Virology 445 (2013) 2–10

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Cross-roads in the classification of papillomaviruses

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ARTICLE INFO

Article history:
Received 1 March 2013
Returned to author for revisions
2 April 2013
Accepted 25 April 2013
Available online 16 May 2013

Keywords:
Classification
Human
Papillomaviruses
Metagenomics

ABSTRACT

Acceptance of an official classification for the family *Papillomaviridae* based purely on DNA sequence relatedness, was achieved as late as 2003. The rate of isolation and characterization of new papillomavirus types has greatly depended on and subjected to the development of new laboratory techniques. Introduction of every new technique led to a temporarily burst in the number of new isolates. In the following, the bumpy road towards achieving a classification system combined with the controversies of implementing and accepting new techniques will be summarized. An update of the classification of the 170 human papillomavirus (HPV) types presently known is presented. Arguments towards the implementation of metagenomic sequencing for this rapidly growing family will be presented.

Contents

Classical virology developed because the majority of human viruses could be isolated from lesions or body fluids by exposing the samples to in vitro propagation followed by purification and characterization of virus particles. Taxonomic criteria used by the International Committee on Taxonomy of Viruses (ICTV) for species demarcation were based on genome sequence relatedness, natural host range, cell and tissue tropism, pathogenicity and cytopathology, mode of transmission, as well as physiochemical and antigenic properties (van Regenmortel et al., 1997). Papillomaviruses have posed an exception to the classical rules applied for establishing a classification system right from the initial reports when two groups began isolating papillomaviruses from human tissues (Gissmann et al., 1977; Gissmann and zur Hausen, 1976; Favre et al., 1975; Orth et al., 1978) and when the diversity of human papillomaviruses became evident. An adequate tissue culture system to propagate these viruses on a large scale was not available. Similarly, these viruses could not be transmitted to laboratory animals. The availability and development of new laboratory techniques at that time through to today, have dictated not only the rate with which new papillomaviruses were identified, but have also influenced the level of success in isolating and characterizing full-length genomes of members of the family *Papillomaviridae*. In parallel, continuous discussions at International Papillomavirus meetings amongst researchers in the field on defining and accepting new papillomavirus types have stimulated reconsideration of definitions and conditions. The ICTV was approached for recognition of an official classification system for the papillomaviruses as early as the 1980s. It was however only in 2003 when the present classification based on sequence similarities (de Villiers et al., 2004) was finally accepted. This review will describe the milestones, as well as the many cross-roads, which had to be dealt with. The most recent cross-road brought along by the application of modern techniques (metagenomics) applied for identifying and isolating new viruses will be discussed.

The existence of multiple human papillomavirus types was initially recognized by digestion of double stranded DNA genomes using a very limited number of available restriction enzymes. DNA was purified from papillomavirus particles obtained from warts. Physical maps of the generated genome fragment lengths were compiled (Gissmann et al., 1977; Gissmann and zur Hausen, 1976; Favre et al., 1975; Orth et al., 1978). Other laboratories soon followed leading to each laboratory having its own nomenclature

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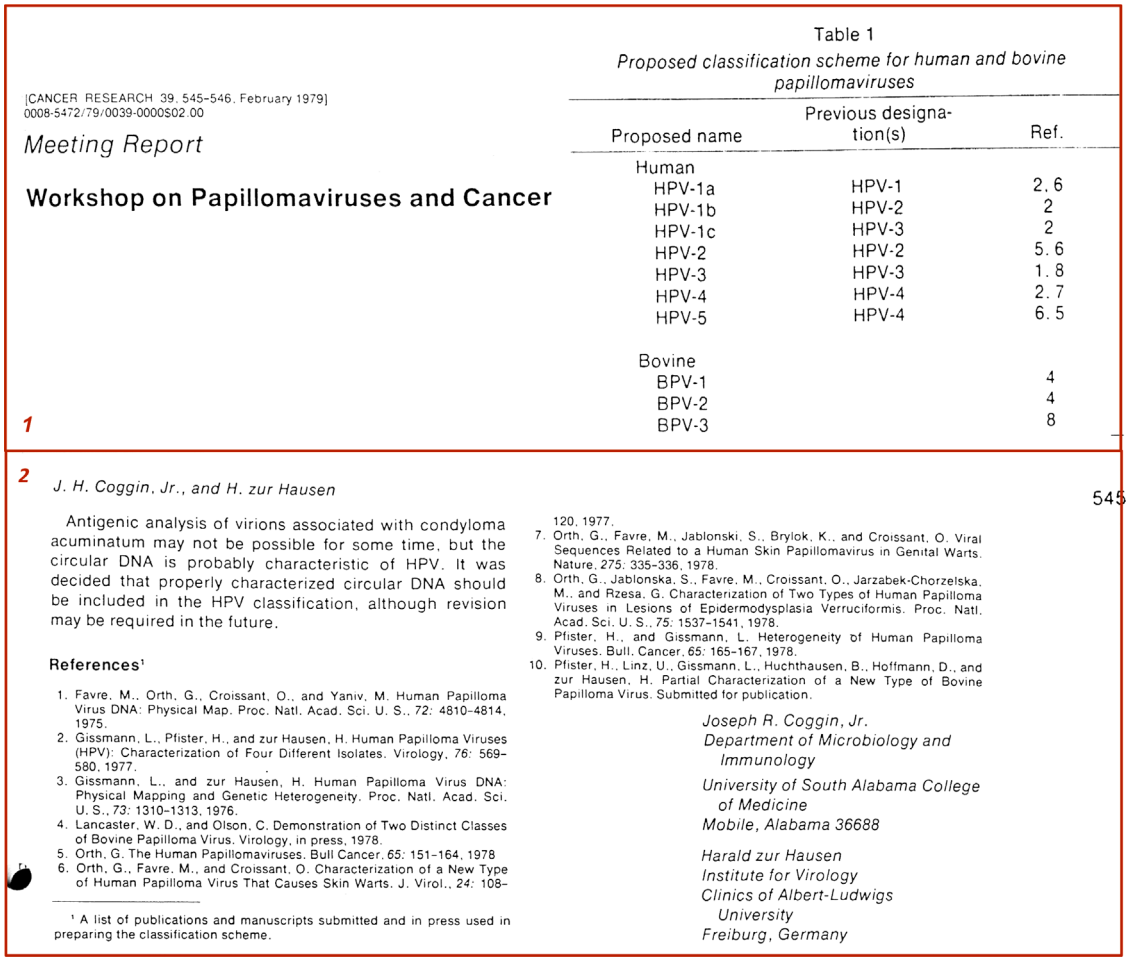
http://dx.doi.org/10.1016/j.virol.2013.04.023



[CANCER RESEARCH 39, 545–546, February 1979]
0008-5472/79/0039-0000$02.00

Meeting Report

Workshop on Papillomaviruses and Cancer

Table 1
Proposed classification scheme for human and bovine papillomaviruses

Proposed name	Previous designation(s)	Ref.
Human		
HPV-1a	HPV-1	2, 6
HPV-1b	HPV-2	2
HPV-1c	HPV-3	2
HPV-2	HPV-2	5, 6
HPV-3	HPV-3	1, 8
HPV-4	HPV-4	2, 7
HPV-5	HPV-4	6, 5
Bovine		
BPV-1		4
BPV-2		4
BPV-3		8

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Antigenic analysis of virions associated with condyloma acuminatum may not be possible for some time, but the circular DNA is probably characteristic of HPV. It was decided that properly characterized circular DNA should be included in the HPV classification, although revision may be required in the future.

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[1] A list of publications and manuscripts submitted and in press used in preparing the classification scheme.

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Fig. 1. Excerpt of a report on the first workshop on papillomaviruses held in Alabama in 1979.

for its own isolates. It was decided at one of the first workshops on papillomaviruses (Coggin and zur Hausen, 1979) to unify the nomenclature—human papillomavirus HPV followed by a number—providing priority to publication date, thereby regarding the first isolation as the prototype. Sequence variations reflected in restriction fragment patterns defined subtypes (Fig. 1). Furthermore, a new HPV type would be defined as a ~7.9-kilobase closed circular, double-stranded DNA molecule which showed less than 50% similarity to any known HPV type. This similarity was to be determined by using liquid hybridization under stringent conditions. The development of the cloning methodology resulted in the complete genome of HPV1 being cloned in 1980 (Danos et al., 1980; Heilmann et al., 1980), followed by applying the first DNA sequencing methods available to generate the HPV1 DNA sequence (Danos et al., 1982).

A genome organization for HPV was established by initial colinear DNA comparisons to, and subsequent DNA sequencing of the bovine papillomavirus type 1 (BPV1) (Chen et al., 1982). All HPV types contained at least six early genes (E1–E7) and two late genes (L1, L2) as well as an upstream regulatory region.

Reports of isolation of papillomaviruses HPV16 and HPV18 and their possible association with cervical cancer (Dürst et al., 1983; Boshart et al., 1984) stimulated papillomavirus research on an international level. Rapid increase in the isolation and characterization of new HPV types called for the decision made at the annual Papillomavirus Workshop held in Kuopio (1985), to establish Reference Centers where the numerous HPV types would be deposited to enable comparisons between new isolates and the already existing HPV types prior to allocating a subsequent HPV number. Reference Centers in France, the United States and Germany were initially planned, but only the Reference Center in Heidelberg, Germany came to bearing. All HPV isolates have since then been collected here and kept as reference stocks. Agreement amongst scientists in the field was that information (relatedness, origin) on submitted clones would be kept confidential until the scientist involved would publish the information. By 1989, 60 new HPV types, as well as a number of duplicates of known types, had been isolated and characterized (reviewed in de Villiers (1989)).

HPV types were commonly grouped as either *cutaneous* or *mucosal*, depending on the type of tissue of origin. Available technologies greatly influenced the spectrum of HPV types isolated. Labeled HPV DNA was used as probe for non-stringent hybridization against cellular DNA to identify putative new HPV types. Phage libraries of restricted cellular DNA were screened accordingly. Other factors determining spectrum, sensitivity and specificity of detection included viral copy numbers present in a lesion and the degree of relatedness between HPV types. A small fragment of the HPV14 prototype was never cloned, because it had been missed in the original cloning (Kremsdorf et al., 1984) and no other HPV14 isolate was ever submitted to the Reference Center. In addition, the increasing spectrum of HPV types and clinical lesions harboring papillomavirus DNA, stressed the huge deficit in understanding cell and tissue tropism, pathogenicity, cytopathology and antigenic properties of these virus infections. This delayed larger studies aimed at collecting epidemiological data. Sequencing of complete genomes of HPV types was very slow and tedious and results very often included several sequencing errors which were corrected by later re-sequencing of HPV prototypes. HPV prototype

sequences were generated and updated in a major effort in the early 90s (Delius and Hofmann, 1994). Interestingly, the sequences of HPV isolates harboring interrupted E1 ORFs were confirmed. Its biological meaning remains to be defined. It is clear that the prototypes in the Reference Center do not necessarily represent the most prevalent variant of a specific HPV type in a clinical context. To define the latter, large studies are required concentrating in isolating the respective HPV types and collecting the respective clinical and pathological data. This will probably never be achieved as present information for the vast majority of HPV types does not extend beyond the report of their isolation.

Availability of the polymerase chain reaction and self-assembly of viral capsid proteins in vitro (Salunke et al., 1986) initiated the next phase in papillomavirus research. The conserved L1 gene played the major role in designing primers for use in PCR reactions (Manos et al., 1989), as well as in synthesizing virus-like particles. Earlier in vitro data (reviewed in zur Hausen (1989, 2009)) could now be confirmed by larger epidemiological studies screening genital lesions revealing high relative risks for HPV 16 and HPV 18 infections in the pathogenesis of anogenital cancers (Munoz et al., 1992; Schiffman et al., 1993). PCR using L1 consensus primers led to the identification of a large number of putative new HPV types. Gradually reports appeared describing the use of PCR primers covering the E6 and E7 genes. This made it difficult to identify whether amplicons from different regions of the genome belonged to the same putative HPV type. The selection of primers, whether degenerate or consensus primers, definitely distorted the true pattern of prevalence and lesion association, mainly because of the varying sensitivity and specificity with which respective primer combinations would amplify specific papillomavirus types. It was virtually impossible to develop primers amplifying all known papillomaviruses with equal sensitivity and specificity. Grave reservations on accepting full-length genomes generated by PCR amplification were however expressed by both the community and ICTV members. It was feared that over-amplification could lead to inclusion of artifacts and the ICTV did not accept *artificially created viruses* (reviewed in de Villiers (2001)). Ongoing analyses of amplicons obtained from samples of healthy skin of both humans and animals revealed an enormous diversity of yet unknown papillomaviruses (Antonsson et al., 2000, 2003; Antonsson and Hansson, 2002), stressing the necessity to accept new techniques to generate full-length genomes.

At the same time intensive debating was ongoing among the papillomavirologist community whether data from partial PCR amplicons should be recognized as new HPV types or whether the existing rule of submission of the complete genome to the Reference Center should be upheld. The importance of availability of full-length genomes was reiterated, although it was decided to modify the definition of a new papillomavirus type to the DNA sequences of the L1, E6 and E7 ORFs each having less than 90% similarity to the closest related known papillomavirus type. The decision to include the E6 and E7 genes was motivated by their involvement in immortalization and was a reflection of the dominating interest in HPV types involved in mucosal lesions. It soon became evident that this definition could not hold and was again modified in 1995 at the Papillomavirus Workshop held in Quebec, to include the sequence of only L1 ORF being less than 90% similar to the closest known HPV type. The reason was the recognition of E7 ORF of the newly characterized HPV 77 having 97% DNA identity to E7 of HPV 29, whereas E6 and L1 ORFs shared less than 90% to the corresponding ORFs of HPV 29 (Delius et al., 1998). Table 1 lists HPV types (starting with HPV70) initially identified as partial PCR fragments.

Table 1
Update on names, accession numbers, references, names of partial fragments, as well as newly isolated HPVs.

HPV			Other name (partial PCR fragment or full-length isolate)
Type:	**Accession number**	**Reference**	
HPV 70	U21941	Forslund and Hansson (1996)	CP141-7
HPV 71	AB040456	Matsukura and Sugase (2001)	CP8061
HPV 74	AF436130	Terai and Burk (2002)	AE10
HPV 75	Y15173	Delius et al., 1998)	vs40-1
HPV 76	Y15174	Delius et al. (1998)	CR148-59
HPV 77	Y15175	Delius et al. (1998)	vs93-1
HPV 78	AB793779	Sata et al. (unpublished)	FAIMVS3, XS2
HPV 79	replaced by HPV 91 (AF070938)	Terai and Burk (2002)	JC9813-A
HPV 81	AJ620209	Matsukura and Sugase (2001)	CP8304
HPV 83	AF151983	Brown et al. (1999)	LVX82, MM7
HPV 84	AF293960	Terai and Burk (2001)	MM8, PAP155
HPV 85	AF131950	Chow and Leong (1999)	HLT7474
HPV 89	AF436128	Terai and Burk (2002)	CP6108, AE6, X06/X08
HPV 90	AY057438	Terai and Burk (2002)	AE11, Han831, Han353, JC9710
HPV 91	AF419318	Terai and Burk (2002)	AE13, JC9813
HPV 92	AF531420	Forslund et al (2003)	FAIMVS2
HPV 93	AY382778	Vasiljevic et al. (2007)	FAIMVS6
HPV 94	AJ620211	de Villiers and Gunst (2009)	DL40
HPV 95	AJ620210	Egawa et al. (2005)	
HPV 96	AY382779	Vasiljevic et al. (2007)	FA47
HPV 98	FM955837	de Villiers and Gunst (2009)	GA1-3
HPV 99	FM955838	de Villiers and Gunst (2009)	GA3-1
HPV 100	FM955839	de Villiers and Gunst ((2009)	DL267, vs73-1, FA53
HPV 104	FM955840	de Villiers and Gunst (2009)	DL253, FA75
HPV 105	FM955841	de Villiers and Gunst (2009)	DL294
HPV 107	EF422221	Vasiljevic et al. (2008)	FA85, DL285
HPV 109	EU541441	Ekström et al. (2010)	FA137
HPV 110	EU410348	Vasiljevic et al. (2008)	FA5
HPV 111	EU410349	Vasiljevic et al. (2008)	FA51
HPV 112	EU541442	Ekström et al. (2010)	FA164
HPV 113	FM955842	de Villiers and Gunst (2009)	DL250
HPV 114	GQ244463	Ekström et al. (2010)	SW1
HPV 115	FJ947080	Chouhy et al. (2010)	A3, GC02

Table 1 (*continued*)

HPV			Other name (partial PCR fragment or full-length isolate)
Type:	**Accession number**	**Reference**	
HPV 116	FJ804072	Li et al. (2009)	
HPV 117	GQ246950	Köhler et al. (2010)	
HPV 118	GQ246951	Köhler et al. (2011)	
HPV 119	GQ845441	Bottalico et al. (2012)	FA155
HPV 120	GQ845442	Bottalico et al. (2012)	SIBX-3a, FA16
HPV 121	GQ845443	Bottalico et al. (2012)	
HPV 122	GQ845444	Bottalico et al. (2012	vs92-1
HPV 123	GQ845445	Bottalico et al. (2012)	FA136
HPV 124	GQ845446	Bottalico et al. (2012)	FA23
HPV 125	FN547152	Kovanda et al. (2011b)	SIBX9
HPV 126	AB646346	Egawa et al. (2012)	
HPV 127	HM011570	Schowalter et al. (2010)	R3a
HPV 128	GU225708	Köhler et al. (2011)	
HPV 129	GU233853	Köhler et al. (2011)	
HPV 130	GU117630	Köhler et al. (2011)	FA147
HPV 131	GU117631	Köhler et al. (2011)	
HPV 132	GU117632	Köhler et al. (2011)	FA78
HPV 133	GU117633	Köhler et al. (2011)	FA44
HPV 134	GU117634	Köhler et al. (2011)	FA1.2
HPV 135	HM999987	Bottalico et al. (2012)	FA28, NJ3500
HPV 136	HM999988	Bottalico et al. (2012	FA8, CL3865
HPV 137	HM999989	Bottalico et al. (2012)	NJ2801H
HPV 138	HM999990	Chen et al. (1982)	FA20, GH13
HPV 139	HM999991	Chen et al. (1982)	GH1646
HPV 140	HM999992	Bottalico et al. (2012)	NJ2801C1
HPV 141	HM999993	Bottalico et al. (2012)	FA73, NJ2801Cn
HPV 142	HM999994	Chen et al. (1982)	FA67, GH1302
HPV 143	HM999995	Bottalico et al. (2012)	FAIVSM10, CL3220
HPV 144	HM999996	Bottalico et al. (2012)	FA83, CL3740
HPV 145	HM999997	Bottalico et al. (2012)	IA16, CL4112
HPV 146	HM999998	Chen et al. (1982)	FA35, GH1677
HPV 147	HM999999	Chen et al. (1982)	FA13, GH659
HPV 148	GU129016	Köhler et al. (2011)	
HPV 149	GU117629	Köhler et al. (2011)	
HPV 150	FN677755	Kovanda et al. ((2011a)	SIBX1, FA22
HPV 151	FN677756	Kovanda et al. (2011a)	SIBX2
HPV 152		Chen and Burk (personal communication)	FA18
HPV 153	JN171845	Sturegard et al. (2013)	
HPV 154		Ore and Forslund, (personal communication)	FAD13
HPV 155	JF906559	Ekström et al. (2011)	SE42
HPV 156	JX429973	Chouhy et al. (2013)	GC01
HPV 157		Chouhy et al. (2010)	GC08
HPV 158		Chouhy et al. (2010)	GC23
HPV 159	HE963025	Kocjan et al. (in preparation)	SIBX8
HPV 160		Kiyono (personal communication)	
HPV 161	JX413109	Li et al. (2012)	KC1
HPV 162	JX413108	Li et al. (2012)	KC2
HPV 163	JX413107	Li et al. (2012)	KC3
HPV 164	JX413106	Li et al. (2012)	KC4
HPV 165	JX444072	Li et al. (2012)	KC7
HPV 166	JX413104	Li et al. (2012)	KC9
HPV 167		Li et al. (2012)	KC10
HPV 168		Li et al. (2012)	KC15, SE64
HPV 169	JX413105	Li et al. (2012)	KC6, vs202-8
HPV 170	JX413110	Li et al. (2012)	KC8

The need for a taxonomic classification for papillomaviruses remained evident. Biological and pathological, as well as some functional information for the more prevalent HPV types was continuously extended, whereas rare HPV types did not receive any subsequent attention (reviewed in de Villiers (1989, 1994, 1997, 1998, 2001) and de Villiers et al. (2004)). The only consistent information available for all papillomavirus types was sequence data. This was utilized to define relationships between papillomavirus types. The conserved region in L1 ORF (Chan et al., 1992) and the E6 ORF (van Ranst et al., 1992) was used to obtain phylogenetic information. Subsequent studies demonstrated that the use of the conserved region of the L1 gene was sufficient, especially in view of no evidence of recombination between papillomavirus types (Bernard et al., 1994). A stretch of highly conserved amino acid residues was contained in a 291 bp segment of the L1 ORF (spanned by the MY09 and MY11 primers). This was used to construct phylogenetic trees resulting in taxonomic supergroups A to E. Different taxonomic levels were recognized splitting each supergroup into groups. Supergroup A (genital HPVs) contained 11 groups and supergroup B (EV HPV types) 2 groups. HPV 1, HPV63 and HPV41 sorted in supergroup E together with a few animal papillomaviruses (Chan et al., 1995). The commonly used grouping of *mucosal* versus *cutaneous* HPV types was not upheld in the phylogeny, confirming earlier reported analyses of clinical lesions (reviewed in de Villiers (1994) and de Villiers et al. (2004)).

Although these phylogenetic groupings or clusters presented in our application were not accepted by the ICTV in 2001, this analysis (Chan et al., 1995) formed the base for the present classification of

papillomaviruses. Fortunately Claude Fauquet (then member of the ICTV) initiated steps to include sequence information in the taxonomic guidelines specified by the ICTV (Fauquet et al., 2003). Being actively involved in the family *Geminiviridae*, the family *Papillomaviridae* interested him and he approached us with a phylogenetic analysis of all complete genomes of papillomaviruses available at the time in the databanks. This analysis proved to be almost identical to phylogeny generated from the complete L1 sequence, which helped to convince him that we could continue using L1 as criterium for our classification. His pairwise nucleotide sequence comparisons (de Villiers et al., 2004) underlined our previous definition of a new papillomavirus type. An important aspect from our side was to maintain continuity of established criteria regularly used in papillomavirus research. Rules of the International Code of Virus Classification and Nomenclature include "aiming for stability" (rule 2.1(I)) and "existing names should be retained wherever possible" (rule 3.9) (http://www.ictvonline.org). The groupings mentioned above (Chan et al., 1995) had very rapidly been accepted by scientists leading to e.g. "HPV16 belonging to A9" being common use in publications. Here rule 3.25 stated that "newly designated combinations of letters and numerals alone are not legal". We therefore proposed to use the Greek alphabet which had already been accepted by the ICTV in a different context for other virus families. These considerations (sequence of full-length L1 ORF, Greek alphabet) were included in our proposal submitted in 2003. Claude Fauquet was crucial in preparing the proposal (including 96 HPV types and 22 animal viruses), as well as influencing the final decision by the ICTV in 2003 in which the classification of the family *Papillomaviridae* was accepted as it stands today (de Villiers et al., 2004). A counter-argument of the limited number of letters in the Greek alphabet was later circumvented (Bernard et al., 2010). The exact criteria for classification, as well as proposed future amendments, are extensively reviewed in de Villiers et al. (2004) and Bernard et al. (2010).

Isolating and characterizing new HPV types had been ongoing. Presently 170 HPV types have been accepted. Long-PCR amplification with specific primers designed on initially isolated partial PCR fragments was tedious. The introduction of multiple primed rolling

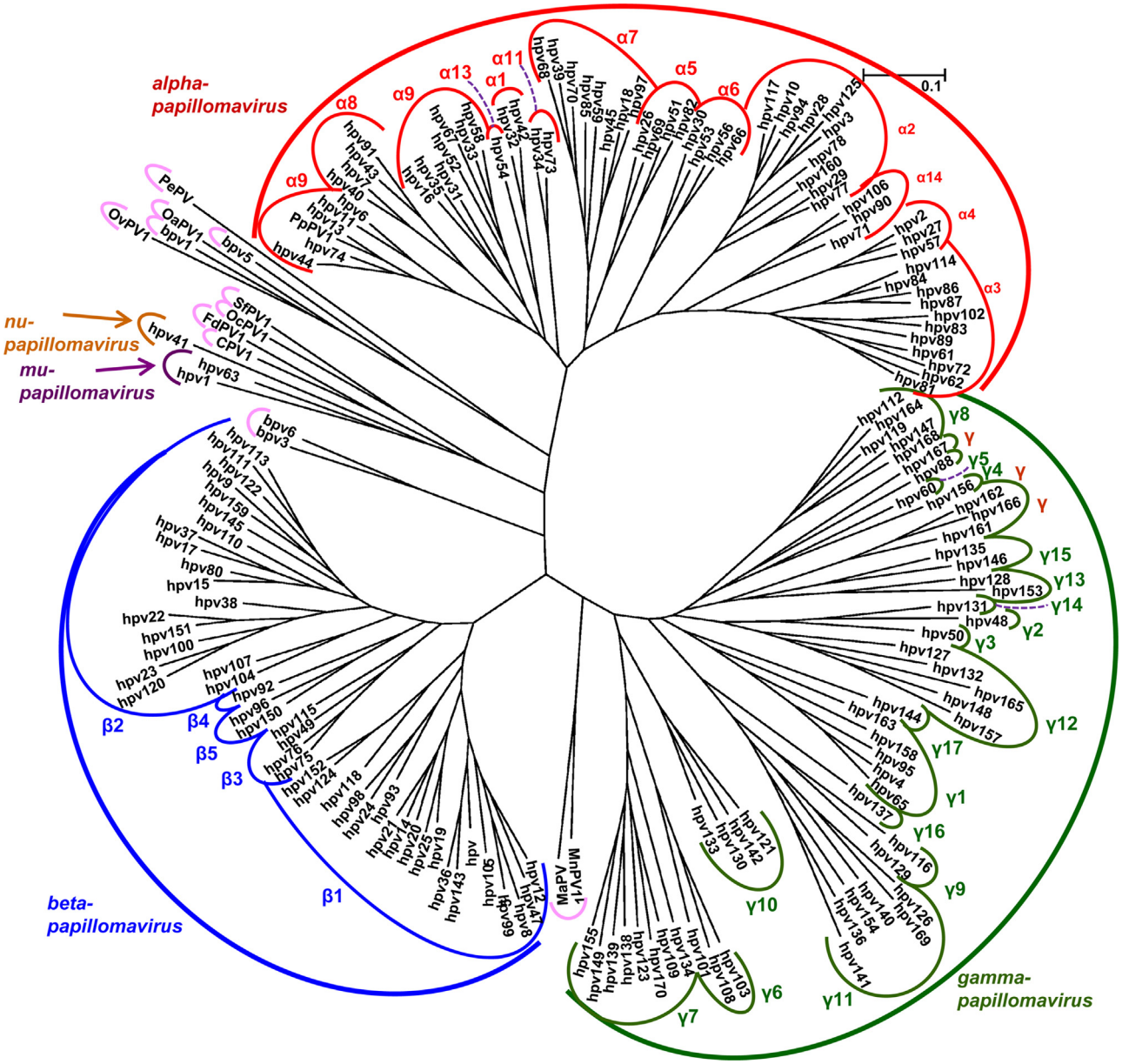

Fig. 2. Phylogenetic analysis based on the L1 ORF sequences of 170 HPV types, as well as single animal papillomaviruses, using the maximum likelihood method. The tree was constructed using the MEGA5.1 program.

circle amplification (RCA) for papillomaviruses circumvented obstacles (Rector et al., 2004) and resulted in a sudden burst in numbers of newly isolated and characterized HPV types. Initial RCA amplification with subsequent PCR amplification using specific primers was helpful in cases of samples with extremely low HPV copy numbers (de Villiers and Gunst, 2009). Reports on a relative high prevalence of unknown HPV types on the surface of the skin (Antonsson et al., 2000, 2003; Antonsson and Hansson, 2002) and reports on smaller studies investigating the role of papillomaviruses in the pathogenesis of non-melanoma skin cancer, led to a general shift in interest in papillomavirus research. This and the use of RCA led to an increase in HPV types in the genus Betapapillomavirus from 25 (2004) to 45 (2013) and in genus Gammapapillomavirus from 7 (2004) to 54 in 2013. In contrast, HPV types in genus Alphapapillomavirus increased from 59 to 66 during this time period. An updated phylogenetic analysis including all 170 HPV types, as well as single animal papillomaviruses, is presented in Fig. 2. HPV161, HPV162 and HPV166 together represent a new species and HPV156 and HPV 167 each a separate new species. No additional species for genera Alpha- or Betapapillomavirus were recognized.

Gathering of clinical and biological information for many of the HPV types in genus Alphapapillomavirus was supported and stimulated by molecular biological data—and later epidemiological—demonstrating the importance of individual HPV types in the pathogenesis of genital cancer. Presently molecular data on HPV types associated with non-melanoma skin cancer are scarce and epidemiological data are not convincing for a role at all. Molecular studies have highlighted the diversity of the HPV types in the genera Beta- and Gamma-papillomavirus—single HPV types each follow individual pathways or interactions in the cell (de Villiers et al., 1999; Fei and De Villiers, 2012; Massimi et al., 2008; Underbrink et al., 2008). The tremendous and rapid expansion of isolated HPV types of the Beta- and Gammapillomavirus pinpoints the urgent need for molecular and biological data of these HPV types if continuous future isolation is to be meaningful.

The genome organization of HPV101, HPV 103 and HPV 108 (γ6) differs from all other HPV types in that they do not have an E6 gene (similar to a number of animal papillomaviruses). HPV108 E7 induces dysplastic modifications in organotypic raft cultures (Nobre et al., 2009). It is not difficult to envision the existence of other, similarly different, but yet unidentified HPV types. In addition, presently unknown HPV types may exist which have even other genome organizations. HPV1 and HPV63 (genus mu-papillomavirus), as well as HPV41 (genus nu-papillomavirus), remain the only representatives of these respective genera harboring HPV types. It is extremely unlikely, based on our present knowledge of HPV types, that these genera contain only 1 or 2 papillomavirus types. Identification of additional very distantly related papillomaviruses calls for more diverse methods of identification and isolation.

A vast spectrum and number of viruses are being identified by high throughput sequencing of biological samples (metagenomic sequencing and analysis). It allows for unbiased detection of known and unknown viruses (and other organisms). Heavy debate on different levels is again continuing whether viruses identified by this method should be accepted or not. The genomes of HPV116 (γ9) (Li et al., 2009) and HPV155 (γ7) (Ekström et al., 2011; Bzhalava et al., submitted for publication) were originally identified by metagenomics. These HPV types were subsequently re-amplified from the respective samples, cloned and submitted to the Reference Center. The genomes of 13 putative HPVs have been identified by metagenomic sequencing during the past year (Mokili et al., 2013; Foulongne et al., 2012; Johansson et al., 2013; Phan et al., 2013). These putative HPV genomes are listed in Table 2 and the affix *m* indicates the mode (metagenomics) of their identification. In one case mCG1 was identified at the level of 98% identity to the prototype HPV146 (Foulongne et al., 2012), and in another the full-length genome mFA69 (Johansson et al., 2013) of a partial PCR fragment FA69 (Antonsson et al., 2003) was generated. mKN1 is the full-length genome of partial PCR fragment vs19-6 (Shamanin et al., 1996), mCG1 that of FA35 (Antonsson et al., 2003) and mCG2 of FA106 (Olsson et al., unpublished). All these papillomaviruses form part of genus Gammapapillomavirus (Fig. 3) and 4 of them represent 3 new species. HPV156 represents a new species together with mFi864 (Phan et al., 2013), whereas mFD1 and mKN1 (Foulongne et al., 2012) together represent another new species. HPV60 has to date been the only representative of species γ4, but is now joined by mFS1 (Foulongne et al., 2012) and mSE87 (Johansson et al., 2013). It is evident many of these newly identified HPV types are not clustering with known HPV types, therefore indicating that identifying new HPV types by using metagenomic sequencing will most probably open to date unreachable corridors for papillomavirus research. Ways to obtain these putative HPV types in the Reference Center in order to make it available for the community needs to be discussed.

Accepting putative new HPV types only after submission of the cloned full-length genome to the Reference Center has its merits and has proved to be very successful over 28 years. The main advantage is the availability of such a clone to the community and the necessity to verify a submitted clone has in some instances been evident. The situation for animal papillomaviruses is very different. Lack of collaboration during the same time period has made access to newly isolated/cloned animal papillomaviruses almost impossible. Attempts to establish a Reference Center for animal papillomavirus has thus far been without success, leading to the present acceptance of new animal papillomaviruses based

Table 2
Human papillomaviruses identified by metagenomic sequencing and available in the databanks. Accession numbers, references and closest related identified or known HPV type, as well as partial fragment originally described.

Name (m=metagenomics)	Accession number	Reference	Closest related HPV type	Other name/PCR fragment
mFA69	KC108722	Johansson et al. (2013)	HPV121 82%	FA69
mSE87	KC108721	Johansson et al. (2013)	mFS1 79% HPV60 68%	
mfi864	KC311731	Phan et al. (2013)	HPV156 69%	
mSD2	SRA051429	Mokili et al. (2013)	HPV135 74%	
mKN1	JF966371	Foulongne et al. (2012)	mFD1 74% HPV65 69%	vs19-6
mKN2	JF966372	Foulongne et al. (2012)	HPV161 65%	
mFS1	JF966373	Foulongne et al. (2012)	mSE87 79% HPV60 69%	
mKN3	JF966374	Foulongne et al. (2012)	HPV141 70%	
mFD1	JF966375	Foulongne et al. (2012)	mKN1 74% HPV142 68%	
mFD2	JF966376	Foulongne et al. (2012)	HPV129 71%	
mCG1	JF966377	Foulongne et al. (2012)	***HPV146 98%***	FA35
mCG2	JF966378	Foulongne et al. (2012)	HPV148 78%	FA106
mCG3	JF966379	Foulongne et al. (2012)	mCG2 72% HPV148 71%	

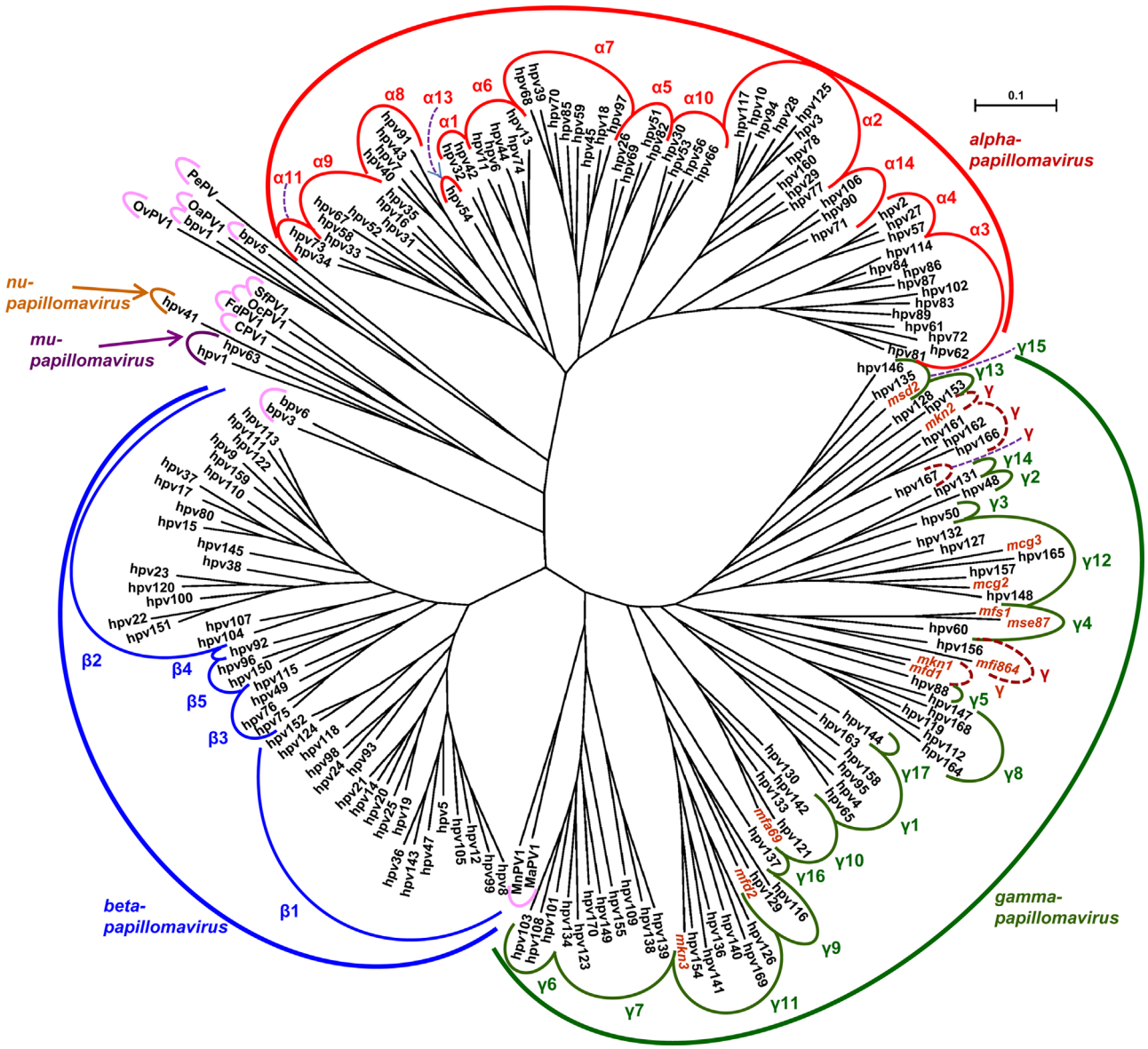

Fig. 3. Phylogenetic tree based on the L1 ORF sequences of 170 HPV types, single animal papillomaviruses and newly identified human papillomaviruses using metagenomic sequencing. The maximum likelihood method was used for the analysis and the tree was constructed using the MEGA5.1 program.

solely on sequence data. The difference in conditions for acceptance by the community between human versus animal papillomaviruses is not acceptable and should be reconsidered. If it is not modified, the reluctance to accept and include human papillomavirus types generated by metagenomics is greatly unjustified. A (long awaited) statement on the ICTV website: *"The advent of nucleotide sequence determination has revolutionized biology and largely rationalized taxonomy, including that of viruses"* (http://www.ictvonline.org/virusTaxonomy) demonstrates that change can be accepted and should be helpful in overcoming yet another hurdle in the history of the family *Papillomaviridae* on the road to updating the classification.

Acknowledgments

The author wishes to thank the following colleagues for permission to include their unpublished data: Dr. T. Matsukura, Dr. D. Bzhalava, Drs. D. Couchy and A. Giri, Dr. B Kocjan, Dr. T. Kiyono, Dr. J. Li, Dr. R.D. Burk and Dr. O. Forslund.

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