxvirinae* (vertebrate viruses) and *Entomopoxvirinae* (insect viruses). Viruses of the subfamily *Chordopoxvirinae* have a broad host range: mammals, birds, reptiles, fishes. Some viruses of this subfamily, in particular *Orthopoxvirus* species (monkeypox virus, cowpox virus and others), can cause zoonotic infections in humans upon their contact with infected animals. In view of the current situation with monkeypox virus in Russia and globally (several cases of monkeypox were registered among Russian tourists visiting southern countries in the spring of 2026), a phylogenetic study of the genus *Orthopoxvirus* would be relevant. Correspondingly, we have carried out a comparative study of 49 DNA-dependent DNA polymerases of *Chordopoxvirinae* viruses, including species from the genus *Orthopoxvirus*. Based on the multiple alignment with MUSCLE, a phylogenetic tree was inferred using the UPGMA method in MEGA6. The branch support level was determined using 1000 bootstrap replicates. The closest sequences to DNA polymerase of monkeypox virus were sequences of several similar enzymes from the group of cowpox virus, and a slightly more distant sequence was DNA polymerase of ectromelia virus, which causes mousepox. The overall consistency of the results of this comparative analysis suggests that the amino acid sequences of *Orthopoxvirus* B-type DNA polymerases can be used to determine taxonomy of viral isolates. In the context of biotechnology and genetic engineering, all these DNA polymerases can be considered equivalent.

Keywords: poxviruses, DNA polymerase, phylogenetic analysis, monkeypox virus, UPGMA

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The importance of this phylogenetic study of poxviruses, which is based on the comparative analysis of their DNA polymerases, is dictated by the current monkeypox epidemiological situation in Russia and worldwide. In the spring of 2026, four cases of monkeypox were registered: two patients were hospitalized in the Moscow region (https://ria.ru/20260417/situatsiya-2087467516.html) and St.Petersburg (https://www.fontanka.ru/2026/02/15/76266151/). As stated by the head of Rospotrebnadzor (Federal Service for the Oversight of Consumer Protection and Welfare) Anna Popova, the situation with monkeypox is a

matter of serious concern: for the entire history of epidemiological monitoring of this virus, it has never been observed to spread so wide. "The situation with the plague is, of course, difficult, and monkeypox is still ongoing. We are watching how volatile the virus is going to be and what happens next," TASS quotes her. The head of Rospotrebnadzor also noted that the number of worldwide monkeypox infections reached the maximum. In connection with this, the agency is monitoring the risks of the pathogen spread in Russia.

Monkeypox is a rare disease caused by a virus from the genus *Orthopoxvirus*. The incubation period usually ranges from 5 to 21 days. The main symptoms include swollen lymph nodes, fever, headache, and nausea. On the 3rd to 5th day after infection, a characteristic rash may appear: emerging first are spots and then swollen zones, in which blisters filled with transparent fluid form. Over time, the rash develops into crusts and falls off. The disease can also be asymptomatic.

Poxviruses are representatives of the family *Poxviridae*. It belongs to the realm *Varidnaviria*, kingdom *Bamfordvirae*, phylum *Nucleocytoviricota* (nucleocytoplasmic large DNA viruses), class *Pokkesviricetes*, order *Chitovirales* (https://ictv.global/taxonomy). The *Poxviridae* family has two subfamilies: *Chordopoxvirinae* (vertebrate viruses) and *Entomopoxvirinae* (insect viruses) (https://ictv.global/report/chapter/poxviridae/poxviridae). The hosts of poxviruses are wild and domestic vertebrates, including mammals, birds, reptiles, and fishes. Poxviruses can cause epidemic and epizootic outbreaks [Silva et al., 2020]. Along with monkeypox virus, other species from the genus *Orthopoxvirus*, in particular cowpox virus and vaccinia virus, can cause zoonotic infections when humans come into contact with infected animals [Reynolds et al., 2018]. Species from the genus *Parapoxvirus*, such as bovine papular stomatitis virus, pseudocowpox virus and orf virus, are also causative agents of a number of zoonotic infections [Sindryakova et al., 2024; Lostia et al., 2026]. Avipoxviruses infect various species of birds, including sparrows, pigeons, flamingos, penguins, etc. [Weli, Tryland, 2011; Giotis, Skinner, 2019].

Some poxviruses are capable to infect multiple hosts [Haller et al., 2014]. At least 11 species can infect humans [Oliveira et al., 2017]. The best-studied members of the genus *Orthopoxvirus* are the smallpox virus, which has caused numerous pandemics, and the vaccinia virus, which is used to vaccinate against smallpox [Jones et al., 2009].

Poxvirus genomes are large in comparison with the genomes of other animal viruses. They contain linear double-stranded DNA with a length from 120 to 450 thousand bp. Their virions are brick- or ovoid-shaped. The replication of poxvirus DNA occurs in the cytoplasm of the infected cell.

The genomes of nucleocytoplasmic large DNA viruses include 5 core genes: genome-packaging ATPase, major capsid protein, bifunctional primase-helicase, B-family DNA polymerase, and viral late gene transcription factor 3 [Koonin, Yutin, 2018].

In this work, we have constructed a phylogenetic tree based on the alignment of the amino acid sequences of DNA polymerases of 49 vertebrate-infecting poxviruses (subfamily *Chordopoxvirinae*). Amino acid sequences were aligned using MUSCLE [Edgar, 2004]. The phylogenetic tree was inferred in MEGA6 [Tamura et al., 2013] using the UPGMA method [Sokal, Michener, 1958]. The branch support level was determined using bootstrap [Felsenstein, 1985] (1000 replicates). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the JTT matrix-based method [Jones et al., 1992] and expressed in the number of amino acid substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 3). The analysis involved 49 amino acid sequences. All positions with less than 95% site coverage were eliminated (i.e., less than 5% of alignment gaps, missing data, and ambiguous bases were allowed at any position). There was a total of 967 positions in the final dataset. Evolutionary analysis was conducted in MEGA6 [Tamura et al., 2013].

Figure 1 shows that DNA polymerases viruses belonging to the genus *Orthopoxvirus* are grouped in a single clade with 100% bootstrap support (marked in brown). This clade consists of two separate clusters, for which the bootstrap support is 100% and 99%, respectively. The first cluster includes DNA polymerases of buffalopox, rabbitpox, cowpox, horsepox, monkeypox, variola (smallpox), and ectromelia (mousepox) viruses. The second cluster contains 2 proteins of raccoonpox and skunkpox viruses. The neighboring clade with *Orthopoxvirus* DNA polymerases includes 2 proteins from representatives of the genus *Centapoxvirus*: Murmansk poxvirus (isolated from a root vole) и yokapox virus, which was isolated from mosquitoes collected in the Central African Republic (its vertebrate host is still unknown). The node containing the genera *Orthopoxvirus* and *Centapoxvirus* (corresponds to the *Ch*-A1 subgroup from [Yu et al., 2021]) also has the highest level of bootstrap support (100%), and the neighboring clade included 2 sequences of DNA polymerases of bat-associated viruses belonging to the genus *Vespertilionpoxvirus*: hypsugopox and eptesipox.

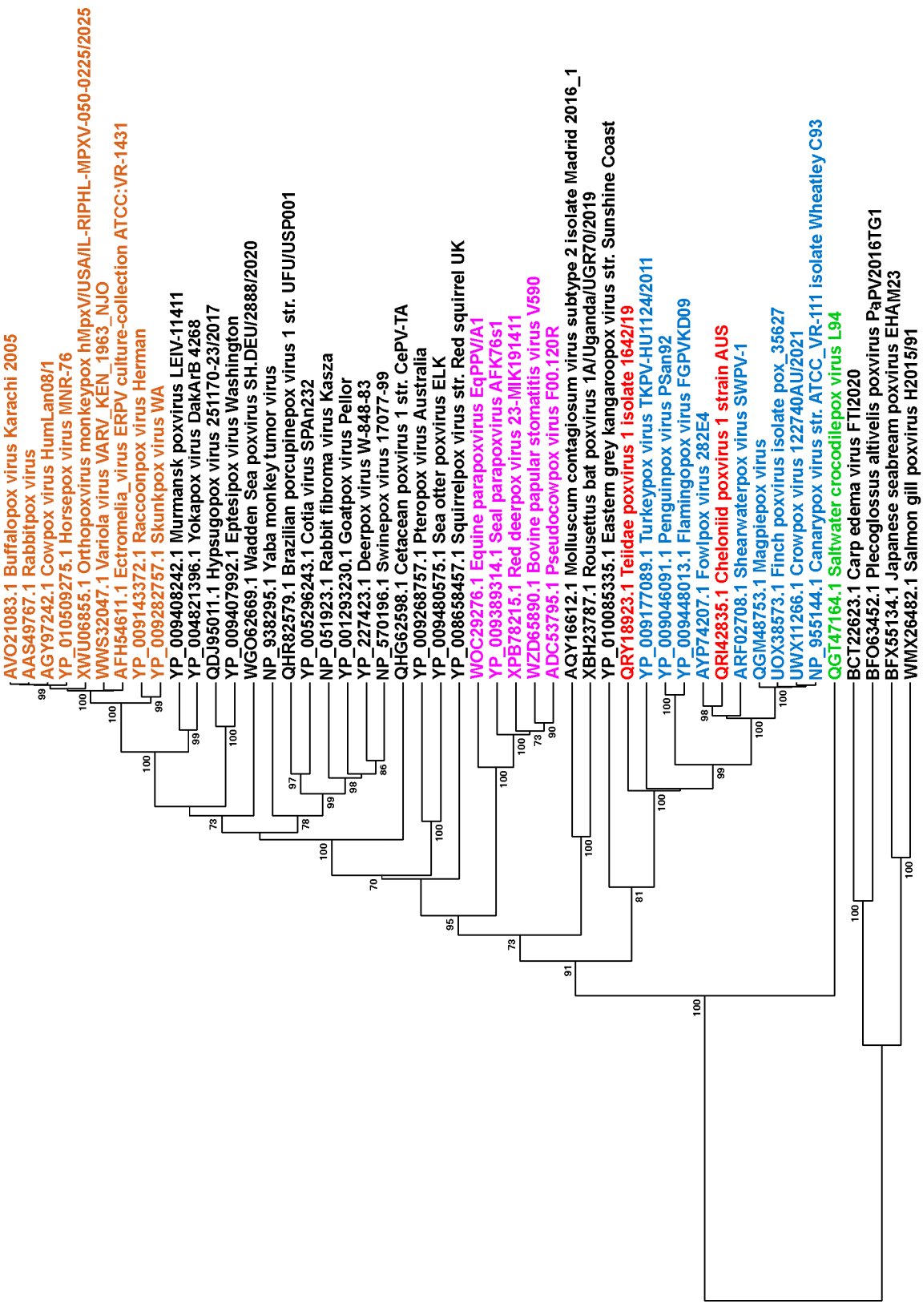

Fig. 1. Evolutionary history of 49 amino acid sequences of *Chordopoxvirinae* DNA polymerases with 5`→3`-polymerase and 3`→5`-exonuclease proofreading activities was inferred using the UPGMA method [Sokal, Michener, 1958]. The accession numbers are given before the virus names. Other explanations in the text.

DNA polymerases of viruses from the genus *Parapoxvirus* are grouped into a single cluster with 100% bootstrap support (indicated in purple in Fig. 1). It contains 5 proteins from equine parapoxvirus, seal parapoxvirus, red deerpox virus, bovine papular stomatitis virus, and pseudocowpox virus.

It was found that the clade with DNA polymerases of viruses from the genus *Avipoxvirus* (indicated in blue in Fig. 1), whose natural hosts are various birds (turkeypox, penguinpox, flamingopox, fowlpox, shearwaterpox, magpiepox, finch poxvirus, crowpox, canarypox), included 2 proteins of reptile viruses (indicated in red in Fig. 1), namely Teiidae poxvirus 1 (isolated from the lizard *Crocodilurus amazonicus*) and Cheloniid poxvirus 1 (isolated from the turtle *Chelonia mydas*). As indicated in the papers describing these two viruses [Seitz et al., 2021; Sarker et al., 2021], they cluster with avipoxviruses. The DNA polymerase of saltwater crocodilepox virus, which belongs to the genus *Crocodylidpoxvirus*, is located on a separate branch (indicated in green in Fig. 1).

The 4 proteins of fish viruses form an outgroup in relation to the overwhelming majority (45) of DNA polymerases. They form 2 separate clusters with 100% bootstrap support. The first cluster includes proteins of Carp edema virus and Plecoglossus altivelis poxvirus; the second cluster, proteins of Japanese seabream poxvirus and salmon gill poxvirus. Correspondingly, DNA polymerases of fish poxviruses differ the most from those of mammalian, avian, and reptilian poxviruses – due to the early divergence of fish poxviruses from the common ancestor of vertebrate poxviruses.

Conclusion

A comparative study of 49 DNA-dependent B-type DNA polymerases of vertebrate poxviruses (subfamily *Chordopoxvirinae*) was conducted. The amino acid sequences were selected from GenBank by BLASTp comparison with the reference sequence of DNA polymerase of monkeypox virus. The stepwise analysis of the protein sequences using multiple sequence alignment (MUSCLE) and the subsequent construction of an evolutionary tree using the unweighted pairwise grouping method with arithmetic mean (UPGMA) showed a fairly high similarity between the majority of DNA polymerases of the *Chordopoxvirinae* subfamily, as evidenced by the high level of branch support according to the results of bootstrap.

Clustering of *Orthopoxvirus* DNA polymerases in this UPGMA analysis has a high level of statistical significance (bootstrap support of 100% or near 100%). The monkeypox enzyme is in the center of a radiation of enzymes from buffalopox, rabbitpox, cowpox, horsepox, variola (smallpox), and ectromelia (mousepox) viruses, with greater similarity to enzymes of mammal viruses, namely buffalopox, rabbitpox, cowpox, and horsepox, than to enzymes of other poxviruses. According to our results, the enzymes of viruses from the genus *Centapoxvirus* are most closely related to *Orthopoxvirus* DNA polymerases, which is consistent with the results obtained in the work [Yu et al., 2021] based on synteny analysis, phylogenetic analysis of concatenated alignments of 22 genes of poxvirus core genome using the maximum likelihood method, SNP analysis of core genes, and the alignment-free method. It should be noted that DNA polymerase was among the 22 core genes identified in the analysis of 60 poxvirus genomes [Yu et al., 2021].

In general, it can be said that the phylogenetic tree constructed from the amino acid sequences of DNA polymerases using the UPGMA method corresponds to the modern classification of *Poxviridae* based on concatenated alignments of 25 conserved proteins (https://ictv.global/report/chapter/poxviridae/poxviridae). DNA-dependent B-family DNA polymerases, characteristic of this group of viruses, are also promising enzymes for use in genetic engineering and applied molecular biology [Irwin et al., 2012; Czarnecki, Traktman, 2017]. The modern methods of gene synthesis and recombinant DNA technologies make it possible to carry out bacterial production of virtually any protein. One of the reasons of these DNA polymerases being prospective is their high accuracy in copying matrix DNA. The phylogenetic analysis of these enzymes shows that all *Orthopoxvirus* DNA polymerases should be considered equivalent for biotechnology. However, selecting the suitable enzyme would require a careful preliminary assessment of differences in their individual amino acid residues – by structural modeling with the prediction of differences in their biochemical properties and verification of these differences experimentally.

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