Originally published as:

Scuda, N., Hofmann, J., Calvignac-Spencer, S., Ruprecht, K., Liman, P., Kühn, J., Hengel, H., Ehlers, B.
A novel human polyomavirus closely related to the African green monkey-derived lymphotropic polyomavirus
(2011) Journal of Virology, 85 (9), pp. 4586-4590.

DOI: 10.1128/JVI.02602-10

A novel human polyomavirus closely related to the African green monkey derived lymphotropic polyomavirus (LPV)

Nelly Scuda[1], Jörg Hofmann[2], Sébastien Calvignac-Spencer[3], Klemens Ruprecht[4], Peter Liman[5], Joachim Kühn[6], Hartmut Hengel[7], Bernhard Ehlers[1]

[1] Division of Viral Infections, Robert Koch Institute, Berlin, Germany
[2] Institute of Virology, Charité - Universitätsmedizin Berlin, Berlin, Germany
[3] Research Group Emerging Zoonoses, Robert Koch Institute, Berlin, Germany
[4] Department of Neurology, Charité - Universitätsmedizin Berlin, Berlin, Germany
[5] Department of Nephrology and Intensive Care, Charité - Universitätsmedizin Berlin, Berlin, Germany
[6] Institute of Medical Microbiology, Clinical Virology, University of Muenster, Muenster, Germany
[7] Institute for Virology, Heinrich-Heine-University, Düsseldorf, Germany

* Dr. B. Ehlers
Division of Viral Infections
Robert Koch Institute
Nordufer 20
13353 Berlin
email: EhlersB@rki.de
phone: ++4930187542347
fax: ++4930187542598

J. Virol. doi:10.1128/JVI.02602-10
JVI Accepts, published online ahead of print on 9 February 2011

A novel human polyomavirus was identified with generic PCR in a kidney transplant patient under immunosuppressive treatment. Its genome was completely amplified and sequenced. In phylogenetic analyses it appeared as the closest relative to the African green monkey-derived lymphotropic polyomavirus (LPV). Further investigation of clinical samples from immunocompromised patients with specific nested PCR revealed additional positive samples indicating that the virus naturally infects humans. The virus was tentatively named Human Polyomavirus 9 (HPyV9). The previously observed seroreactivity to LPV in human populations might find a partial explanation in the circulation of HPyV9.

Humans are known to be infected with a minimum of eight distinct polyomaviruses (PyVs): BK PyV (BKV (8)), JC PyV (JCV(14)), KI PyV (KIV (1)), WU PyV (WUV (9)), Merkel cell polyomavirus (MCPyV (6)), HPyV6 and HPyV7 (15) and Trichodysplasia spinulosa polyomavirus (TSV (17)). These members of the *Polyomaviridae* family are small DNA viruses which generally infect individuals in a persistent manner (from their early childhood on) but asymptomatically. In immunocompromised individuals, however, PyV reactivation has been associated with pathological conditions: JCV with progressive multifocal leukoencephalopathy, BKV with nephropathy and cystitis (both reviewed in: (12)), MCPyV with Merkel cell carcinoma (MCC), a rare but aggressive human tumor of the skin (6), and the *Trichodysplasia spinulosa* - associated virus (TSV) with the eponymous pathology (17). Given the growing disease burden entailed by acquired immunodeficiencies (mostly medically induced in industrialized countries, as well as a side effect of the spread of HIV-1 infections), human PyVs are now increasingly considered as emerging opportunistic pathogens. Gaining more insight into their diversity, prevalence and etiopathogenesis is therefore essential. Given the accelerated rate of human PyV discovery over the last few years it appears very likely that further, still unknown PyVs are actually circulating in human populations. In support of this notion, sero-epidemiological studies have revealed that up to 30% of human sera had strong reactions to antigens derived from a PyV initially identified in African green monkeys (*Chlorocebus aethiops*), the lymphotropic polyomavirus (LPV or AGMPyV) (4, 10, 16, 18, 19), a fact which could not be explained by cross-reactivity to already known human PyVs (18). In addition, we recently identified m ore than 20 novel PyVs in a number of non human primates

(NHP), including apes ((13) and N. Scuda, F. Leendertz and B. Ehlers, unpublished data). These new PyVs nearly span the entire known diversity of mammalian PyVs, which suggests that primates as a whole, including humans, are infected with a plethora of PyVs. To further test this hypothesis, we screened DNA extracts from 597 clinical samples for the presence of PyVs. The majority of the samples were from immunocompromised transplant recipients, leukoencephalopathy (LEP) patients and HIV-positive patients (serum, plasma, urine or whole blood) that had originally been collected for BKV and herpes virus diagnostics at three German university hospitals (Table 1). In addition, cerebrospinal fluid (CSF) samples from LEP patients, bronchoalveolar lavage (BAL) from patients with pneumonia and fecal samples from children with diarrhoea were tested (Table 1). A generic PCR assay using degenerate and deoxyinosine-substituted primers was used as described previously (13). The primers had been designed to target conserved regions in the VP1 genes of the known PyVs (except the highly divergent PyV group represented by KIV, WUV, HPyV6 and HPyV7). From 85/597 samples, VP1 sequences could be determined as shown by sequencing and BLAST analysis (2) in GenBank. All of these PyVs except one were known: BKV (n=56), JCV (n=18), MCPyV (n=8) and TSV (n=2) (Table 1). The PyV sequences were amplified from plasma (12/176), urine (71/179) and BAL (1/21) samples. CSF (n=74), whole blood (n=21), serum (n=87) and fecal (n=38) samples were negative (Table 1). Most importantly, an unknown PyV sequence was amplified from the serum of a kidney transplant patient under immunosuppressive treatment. In BLAST analysis it revealed the closest similarity to the VP1 gene of LPV (pairwise nucleic acid identity: 83%). To obtain additional sequence information of the novel PyV, a 950 bp genome fragment spanning parts of the VP3 and VP1 genes was amplified. For this purpose, two degenerate sense primers derived from the VP3 sequence of LPV and two specific antisense primers deduced from the novel VP1 sequence were used in a nested PCR. Based on the resulting sequence, primers were designed tail-to-tail for the amplification and sequencing of the remaining part of the genome, and used in nested long-distance (LD-) PCR (all primer sequences and PCR reaction conditions are shown in Tables S1 and S2 of the supplemental material). A final circular genome of 5026 bp was obtained, and confirmed to be more similar to the LPV genome (pairwise nucleic acid identity: 76%) than to those of other PyVs (<60%). Since eight human PyVs are presently known, the virus from which the genome originated was tentatively named Human Polyomavirus 9 (HPyV9). Examination of putative open reading frames (ORFs) showed that the genome of HPyV9 has a typical PyV structure with an early region encoding regulatory proteins (small t and large T antigens) and a late region coding for structural proteins (VP1, VP2 and VP3) separated by a non-coding control region (NCCR) (Figure 1A). HPyV9 lacked any ORF encoding an agnoprotein. The ORF locations on the viral genome and their similarity to LPV and other human PyVs are listed in the Table S3 in the supplemental material. Over the entire genome length HPyV9 and LPV revealed a high similarity (see Figure S4 in the supplemental material) including functionally important sites in the NCCR and the large T and VP1 proteins (Figures 1B-1D, respectively). Phylogenetic analyses were performed using a concatenated dataset of VP1, VP2 and large T sequences including HPyV9 as well as a representative collection of published PyVs (Table S5 of the supplemental material). Preparation of the dataset as well as downstream analyses, including maximum likelihood (ML) and Bayesian tree reconstructions were conducted as described previously (13). The resulting tree evidenced the close relationship of HPyV9 and LPV, which formed a highly supported monophyletic group - bootstrap (Bp) and posterior probability (pp) values: 100 and 1 (Figure 2). To determine whether individuals other than the index case were infected with HPyV9, all 597 samples were re-screened with HPyV9-specific nested PCR using primers deduced from the VP1 sequence (see Tables S1 and S2 in the supplemental material). In four additional samples (serum, plasma, whole blood and urine), VP1 sequences of HPyV9 were detected (100% nucleic acid sequence identity to the index VP1 sequence). All CSF and BAL samples were negative (Table 1). In attempting to amplify complete genomes sequences from these samples, PyV genomes were pre-amplified by using the Templiphi 100 rolling circle amplification kit, according to the instructions of the manufacturer (GE Healthcare, Germany), prior to perform the VP3/VP1-PCR and the LD-PCR. By this approach, another complete HPyV9 genome could be amplified (from the plasma sample of a renal transplant patient; data not shown). It revealed 100% nucleic acid identity to the index genome. These findings qualify HPyV9 as a human virus, however with a low prevalence in the selected samples (as revealed by DNA-based assays). Further studies are required to identify the sites of HPyV9 replication and persistence as well as to determine HPyV9 pathogenicity in immunocompetent and immunocompromised individuals. Notably, HPyV9 exhibited very close sequence relatedness to LPV, a fact that deserves closer attention. Sero-epidemiological studies have indeed revealed that up to 30% of adult human sera contain antibodies against LPV (3, 4, 10, 16). Viscidi et al. studied the serological cross reactivity between BKV, JCV, SV40 and LPV capsids using enzyme immunoassays (EIA) based on virus-like particles. Their data indicated that LPV-reactive antibodies found in human sera could not be attributed to cross-reacting BKV or JCV antibodies (18). Based on competition assays, Kean et al. could exclude serological crossreactivity between VP1 proteins of LPV and

MCPyV (10). Taken together, these results pointed at LPV having a serologically related human counterpart. The capsid protein VP1 is the major structural component of polyomavirus particles. It contains antigenic determinants and is involved in the interaction of PyVs with host cells through its outfacing loops. Therefore the similarity between the HPyV9 and LPV VP1 proteins, even in the highly variable loop regions (Figure 1D), is remarkable, and serological cross-reactivity appears as a reasonable possibility. This hypothesis is supported by the finding that cross-reactivity has been observed between VP1 capsomers of JCV and BKV on the one hand and SV40 on the other hand (10), despite the fact that SV40 VP1 is more distantly related to the JCV/BKV VP1 (pairwise amino acid sequence identities: 75% and 81%, respectively) than HPyV9 to LPV (87%). Against this line of argumentation stand two observations. First, WUV and KIV VP1 capsomers show no evidence of cross-reactivity, while their VP1 amino acid sequences are approximately as distant from each other as JCV/BKV from SV40 (10). Then, it was also shown that antibodies raised against one or the other strains of BKV could occasionally discriminate subgroups in neutralization assays (11) while the maximum VP1 genetic distance within BKV is largely outscored by the genetic distance between LPV/AGMPyV and HPyV9 VP1 (Figure S6 of the Supplementary Material). All in all, these contrasted results prevent any firm prediction of cross-reactivity that would be solely based on genetic make-up. However, we propose that HPyV9 could at least be considered one good candidate for the frequent LPV-like serological reactivity observed in human populations, acknowledging that other PyVs might be involved as well. Another good candidate PyV could be LPV itself whose short DNA fragments were recently detected in CSF and blood of AIDS patients as well as in blood of healthy subjects (5). Other authors analysed patients suffering from progressive multifocal leukoencephalopathy, and did not detect LPV while using specific primers (7). Our data do not provide evidence of LPV infection in immunocompromised patients either. If the prevalence of both HPyV9 and LPV infection in human populations can be confirmed to be at low levels, the existence of other antigenically related, yet unknown human PyVs must be postulated. Further investigation of this question, notably involving differential serological testing of HPyV9 and LPV, should clarify this issue. The genome sequence of HPyV9 was deposited i n Genbank under the accession number HQ696595.

Acknowledgements

The authors are grateful for the excellent technical assistance by Sonja Liebmann, Cornelia Walter and Nezlisah Yasmum as well as for helpful discussions with Fabian H. Leendertz. The supply with clinical samples by Marina Höhne, Sebastian Voigt, Ioannis Anagnostopoulos, Ulrike Blume-Peytavi and Mona Embarek is kindly acknowledged.

References

1. **Allander, T., K. Andreasson, S. Gupta, A. Bjerkner, G. Bogdanovic, M. A. Persson, T. Dalianis, T. Ramqvist, and B. Andersson.** 2007. Identification of a third human polyomavirus. J Virol **81:**4130-6.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J Mol Biol **215:**403-10.
3. **Brade, L., N. Mueller-Lantzsch, and H. Zur Hausen.** 1980. B-lymphotropic papovavirus and possibility of infections in humans. J Med Virol **6:**301-308.
4. **Brade, L., N. Muller-Lantzsch, and H. zur Hausen.** 1981. B-lymphotropic papovavirus and possibility of infections in humans. J Med Virol **6:**301-8.
5. **Delbue, S., S. Tremolada, E. Branchetti, F. Elia, E. Gualco, E. Marchioni, R. Maserati, and P. Ferrante.** 2008. First identification and molecular characterization of lymphotropic polyomavirus in peripheral blood from patients with leukoencephalopathies. J Clin Microbiol **46:**2461-2.
6. **Feng, H., M. Shuda, Y. Chang, and P. S. Moore.** 2008. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science **319:**1096-100.
7. **Focosi, D., F. Maggi, E. Andreoli, L. Lanini, L. Ceccherini-Nelli, and M. Petrini.** 2009. Polyomaviruses other than JCV are not detected in progressive multifocal leukoencephalopathy. J Clin Virol **45:**161-2.
8. **Gardner, S. D., A. M. Field, D. V. Coleman, and B. Hulme.** 1971. New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet **1:**1253-7.
9. **Gaynor, A. M., M. D. Nissen, D. M. Whiley, I. M. Mackay, S. B. Lambert, G. Wu, D. C. Brennan, G. A. Storch, T. P. Sloots, and D. Wang.** 2007. Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoS Pathog **3:**e64.
10. **Kean, J. M., S. Rao, M. Wang, and R. L. Garcea.** 2009. Seroepidemiology of human polyomaviruses. PLoS Pathog **5:**e1000363.
11. **Knowles, W. A., P. E. Gibson, and S. D. Gardner.** 1989. Serological typing scheme 233 for BK-like isolates of human polyomavirus. J Med Virol **28:**118-23.
12. **Krumbholz, A., O. R. Bininda-Emonds, P. Wutzler, and R. Zell.** 2009. Phylogenetics, evolution, and medical importance of polyomaviruses. Infect Genet Evol **9:**784-99.
13. **Leendertz, F. H., N. Scuda, K. N. Cameron, T. Kidega, K. Zuberbuhler, S. A. Leendertz, E. Couacy-Hymann, C. Boesch, S. Calvignac, and B. Ehlers.** 2011. African great apes are naturally infected with polyomaviruses closely related to merkel cell polyomavirus. J Virol **85:**916-24.
14. **Padgett, B. L., D. L. Walker, G. M. ZuRhein, R. J. Eckroade, and B. H. Dessel.** 1971. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet **1:**1257-60.
15. **Schowalter, R. M., D. V. Pastrana, K. A. Pumphrey, A. L. Moyer, and C. B. Buck.** 2010. Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. Cell Host Microbe **7:**509-15.
16. **Takemoto, K. K., and K. Segawa.** 1983. A new monkey lymphotropic papovavirus: characterization of the virus and evidence of a related virus in humans. Prog Clin Biol Res **105:**87-96.
17. **van der Meijden, E., R. W. Janssens, C. Lauber, J. N. Bouwes Bavinck, A. E. Gorbalenya, and M. C. Feltkamp.** 2010. Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromized patient. PLoS Pathog **6:**e1001024.
18. **Viscidi, R. P., and B. Clayman.** 2006. Serological cross reactivity between polyomavirus capsids. Adv Exp Med Biol **577:**73-84.
19. **zur Hausen, H., and L. Gissmann.** 1979. Lymphotropic papovaviruses isolated from African green monkey and human cells. Med Microbiol Immunol **167:**137-53.

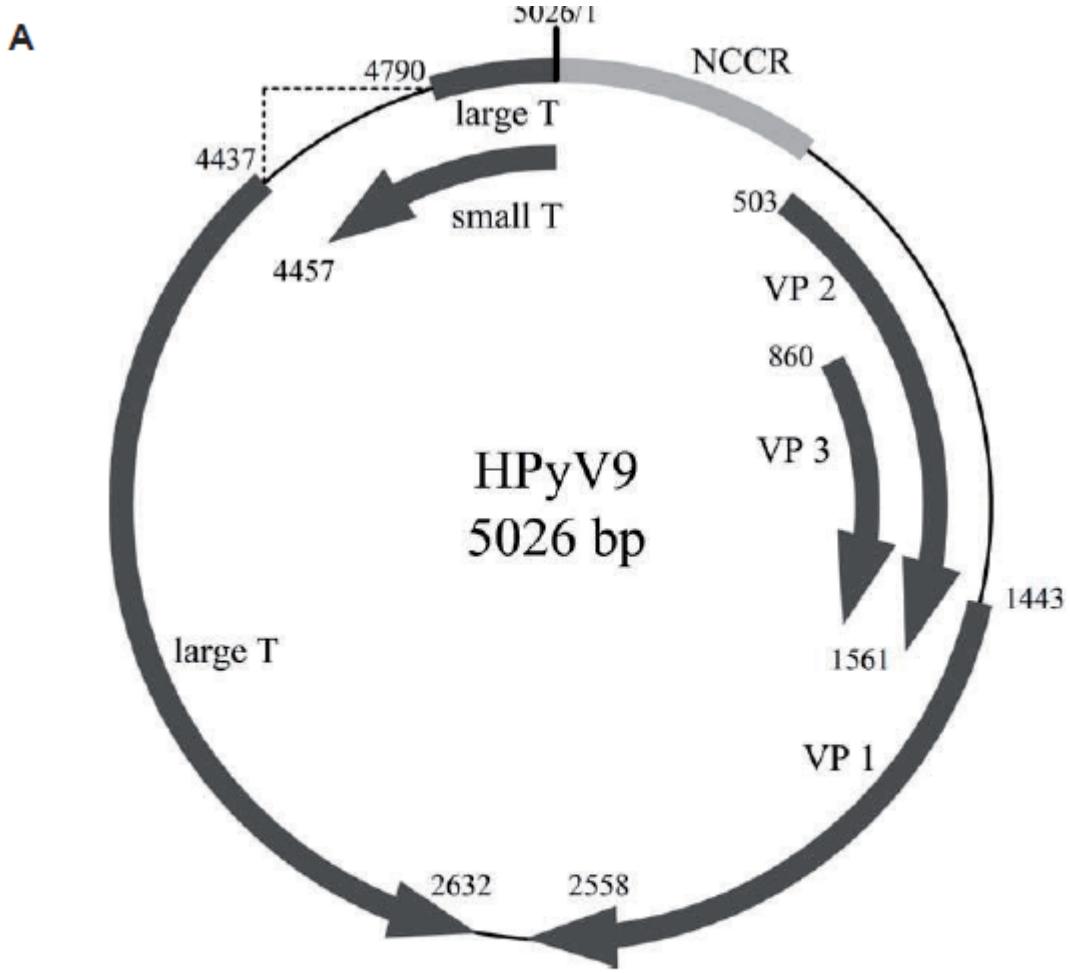
Tables and Figures

Table 1 Analysis of selected human samples for polyomaviruses and HPyV9 with PCR

Sample type	Source	No. of samples tested	PyV detected (no.) by generic PCR	No. of HPyV9-positive by specific PCR	HPyV9 genome amplified
Plasma	kidney transplant recipients [a]	76	BKV (5), MCPyV (1)	1	+
	patients with HIV [b]	50	MCPyV (1)	- [c]	-
	immunocompromised adults [d]	25	BKV (3)	-	-
	immunocompromised paediatric patients [d]	25	BKV (2)	-	-
Serum	patients with leukoencephalopathy [e]	47	-	1	-
	kidney transplant recipients [a]	41	HPyV9 (1)	1	+
Urine	kidney transplant recipients [a,f]	38	BKV (6), MCPyV (1)	1 [g]	-
	immunocompromised patients with cancer [a]	26	BKV (6), JCV (4), TSV (1)	-	-
	stem cell transplant recipients [a]	14	BKV (6)	-	-
	patients with multiple sclerosis [h]	9	JCV (2)	-	-
	other [a]	92	BKV (28), JCV (12), MCPyV (4), TSV (1)	-	-
Whole blood	transplant recipients [e]	20	-	-	-
	acute myeloid leukemia patient	1	-	1	-
Bronchoalveolar lavage	patients with pneumonia [e]	21	MCPyV (1)	-	-
Feces	paediatric clinical samples, gastroenteritis	38	-	-	-
Cerebrospinal fluid	patients with leukoencephalopathy [k]	36	-	-	-
	other [e]	38	-	-	-

[a] samples collected for BKV diagnosis
[b] plasma with active cytomegalovirus infection
[c] -: PCR-negative
[d] samples collected for cytomegalovirus diagnosis
[e] samples collected for herpesvirus diagnosis
[f] 25-100µl eluate from 10-30 ml urine (n=27) or from 0,2 ml urine (n=11)
[g] patient with a double infection (MCPyV + HPyV9)
[h] patients under Tysabri® (natalizumab) therapy
[k] samples collected for JCV diagnosis

Figure 1 Genome map of HPyV9 and comparison of encoded proteins with those of LPV. (A) Genome organization of HPyV9. Putative coding regions for VP1 to VP3, small T antigen, and large T antigen are marked by arrows. (B) Part of the non-coding control region (NCCR) of HPyV9 was aligned with that of LPV using ClustalW (as implemented in MacVector 10.6). Conserved regions are outlined and shaded. T-antigen binding elements (GAGGC or the complement GCCTC) are underlined. (C) Comparison of HPyV9 and LPV large T proteins. The CR1, J, RB-binding, *ori*-binding and Zn-Finger domains are underlined. (D) Comparison of VP1 proteins. VP1 loops are underlined. Identical amino acids (in bold type) are outlined and shaded, similarities (in bold type) are outlined only, and mismatches are in normal, lower type.



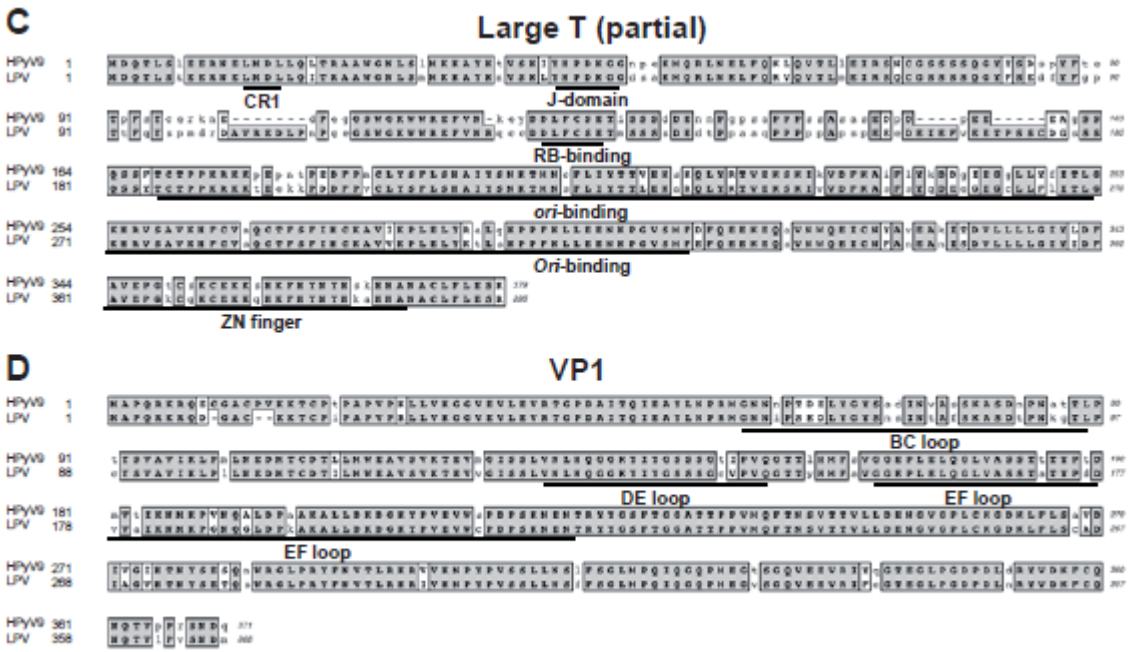


Figure 2 Bayesian chronogram deduced from the analysis of a 687 amino acid alignment of concatenated VP1, VP2 and large T sequences. PyVs identified from human hosts are in red, from non-human primates in blue. The clade formed by HPyV9 and LPV is highlighted by grey line color. Statistical support for branch is given where Bp≥70 and pp≥0.95. Bp values are shown after the slash bar, pp values before the slash bar. The scale axis is in amino acid substitution per site. This chronogram was rooted using a relaxed clock. A maximum likelihood analysis of the same dataset concluded to a similar topology and is thus not shown here.