Characterization of *Eptesipoxvirus*, a novel poxvirus from a microchiropteran bat

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**Abstract** The genome of *Eptesipoxvirus* (EPTV) is the first poxvirus genome isolated from a microbat. The 176,688 nt sequence, which is believed to encompass the complete coding region of the virus, is 67% A+T and is predicted to encode 191 genes. 11 of these genes have no counterpart in GenBank and are therefore unique to EPTV. The presence of a distantly related ortholog of *Vaccinia virus* F5L in EPTV uncovered a link with fragmented F5L orthologs in *Molluscum contagiosum* virus/squirrelpox and clade II viruses. Consistent with the unique position of EPTV approximately mid-point between the orthopoxviruses and the clade II viruses, EPTV has 11 genes that are specific to the orthopoxviruses and 13 genes that are typical, if not exclusive, to the clade II poxviruses. This mosaic nature of EPTV blurs the distinction between the old description of the orthopoxviruses and clade II groups. Genome annotation and characterization failed to find any common virulence genes shared with the other poxvirus isolated from bat (pteropoxvirus); however, EPTV encodes 3 genes that may have been transferred to or from deerpox and squirrelpox viruses; 2 of these, a putative endothelin-like protein and a MHC class I-like protein are likely to have immunomodulatory roles.

**Keywords** Poxvirus · Next-generation sequencing · NGS · Batpox · Eptesipoxvirus

**Introduction**

Poxviruses have dsDNA genomes that range from 130 to 360 kb and are sufficiently large that they can be seen by light microscopy. The *Poxviridae* family is divided into the *Entomopoxvirinae* subfamily of viruses that infect insects and the *Chordopoxvirinae* subfamily of viruses that infect vertebrates. According to the International Committee on Taxonomy of Viruses (ICTV) 2016 Release [1], 11 genera have been created to classify the Chordopoxviruses, but several viruses that are unclassified are likely to require new genera. Many of these genera contain only a few virus species, the exceptions are the *Orthopoxvirus* and *Avipoxvirus* genera. Orthopoxviruses have been extensively studied as models of poxvirus biology and the host immunological response because this group includes variola virus and vaccinia virus that are the agent of smallpox and the virus used as the smallpox vaccine, respectively. In contrast, avipoxvirus infections that have been recognized in a large number of wild and domestic birds have been widely surveyed by PCR techniques, but there is very limited sequence data available for these isolates.

As many of the individual virus names suggest, poxviruses infect a large array of hosts. Many poxviruses have been named after the animal from which they were first isolated; however, these animals may not be the natural reservoir hosts for these pathogens. For example, there are 3 groups of orthopoxviruses that have been named cowpox,
but these appear to be distinct species and are thought to be typically spread by rodents and possibly other small mammals [2]. Others such as goatpox virus, sheeppox virus, and lumpy skin disease virus (cattle) do not have a strictly limited host range and have been used as vaccines in the different host species [3]. Thus, it is important to note that the natural hosts of bat-isolated poxviruses may not be bats. However, poxviruses found in bats are of special interest because bats are associated with a number of known zoonotic disease viruses, including the coronaviruses that cause SARS [4] and MERS [5], the rhabdovirus that causes rabies [6], the filoviruses ebola [7], and Marburg viruses [8], and Australian bat lyssaviruses that cause rabies-like symptoms [9]. In 2013, 3 reports described the association of poxviruses with bats on 3 continents. *Eptesipoxivirus* (EPTV) was isolated in North America [10], *Eidolon helvum poxvirus 1* in West Africa [11], and a third virus was identified only by electron microscopy in South Australia [12]. Since only small amounts of sequence data were obtained from the first 2 viruses, the first complete genome of a bat-associated poxvirus was not published until 2016, from a Pteropox virus isolated in North Western Australia [13].

Bats, which have been categorized into approximately 1200 species, form the second most diverse animal taxon (Chiroptera order) after rodents. So far, bat-isolated poxviruses have been isolated from both the megabat and microbat suborders. These suborders differ significantly; megabats are large fruit bats with big eyes, small ears (always lack tails) that are found in tropical regions, whereas the small microbats have the opposite features, eat insects, and use echolocation [14, 15]. Thus, bats comprise a huge and diverse mammalian reservoir for poxviruses that may pose a risk to humans through zoonotic transmission [16]. Additionally, bat poxviruses may provide a genetic reservoir that, through recombination with other mammalian poxviruses, could generate new human pathogens [17]. It has been hypothesized that recombination events may have been involved in the development of variola virus as a human-specific pathogen [18]. Here, we aim to expand the available genome data and advance the study of bat-associated poxviruses. This paper presents the complete genomic sequence and annotation of EPTV, and establishes its potential relationship to other recognized poxvirus genera.

**Materials and methods**

**Virus isolation and DNA preparation**

The virus was isolated from a big brown bat (*Eptesicus fuscus*) that was treated at a wildlife rehabilitation center in the state of Washington due to joint swellings which impeded its ability to fly. Samples from an elbow joint were homogenized in sterile phosphate-buffered saline using a tissue grinder. Green monkey kidney epithelial cells (BSC40) were infected with 10 microliters of the homogenate and harvested after 95% of the cells were infected [10]. Viral nucleic acid was extracted from the harvested material using the Qiagen EZ1 Advanced XL. The sequencing library was prepared for use with the Illumina platform (HiSeq 2500; www.illumina.com/).

**Sequence quality control and assembly**

The single-ended raw Illumina EPTV sequence read file was subjected to quality control using Taxonomer, a metagenomics tool that assigns sequencing reads to taxonomic categories (taxonomer.iobio.io) [19]. At least 40% of the sequences were determined to be non-viral contamination (human, bacterial, and unknown sequences). Initially, the raw sequence reads that mapped to human genome (build 38) using the Burrows–Wheeler Alignment (BWA) tool [20] were removed, and the remaining reads were extracted using SAMtools and Fastqutils [21] prior to assembly by MIRA [22]. Second, using the same protocol, a separate assembly trial was performed with *Eptesicus fuscus* (bat host) sequences removed. However, both trials failed to generate a complete poxvirus genome contig, probably due to excessive read removals. Therefore, the final assembly was performed directly with the original raw read file with no decontamination process using designated “genome, denovo, accurate” parameters in MIRA. This contig was mapped to the original read file using the Tanoti short read aligner (http://www.bioinformatics.cvr.ac.uk/tanoti.php), and subsequently visualized in Tablet [23] for coverage analyses and manual base-calling.

**Viral Bioinformatics Resource Centre**

Genome annotation and analysis was performed with tools from the bioinformatics suite developed at the Viral Bioinformatics Resource Centre [24] (http://virology.uvic.ca/). The Genome Annotation Transfer Utility (GATU) [25] uses a reference genome to automatically annotate EPTV genes with clear orthologs in the reference. Other possible genes are presented to the annotator for further characterization and to make the final decision on annotation. Generally, our approach to annotation was conservative with ORFs smaller than 50 codons or overlapping with known genes being ignored. EPTV genes were preferentially referenced to VACV-Cop, with DPV-W1170_84 and CPXV-BR used second and third when an ortholog group does not exist in the prior species, respectively.
Virus orthologous clusters (VOCs) [26] and Base-By-Base (BBB) [27] were used to create MSAs of DNA and proteins.

**Phylogenetic analyses and poxviral sequences used**

Sequences were extracted from the following virus strains using the VOCs database: EPTV-WA (KY747497); PTPV-Aus (KU980965); BPSV-BV-AR02 (NC_005337); CNPV-VR111 (NC_005309); COTV-SPAn232 (HQ647181); CPXV-BR (NC_003663); CRV-ZWE (NC_008030); DPV-W1170_84 (AY689437); FWPV-Iowa (NC_001888); GTPV-Pellor (NC_004003); MOCV-st1 (NC_001731); MYXV-Lau (NC_001132); ORFV-SA00 (NC_005336); RFV-Kas (NC_001266); RCNV-Herman (KP143769); SQPV-Red_squirrel_UK (HE601899); SWPV-Neb (NC_003389); TANV-KEN (NC_009888); TPKV-HU1124 (NC_028238.1); VARV-GRB44_harv (DQ441444); YMTV-Amano (NC_005179); YLDV-Davis (NC_002642); YK-V-DakArB_4268 (HQ849551).

Seven conserved poxviral proteins (previously used in [10] and [13]): RPO147, RAP94, mRNA capping enzyme large subunit, P4a precursor, RPO132, VETF-L, and DNA primase were extracted from VOCs and aligned using MUSCLE [28] in Base-By-Base. MEGA6.06 was used to create a Maximum-likelihood tree using the LG model with the G + I rate parameter [29].

**Results**

**Genome assembly and gene annotation**

The initial assembly of the unfiltered Illumina single read sequencing data by MIRA [22] generated 2 large poxvirus-specific contigs (121,151, and 43,742 nt), which overlapped by 54 nt. After manually joining the 2 contigs and correction/matching of the genome Inverted Terminal Repeats (ITRs), a draft genome of 176,491 nt containing 2 copies of 11,999 nt ITRs was obtained. The mapping of raw reads against this draft sequence gave an average coverage of 150x, which was consistent throughout the genome except for 1 region (134,421–134,514 nt). This region, which showed a dramatic increase in coverage of 150x, which was consistent throughout the genome except for 1 region (134,421–134,514 nt). This region, which showed a dramatic increase in coverage of 411x, was found to consist of 2 tandem repeats of about 54 nt each. Since the Illumina reads, which were approximately 71 nt on average, were unable to resolve the true number of repeats in the region, this region was amplified by PCR and re-sequenced using the Sanger method [30]. This process determined that this region consisted of six repeats rather than 2 and created a final EPTV genome sequence of 176,688 nt.

A conservative approach was taken to genome annotation to avoid over-annotation of ORFs that are unlikely to represent functional genes. ORFs less than 50 codons or overlapping more than 25% with well-characterized genes were not considered for annotation unless supported by other evidence. A total of 191 genes were annotated (Table 1), of which 13 are associated with each of the ITRs. EPTV-013 and EPTV-179 span the ITR junctions, the former being a N-terminus truncated version of the EPTV-179 gene that is predicted to encode an ankyrin-like protein. The GenBank accession number for the genome sequence of EPTV-WA is KY747497.

**Phylogeny**

The relationship between EPTV and the other orthopoxviruses was determined by (1) using MUSCLE [28] to align the result of concatenation of 7 conserved core proteins, (2) manual editing of the multiple sequence alignment (MSA) in base-by-base [27], (3) generation of an unrooted phylogenetic tree by MEGA v6.06 using maximum-likelihood [29]. The resulting radiation tree illustrates that EPTV branches off the backbone independent of all other viruses (Fig. 1). The genetic distance supports that EPTV should be placed into a new genus, which was previously proposed to be called *Chiropoxvirus* [10]. Thus, the 3 poxviruses isolated from bats (EPTV, PTPV, EHPV1) on different continents, each belong to a different poxvirus genus. Although bootstrap analysis strongly supports the position of EPTV in this tree, this is at odds with an earlier paper [10] which had indicated that EPTV was more closely related to Cotia virus (COTV) and that the pair branched off the backbone to form their own clade. When we analyzed the concatenated protein sequences used by Emerson et al. [10], we found that the protein sequences of the P4a precursor were shuffled, and placed with the concatenated sequences of the wrong virus species. Importantly, this switched the COTV and EPTV proteins with orthologous sequences from ORFV and BPSV, respectively, and this was sufficient, when the tree was generated, to create the impression that COTV and EPTV were within a single clade due to the close relationship of the parapoxviruses. Since the inclusion of the GC-rich viruses in the MSA results in the generation of a very large number of gaps and these frequently introduce mis-alignment around the indels, we also rebuilt the MSA omitting these sequences and recalculated the tree. The relationship between EPTV and neighboring viruses was identical to that shown in Fig. 1.

EPTV branches off the common backbone of the phylogenetic tree between the viruses that have been referred to as “clade II” viruses and the orthopoxviruses. The term clade II was coined to enable distinction between the
| Gene # | ORF position | AA #  | Putative gene function                      | Orthologs                  |
|--------|--------------|-------|---------------------------------------------|----------------------------|
| EPTV-001 | 620-141 | 159 | Hypothetical protein                       | VACV-Cop-B15R              |
| EPTV-002 | 1694-732 | 320 | Serpin 2/CrmA (host range)                  | VACV-Cop-B15R              |
| EPTV-003 | 2410-1727 | 227 | Hypothetical protein                       | DPV-B-009                  |
| EPTV-004 | 3472-2474 | 332 | IL-1 receptor-like protein                  | DPV-B-015                  |
| EPTV-005 | 3989-3510 | 159 | Hypothetical protein                       | DPV-B-015                  |
| EPTV-006 | 4934-4029 | 301 | Tyrosine protein kinase-like protein        | DPV-B-015                  |
| EPTV-007 | 5683-4991 | 230 | ER-localized apoptosis regulator (host range)| VACV-Cop-B9R               |
| EPTV-008 | 6245-5769 | 158 | Hypothetical protein                       | VACV-Cop-B15R              |
| EPTV-009 | 6804-6325 | 159 | Hypothetical protein                       | VACV-Cop-B15R              |
| EPTV-010 | 8696-6963 | 577 | Ankyrin repeat-containing protein (host range)| VACV-Cop-K3L               |
| EPTV-011 | 11040-9211 | 609 | Ankyrin repeat-containing protein           | VACV-Cop-K3L               |
| EPTV-012 | 11599-11090 | 169 | IFN resistance, eIF2a-like PKR inhibitor (host range)| VACV-Cop-K3L               |
| EPTV-013 | 11992-11636 | 118 | Ankyrin repeat protein fragment             | DPV-B-019                  |
| EPTV-014 | 12864-12010 | 284 | Monoglyceride lipase                       | VACV-Cop-K5L/K6L           |
| EPTV-015 | 13183-12941 | 80  | Secreted EGF-like protein                  | VACV-Cop-C11R              |
| EPTV-016 | 13689-13189 | 166 | Mitochondria anti-apoptotic factor (host range)| DPV-B-022                  |
| EPTV-017 | 14167-13742 | 141 | dUTPase                                    | VACV-Cop-F2L               |
| EPTV-018 | 14598-14194 | 134 | IFN-inducible protein (host range)         | VACV-Cop-F2L               |
| EPTV-019 | 15616-14642 | 324 | Ribonucleotide reductase small subunit      | VACV-Cop-F4L               |
| EPTV-020 | 16786-15656 | 376 | F5L membrane protein                       | VACV-Cop-F5L               |
| EPTV-021 | 17026-16808 | 72  | Hypothetical protein                       | Unique to EPTV             |
| EPTV-022 | 17287-17063 | 74  | Hypothetical protein                       | Unique to EPTV             |
| EPTV-023 | 17537-17346 | 63  | Cytoplasmic protein                        | VACV-Cop-F8L               |
| EPTV-024 | 18031-17645 | 128 | Hypothetical protein                       | VACV-Cop-F15L              |
| EPTV-025 | 18714-18067 | 215 | S-S bond formation pathway protein         | VACV-Cop-F9L               |
| EPTV-026 | 20017-18704 | 437 | Ser/Thr protein kinase                     | VACV-Cop-F10L              |
| EPTV-027 | 21337-20039 | 432 | RhoA signalling inhibitor, virus release protein| VACV-Cop-F11L              |
| EPTV-028 | 23302-21362 | 646 | EEV maturation protein                     | VACV-Cop-F12L              |
| EPTV-029 | 24456-23341 | 371 | Palmitoylated EEV membrane glycoprotein     | VACV-Cop-F13L              |
| EPTV-030 | 24703-24476 | 75  | F14L conserved protein                     | VACV-Cop-F14L              |
| EPTV-031 | 24950-24750 | 66  | Hypothetical protein                       | Unique to EPTV             |
| EPTV-032 | 25594-25148 | 148 | F15L conserved protein                     | VACV-Cop-F15L              |
| EPTV-033 | 26362-25682 | 226 | Conserved non-functional serine recombinase | VACV-Cop-F16L              |
| EPTV-034 | 26423-26737 | 104 | DNA-binding phosphoprotein (VP11)           | VACV-Cop-F17R              |
| EPTV-035 | 28149-26731 | 472 | Poly (A) polymerase catalytic subunit (VP55)| VACV-Cop-E1L               |
| EPTV-036 | 30364-28166 | 732 | IEV morphogenesis                          | VACV-Cop-E2L               |
| EPTV-037 | 31138-30422 | 238 | dsRNA-binding PKR inhibitor (host range)   | VACV-Cop-E3L               |
| EPTV-038 | 31903-31175 | 242 | RNA polymerase subunit (RPO30)             | VACV-Cop-E3L               |
| EPTV-039 | 32072-33775 | 567 | IMV protein, virion morphogenesis          | VACV-Cop-E4L               |
| EPTV-040 | 33799-34611 | 270 | ER-localized membrane protein, virion core protein| VACV-Cop-E8R               |
| EPTV-041 | 34728-34608 | 1006 | DNA polymerase                            | VACV-Cop-E9L               |
| EPTV-042 | 37661-37951 | 96  | Sulphhydril oxidase (FAD-linked)            | VACV-Cop-E9L               |
| EPTV-043 | 38365-37943 | 140 | Virion core protein                        | VACV-Cop-E11L              |
| EPTV-044 | 40424-38349 | 691 | Virulence, modulates Raf/MEK/ERK pathway    | VACV-Cop-O1L               |
| EPTV-045 | 40795-40481 | 104 | Nonessential glutaredoxin                  | VACV-Cop-O2L               |
| EPTV-046 | 41842-40910 | 310 | DNA-binding core protein                   | VACV-Cop-O1L               |
| EPTV-047 | 42064-41843 | 73  | IMV membrane protein                       | VACV-Cop-O1L               |
| EPTV-048 | 42877-42065 | 270 | ssDNA-binding phosphoprotein               | VACV-Cop-O3L               |
| EPTV-049 | 45218-42930 | 762 | Ribonucleotide reductase large subunit      | VACV-Cop-O4L               |
| EPTV-050 | 45491-45255 | 78  | IMV protein (VP13)                         | VACV-Cop-O5L               |
| EPTV-051 | 46654-45509 | 381 | Telomere-binding protein                   | VACV-Cop-O6L               |
| EPTV-052 | 47933-46647 | 428 | Viral core cysteine protease               | VACV-Cop-O7L               |
| EPTV-053 | 47939-49978 | 679 | RNA-helicase, DExH-NPH-II | VACV-Cop-18R |
| EPTV-054 | 51755-49965 | 596 | Insulin metalloproteinase-like protein | VACV-Cop-Q1L |
| EPTV-055 | 52084-51752 | 110 | Entry/fusion complex component | VACV-Cop-Q3L |
| EPTV-056 | 52746-52078 | 222 | Late transcription elongation factor (VLTF) | VACV-Cop-Q2R |
| EPTV-057 | 53090-52713 | 125 | Thioredoxin-like protein | VACV-Cop-Q6L |
| EPTV-058 | 53093-54049 | 438 | FEN1-like nuclease | VACV-Cop-Q6R |
| EPTV-059 | 54009-54600 | 63 | RNA polymerase subunit (RPO7) | VACV-Cop-Q5.5R |
| EPTV-060 | 54604-55143 | 179 | NLPc/P60 superfamily protein | VACV-Cop-Q6R |
| EPTV-061 | 55109-56212 | 367 | Virion structural phosphoprotein, early morphogenesis | VACV-Cop-Q7L |
| EPTV-062 | 56241-57023 | 260 | Late transcription factor (VLTF-1) | VACV-Cop-Q4R |
| EPTV-063 | 57039-58064 | 341 | Myristylated entry/fusion protein | VACV-Cop-Q9R |
| EPTV-064 | 58065-58814 | 249 | Myristylated IMV envelope protein | VACV-Cop-Q5R |
| EPTV-065 | 58847-59116 | 89 | Crescent membrane/immature virion protein | VACV-Cop-Q1R |
| EPTV-066 | 60073-59108 | 321 | Internal virion protein | VACV-Cop-Q3L |
| EPTV-067 | 60098-60856 | 252 | DNA-binding virion protein (VP8) | VACV-Cop-Q4R |
| EPTV-068 | 60871-61275 | 134 | IMV protein, entry/fusion | VACV-Cop-Q5R |
| EPTV-069 | 61217-61663 | 148 | IMV membrane protein, virion morphogenesis | VACV-Cop-Q1R |
| EPTV-070 | 61689-62219 | 176 | Thymidine kinase | VACV-Cop-Q2R |
| EPTV-071 | 62305-62916 | 203 | Type I IFN inhibitor (host range) | VACV-Cop-Q7L |
| EPTV-072 | 62991-63992 | 333 | Poly (A) polymerase small subunit (VP39) | VACV-Cop-Q4R |
| EPTV-073 | 64464-64873 | 135 | IMV membrane protein, entry/fusion | VACV-Cop-Q4R |
| EPTV-074 | 6473-64466 | 135 | RNA polymerase subunit (RPO22) | VACV-Cop-Q4R |
| EPTV-075 | 64979-68836 | 1285 | RNA polymerase subunit (RPO147) | VACV-Cop-Q6R |
| EPTV-076 | 69351-69937 | 190 | Entry/fusion IMV protein | VACV-Cop-Q5R |
| EPTV-077 | 69365-69944 | 337 | IMV heparin-binding surface protein (p35) | VACV-Cop-Q3L |
| EPTV-078 | 73348-70961 | 795 | RNA polymerase-associated protein (RAP94) | VACV-Cop-Q4R |
| EPTV-079 | 73557-74210 | 217 | Late transcription factor (VLTF-4) | VACV-Cop-Q4R |
| EPTV-080 | 74229-75051 | 313 | DNA topoisomerase type I | VACV-Cop-Q6R |
| EPTV-081 | 75022-75651 | 149 | Crescent membrane/immature virion protein | VACV-Cop-Q7R |
| EPTV-082 | 75694-78228 | 844 | mRNA capping enzyme large subunit | VACV-Cop-Q1R |
| EPTV-083 | 78633-78190 | 147 | Virion core protein | VACV-Cop-Q2L |
| EPTV-084 | 78632-79375 | 247 | Virion core protein | VACV-Cop-Q3R |
| EPTV-085 | 79372-80028 | 218 | Uracil DNA glycosylase, DNA pol processivity factor | VACV-Cop-Q4R |
| EPTV-086 | 80062-84245 | 787 | NTPase, DNA primase | VACV-Cop-Q5R |
| EPTV-087 | 82422-84329 | 635 | Early transcription factor small subunit (VETF-s) | VACV-Cop-Q6R |
| EPTV-088 | 84363-84875 | 170 | RNA polymerase subunit (RPO18) | VACV-Cop-Q7R |
| EPTV-089 | 85682-8410 | 290 | Carboxic anhydrase, GAG-binding MV membrane protein | VACV-Cop-Q8L |
| EPTV-090 | 85740-86408 | 222 | mRNA decapping enzyme | VACV-Cop-Q9R |
| EPTV-091 | 86386-87171 | 261 | mRNA decapping enzyme | VACV-Cop-Q10R |
| EPTV-092 | 89052-87145 | 635 | ATPase, NPH1 | VACV-Cop-Q11L |
| EPTV-093 | 89948-89085 | 287 | mRNA capping enzyme small subunit | VACV-Cop-Q12L |
| EPTV-094 | 91661-89982 | 559 | Trimeric virion coat protein (rifampicin resistance) | VACV-Cop-Q13L |
| EPTV-095 | 92142-91687 | 151 | Late transcription factor (VLTF-2) | VACV-Cop-Q11L |
| EPTV-096 | 92847-92173 | 224 | Late transcription factor (VLTF-3) | VACV-Cop-Q12L |
| EPTV-097 | 93074-92844 | 76 | S-S bond formation pathway protein | VACV-Cop-Q2.5L |
| EPTV-098 | 95085-93094 | 663 | P4b precursor | VACV-Cop-Q3L |
| EPTV-099 | 95713-95141 | 190 | Putative membrane-associated virion core protein (p39) | VACV-Cop-Q4L |
| EPTV-100 | 95751-96260 | 169 | RNA polymerase subunit (RPO19) | VACV-Cop-Q5R |
| EPTV-101 | 97375-96257 | 372 | Virion morphogenesis core protein | VACV-Cop-Q6L |
| EPTV-102 | 99543-97399 | 714 | Early transcription factor large subunit (VETF-L) | VACV-Cop-Q7L |
| EPTV-103 | 99610-100485 | 291 | Intermediate transcription factor (VITF-3s) | VACV-Cop-Q8R |
| EPTV-104 | 100724-100482 | 80 | IMV membrane protein, early morphogenesis | VACV-Cop-Q9L |
| EPTV-105 | 103451-100725 | 908 | P4a precursor | VACV-Cop-Q10L |
| EPTV-106 | 103466-104401 | 311 | Viral membrane formation | VACV-Cop-Q11R |
| EPTV-107 | 104910-104398 | 170 | Virion core and cleavage processing protein | VACV-Cop-Q12L |
| EPTV-108 | 105224-105003 | 73 | IMV membrane protein, virion maturation | VACV-Cop-Q13L |
| EPTV-109 | 105572-105291 | 93 | IMV membrane protein, essential | VACV-Cop-Q14L |
| EPTV-111 | 105750-105589 | 53 | IMV membrane protein, non-essential | VACV-Cop-A14.5L |
| EPTV-112 | 106033-105740 | 97 | Core protein | VACV-Cop-A15L |
| EPTV-113 | 107159-106017 | 380 | Myristylated protein, essential for entry/fusion | VACV-Cop-A16L |
| EPTV-114 | 107747-107160 | 195 | IMV membrane protein | VACV-Cop-A17L |
| EPTV-115 | 107762-109201 | 479 | DNA helicase, transcript release factor | VACV-Cop-A18R |
| EPTV-116 | 109409-109191 | 72 | Zn finger-like protein, late morphogenesis | VACV-Cop-A19L |
| EPTV-117 | 109754-109410 | 114 | IMV membrane protein, entry/fusion | VACV-Cop-A21L |
| EPTV-118 | 109753-111030 | 425 | DNA polymerase processivity factor | VACV-Cop-A20R |
| EPTV-119 | 111014-111565 | 183 | Holliday junction resolvase | VACV-Cop-A22R |
| EPTV-120 | 111562-112722 | 300 | Intermediate transcription factor (VITF-3L) | VACV-Cop-A22R |
| EPTV-121 | 112719-116219 | 1166 | RNA polymerase subunit (RPO132) | VACV-Cop-A24R |
| EPTV-122 | 119230-116222 | 1002 | A-type inclusion protein | VACV-Cop-A25L |
| EPTV-123 | 121205-119274 | 643 | P4c precursor | VACV-Cop-A26L |
| EPTV-124 | 121605-121261 | 114 | IMV membrane protein, fusion | VACV-Cop-A27L |
| EPTV-125 | 122022-121606 | 138 | IMV membrane protein, entry | VACV-Cop-A28L |
| EPTV-126 | 122938-122036 | 300 | RNA polymerase subunit (RPO35) | VACV-Cop-A29L |
| EPTV-127 | 123149-122922 | 75 | Hypothetical protein | Unique to EPTV |
| EPTV-128 | 123348-134403 | 351 | 3 β-hydroxysteroid dehydrogenase/δ 5→4 isomerase | VACV-Cop-A44L |
| EPTV-129 | 124635-123855 | 166 | Hypothetical protein | Unique to EPTV |
| EPTV-130 | 124771-125334 | 187 | C-type lectin-like EEV membrane phosphoglycoprotein | VACV-Cop-A33R |
| EPTV-131 | 125386-125886 | 166 | C-type lectin like IEV/EEV membrane glycoprotein | VACV-Cop-A34R |
| EPTV-132 | 125923-126441 | 172 | MHC class II antigen presentation inhibitor | VACV-Cop-A35R |
| EPTV-133 | 126481-127326 | 254 | ATPase/DNA packaging protein | VACV-Cop-A37L |
| EPTV-134 | 127368-128099 | 132 | Myristylated protein | VACV-Cop-A38L |
| EPTV-135 | 128142-128972 | 276 | Hypothetical protein | Unique to EPTV |
| EPTV-136 | 128996-129259 | 87 | Hypothetical protein | Unique to EPTV |
| EPTV-137 | 129840-129256 | 194 | Truncated CD47-like protein, integral membrane protein | VACV-Cop-A38L |
| EPTV-138 | 130325-131070 | 250 | Hypothetical protein | Unique to EPTV |
| EPTV-139 | 131927-131070 | 285 | Chemokine-binding protein | VACV-Cop-A41L |
| EPTV-140 | 132054-132457 | 133 | Profilin-like protein, ATI-localized | VACV-Cop-A42R |
| EPTV-141 | 132843-132457 | 128 | Hypothetical protein | Unique to EPTV |
| EPTV-142 | 133074-134687 | 155 | BTB kelch-domain protein | Unique to EPTV |
| EPTV-143 | 134715-135509 | 264 | Hemagglutinin | VACV-Cop-A48R |
| EPTV-144 | 135685-137770 | 561 | DNA ligase-like protein | VACV-Cop-A50R |
| EPTV-145 | 136085-136053 | 525 | BTB kelch-domain protein | VACV-Cop-A55R |
| EPTV-146 | 136085-136053 | 195 | Thymidylate kinase | VACV-Cop-A44L |
| EPTV-147 | 136085-137770 | 561 | DNA ligase-like protein | VACV-Cop-A50R |
| EPTV-148 | 137817-139394 | 128 | Hypothetical protein | Unique to EPTV |
| EPTV-149 | 140099-140596 | 165 | Hypothetical protein | Unique to EPTV |
| EPTV-150 | 140648-141679 | 343 | Hypothetical protein | Unique to EPTV |
| EPTV-151 | 141743-142393 | 216 | Toll/IL-1 receptor-like protein, NFkB signaling inhibitor | VACV-Cop-A52R |
| EPTV-152 | 142985-142518 | 155 | Hypothetical protein | Unique to EPTV |
| EPTV-153 | 143074-144687 | 537 | BTB kelch-domain protein | VACV-Cop-A55R |
| EPTV-154 | 144715-145509 | 264 | Hemagglutinin | VACV-Cop-A56R |
| EPTV-155 | 145529-146464 | 311 | Ser/Thr protein kinase | VACV-Cop-A55R |
| EPTV-156 | 146495-147493 | 323 | IL-1 receptor antagonist | VACV-Cop-C10L |
| EPTV-157 | 147510-148409 | 999 | KilA-N/RING finger protein (host range) | CPXV-8R-023 |
| EPTV-158 | 148441-149031 | 196 | Partial schlafen-like protein | VACV-Cop-B28 |
| EPTV-159 | 149126-149839 | 237 | EEV type-1 membrane glycoprotein (host range) | VACV-Cop-B5R |
| EPTV-160 | 149954-150385 | 143 | Anti-apoptotic Bcl-2-like protein | VACV-Cop-N1L |
| EPTV-161 | 150436-151284 | 282 | dsRNA binding PKR inhibitor | VACV-Cop-E3L |
| EPTV-162 | 151349-152353 | 334 | Serpin 1 (host range) | VACV-Cop-C12L |
| EPTV-163 | 152461-152919 | 152 | Hypothetical protein | VACV-Cop-B15R |
| EPTV-164 | 152947-153822 | 291 | Tyrosine protein kinase-like protein | VACV-Cop-B16R |
| EPTV-165 | 153877-154884 | 335 | IL-1 beta-receptor | VACV-Cop-B16R |
| EPTV-166 | 154911-156857 | 648 | Ankyrin repeat protein | VACV-Cop-B16R |
| EPTV-167 | 156857-157681 | 648 | Ankyrin repeat protein | VACV-Cop-B16R |
| EPTV-168 | 157681-158504 | 648 | Ankyrin repeat protein | VACV-Cop-B16R |
monophyly of the “clade II” capri-, sui-, cervid-, yata-, leporipoxviruses, and COTV, to that of the ancient clade containing the orthopoxviruses [31]; clade II has also been alternatively referred to as the CSYLC clade [32]. Calculation of the average % amino acid (aa) identity from the MSA used to generate Fig. 1 reveals that EPTV is slightly more similar to clade II viruses (Fig. 1; 73–76%) than orthopoxviruses (Fig. 1; 71–72%); the exception is COTV, which has a very long branch. This phylogenetic relationship was also supported by comparison of the EPTV proteins to all other poxvirus proteins using BLASTP [33]; the “best match” was DPV in 25% of the searches. Other “best matches” were usually clade II viruses, but approximately 20% of searches yielded an orthopoxvirus as the “best

Ortholog families are represented by VACV-Cop designations; where the ortholog is lacking in VACV-Cop, the DPV-84 and CPXV-BR gene numbers are used, respectively. Inverted Terminal Repeat regions are bolded

| EPTV-169 | 156942-157568  | 208 | Ankyrin repeat protein | DPV-84-014 |
| EPTV-170 | 157626-158333  | 235 | Alpha-amanitin target protein | VACV-Cop-N2L |
| EPTV-171 | 158383-159060  | 225 | NFKβ inhibitor | VACV-Cop-M2L |
| EPTV-172 | 159108-159341  | 77  | Endothelin precursor | DPV-84-006 |
| EPTV-173 | 159392-160078  | 228 | NFKβ inhibitor | VACV-Cop-M2L |
| EPTV-174 | 160085-160876  | 263 | Secreted complement binding protein (host range) | VACV-Cop-C3L |
| EPTV-175 | 160944-161372  | 142 | IL-18 binding protein | DPV-84-021 |
| EPTV-176 | 161449-161637  | 62  | Hypothetical protein | Unique to EPTV |
| EPTV-177 | 161603-162202  | 199 | Truncated TNFα-receptor (host range) | VACV-Cop-B28R |
| EPTV-178 | 162245-163201  | 318 | MHC class I-like protein | SQPV-0040 |
| EPTV-179 | 163239-165053  | 604 | Ankyrin repeat protein | DPV-84-019 |
| EPTV-180 | 165090-165599  | 169 | IFN resistance, elF2α-like PKR inhibitor (host range) | VACV-Cop-K3L |
| EPTV-181 | 165649-167478  | 609 | Ankyrin repeat protein | DPV-84-019 |
| EPTV-182 | 167993-169726  | 577 | Ankyrin repeat protein (host range) | CPXV-BR-025 |
| EPTV-183 | 169885-170364  | 159 | Hypothetical protein | VACV-Cop-B15R |
| EPTV-184 | 170444-170920  | 158 | Hypothetical protein | Unique to EPTV |
| EPTV-185 | 171006-171698  | 230 | ER-localized apoptosis regulator (host range) | VACV-Cop-B9R |
| EPTV-186 | 171755-172660  | 301 | Tyrosine protein kinase-like protein | DPV-84-158 |
| EPTV-187 | 172700-173179  | 159 | Hypothetical protein | DPV-84-159 |
| EPTV-188 | 173217-174215  | 332 | IL-1 receptor-like protein | DPV-84-015 |
| EPTV-189 | 174279-174962  | 227 | Hypothetical protein | DPV-84-009 |
| EPTV-190 | 174995-175957  | 159 | Hypothetical protein | VACV-Cop-B13R |
| EPTV-191 | 176069-176548  | 159 | Hypothetical protein | VACV-Cop-B15R |

Table 1 continued

Fig. 1 Maximum-likelihood phylogenetic tree of EPTV with representatives of the Chordopoxvirinae subfamily; the MSA consisted of concatenated sequences of 7 conserved proteins: RPO147, RAP94, mRNA capping enzyme (large subunit), P4a precursor, RPO132, VETF-L, and DNA primase

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match.” This type of comparison is somewhat over-simplified because it does not consider whether genes actually exist in the various genera; however, the trend prompted us to use DPV gene numbers as a reference in the absence of a VACV-Cop ortholog (Table 1).

The comparison of EPTV proteins and gene sequences to their orthologs in other poxviruses reveals the importance of understanding exactly what data are being compared when evaluating relationships between viruses. Although the essential genes present in the various poxviruses are likely to have evolved along identical paths and give similar phylogenetic trees, examination of DNA and protein sequences of different genes can produce considerably different views as to the divergence between viruses. As an illustration, Fig. 2 shows the aa and nt % identity between 6 EPTV and VACV-Cop orthologous gene pairs. The aa identity ranges from 30 to 83%, whereas the nt identity ranges from 68 to 75%. These results show (1) different selection pressures are acting on different genes within a genome, (2) the huge variation in aa identity observed between different orthologs from the same pair of viruses, and (3) the compression in the difference values when comparing DNA sequences rather than protein sequences due to the 4 and 20 letter codes of DNA and proteins, respectively. This illustrates why phylogenetic trees are usually constructed from conserved essential genes: using DNA sequences if the organisms are closely related, and with aa sequences if they are more divergent.

Unique EPTV genes

Given the >75% aa identity between some EPTV proteins and their orthologs, it would appear straightforward to determine which genes are unique to EPTV; however, as noted above and in Fig. 2, there is considerable variation between the similarity of the different ortholog pairs. Therefore, instead of choosing an arbitrary cut-off for assigning orthologs, we evaluated % identity, % similarity (allowing chemically similar aa to be matched), protein motifs, secondary structure prediction, synteny, and gene size. Hence, 21 EPTV proteins were predicted to have poxviral orthologs despite having <30% aa identity (Online Resource 1). Further, eleven EPTV genes: -008 (ITR: -184), -021, -024, -031, -136, -142, -143, -145, -151, -154, -176 could not be matched confidently with any poxvirus counterpart. However, several of these genes are located in positions in the genome where orthologs are missing when compared to the arrangement of genes in VACV-Cop (Table 1). Although it is possible that the EPTV orthologs have diverged to such an extent that no recognizable sequence patterns are discernable, it is also possible that genes have been replaced in EPTV. Interestingly, none of this set of EPTV sequences have any significant similarity to any of the sequences in the bat genome database [34], as well as the current databases when analyzed by the BLAST suite of programs [33]. A further question concerning the annotation of these ORFs is whether they are likely to be functional genes. The size of these unique ORFs ranges from 62 to 165 codons, five being in the set of twelve smallest ORFs annotated in EPTV (Table 1). This, together with the fact that several of these predicted proteins have isoelectric points in the extreme tails (low and high) of the distribution observed for known poxvirus proteins (not shown), suggests that some of these annotated ORFs may not represent functional genes [35]. Others may provide EPTV with functions specific to promotion of virus replication within its host.

Fig. 2 Amino acid (dark gray) and nucleotide (light gray) percent identities between EPTV and VACV-Cop gene sets
Relationship with clade II poxviruses

As noted above, the % aa identity from the MSA of seven core proteins indicates that EPTV is slightly more similar to the clade II poxviruses. As shown in Table 1, EPTV possesses 7 genes that appear to only have clade II orthologs; however, some of these genes have one or more paralogs with different distributions among the various viruses that complicate the assignment of divergent genes to particular ortholog families. The EPTV genes -004 (-188; ITR), -018, -150, and -169 are predicted to have functions associated with virulence/host range (Table 1). EPTV genes -003 (-189; ITR), -005 (-187; ITR) have no known function, but are conserved in DPV and are 227 and 159 codons long, respectively; this suggests that they are likely functional genes in EPTV. EPTV-134 is unusual in that it is predicted to encode a clade II-specific structural protein that spans the outer membrane of intracellular enveloped virus (IEV) particles [36]. This clade II-specific IEV protein is located at the same position as orthopoxvirus ortholog A36R, which also encodes an IEV protein. Experimental evidence shows these clade II IEV orthologs (DPV-84-136) as functional orthologs of A36R, despite very low sequence identity, also induce actin tail formation, albeit via a different mechanism [37]. Using ScanSite3 [38], EPTV-134 is predicted to have five Nck-binding tyrosine motifs and 1 Grb2 binding motif; thus, it has the same signatures as DPV-84-136 [37] and is therefore likely to represent an ortholog that is otherwise clade II specific.

Further support for the closer association of EPTV with clade II viruses is that EPTV orthologs of VACV-Cop-C7L (-071) and -E7R (-138) genes (Table 1) are in positions that are syntenic only with their positions in clade II viruses [32].

Relationship with orthopoxviruses

EPTV also possesses several genes that are otherwise orthopoxvirus specific. EPTV-010 (ITR: -182) is an ortholog of CPXV-BR-025, which encodes an ankyrin-like protein (577 aa). It is curious that the neighboring EPTV-011 gene also encodes an ankyrin-like protein (609 aa), which is an ortholog of DPV-84-019 that is associated with clade II, avipox, and parapox viruses. Despite the physical proximity of these 2 genes, the aligned predicted protein sequences are only 23% identical. In addition, EPTV-010 and EPTV-011 have a different number of ankyrin repeats, 3 and 9, respectively. Although it is assumed that gene duplication events have created families of paralogous genes in poxviruses, at this level of sequence diversity it is very difficult to differentiate this from the possibility that some may be the result of separate gene capture events. COTV is the only other clade II chordopoxvirus (to date) that is believed to have a mixture of ankyrin orthologs from both orthopoxviruses and clade II viruses [26]. Together with COTV, EPTV also encodes a VACV-C3L homolog (secreted complement binding protein) that is otherwise exclusive to the orthopoxviruses.

Additional EPTV genes that are otherwise orthopoxvirus specific are scattered throughout the genome: EPTV-045, -141, -146, and -167 are orthologs of VACV-O2L, -A42R, -A47L, and -B16R, and encode a glutaredoxin-like protein, a profilin-like protein, the immunoprevalent protein, and an IL-1 beta-receptor-like protein, respectively. EPTV also encodes a chemokine-binding protein (EPTV-140) as well as a partial schlafen-like protein (EPTV-160) that have only been found in orthopoxviruses, yokapoxvirus (YKV), and the recently sequenced pteropoxvirus [13, 39].

Variably present genes

Above, we tried to categorize EPTV genes by which other viruses have orthologs; however, when the presence of a particular gene is inconsistent through different genera, it is also instructive to determine those genera that do not contain an ortholog. For example, the EPTV-122, -123, and -141 proteins that create or localize to an A-type inclusion body are absent from the clade II viruses. Similarly, EPTV encodes a ribonucleotide reductase large subunit (I4L) that is missing from all clade II viruses except swinepox (SWPV). In contrast, EPTV-006 (-185), -011 (-181), -016, -133, -166, -168, -175, and -179 are absent from the orthopoxviruses, but variably present in the clade II viruses and other chordopoxviruses (Table 1). This mosaic nature of EPTV genes makes the elucidation of evolutionary events interesting but more difficult.

A link between diverged F5L ortholog families

Although EPTV-019 (ribonucleotide reductase, small subunit) and EPTV-025 (S–S bond formation pathway protein) are clear orthologs of VACV-F4L and -F9L, the genes between these pairs have varied relationships. This is not unexpected because the region between F4L and F9L is also highly diverse between chordopoxviruses species, and genes here retain little or no identity with a VACV reference genome. EPTV-020 shows similarity with VACV-F5L (membrane protein) and MOCV/SQPV-003 throughout the sequence (26–29% aa ID with functional aa conserved), and peaks in identity with clade II DPV-84-027 ortholog group at the C-terminus. However, the 3 groups of protein showed no discernible similarity with each other prior to the discovery of EPTV-020 sequence (elaborated below). EPTV-021 (hypothetical gene), which could
encode a 72 aa polypeptide, is unique to EPTV and replaces VACV-F5L, that is itself a small hypothetical gene. Although EPTV-022 and -023 are predicted to encode small proteins of unknown function, they have some similarity to the gene products of VACV-F7L and F8L. EPTV-024, which is capable of encoding a 128 aa polypeptide, is another gene that is unique to EPTV.

Discovery of EPTV-020 revealed MOCV003L/SQPV003 and clade II DPV-84-027 ortholog family as divergent F5L proteins. Previously, the MOCV/SQPV orthologs had only 17% aa ID with VACV-F5L, while the clade II orthologs (DPV-84-027) that replaced the F5L position in the genome appeared to lack detectable sequence similarity to the established orthopox F5L orthologs. However, they actually share up to 39.5% aa identity with EPTV-020 at the C-terminal region, rather than just 22% aa identity with orthopoxviruses. This suggests that these F5L-positioned clade II orthologs (DPV-84-027) are likely N-terminus truncated versions of F5L orthologs with subsequent divergence. This is significant because it demonstrates a closer relationship between EPTV and clade II that is not visible in the phylogenetic tree or the 1% difference in identity observed in the MSA. Furthermore, this relationship, uncovered by analysis of the EPTV-020 sequence, supports the idea that the clade II gene at the F5L position evolved from the same ancestral F5L rather than being acquired by an independent gene capture event.

Other genes of interest

EPTV-172 encodes an endothelin-like polypeptide; only DPV has a similar gene. Eukaryotic endothelin is part of a multi-gene family that, along with at least 4 receptors, acts as vaso-constrictors [40]. However, as noted by others [41] there is currently no experimental evidence as to whether this viral ortholog acts in a manner similar to the endothelins or as an antagonist. Blocking endothelin function by binding a receptor without initiating signaling might be favorable to the virus due to a reduction of inflammation [42].

EPTV-139 is predicted to encode a cysteine-rich protein that has similarity to only 1 other poxvirus protein, SQPV-130. It is curious to see an AT-rich poxvirus, like EPTV, share a gene exclusively with a GC-rich virus like SQPV. SQPV-130 is a hypothetical protein of unknown function that was previously erroneously annotated as containing a death effector domain (Dr. C. McInnes, personal communication). Although these poxvirus proteins have only 23% aa identity, 10 cysteines are conserved over the 250 aa alignment despite the fact that these EPTV and SQPV genes have an A+T nucleotide composition of 82 and 30%, respectively. The proteins are not predicted to have N-terminal signal sequences, but since poxviruses encode proteins that create S–S bonds within the cytoplasm [43], these proteins may still be structurally dependent on these cysteines.

Although aa identity is low (25%), the EPTV-178 protein is clearly a member of the MHC class I family by virtue of the conservation of a characteristic pattern of cysteine residues. Several other poxviruses have similar genes, but it appears that at least some have been independently acquired, since, despite low identity scores, EPTV-178 and SQPV-004 proteins score best in BLASTP searches against microbat and squirrel MHC class I proteins, respectively. COTV also has an independently acquired MHC class I protein most similar to a homolog in a Southern/Central American wild cat [32].

Discussion

The reconstructed phylogeny shows that, unlike what was previously suggested, EPTV does not form a sister clade with COTV. Instead, it branches off the common backbone from the rest of the A+T % rich chordopoxviruses and forms its own genus between clade II poxviruses and orthopoxviruses. This effectively blurs the distinction between orthopoxviruses and clade II viruses. The term “clade II” arose to distinguish a clade of viruses (capri-, sui-, cervid-, yata-, leporipoxviruses, and COTV) from the orthopoxviruses which diverged at their last common node [31]. However, given the phylogenetic position of EPTV and its mosaic collection of orthologous clade II and orthopoxvirus genes (this characteristic is also exhibited by COTV to some extent) [32], the term may have outlived its usefulness. EPTV along with the recently sequenced pteropoxvirus [13] demonstrate the value of sequencing further poxvirus genomes to increase the information in phylogenetic trees.

In terms of genome statistics, the 176,688 nt genome size for EPTV is considered medium length among the chordopoxviruses. It is predicted to encode 191 genes and has one of the larger ITRs sequenced (10,000 nt). It should be noted that EPTV and COTV genomes are the most A+T % rich of the chordopoxvirus genomes (76.4%). The reduced synteny, including gene loss, at the right end of the EPTV genome compared to VACV-Cop reflects the fact that many VACV genes in this region may be non-essential. Three immunomodulatory genes suspected to be the result of horizontal gene transfer have been found in this region (EPTV-139, -172, and -178), including 1 predicted to encode a MHC class I protein (Table 1).

Further examples of genomic variability towards the terminal regions of the EPTV genome include the presence of 3 and 8 novel EPTV ORFs at the left and right ends, respectively. Although these “unique” EPTV sequences
may encode new virulence factors, their small sizes cast doubt as to whether they produce novel polypeptides. However, it is likely that the process of generating novel genes (not paralogs) in poxviruses begins small, for example, the creation of a promoter region adjacent to a novel ORF in new DNA acquired by a horizontal transfer event or in a fragmented pseudogene (non-functional). Such an initiating event could be followed by the gain of further protein domains [44, 45].

With regard to the potential for cross-species infections, EPTV possesses 11 out of 12 sets of host range genes previously reviewed [46, 47] (missing K1L, which is only found in orthopoxviruses). Of these 11, unique sequence extensions and truncations are found associated with EPTV orthologs of PKR inhibitors (K3L and E3L), KilA-N/RING domain protein (p28/N1R) involved in ubiquitin or apoptosis inhibition, and tumor necrosis factor receptor family 2 (B28R). It is unknown how these altered sequences may impact the ability of the encoded protein to avert different host immune responses. For example, SPPV with a deletion of the kelch-like protein (SPPV-019) restored 100% survival rate in infected sheep, but the same deletion failed to reduce virulence in a VACV model [48]. Therefore, the mere possession of host range proteins alone cannot serve as a marker or indicator for cross-species infection without considering the diversity of viral sequences and host immune systems, as well as any combinatorial effect of gene sets. However, we can foresee some beneficial role for EPTV in having either an increased virulence that promotes infections, or an attenuation that could contribute to the host serving as a reservoir of different viruses. The latter seems to align with the fact that majority of bat-borne viruses exhibit non-severe nor fatal symptoms in their reservoir hosts [49].

The discovery of multiple poxviruses in bats leads to inquiries about the possibility of coevolution between the host and virus [50]. Bats, whose species have diverged 50 million years ago, include diverse species in the suborders of “megabats” and “microbats,” differing both on the behavioral and molecular levels [14, 15]. We suggest that bat poxviruses, like other poxviruses shown to date, do not usually co-evolve with their hosts [51]. First of all, there is no evidence yet to suggest the individual bats as the natural hosts of these poxviruses (EPTV, PTPV, and EHPV1); surveys have yet to be conducted on these geographically distinct bats that screen for the prevalence of a long-harbing poxvirus pool in the population. Secondly, given bats are indeed common hosts of these poxviruses, the phylogeny (Fig. 1) clearly shows EPTV and PTPV branch off into individual genera that are not in proximity to each other on the phylogenetic tree the way bats from different suborders usually group; EHPV, represented by a partial RPO18 amino acid sequence, branches off yet another separate genus close to molluscum contagiosum virus (MOCV). This is consistent with the poxviral phylogenetic analysis where capripox- and parapoxviruses do not cluster into the same branch despite common hosts in even-toed hoofed mammals [51].

It is notable that A+T-rich EPTV (76%) groups with other A+T-rich viruses, while PTPV (66% A+T) and EHPV (N/A) both group closer to G+C-rich viruses. In fact, PTPV has been noted to possess 3 genes, also in the same position, observed only in G+C-rich viruses. Although this is a small sample size, it is intriguing that PTPV and EHPV were isolated from megabat hosts (Pteropus scapulatus and Eidolon helvum), whereas EPTV was isolated from a microbat (Eptesicus fuscus). However, there is no evidence for a correlation between virus and host genome nucleotide composition.

Lastly, we found no evidence of similarities between the genes shared by the bat-borne EPTV and PTPV that suggest common virulence mechanisms in bat hosts. Consistent with this theme, the clinical symptoms of EPTV in Eptesicus fuscus manifest in the form of joint swelling and increased lethargy [10], whereas Pteropus scapulatus infected with PTPV presented lesions on wing membranes [13]; neither type of symptoms was directly linked to fatality. Thus, it appears that these “bat-isolated poxviruses” do not have any common genes based on their related hosts.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics statement This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent No human subjects were involved in this study.

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