Supplementary Methods

DNA sequence analysis: Whole genome sequencing of extracted DNA from the original swab cell culture (BSC40®) was conducted (Otogenetics Corporation; Atlanta, Georgia, USA) by using the Illumina® HiSeq2000 platform (Illumina; San Diego, California, USA). Nine genes located within the central, conserved region of the genome (*Vaccinia virus* Copenhagen strain homologues A7L, A10L, A24R, D1R, D5R, E6R, E9L, H4L, and J6R) were chosen for phylogenetic inference (GenBank Accession numbers KX914668-76) [1]. In addition to the virus isolate, taxonomic sampling included 52 representatives from all recognized genera within *Chordopoxvirinae* and 2 outgroup taxa of the subfamily *Entomopoxvirinae* (*Melanoplus sanguinipes entomopoxvirus*, *Amsacta moorei entomopoxvirus*). Each of the 9 loci included in the analysis was individually aligned by using the translation alignment method in Geneious® v8.1 (Biomatters Incorporated; San Francisco, California, USA). Genes were concatenated by using BioEdit® v7.0.9 (Ibis Biosciences; Carlsbad, California, USA) and the resulting alignment was analyzed by using MrBayes v3.1.2 [2] with the following parameters: GTR+I+G model of molecular evolution with 10 million generations, 2 runs with 4 chains each, and a sample frequency of 1,000. The first 2,500 trees were discarded as burn-in and the remaining trees were used to generate a majority-rule consensus tree and posterior probabilities. Genetic p-distances were calculated by using MEGA v6 [3]. The nexus alignment used for the phylogenetic analysis is available on the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.n0d08).

Transmission electron microscopy: Virus morphology was observed from the original swab cell culture (BSC40) by using transmission electron microscopy. Twenty-four hours

postinfection, BSC40 cells were harvested and the cell pellet was fixed by using buffered glutaraldehyde and subsequently processed for thin-section electron microscopy [4].

Small mammal trapping: Twenty-five Museum Special snap traps and 5 Victor rat traps (Woodstream Corporation; Lititz, Pennsylvania, USA) were deployed continuously at each of the two sampling sites (around the perimeter of the patient's home and at a site approximately 1 km away where she and her partner were building a new home) from approximately 16:00 h on October 23 to 09:00 h on October 25 and checked each dawn and dusk. As part of each check, traps were rebaited (peanut butter and rolled oats) as necessary, and the carcasses of trapped animals were collected. Eight traps deployed around the patient's home at dusk on 24 October were not recovered at dawn on 25 October. Three samples were collected from each trapped animal, including a blood sample on a Nobuto filter paper strip (Advantec; Dublin, California, USA), a liver tissue sample, and a swab of the oral cavity by using a HydraFlock® Dacron swab (Puritan Medical; Guilford, Maine, USA). Trapped animals are archived as voucher specimens (study skins, prepared skulls and skeletons, and duplicate tissue samples) at the University of Alaska Museum (UAM) Mammal Collection and Genomic Resources facility (see Supplementary Table 3 for institutional catalog number). All activities associated with small mammal trapping were conducted in accordance with the American Society of Mammalogists' guidelines for the use of mammals in research [5], assurance 152295-13 issued by the Institutional Animal Care and Use Committee of the University of Alaska Fairbanks, and scientific permit 15-078 issued by the Alaska Department of Fish and Game. For reference, small-mammal species known to occur in the geographic area where small mammal trapping was conducted include the cinereus shrew (*Sorex cinereus*), northern red-backed vole (*Myodes*

rutilus), North American red squirrel (*Tamiasciurus hudsonicus*), meadow vole (*Microtus pennsylvanicus*), root vole (*Microtus oeconomus*), meadow jumping mouse (*Zapus hudsonius*), common muskrat (*Ondatra zibethicus*), woodchuck (*Marmota monax*), northern flying squirrel (*Glaucomys sabrinus*), snowshoe hare (*Lepus americanus*), ermine (*Mustela erminea*), least weasel (*Mustela nivalis*), and little brown myotis (*Myotis lucifugus*) [6].

References

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