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VIROLOGY DIVISION NEWS

A taxonomy update for the family *Polyomaviridae*

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Abstract Many distinct polyomaviruses infecting a variety of vertebrate hosts have recently been discovered, and their complete genome sequence could often be determined. To accommodate this fast-growing diversity, the International Committee on Taxonomy of Viruses (ICTV) *Polyomaviridae* Study Group designed a host- and sequence-based rationale for an updated taxonomy of the family *Polyomaviridae*. Applying this resulted in numerous recommendations of taxonomical revisions, which were accepted by the Executive Committee of the ICTV in December 2015. New criteria for definition and creation of polyomavirus species were established that were based on the observed distance between large T antigen coding sequences. Four genera (*Alpha-*, *Beta*, *Gamma-* and *Deltapolyomavirus*) were delineated that together include 73 species. Species naming was made as systematic as possible – most species names now consist of the binomial name of the host species followed by *polyomavirus* and a number reflecting the order of discovery. It is hoped that this important update of the family taxonomy will serve as a stable basis for future taxonomical developments.

The taxonomic changes summarized here have been submitted as an official taxonomic proposal to the International Committee on Taxonomy of Viruses (ICTV) (http://www.ictvonline.org) and have now been accepted but not yet ratified. These changes therefore may differ from any new taxonomy that is ultimately approved by the ICTV.

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Introduction

When it was created, the family *Polyomaviridae* included only a handful of polyomavirus species, whose members had all been discovered by the early 1980s [21]. The situation has now changed dramatically: sequences attributed to relatives of these early polyomaviruses have been

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published at a much accelerated pace [5, 22], and by September 2015, more than 1200 complete polyomavirus genome sequences representing roughly 100 genetically and biologically distinct polyomaviruses had been deposited in public databases. Nearly all of them were made publicly available in the years 2000–2015, and a number of novel polyomaviruses were published while this report was being prepared.

This sudden acceleration found its roots in technological improvements that made polyomavirus discovery much easier, though still a laborious task (reviewed in [5]). Concomitantly, the first demonstration of the oncogenic potential of a polyomavirus in humans, Merkel cell PyV [6], considerably rekindled interest in this viral family. With the ever-growing body of data, new questions will emerge that will likely result in maintaining a firm foot on the discovery throttle. In this respect, it is striking to observe that even for the few well-sampled non-human mammalian hosts, e.g., chimpanzees, increasing the sample size often results in the identification of new polyomaviruses [4, 9, 13, 16, 19]. Cataloguing the diversity of this family will be a work in progress for many years. Ideally, taxonomy should accompany and help this work.

To enable taxonomic classification, pieces of information have to be identified that are frequently available and that we consider suitable to build a stable and consistent taxonomic system upon. For most novel polyomaviruses, their host and their nucleic acid sequence are the only characters within immediate reach; it is reasonable to anticipate that this will be a long-lasting *status quo*. Therefore, designing a host- and sequence-based taxonomy of the family *Polyomaviridae* seems to be the best way forward. A first step in this direction had been made by the International Committee on Taxonomy of Viruses (ICTV) *Polyomaviridae* Study Group (SG) with the suggestion that entities with >19 % whole-genome divergence be considered members of separate species. In addition, the SG had proposed to create three genera within the family (*Avi-, Wuki-,* and *Orthopolyomavirus*) [11]. However, this approach has not been adopted by the ICTV because it did not account for the observation that some polyomaviruses are recombinants, and the phylogenetic analyses underlying the genus definition were based on different genes. In consideration of the committee's criticisms, the SG developed novel host- and sequence-based criteria for species demarcation and genus delineation. In addition, a standardized scheme for species naming was established. These taxonomical updates were accepted by the Executive Committee of the ICTV in December 2015 and are described in this article.

Criteria for definition of polyomavirus species

Briefly, the five delineation criteria aim at ensuring that: (1) nucleic acid sequence information is public and verified and unambiguously identifies a polyomavirus (C1-C2), (2) a plausible host is known (C3), and (3) the genetic (and possibly biological) divergence qualifies the new entity as a member of a species distinct from members of all species already recognized (C4-C5). Complying with C1 to C4 is enough to justify the creation of a new species; in cases where C1 to C3 are fulfilled but C4 is not, a new species may still be validated by applying C5. The five delineation criteria are as follows:

C1. The complete genome sequence is available in public databases and published in a peer-reviewed journal or an edited journal announcing the availability of genome sequences.
Note: As the binomial host species name is part of the polyomavirus species name (see below), information on the host of the virus and details regarding how the host was determined are required. Such information is usually included in publications but frequently is not available in sequence database entries.

C2. The genome displays an organization typical for polyomaviruses, i.e., a dsDNA genome with an early region and a late region encoding the T antigens and the structural viral proteins on opposite strands, respectively. Both regions are separated by a non-coding control region.
Note: This criterion was set up to exclude recombinant viruses that associate polyomavirus-related coding regions with genomic elements from other viruses, e.g., bandicoot papillomatosis viruses [1, 23].

C3. Sufficient information on the natural host is available.
Note: In cases where the host cannot be firmly identified by host morphology, molecular methods should be applied, e.g., mitochondrial cytochrome b typing.

C4. The observed genetic distance to members of the most closely related species is >15 % for the large T antigen (LTAg) coding sequence.
Note: Under this criterion, all publicly available genomes of frequently sequenced polyomaviruses fall into their respective species (e.g., BKPyV,

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Table 1 Polyomavirus species

No.	PyV species[a,b]	Polyomavirus name (abbreviation)[c]	Isolate/strain	Common host name	Genome length (bp)	GenBank accession number	NCBI Reference Sequence Database accession number	% identity to PyV species (species no.)[d]
	Genus *Alphapolyomavirus*							
1	*Acerodon celebensis polyomavirus 1*	Bat polyomavirus 5b (BatPyV5b)	5b-2	Sulawesi flying fox	5040	AB972940		88 (*35*)
2	*Artibeus planirostris polyomavirus 2*	Bat polyomavirus 3a (BatPyV3a)	A1055	Flat-faced fruit bat	5019	JQ958886		86 (*36*)
3	*Artibeus planirostris polyomavirus 3*	Bat polyomavirus 4a (BatPyV4a)	R104	Flat-faced fruit bat	5371	JQ958887		74 (*6*)
4	*Ateles paniscus polyomavirus 1*	*Ateles paniscus* polyomavirus 1 (ApanPyV1)	1960	Spider monkey	5273	JX159987	NC_019853	69 (*13*)
5	*Cardioderma cor polyomavirus 1*	*Cardioderma* polyomavirus 1 (*Cardioderma* PyV)	KY336	Heart-nosed bat	5372	JX520659	NC_020067	60 (*22*)
6	*Carollia perspicillata polyomavirus 1*	Bat polyomavirus 4b (BatPyV4b)	C1109	Seba's short-tailed bat	5352	JQ958889		74 (*3*)
7	*Chlorocebus pygerythrus polyomavirus 1*	Vervet monkey polyomavirus 1 (VmPyV1)	VMS96	Vervet monkey	5157	AB767298	NC_019844	87 (*31*)
8	*Chlorocebus pygerythrus polyomavirus 3*	Vervet monkey polyomavirus 3 (VmPyV3)	VMS95/ VMV97	Vervet monkey	5055	AB767297	NC_025898	86 (*30*)
9	*Dobsonia moluccensis polyomavirus 1*	Bat polyomavirus 5a (BatPyV5a)	5a	Moluccan naked-backed fruit bat	5075	AB972945	NC_026768	74 (*35*)
10	*Eidolon helvum polyomavirus 1*	*Eidolon* polyomavirus 1 (*Eidolon* PyV 1)	KY270	Straw-colored fruit bat	5294	JX520660	NC_020068	57 (*12*)
11	*Gorilla gorilla polyomavirus 1*	*Gorilla gorilla gorilla* polyomavirus 1 (GgorgPyV1)	5766	Western gorilla	5300	HQ385752	NC_025380	87 (*25*)
12	*Human polyomavirus 5*	Merkel cell polyomavirus (MCPyV)	R17b	Human	5387	HM011556	NC_010277	82 (*25*)
13	*Human polyomavirus 8*	*Trichodysplasia spinulosa* polyomavirus (TSPyV)	Skin	Human	5232	GU989205	NC_014361	82 (*33*)
14	*Human polyomavirus 9*	Human polyomavirus 9 (HPyV9)	2540	Human	5026	HQ696595	NC_015150	78 (*28*)
15	*Human polyomavirus 12*	Human polyomavirus 12 (HPyV12)	1403	Human	5033	JX308829	NC_020890	49 (*1*)
16	*Human polyomavirus 13*	New Jersey polyomavirus (NJPyV)	NJ-PyV-2013	Human	5108	KF954417	NC_024118	82 (*23*)
17	*Macaca fascicularis polyomavirus 1*	*Macaca fascicularis* polyomavirus 1 (MfasPyV1)	2085	Crab-eating macaque	5087	JX159986	NC_019851	81 (*30*)
18	*Mesocricetus auratus polyomavirus 1*	Hamster polyomavirus (HaPV)	Berlin-Buch	Syrian hamster	5372	JX036360	NC_001663	47 (*9*)

Table 1 continued

No.	PyV species[a,b]	Polyomavirus name (abbreviation)[c]	Isolate/strain	Common host name	Genome length (bp)	GenBank accession number	NCBI Reference Sequence Database accession number	% identity to PyV species (species no.)[d]
19	*Molossus molossus polyomavirus 1*	Bat polyomavirus 3b (BatPyV3b)	B1130	Velvety free-tailed bat	4903	JQ958893		69 (*36*)
20	*Mus musculus polyomavirus 1*	Mouse polyomavirus (MPyV)	BG	House mouse	5307	AF442959	NC_001515	46 (*18*)
21	*Otomops martiensseni polyomavirus 1*	*Otomops* polyomavirus 1 (Otomops PyV 1)	KY156	Martienssen's free-tailed bat	4914	JX520658	NC_020066	69 (*19*)
22	*Otomops martiensseni polyomavirus 2*	*Otomops* polyomavirus 2 (Otomops PyV 2)	KY157	Martienssen's free-tailed bat	5176	JX520664	NC_020071	60 (*5*)
23	*Pan troglodytes polyomavirus 1*	Chimpanzee polyomavirus (ChPyV)	Bob	Common chimpanzee	5086	FR692334	NC_014743	82 (*16*)
24	*Pan troglodytes polyomavirus 2*	*Pan troglodytes verus* polyomavirus 1a (PtrovPyV1a)	6444	Common chimpanzee	5303	HQ385746	NC_025368	81 (*25*)
25	*Pan troglodytes polyomavirus 3*	*Pan troglodytes verus* polyomavirus 2a (PtrovPyV2a)	6512	Common chimpanzee	5309	HQ385748	NC_025370	87 (*11*)
26	*Pan troglodytes polyomavirus 4*	*Pan troglodytes verus* polyomavirus 3 (PtrovPyV3)	3161	Common chimpanzee	5333	JX159980	NC_019855	80 (*27*)
27	*Pan troglodytes polyomavirus 5*	*Pan troglodytes verus* polyomavirus 4 (PtrovPyV4)	3147	Common chimpanzee	5349	JX159981	NC_019856	81 (*32*)
28	*Pan troglodytes polyomavirus 6*	*Pan troglodytes verus* polyomavirus 5 (PtrovPyV5)	5743	Common chimpanzee	4994	JX159982	NC_019857	78 (*14*)
29	*Pan troglodytes polyomavirus 7*	*Pan troglodytes schweinfurthii* polyomavirus 2 (PtrosPyV2)	6350	Common chimpanzee	4970	JX159983	NC_019858	67 (*14*)
30	*Papio cynocephalus polyomavirus 1*	Yellow baboon polyomavirus 1 (YbPyV1)	BS20	Yellow baboon	5064	AB767294	NC_025894	86 (*8*)
31	*Piliocolobus rufomitratus polyomavirus 1*	*Piliocolobus rufomitratus* polyomavirus 1 (PrufPyV1)	4601	Red colobus	5140	JX159984	NC_019850	87 (*7*)
32	*Pongo abelii polyomavirus 1*	Sumatran orang-utan polyomavirus (OraPyV-Sum)	PI	Sumatran orangutan	5358	FN356901		81 (*27*)
33	*Pongo pygmaeus polyomavirus 1*	Bornean orang-utan polyomavirus (OraPyV-Bo)	BO	Bornean orangutan	5168	FN356900	NC_013439	82 (*13*)
34	*Procyon lotor polyomavirus 1*	Raccoon polyomavirus (RacPyV)	R45	Raccoon	5016	JQ178241	NC_023845	50 (*24*)
35	*Pteropus vampyrus polyomavirus 1*	Bat polyomavirus 5b (BatPyV5b)	5b-1	Large flying fox	5047	AB972944	NC_026767	88 (*1*)
36	*Sturnira lilium polyomavirus 1*	Bat polyomavirus 3a (BatPyV3a)	B0454	Little yellow-shouldered bat	5058	JQ958888		86 (*2*)

Table 1 continued

No.	PyV species[a,b]	Polyomavirus name (abbreviation)[c]	Isolate/strain	Common host name	Genome length (bp)	GenBank accession number	NCBI Reference Sequence Database accession number	% identity to PyV species (species no.)[d]
	Genus *Betapolyomavirus*							
37	*Acerodon celebensis polyomavirus 2*	Bat polyomavirus 6a (BatPyV6a)	6a	Sulawesi flying fox	5019	AB972941	NC_026762	66 (*43*)
38	*Artibeus planirostris polyomavirus 1*	Bat polyomavirus 2c (BatPyV2c)	A504	Flat-faced fruit bat	5187	JQ958890		74 (*58*)
39	*Cebus albifrons polyomavirus 1*	*Cebus albifrons* polyomavirus 1 (CalbPyV1)	2141	White-fronted capuchin	5013	JX159988	NC_019854	73 (*61*)
40	*Cercopithecus erythrotis polyomavirus 1*	*Cercopithecus erythrotis* polyomavirus 1 (CeryPyV1)	4077	Red-eared guenon	5189	JX159985	NC_025892	86 (*57*)
41	*Chlorocebus pygerythrus polyomavirus 2*	Vervet monkey polyomavirus 2 (VmPyV2)	VMK96	Vervet monkey	5167	AB767299	NC_025896	87 (*57*)
42	*Desmodus rotundus polyomavirus 1*	Bat polyomavirus 2a (BatPyV2a)	AT7	Vampire bat	5201	JQ958892		66 (*59*)
43	*Dobsonia moluccensis polyomavirus 2*	Bat polyomavirus 6b (BatPyV6b)	6b	Moluccan naked-backed fruit bat	5039	AB972947	NC_026770	66 (*37*)
44	*Dobsonia moluccensis polyomavirus 3*	Bat polyomavirus 6c (BatPyV6c)	6c	Moluccan naked-backed fruit bat	5046	AB972946	NC_026769	63 (*37*)
45	*Equus caballus polyomavirus 1*	Equine polyomavirus (EPyV)	CU03	Horse	4987	JQ412134	NC_017982	59 (57)
46	*Human polyomavirus 1*	BK polyomavirus (BK virus; BKV; BKPyV)	Dunlop	Human	5153	V01108	NC_001538	82 (*41*)
47	*Human polyomavirus 2*	JC polyomavirus (JC virus; JCV; JCPyV)	Mad1	Human	5130	J02226	NC_001699	76 (*46*)
48	*Human polyomavirus 3*	KI polyomavirus (KIPyV)	Stockholm 60	Human	5040	EF127906	NC_009238	70 (*49*)
49	*Human polyomavirus 4*	WU polyomavirus (WU virus; WUPyV)	B0	Human	5229	EF444549	NC_009539	70 (*48*)
50	*Loxodonta africana polyomavirus 1*	African elephant polyomavirus 1 (AelPyV1)	DK-1/2011	African elephant	5722	KF147833	NC_022519	54 (*47*)
51	*Macaca mulatta polyomavirus 1*	Simian virus 40 (SV40)		Rhesus monkey	5243	JO2400	NC_001669	70 (*40*)
52	*Mastomys natalensis polyomavirus 1*	*Mastomys* polyomavirus (MasPyV)	NR55	Multimammate mouse	4899	AB588640	NC_025895	62 (*56*)
53	*Meles meles polyomavirus 1*	*Meles meles* polyomavirus 1 (MmelPyV1)	French	European badger	5187	KP644238	NC_026473	64 (62)
54	*Miniopterus africanus polyomavirus 1*	*Miniopterus* polyomavirus (*Miniopterus* PyV)	KY369	African long-fingered bat	5213	JX520661	NC_020069	57 (*51*)
55	*Mus musculus polyomavirus 2*	Mouse pneumotropic virus (MPtV)	Kilham	House mouse	4754	M55904	NC_001505	58 (*52*)

Table 1 continued

No.	PyV species[a,b]	Polyomavirus name (abbreviation)[c]	Isolate/strain	Common host name	Genome length (bp)	GenBank accession number	NCBI Reference Sequence Database accession number	% identity to PyV species (species no.)[d]
56	*Myotis lucifugus polyomavirus 1*	*Myotis* polyomavirus (MyPyV)	VM2008_14	Little brown bat	5081	FJ188392	NC_011310	62 (*52*)
57	*Papio cynocephalus polyomavirus 2*	Yellow baboon polyomavirus 2 (YbPyV2)	BS94/BC94	Yellow baboon	5181	AB767295	NC_025897	86 (*41*)
58	*Pteronotus davyi polyomavirus 1*	*Pteronotus* polyomavirus (Pteronotus PyV)	GTM203	Naked-backed bat	5136	JX520662	NC_020070	78 *(59)*
59	*Pteronotus parnellii polyomavirus 1*	Bat polyomavirus 2b (BatPyV2b)	R266	Mustached bat	5041	JQ958891		78 (*58*)
60	*Saimiri boliviensis polyomavirus 1*	Squirrel monkey polyomavirus (SquiPyV)	Squi106	Black-capped squirrel monkey	5075	AM748741	NC_009951	89 (*61*)
61	*Saimiri sciureus polyomavirus 1*	*Saimiri sciureus* polyomavirus 1 (SsciPyV1)	2033	Common squirrel monkey	5067	JX159989		89 (*60*)
62	*Zalophus californianus polyomavirus 1*	California sea lion polyomavirus 1 (SLPyV, CSLPyV)	CSL6994	Sea lion	5112	GQ331138	NC_013796	64 (*53*)
	Genus *Gammapolyomavirus*							
63	*Anser anser polyomavirus 1*	Goose hemorrhagic polyomavirus (GHPV)	Germany 2001	Goose	5256	AY140894	NC_004800	60 (*66*)
64	*Aves polyomavirus 1*	Budgerigar fledgling disease virus (BFDV)		Parrots, passerines	4981	AF241168	NC_004764	53 (*68*)
65	*Corvus monedula polyomavirus 1*	Crow polyomavirus (CPyV)		Eurasian jackdaw	5079	DQ192570	NC_007922	67 (*66*)
66	*Cracticus torquatus polyomavirus 1*	Butcherbird polyomavirus (Butcherbird PyV)	AWH19840	Butcherbird	5084	KF360862	NC_023008	67 (*65*)
67	*Pygoscelis adeliae polyomavirus 1*	Adélie penguin polyomavirus (AdPyV)	Crozier_2012	Adélie penguin	4988	KP033140	NC_026141	51 (*63*)
68	*Pyrrhula pyrrhula polyomavirus 1*	Finch polyomavirus (FPyV)		Eurasian bullfinch	5278	DQ192571	NC_007923	53 (*64*)
69	*Serinus canaria polyomavirus 1*	Canary polyomavirus (CaPyV)	Ha09	Canary	5421	GU345044	NC_017085	51 (*68*)
	Genus *Deltapolyomavirus*							
70	*Human polyomavirus 6*	Human polyomavirus 6 (HPyV6)	607a	Human	4926	HMO11560	NC_014406	66 (*71*)
71	*Human polyomavirus 7*	Human polyomavirus 7 (HPyV7)	713a	Human	4952	HMO11560	NC_014407	66 (*70*)
72	*Human polyomavirus 10*	MW polyomavirus (MWPyV)	MA095	Human	4927	JQ898291	NC_018102	63 (*73*)
73	*Human polyomavirus 11*	STL polyomavirus (STLPyV)	MA138	Human	4776	JX463183	NC_020106	63 (*72*)
	Not assigned to a genus							
74	*Bos taurus polyomavirus 1*	Bovine polyomavirus (BPyV)	Monkey kidney cell	Cattle	4697	PLYBCG	NC_001442	49 (*61*)

Table 1 continued

No.	PyV species[a,b]	Polyomavirus name (abbreviation)[c]	Isolate/strain	Common host name	Genome length (bp)	GenBank accession number	NCBI Reference Sequence Database accession number	% identity to PyV species (species no.)[d]
75	*Centropristis striata polyomavirus 1*	Black sea bass-associated polyomavirus 1 (BassPyV1)	2835	Black sea bass	7369	KP071318	NC_025790	37 (*23*)
76	*Delphinus delphis polyomavirus 1*	Dolphin polyomavirus 1 (DPyV-1)	Trachea/ 2010	Short-beaked common dolphin	5159	KC594077	NC_025899	54 (*59*)

[a] Cutoff date, 2015-March-30

[b] For each species, only one virus is listed. Especially for human polyomaviruses, genome sequences are available from numerous strains and isolates in public databases that cannot be listed here

[c] Polyomavirus name and abbreviation, as used in the literature

[d] Percentage of LTAg coding sequence identity as compared to the closest related species (in parentheses, species number as indicated in column 1); all percentage values above 85 % are underlined

HPyV6, HPyV7, JCPyV, KIPyV, MCPyV, MWPyV, SV40 and WUPyV genomes). The choice of LTAg as a delineating marker was made to keep this criterion in line with the genus delineation criteria (see below). Observed genetic distances were chosen after having checked that they were very similar to patristic distances (data not shown).

C5. When two polyomaviruses exhibit <15 % observed genetic distance, biological properties (e.g., host specificity, disease association, tissue tropism, etc.) can justify the creation of a new species.

Example 1: Two polyomaviruses are regularly detected in the same host, but C4 is not fulfilled (i.e., they exhibit less than 15 % divergence). Here, both viruses are assigned to the same species (e.g., BKPyV variants; percentage of identity: 93–100 %).

Example 2: Two polyomaviruses are regularly and exclusively detected in separate hosts, but C4 is not fulfilled (i.e., they exhibit less than 15 % divergence). In this case, C5 may result in assigning both viruses to separate species, i.e., C5 overrides C4. This is exemplified by the two polyomaviruses infecting squirrel monkeys of different species (percentage of identity: 89 %; Table 1).

Example 3: Two polyomaviruses are regularly detected in the same host and C4 is fulfilled: the two polyomaviruses are assigned to separate species (e.g., *Pan troglodytes polyomavirus 2* and *3*; percentage of identity: 81 %).

Naming of polyomavirus species

As novel polyomaviruses are discovered at a very fast pace, the SG recommended the implementation of standardized species naming, thereby avoiding the nonsystematic inclusion of patient acronyms, geographical and biological designations, etc. into the species name. It seems clear that polyomaviruses are host-specific. Despite the use of broad-ranging and flexible detection methods, there are no (or very few) reports about any polyomavirus first discovered in an organism and later detected in another. Exceptions may be SV40 and lymphotropic polyomavirus, but the circulation of these monkey viruses in human populations – or their origin– is still a controversial issue [3, 7, 8, 15, 18]. Therefore, the SG decided to include the host species name into the polyomavirus species name. For this purpose, the binomial host species name was preferred to a common host name, as it is unique at the time of polyomavirus species creation. Naming was achieved by a combination of the Latinized host species name and the term "*polyomavirus*", followed by a number. Numbers are consecutive and follow the chronological order of discovery/publication of the according polyomavirus. Example: the virus known in the literature as bovine polyomavirus (BPyV) belongs to the species *Bos taurus polyomavirus 1*.

Only a few exceptions to this naming scheme were accepted. The ability of budgerigar fledgling disease polyomavirus (BFDPyV) to infect multiple avian hosts [10] was accounted for by renaming the corresponding species *Aves polyomavirus 1*. In addition, all species accommodating human polyomaviruses were named *Human*

polyomavirus (instead of *Homo sapiens polyomavirus*), followed by a number. Example: the virus known in the literature as BK polyomavirus (BKV or BKPyV) belongs to the species *Human polyomavirus 1*.

Definition of novel species, renaming or removal of former species

As of 2015-March-30 (cutoff date for preparation of the current taxonomical update), 68 novel polyomavirus species were defined and named, eight species were renamed and five species were removed from the *Polyomaviridae*, since they do not meet the novel species definition criteria. All in all, 76 species were defined, including 13 polyomavirus species with members infecting humans, 10 ape polyomavirus species (7 chimpanzee, 1 gorilla and 2 orangutan polyomavirus species), 13 monkey polyomavirus species, 21 bat polyomavirus species, 4 rodent polyomavirus species, seven species with members identified from other mammals, seven avian polyomavirus species, and one fish polyomavirus species. They are listed with their host and accession number in Table 1. Members of 61 species displayed >15 % divergence from the most closely related polyomavirus of another species. Members of 15 species displayed <15 % divergence (11-14 %) to the most closely related polyomavirus of another species but originated from different host species (Table 1).

Additional mammalian and fish polyomaviruses, including polyomaviruses of five previously ICTV-recognized species that have now been removed from the *Polyomaviridae* (see above) might give rise to additional species within the *Polyomaviridae* in the near future. They are currently excluded from species definition or have been removed from the family because their host species was not reported, their publication happened after the cutoff date, or the report was not validated by peer review (GenBank accession numbers: NC_025811, NC_007611, KM496324, NC_025800, NC_004763, AB972942, NC_026766, NC_015123, NC_020065, NC_010107, NC_010817, KJ641707, KJ641705, KJ577598, NC_025259, NC_026244, NC_026012, NC_026015, NC_026942, NC_026944, NC_027531, NC_027532).

Creation of genera and assignment of polyomavirus species to genera

The tremendous diversity of polyomaviruses naturally calls for the identification of some hierarchy within the taxonomical structure of the family, e.g., through the definition of intermediate taxa such as genera. Some years ago, the SG took a first step in this direction and proposed to delineate three genera [11]. The suggestion to create the genus *Avipolyomavirus* aimed at accounting for the distinctive biological properties that avian polyomaviruses display when compared to mammalian ones: broad host range and tissue tropism, no oncogenicity but marked pathogenicity, individual genomic features [11]. In line with this, phylogenetic analyses consistently supported the reciprocal monophyly of avian and mammalian polyomaviruses. Most mammalian polyomaviruses are only

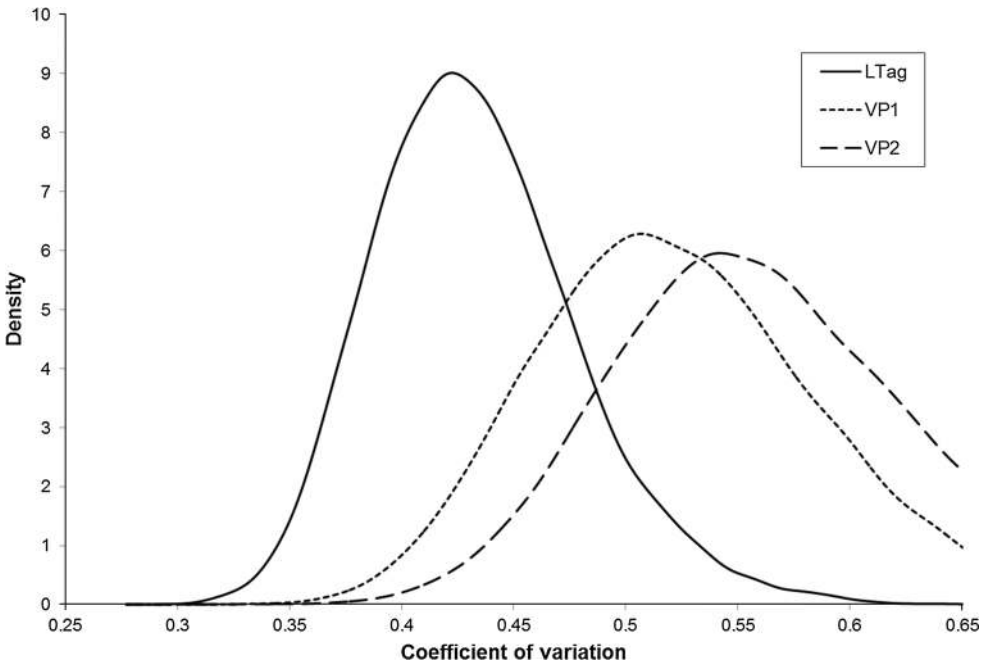

Fig. 1 Bayesian estimates of the coefficient of variation of the amino acid substitution rate (across lineages) in polyomavirus LTAg, VP1, and VP2

known from their sequences, which prevented a sound examination and comparison of their biological properties. It was, however, proposed to create two mammalian genera, respectively coined *Orthopolyomavirus* and *Wukipolyomavirus*, whose existence was essentially based on sequence divergence of the VP1-encoding gene [11]. The addition of new polyomaviruses revealed that these genera were unlikely to reflect evolutionary lineages [14] and alternative taxonomical arrangements were proposed, e.g., grouping all polyomaviruses into a single genus [20] or delineating additional genera [5]. The SG also re-examined this question, keeping in mind the important constraint that, for most novel polyomaviruses, only the host and nucleic acid sequences are known.

There is little evidence for pronounced co-divergence of polyomaviruses with their hosts in family-scale phylogenies [20], but at very deep nodes, phylogenetic trees mostly support the separation of polyomaviruses infecting birds and mammals. Although the lack of observed co-divergence may reflect a mere sampling artifact (and be corrected in the future), at the moment, there is no real possibility to use hosts as a major factor (or virus trait) to delineate genera.

The genomic organization of polyomaviruses is very uniform. Although a number of accessory open reading frames have been described, only a single one (ALTO; [2]) can be regarded as a landmark characterizing a monophyletic group of polyomaviruses. It therefore seems that genomic organization also cannot generally be used as a primary criterion for genus-level delineation.

The unique option left is to use reconstructed evolutionary relationships for the delineation of genera. Although the SG acknowledges that full-genome analyses would in principle be the ideal tool [12], the recent realization that recombination events in some instances can significantly reshuffle long-diverged genomes indicated that caution is needed [14, 20]. The SG therefore recommended using one of the three major coding sequences (LTAg, VP1 or VP2) for the delineation of genera. To the best of the SǴs knowledge, there has not been a report thus far of significant recombination events within these three coding sequences.

The SG proposed that evolutionary relationships derived from analyses of the LTAg amino acid sequences be used for this purpose. Our estimate of amino acid sequence variation rates based on relaxed molecular clock models obtained using BEAST v1.8.2 was lower for LTAg than for VP1 and VP2 (Fig. 1), which facilitates phylogenetic analysis. In addition, more internal branches appeared to be relatively well supported with this same fragment, as notably revealed by overlaying posterior sets of trees generated with BEAST v1.8.2 with DensiTree v2.01 (Fig. 2).

Fig. 2 Superposition of sets of posterior trees. In all cases, 9000 posterior trees were overlaid using DensiTree v2.01. Fuzziness and branch intersections indicate branch length and topological uncertainty

Fig. 3 shows a chronogram derived from an alignment of conserved amino acid blocks (selected with Gblocks v0.1) reconstructed with BEAST v1.8.2 under the best model of amino acid substitution (LG + F + I + G; as determined with ProtTest v3.2), a relaxed clock (lognormal) and a birth-death model of speciation. Branch thickness is proportional to posterior probability support (thin branches are less supported). A similar topology was

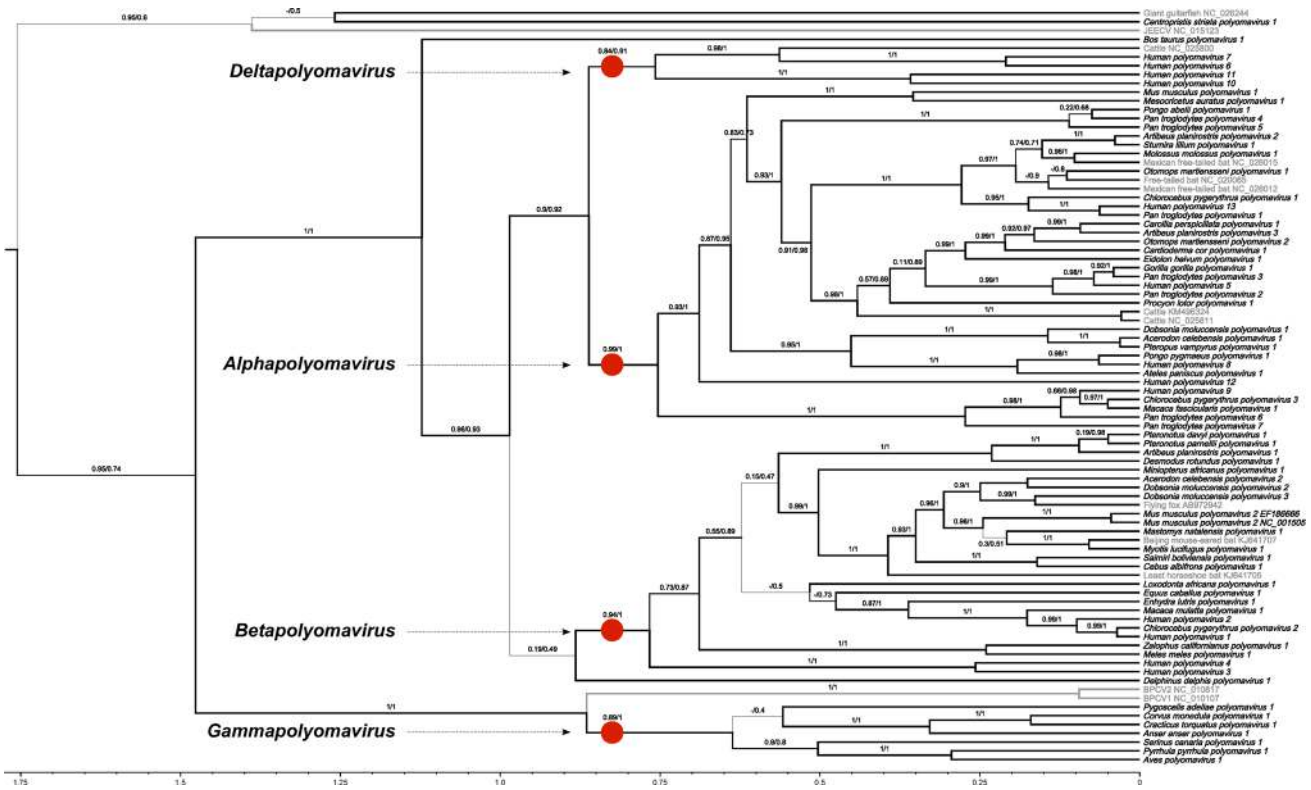

Fig. 3 LTAg-derived Bayesian chronogram of the family *Polyomaviridae*. The branches supporting the existence of the four genera whose creation is recommended by the SG are indicated by a red circle. Branch support is reported above the branches (SH-aLRT/posterior probability). Detailed methods are described in Supplementary File 1. Tips display the names of species (black), the vernacular names followed by accession numbers for viruses not allocated to a polyomavirus species (grey) or, in the case of viruses other than polyomaviruses comprising an LTAg sequence, abbreviations followed by accession numbers (grey). JEECV, Japanese eel endothelial cells-infecting virus; BCPV, bandicoot papillomatosis carcinomatosis virus type 1 and 2 (BPCV1 and 2). Note: as this tree was constructed to enable genus delineation, members of species were excluded that displayed an observed amino acid distance in LTAg of less than 5 % to a member of one of the species included in the tree

supported by an analysis with PhyML v3 using the BEST tree search algorithm. As far as the SG is aware, it comprises sequences representative of most lineages described to date. Members of species were excluded that displayed an observed amino acid distance in LTAg of less than 5 % to a member of one of the species included in the tree, as this tree was constructed to facilitate genus delineation.

Based on this, the SG recommended the creation of four genera. These include four relatively large radiations of polyomaviruses that together include 73 of the 76 species created by the SG. To name these genera, the SG decided to follow the example of other SGs that had to accommodate many species and to create numerous genera, e.g., *Papillomaviridae*. Genus names will therefore be composed of Greek letters followed by "*polyomavirus*", e.g., *Alphapolyomavirus*. Greek letters will be used consecutively, following the order of description of polyomavirus genera.

In brief, members of the genera *Alphapolyomavirus*, *Betapolyomavirus* and *Deltapolyomavirus* are known to infect only mammals; their most recent common ancestors (MRCAs) emerged within approximately the same time frame as the MRCA of members of the genus *Gammapolyomavirus*. This genus (formerly named *Avipoloyomavirus*; [11]) includes all seven polyomavirus species whose members are known to infect birds; its type species is *Aves polyomavirus 1* (Fig. 3; Table 1).

The type species of the genus *Alphapolyomavirus* is *Mus musculus polyomavirus 1* (member: murine polyomavirus, the first polyomavirus discovered). The genus accommodates 36 species whose members infect primates (humans, apes and monkeys), bats, rodents and other mammals (Fig. 3; Table 1). The type species of the genus *Betapolyomavirus* is *Macaca mulatta polyomavirus 1* (member: simian virus 40, the first discovered polyomavirus in this genus). Twenty-six species are included that infect primates (humans and monkeys), bats, rodents and other mammals (Fig. 3; Table 1). The type species of the genus *Deltapolyomavirus* is *Human polyomavirus 6* (member: human polyomavirus 6, the first discovered polyomavirus

in this genus). The genus is currently populated by only four human polyomavirus species (Fig. 3; Table 1).

The three polyomavirus species not assigned to any genus are *Bos taurus polyomavirus 1*, *Centropristis striata polyomavirus 1* and *Delphinus delphis polyomavirus 1*. The phylogenetic placement of the polyomaviruses populating the species *Bos taurus polyomavirus 1* and *Delphinus delphis polyomavirus 1* came with some ambiguity, which prevented their assignment to the new genera (analyses restricted to mammalian polyomaviruses weakly support their sistership, in disagreement with Fig. 3; data not shown). The virus populating the species *Centropristis striata polyomavirus 1* was, at the cutoff date, the only published PyV infecting fish. Other fish polyomavirus genomes were available in GenBank but not yet peer reviewed. The decision was made to wait for their validation before a possible incremental update of the taxonomy focused on non-tetrapod polyomaviruses.

Polyomaviruses discovered in the future: species definition and assignment to genera

The assignment of a future polyomavirus to a certain genus will rely on its unambiguous phylogenetic placement within the corresponding clade, as demonstrated by sound phylogenetic analyses of LTAg amino acid sequences. All datasets and methods used to generate the phylogenetic trees that served as the basis for the genus delineation are available as Supplementary Files 1-7. The SG suggests that authors who are willing to accompany future polyomavirus discoveries with taxonomical claims should check that their methods are mostly in line with the methods and criteria employed here.

Of note, a prerequisite for correct deduction of LTAg amino acid sequences is the proper identification of LTAg splice donor and acceptor sites. Ideally, this is done experimentally. However, as is the case for most of the currently known polyomaviruses, it can also rely on *in silico* analysis only. This is usually done by search for canonical splice donor and acceptor sites (http://www.umd.be/HSF3/HSF.html; [17]), followed by a selection of those that are well conserved between the virus in question and the most closely related known polyomaviruses. In addition, the observation might help that the introns of the members of genus *Gammapolyomavirus* are shortest (184-205 nt), followed by those of genus *Betapolyomavirus* (262-400 nt), genus *Deltapolyomavirus* (346-406 nt), and genus *Alphapolyomavirus* (353-565 nt). This is a rough guide predicting which length an LTAg intron should have, once preliminary BLAST and phylogenetic analysis have revealed the genus to which the novel virus may belong. Where help is needed in phylogenetic analysis of novel polyomaviruses, for publication purposes or for proposals of new species and genera to the ICTV, the SG offers to provide appropriate assistance.

Conclusions

A novel rationale for the taxonomy within the family *Polyomaviridae* was developed. It is mainly based on genomic sequences and host species, information that is available for most of the published polyomaviruses. The new taxonomical criteria have allowed the assignment of the vast majority of polyomaviruses to species and genera. As, after closing the polyomavirus list for preparation of the current taxonomical update (2015-March-30), additional mammalian and fish polyomavirus genomes became publicly available, new polyomavirus taxa, i.e., species and, possibly, genera, can already be seen on the horizon. They will serve as a useful touchstone for this taxonomy's robustness.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

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

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