Supplementary information

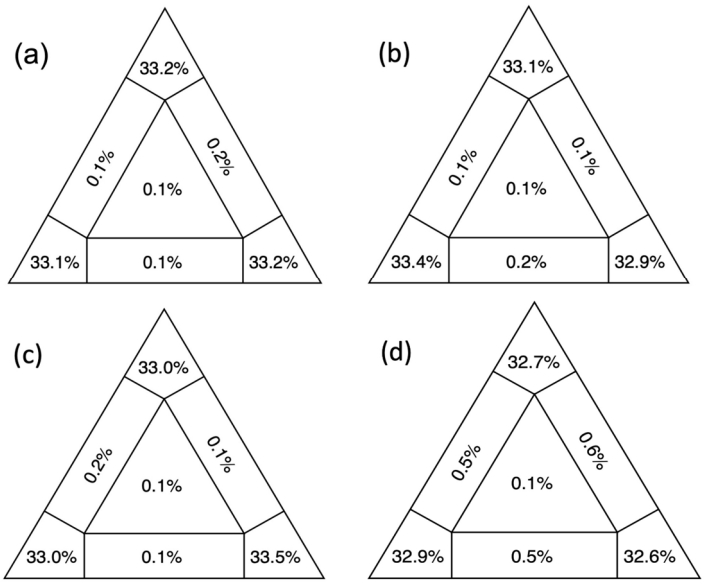


Figure S1. Presence of phylogenetic signal was evaluated by likelihood mapping checking for alternative topologies (tips), unresolved quartets (center) and partly resolved quartets (edges) for 87 OPXV whole genome (a), core genome (b), OPXV orthologous genes (c) and 62 conserved genes.

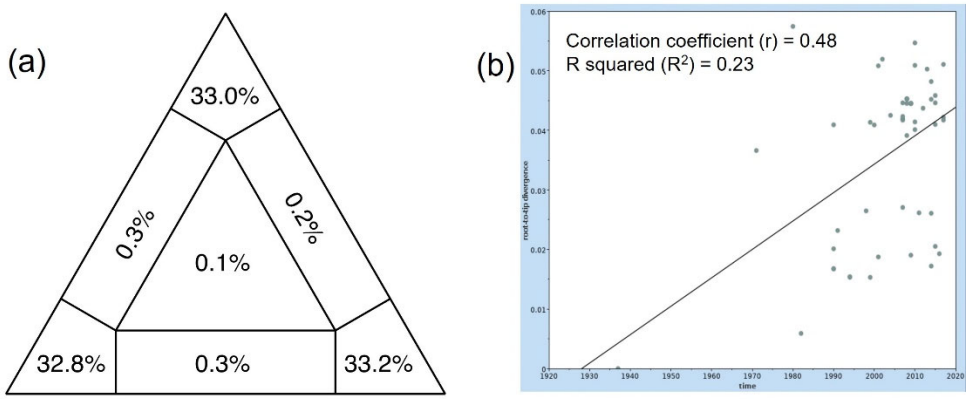
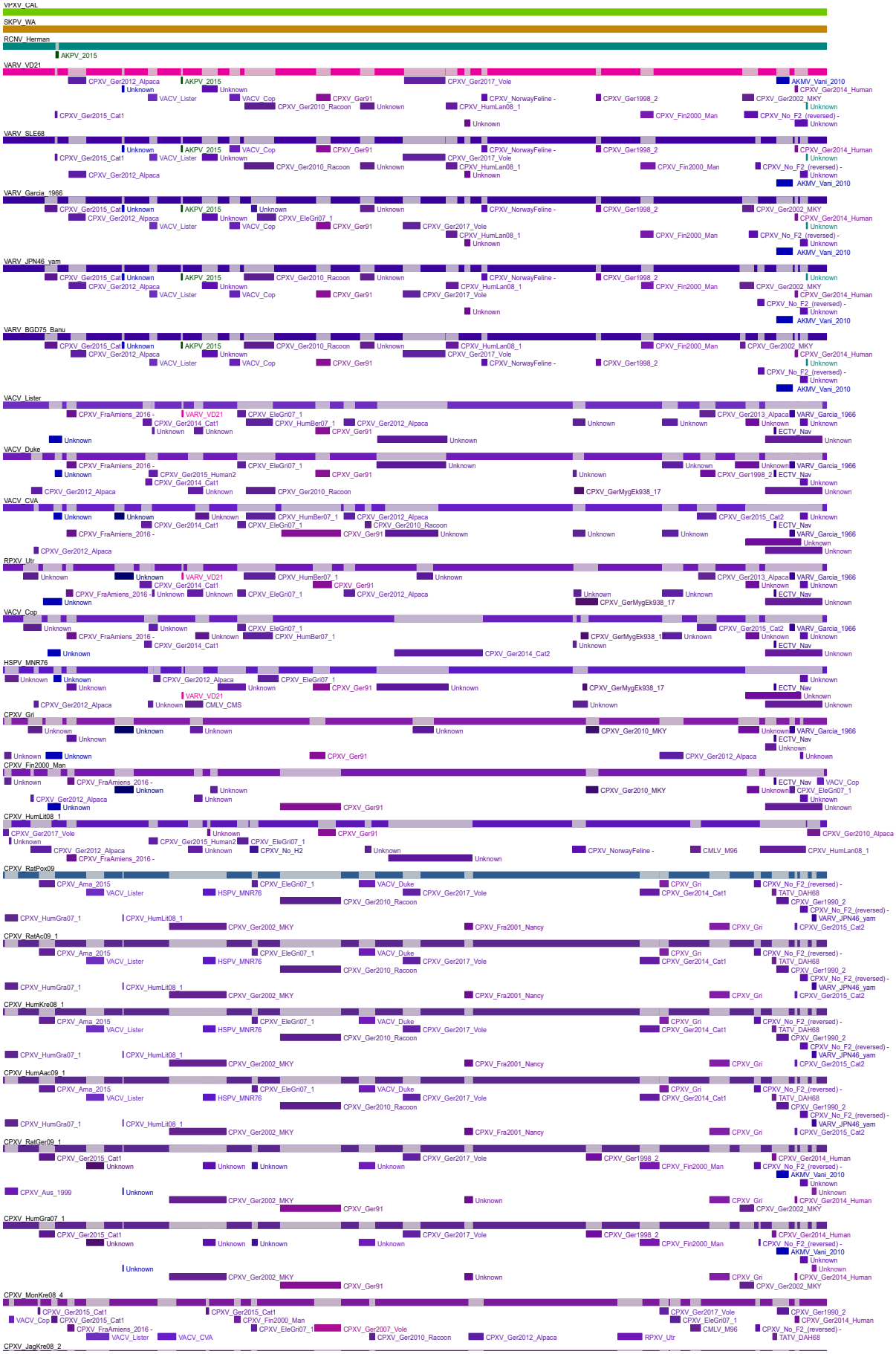


Figure S2. Phylogenetic and temporal signal analyses. (a) Presence of phylogenetic signal was evaluated by likelihood mapping checking for alternative topologies (tips), unresolved quartets (centre) and partly resolved quartets (edges) for 62 conserved genes of 55 CPXV strains. (b) Linear regression of root-to-tip genetic distance in a maximum likelihood phylogeny against sampling time for 62 conserved genes of 55 CPXV strains.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Unknown
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VACV_Lister
VACV_Cop
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CPXV_NorwayFeline -
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CPXV_Ger2010_Racoon
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CPXV_Ger2014_Human
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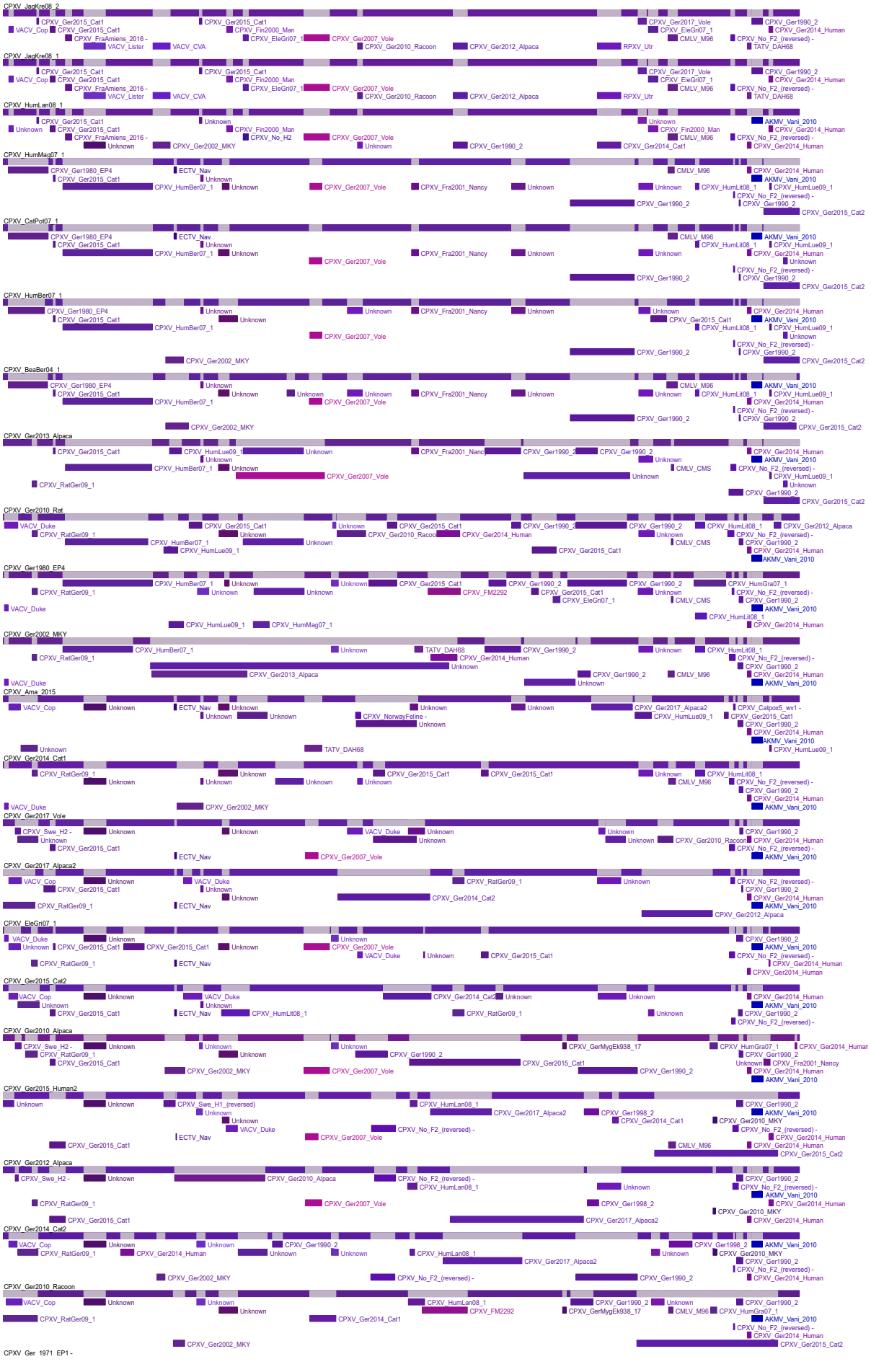
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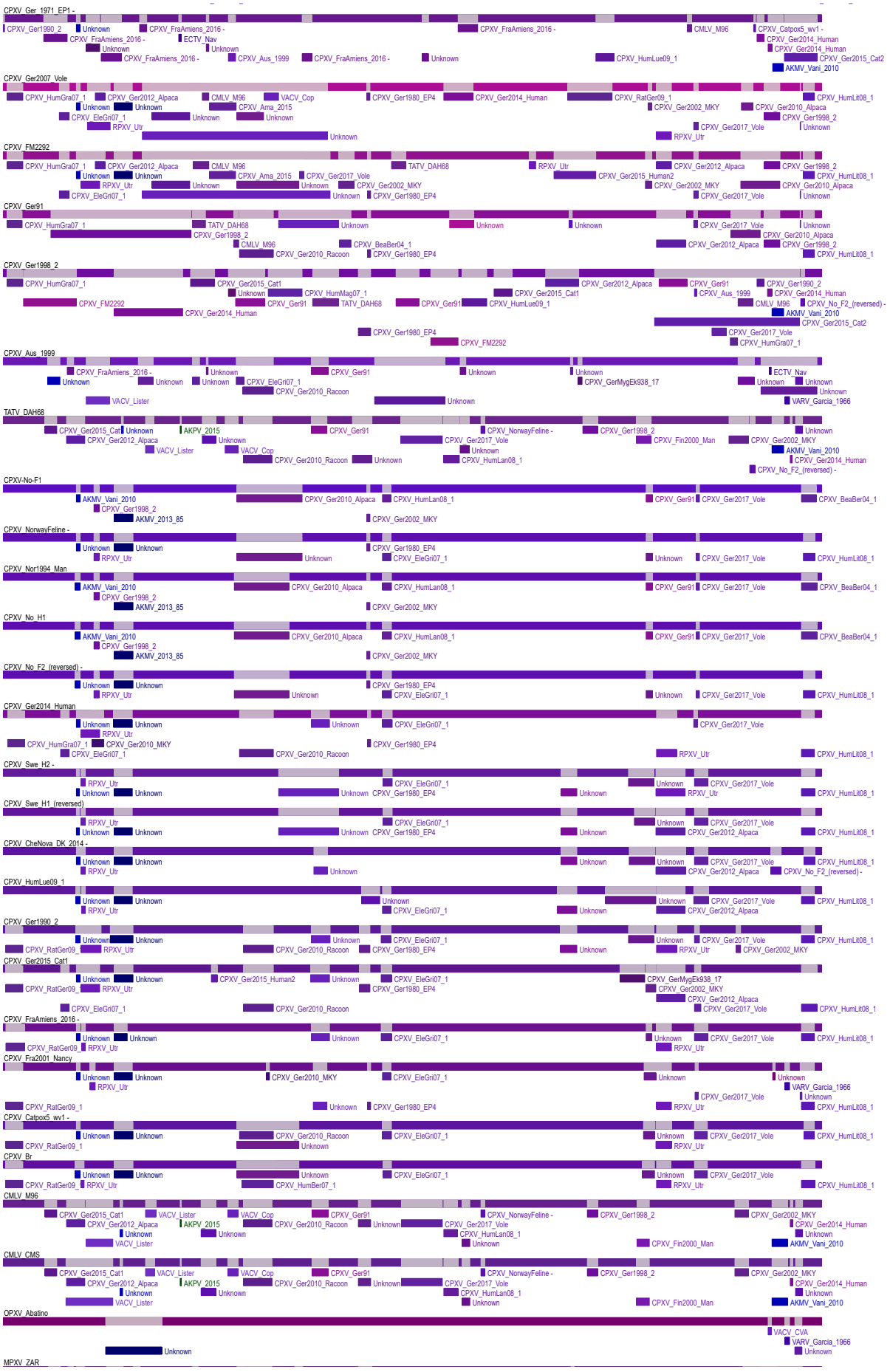
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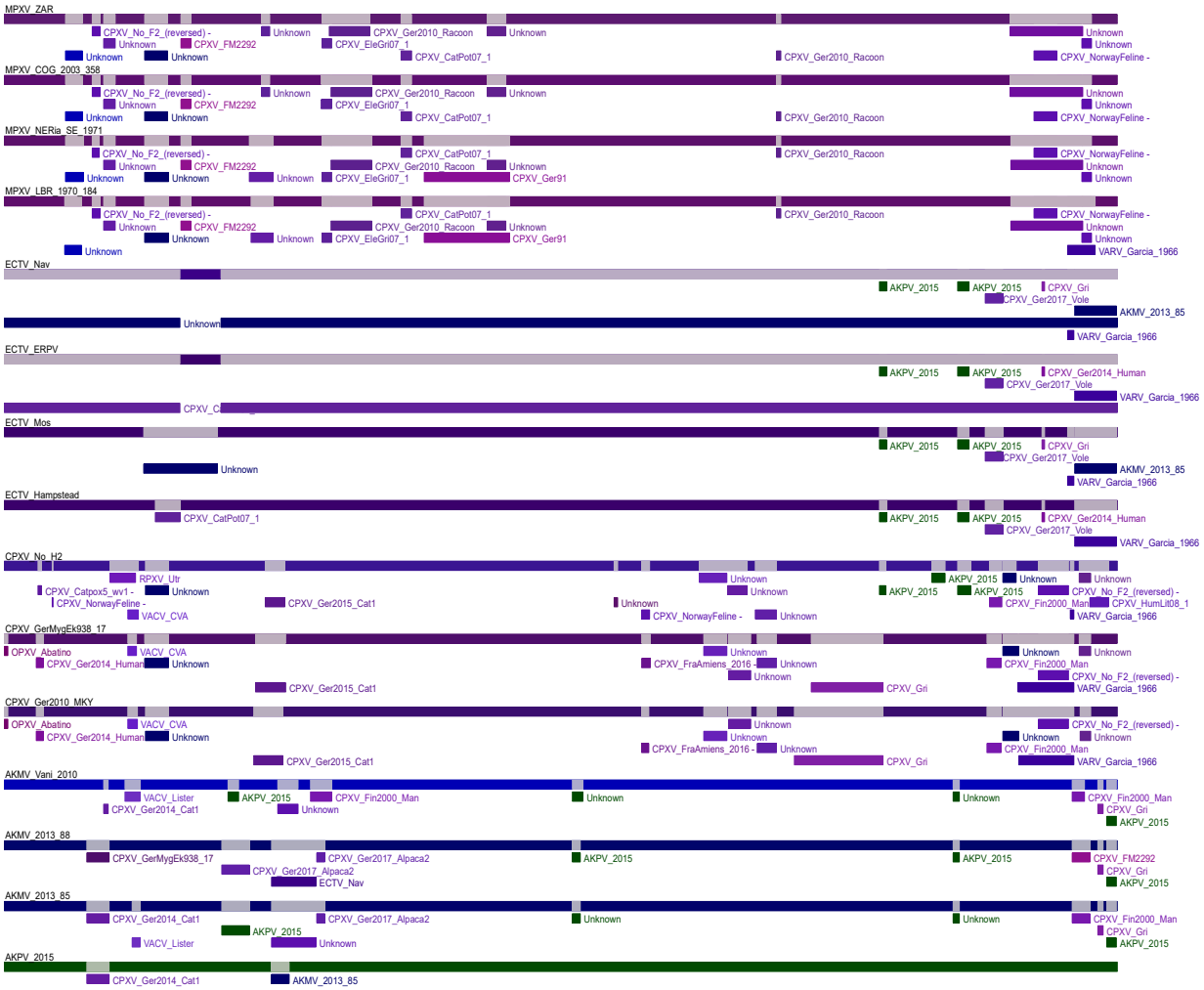

Figure S3. Recombination analysis of 87 orthopoxvirus (OPXV) core genomes with RPD4. Schematic sequence display depicting colour-coded representations of the analyzed sequences and the locations of detected recombination events in the 87 OPXV core genomes. The detected recombination events were detected for at least 5 of 7 methods (RDP, GENECONV, Bootscan, MaxChi, Chimaera, SiScan, and 3Seq) with significant p-values ($p \leq 0.01$).

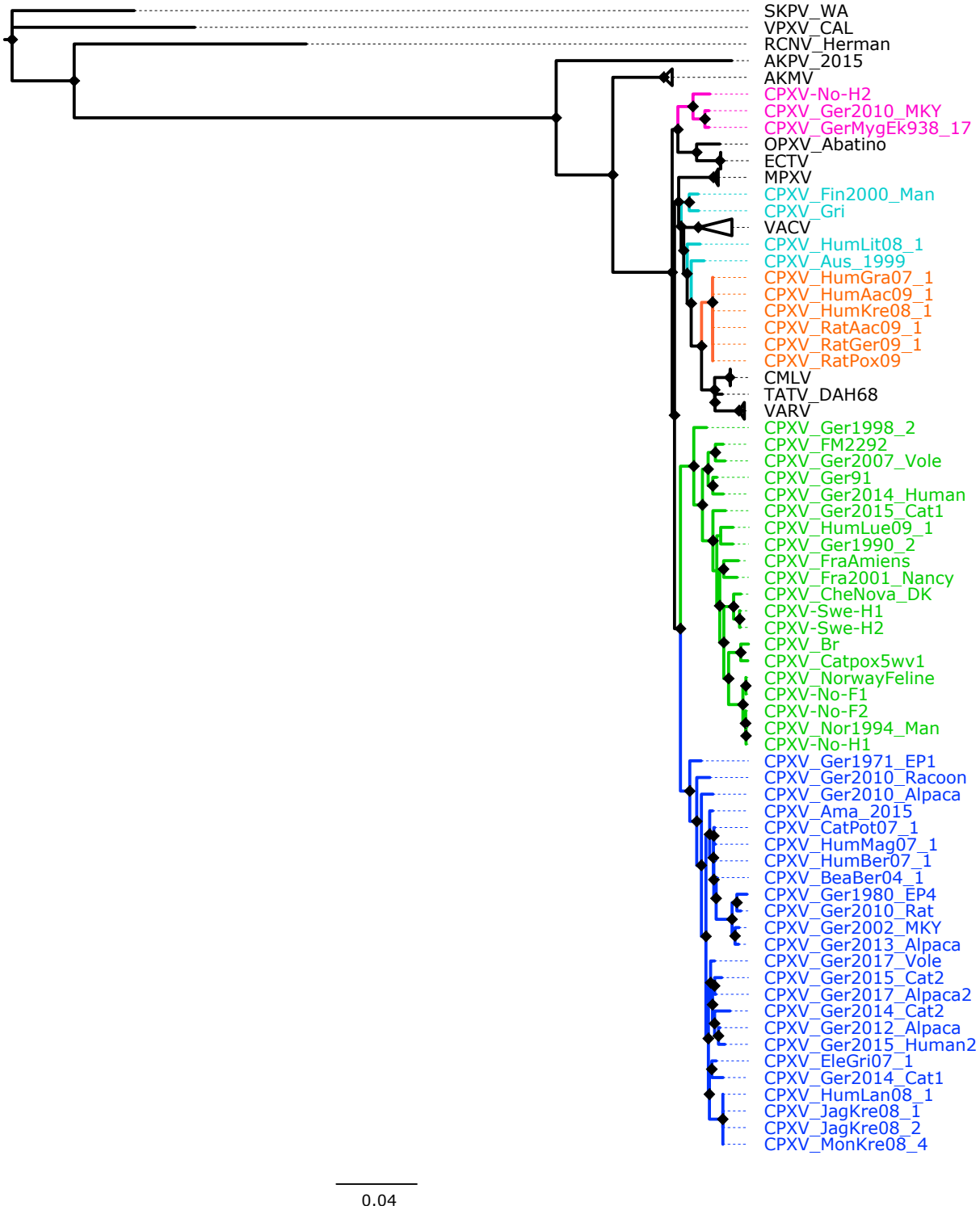

Figure S4. Bayesian inference phylogenetic tree of 62 conserved genes from 87 orthopoxviruses. Diamonds at the nodes indicate posterior probabilities >0.9. The scale bar represents expected substitutions per site. The main five cowpox virus (CPXV) clusters were highlighted in different colors: pink (Ectromelia-Abatino-like CPXV), blue (CPXV-like 1), green (CPXV-like 2), turquoise blue (Vaccinia-like CPXV) and orange (Variola-like CPXV).

Figure S5. Bayesian inference phylogenetic tree of 87 OPXV core genomes. Posterior probabilities are shown on the right side of each node and only posterior probabilities above 0.9. are shown. The scale bar represents expected substitutions per site. The main five cowpox virus (CPXV) clusters were highlighted in different colors: pink (Ectromelia-Abatino-like CPXV), blue (CPXV-like 1), green (CPXV-like 2), turquoise blue (Vaccinia-like CPXV) and orange (Variola-like CPXV).

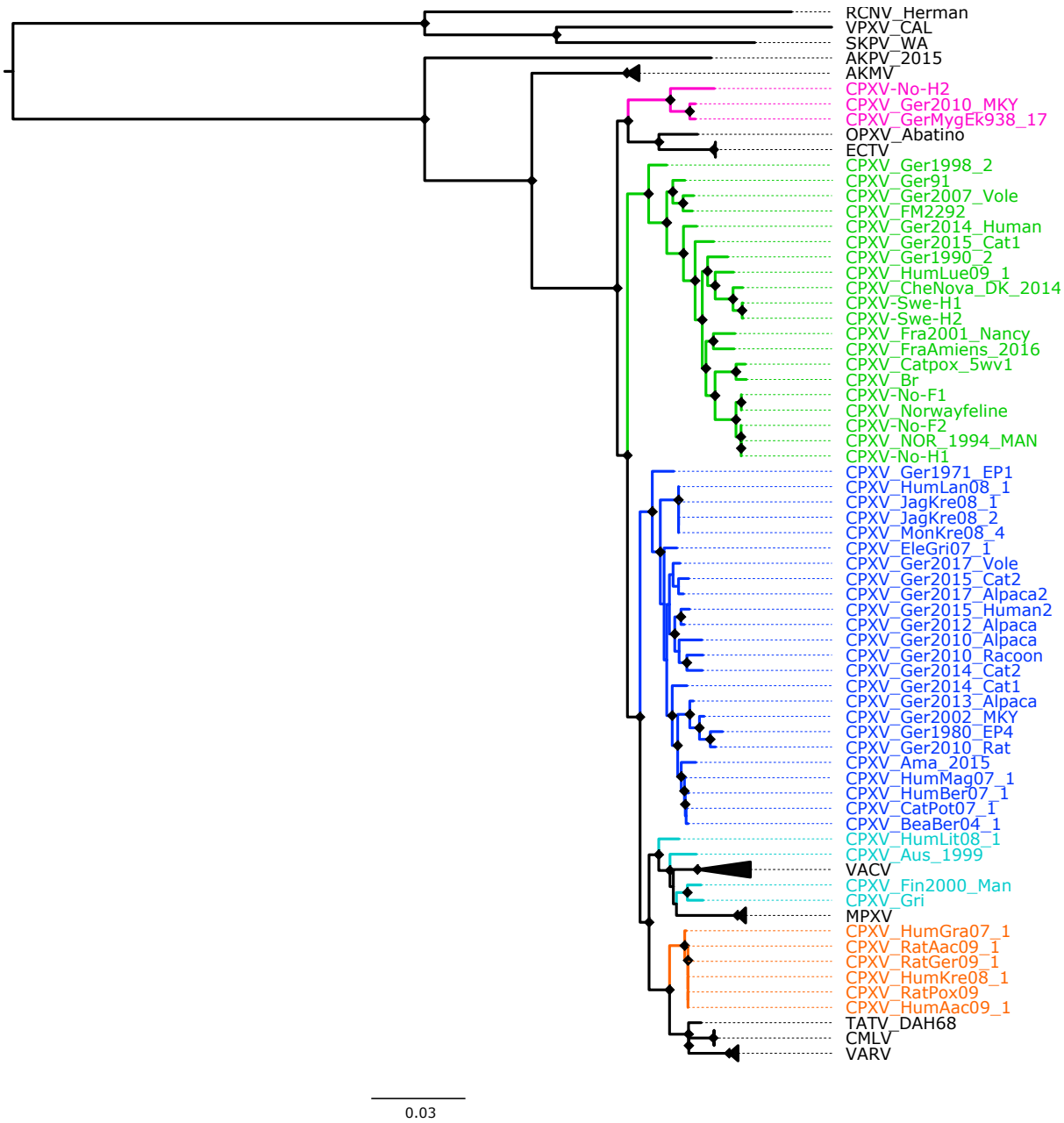

Figure S6. Maximum Likelihood phylogenetic tree of 87 orthopoxvirus whole genomes. Bootstrap values were inferred from 1000 rapid bootstrap replicates. Diamonds at the nodes indicate bootstrap values >80%. The scale bar indicates substitution per site. The main five cowpox virus (CPXV) clusters were highlighted in different colors: pink (Ectromelia-Abatino-like CPXV), blue (CPXV-like 1), green (CPXV-like 2), turquoise blue (Vaccinia-like CPXV) and orange (Variola-like CPXV).

Figure S7. Bayesian inference phylogenetic tree of 87 OPXV whole genomes. Diamonds at the nodes indicate posterior probabilities > 0.9. The scale bar represents expected substitutions per site. The main five cowpox virus (CPXV) clusters were highlighted in different colors: pink (Ectromelia-Abatino-like CPXV), blue (CPXV-like 1), green (CPXV-like 2), turquoise blue (Vaccinia-like CPXV) and orange (Variola-like CPXV).

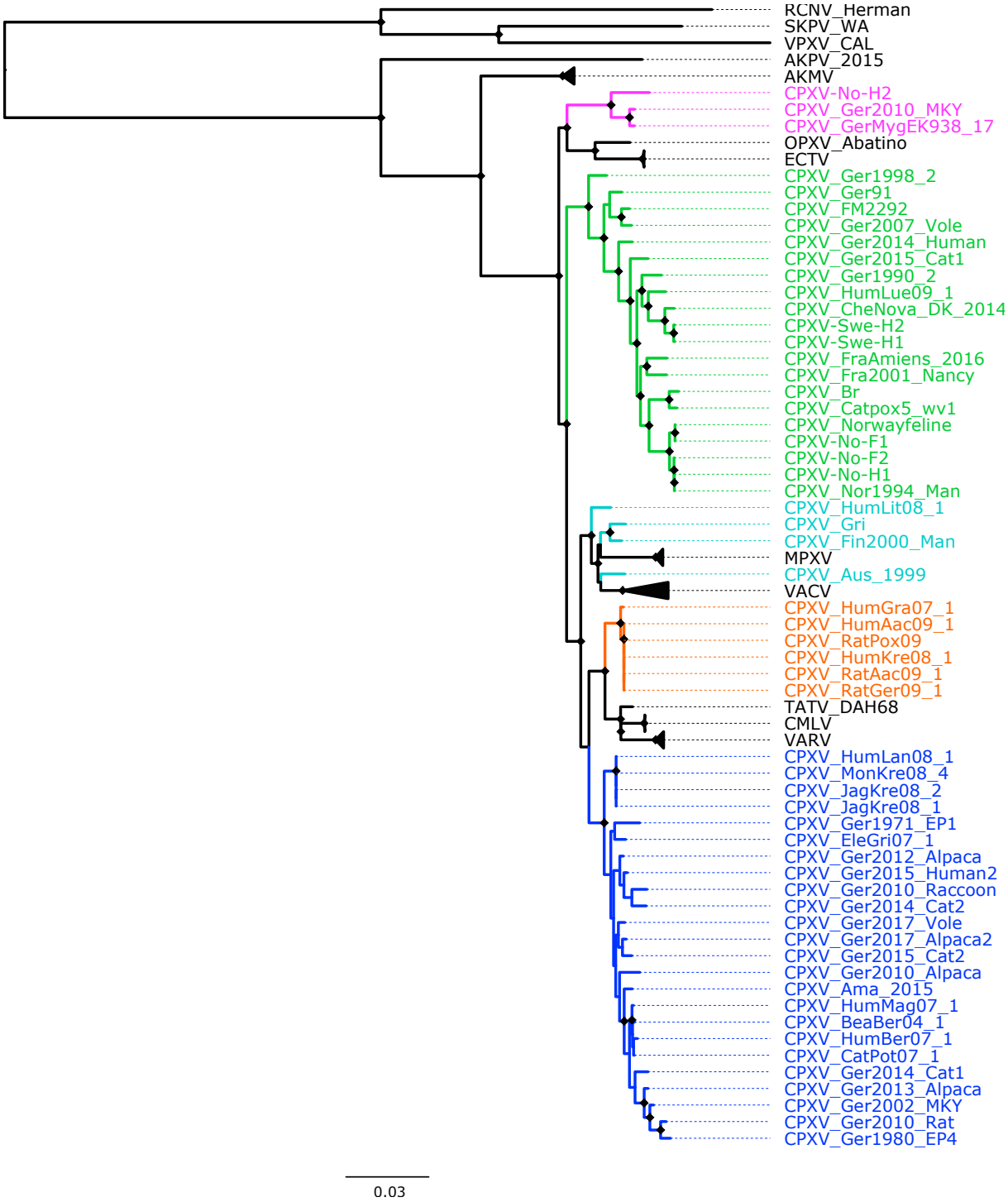

Figure S8. Maximum Likelihood phylogenetic tree based on orthopoxvirus orthologous genes. Bootstrap values were inferred from 1000 rapid bootstrap replicates. Diamonds at the nodes indicate bootstrap values >80%. The scale indicates substitution per site. The main five cowpox virus (CPXV) clusters were highlighted in different colors: pink (Ectromelia-Abatino-like CPXV), blue (CPXV-like 1), green (CPXV-like 2), turquoise blue (Vaccinia-like CPXV) and orange (Variola-like CPXV).

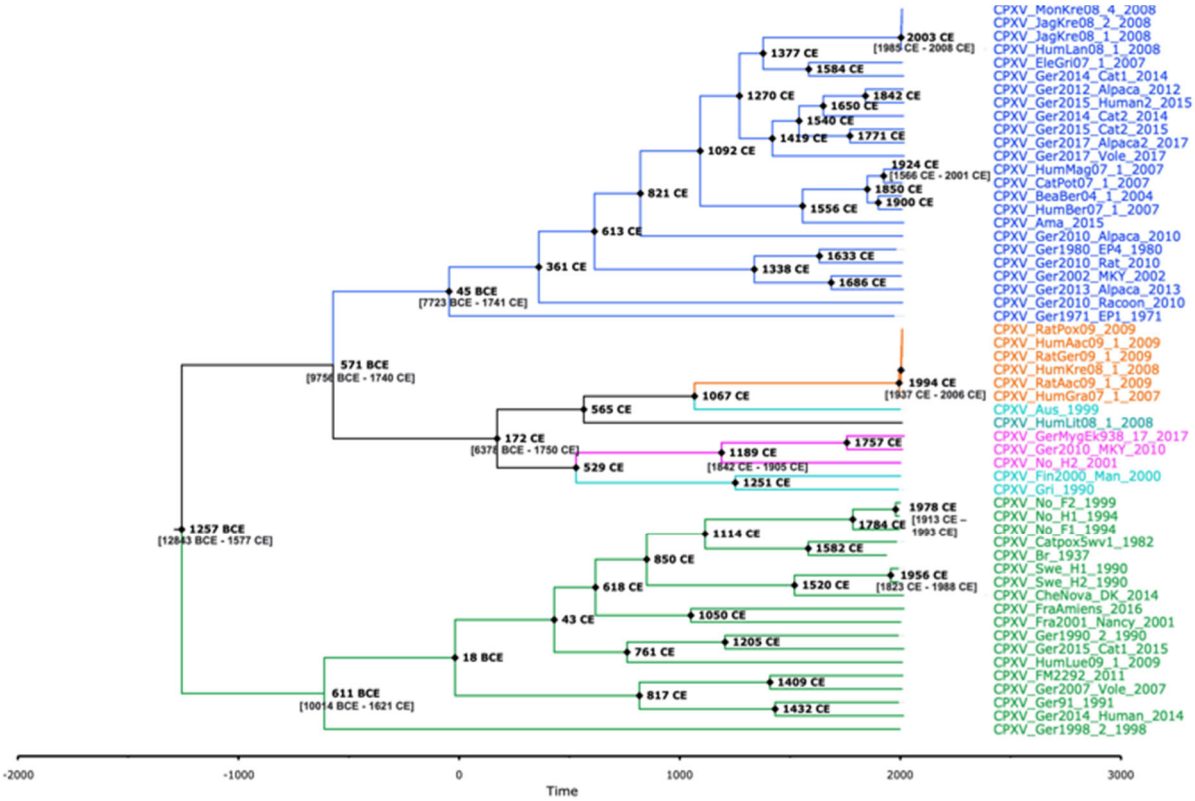

Figure S9. Bayesian maximum clade credibility (MCC) tree of 62 non-recombinant conserved genes of 55 CPXV genomes. The MCC tree was generated using BEAST 1, using a log-normal relaxed clock, coalescent Bayesian skyline population, HKY substitution model and four gamma categories. The numbers on the nodes indicate the time of the most recent common ancestor of the clades. Diamonds at the nodes indicate posterior probability values >0.9. The main five CPXV clusters were highlighted in different colors: pink (Ectromelia-Abatino-like CPXV), blue (CPXV-like 1), green (CPXV-like 2), turquoise blue (Vaccinia-like CPXV) and orange (Variola-like CPXV).

Figure S10. New classification of cowpox virus (CPXV) based on phylogenetic inference (from 87 OPXV whole genomes, core genomes and orthologous genes), patristic and genetic distances. Diamonds at the nodes indicate bootstrap values >80%. The main five CPXV clusters were highlighted in different colors: pink (Ectromelia-Abatino-like CPXV), blue (CPXV-like 1), green (CPXV-like 2), turquoise blue (Vaccinia-like CPXV) and orange (Variola-like CPXV).