1. MPXV

File “MPXV.fasta” contains alignment of five MPXV sequences (four shown in Figure 2A and Last\_common\_ancestor\_of\_CladeI as an outgroup). The 606 MPXV variants used to reconstruct the last common ancestor of the 2022 MPXV outbreak are listed in “606 MPXV variants collected during the 2022 outbreak.txt”. And 26 Clade IIa MPXV variants and 38 Clade I MPXV variants used in this study are shown in the tables below.

**Table 1. The 26 Clade IIa MPXV variants used in this study.**

|  |
| --- |
| MT903346 |
| DQ011157 |
| MT903348 |
| MT903347 |
| DQ011153 |
| KJ642616 |
| AY603973 |
| MT724769 |
| AY753185 |
| AY741551 |
| KP849470 |
| DQ011156 |
| KJ136820 |
| MN346702 |
| MN346703 |
| MN346697 |
| MN346690 |
| MN346692 |
| MN346696 |
| MN346695 |
| MN346699 |
| MN346698 |
| MN346691 |
| MN346694 |
| MN346701 |
| MN346700 |

**Table 2. The 38 Clade I MPXV variants used in this study.**

|  |
| --- |
| JX878417 |
| OP498046 |
| JX878426 |
| JX878408 |
| NC\_003310 |
| JX878418 |
| JX878420 |
| JX878419 |
| JX878412 |
| JX878411 |
| JX878409 |
| JX878410 |
| JX878416 |
| JX878415 |
| JX878414 |
| JX878413 |
| JX878427 |
| JX878421 |
| JX878422 |
| JX878428 |
| KC257460 |
| DQ011155 |
| HM172544 |
| MN702449 |
| MN702446 |
| MN702445 |
| MN702444 |
| DQ011154 |
| MN702451 |
| JX878425 |
| JX878429 |
| JX878407 |
| JX878423 |
| JX878424 |
| MN702452 |
| MN702453 |
| MN702450 |
| MN702447 |

All these MPXV genome sequences were downloaded from <https://data.nextstrain.org/files/workflows/monkeypox/alignment.fasta.xz>. These sequences were aligned against the reference sequence MPXV-M5312\_HM12\_Rivers (NC\_063383) using Nextclade, and inserted nucleotides were trimmed by the Nextstrain database. Therefore, all these genomes have an identical length (197,209 bp, *i.e.*, “pre-aligned”) and are ready for calling base substitutions (see file “MPXV.fasta”). The alignment file of all ~700 MPXV genomes is too large (>100 MB) to be sent through an e-mail (and is not necessary in our view because sequences downloaded from Nextstrain have been pre-aligned). If the authors do need this file, we can put it on github or somewhere similar for downloading.

We built the maximum likelihood tree using IQ-TREE multicore version 2.1.4-beta and reconstructed the last common ancestors using FastML v3.11 under the default parameters. We identified mutations in the evolutionary tree by comparing sequences shown in the file “MPXV.fasta”. The identified mutations within each internal branch (M1–M4) are listed in the file “MPXV\_SNPs.txt”. Some base substitutions close to gaps or within A/T-tracts could be caused by alignment errors or sequencing errors, but they accounted for only a small fraction of the total mutations identified and removing them did not significantly change the mutational profiles.

The branch IDs (the first column in “MPXV\_SNPs.txt”) are shown in Figure 2A. Note that in our original submission, the labels of branches M1–M4 in Figure 2A were not updated when we changed the branch labels in Figure 2B–C, so the branch labeling among these three panels did not match. Please use the updated Figure 2A provided below.

Chart

Description automatically generated

Updated Figure 2, with corrected branch labeling in (A)

2. VARV

We downloaded 55 VARV genome sequences and a camelpox virus genome sequence (NC\_003391.1, as an outgroup) from NCBI Virus (<https://www.ncbi.nlm.nih.gov/labs/virus/vssi/>). We performed multiple sequence alignments using MAFFT v7.505 (results shown in file “VARV.fasta”, all sequences with identical length).

We built the maximum likelihood tree (Figure 2D) using IQ-TREE multicore version 2.1.4-beta and reconstructed the ancestral sequence for each internal node using FastML v3.11 under the default parameters. The VARV sequences and their corresponding clades are shown below.

**Table 3. The 55 VARV variants used in this study.**

|  |  |
| --- | --- |
| Sequence ID | Clade |
| NC\_001611 | P-I |
| DQ437582 | P-I |
| DQ437588 | P-I |
| DQ437581 | P-I |
| DQ441422 | P-I |
| DQ441421 | P-I |
| DQ441420 | P-I |
| DQ441432 | P-I |
| DQ441431 | P-I |
| DQ441430 | P-I |
| DQ441428 | P-I |
| DQ441429 | P-I |
| DQ441442 | P-I |
| DQ437591 | P-I |
| DQ441433 | P-I |
| DQ437586 | P-I |
| DQ437585 | P-I |
| DQ441448 | P-I |
| DQ437580 | P-I |
| DQ437592 | P-I |
| DQ437587 | P-I |
| DQ437583 | P-I |
| DQ441443 | P-I |
| DQ441423 | P-I |
| DQ441444 | P-I |
| DQ441425 | P-I |
| DQ441424 | P-I |
| DQ441441 | P-I |
| DQ441440 | P-I |
| DQ437590 | P-I |
| DQ441439 | P-I |
| DQ441438 | P-I |
| DQ441445 | P-I |
| DQ441427 | P-I |
| DQ441446 | P-I |
| DQ437584 | P-I |
| DQ441435 | P-I |
| DQ441436 | P-I |
| DQ441417 | P-I |
| DQ441418 | P-I |
| L22579 | P-I |
| OL468962 | P-I |
| DQ441447 | P-II |
| DQ441419 | P-II |
| Y16780 | P-II |
| DQ441434 | P-II |
| DQ441437 | P-II |
| DQ441416 | P-II |
| DQ441426 | P-II |
| OL468961 | P-II |
| LR800244 | Viking age samples |
| LR800245 | Viking age samples |
| LR800246 | Viking age samples |
| LR800247 | Viking age samples |
| KY358055 | 17th Century |