

This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections).

For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

# MODULE 1: TITLE, AUTHORS, etc

Code assigned:	2016.005	a-gM		(to be co	mpleted by	ICTV
Short title: One new genus ( <i>H</i> the family <i>Rhabdoviridae</i> . (e.g. 6 new species in the genus <b>Modules attached</b> (modules 1 and 10 are required)	•	ding 12 r  1 ⊠ 6 □	ew specie 2 ⊠ 7 ⊠	3 🖂 8 🖂	eassigned s	5 ☐ 10 ⊠
Author(s):						
Peter J. Walker Nikos Vasilakis Kim R. Blasdell Robert B. Tesh Charles H. Calisher Ralf G. Dietzgen Hideki Kondo Gael Kurath Ben Longdon David M. Stone Noel Tordo Anna E. Whitfield						
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List the ICTV study group(s	· 	n this pro	oposal:			
A list of study groups and contact <a href="http://www.ictvonline.org/subcom">http://www.ictvonline.org/subcom</a> in doubt, contact the appropriate chair (fungal, invertebrate, plant, vertebrate viruses)	mittees.asp . If subcommittee	ICTV I	Rhabdovir	ridae SG		
ICTV Study Group commen	ts (if any) and	response	of the pr	oposer:		
10 members have advised supp	port for the prop	osal; 2 m	nembers h	ave not re	sponded.	
Date first submitted to ICTV:	ent to above):		201	16.005	M	

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ICTV-EC comments and response of the proposer:

### **MODULE 2: NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	201	6.005aM	(assigned by IC	TV officers)					
To crea	ate 12 r	new species within	:						
Subf F	Genus: family: amily: Order:	Hapavirus (new)  Rhabdoviridae  Mononegavirales		Fill in all that apply.  If the higher taxon created (in a later r "(new)" after its pro  If no genus is spec "unassigned" in the					
Name (	of new	species:	Representative iso species please)	Representative isolate: (only 1 per species please)					
La Joya	a hapav	rirus	La Joya virus (LJV	La Joya virus (LJV; J-134)					
Parry (	Creek h	apavirus	Parry Creek virus	Parry Creek virus (PCV; OR189)					
Ord Ri	ver hap	avuirus	Ord River virus (C	ORV; OR1023)	KM204988				
Joinjak	aka haj	pavirus	Joinjakaka virus (J	OIV; AusMK7937)	KM205016				
Marco	hapavii	rus	Marco virus (MCC	OV; BeAn40290)	KM205005				
Gray L	odge ho	apavirus	Gray Lodge virus	(GLOV; BFN3187)	KM205022				
Landjid	a hapav	rirus	Landjia virus (LJA	V; DakAnB769d)	KM205010				
Manito	ba hape	avirus	Manitoba virus (M	IANV; Mn936-77)	KM205008				
Mosque			Mosqueiro virus (I	Mosqueiro virus (MQOV; BeAr185559)					
Hart Po				Hart Park virus (HPV; AR7C)					
Mossur	il hapa	virus	Mossuril virus (M	Mossuril virus (MOSV; SAAr1995)					
Kamese	e hapav	rirus	Kamese virus (KA	Kamese virus (KAMV; MP6186) KM204989					

#### Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - o If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - o If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

# Species demarcation criteria will be defined as follows (see also module 3):

Viruses assigned to different species within the genus *Hapavirus* display several of the following characteristics: A) minimum amino acid sequence divergence of 5% in N proteins; B) minimum sequence divergence of 10% in the L proteins; C) minimum amino acid sequence divergence of 15% in G proteins; D) significant differences in genome organization as evidenced by numbers and locations of ORFs; E) can be distinguished in virus neutralisation tests; and F) occupy different ecological niches as evidenced by differences in hosts and or arthropod vectors.

The viruses assigned to the new genus *Hapavirus* have complex genomes (**Figure 1A**) and form a monophyletic group based on well-supported ML trees generated from complete L

protein sequences (**Figure 2**). Known characteristics of the viruses of taxonomic significance are summarized here. The basis of assignment of the viruses as 15 distinct species in this genus is described under the genus proposal (**module 3**) and the move proposal (**module 7**).

### La Joya hapavirus

La Jolla virus (LJV) was isolated from mosquitoes (*Culex dunni*) collected in 1958 in Panama<sup>1</sup>. Weak cross-reactions have been detected in complement fixation (CF) tests with several vesiculoviruses. The complete LJV genome (15,721 nt) has been sequenced (Figure 1A)<sup>2</sup>. The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L) and seven other long ORFs in independent transcriptional units, each flanked by transcription initiation and polyadenylation sequences<sup>2</sup>. ORFs U1, U2 and U3 are located between the P and M genes; they share significant amino acid sequence homology with each other and with other hapavirus PMIPs (P-M intergenic region proteins)<sup>2</sup>. ORFs U4 and U5 lie between the M and G genes; they appear to encode unique proteins<sup>2</sup>. ORFs U6 and U7 lie between the G and L genes; U6 encodes a protein that has structural characteristics of a class 1a viroporin; U7 appears to encode a unique protein and is followed by a very long (749 nt) 3' non-coding region<sup>2</sup>. There are also short alternative ORFs in different reading frames in the M, G and U7 genes: ORF Gx (15.2 kDa protein) has an initiation codon in very favourable Kozak context, is located proximal to the start of the G ORF and so it appears likely to be expressed. ORF Mx (7.9 kDa protein) and ORF U7x (7.9 kDa protein) are located distally in the transcriptional units, have initiation codons in poor Kozak context and so appear unlikely to be expressed. Phylogenetic analysis of L protein sequences (Figure 2) and amino acid sequence identity in the N, L and G proteins (Figure 3) indicate LJV is most closely related to WONV, ORV and PCV<sup>2</sup>.

## Wongabel hapavirus

We propose the reassignment of the unassigned rhabdovirus species *Wongabel virus* as the species *Wongabel hapavirus* in the new genus *Hapavirus*. The rationale for the reassignment is provided in **Module 7**.

Wongabel virus (WONV) was isolated from biting midges (Culicoides austropalpalis) collected in 1979 in Australia<sup>3</sup>. Low levels of neutralizing antibodies have been detected in sea birds, cattle and wallabies<sup>3, 4</sup>. The complete WONV genome (13,196 nt) has been sequenced<sup>3</sup>. The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L), and five other long ORFs (Figure 1A)<sup>2, 3</sup>. ORFs U1, U2 and U3 are located in independent transcriptional units between the P and M genes, each flanked by transcription initiation and polyadenylation sequences; they demonstrate significant amino acid sequence homology with each other and with other hapavirus PMIPs<sup>2</sup>. WONV U3 has been shown to bind snf5 in the SWI/SNF chromatin remodelling complex and appears to inhibit host cell expression of interferon-stimulated genes<sup>5</sup>. ORF Nx (also called U4) overlaps the 3' end of the N gene; it encodes a polypeptide that displays high identity (63.3-65.3%) with cognate Nx sequences in PCV and ORV; in each virus there is a predicted 'slippery' sequence in the overlap region that may facilitate translation of Nx as an extension of the N ORF<sup>2</sup>. ORF Gy (also called U5) overlaps the 3' end of the G gene; it is predicted to encode a class 1a viroporin. A small ORF (Gx) also in an alternative reading frame in the G gene; however, it is not present in ORV or PCV, the initiation codon is in unfavourable Kozak context, and so the encoded polypeptide (7.4 kDa) may not be expressed in vivo. Phylogenetic analysis of L protein sequences (Figure 2) and amino acid sequence identity in the N, L and G proteins (**Figure 3**) indicate WONV is closely related to PCV, WONV, and more distantly to LJV<sup>2</sup>.

## Parry Creek hapavirus

Parry Creek virus (PCV) was isolated from mosquitoes (*Culex annulirostris*) collected in 1973 in Australia <sup>6,7</sup>. Complete coding regions (13,205 nt) of the PCV genome have been sequenced with only the 3´ and 5´ ends incomplete (**Figure 1A**)². The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L), and five other long ORFs². The genome organisation is similar to that of WONV; it lacks only the small alternative ORF (Gx) in the G gene. Phylogenetic analysis of L protein sequences (**Figure 2**) and amino acid sequence identity in the N, L and G proteins (**Figure 3**) indicate PCV is most closely related to ORV and WONV, and more distantly to LJV².

#### Ord River hapavirus

Ord River virus (ORV) was isolated from mosquitoes (*Culex annulirostris*) collected in 1976 in Australia. Complete coding regions (13,198 nt) of the ORV genome have been sequenced with only the 3´ and 5´ ends incomplete (**Figure 1A**)². The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L), and five other long ORFs². The genome organisation is identical to that of PCV. Phylogenetic analysis of L protein sequences (**Figure 2**) and amino acid sequence identity in the N, L and G proteins (**Figure 3**) indicate ORV is closely related to WONV and PCV, and more distantly to LJV².

No virus neutralisation test data are available on WONV, PCV and ORV. However, amino acid sequence identities in the N, L and G proteins of these viruses are significantly lower than between HPV and FLAV (all isolates) which cross react only weakly in neutralisation tests and are proposed to be assigned to different species (see below and **Figures 3 and 4**). PCV and ORV appear to have similar ecology (each isolated from *Culex annulirostris* mosquitoes at Kunnanurra in Western Australia) but differ from WONV (isolated from *Culicoides austropalpalis* midges in Queensland). On this basis of amino acid sequence identities, we propose WONV, ORV and PCV should also be assigned to different species.

#### Joinjakaka hapavirus

Joinjakaka virus (JOIV) was isolated from a mixed pool of culicine mosquitoes collected in 1966 in Papua New Guinea<sup>1</sup>. Neutralizing antibodies have been detected in cattle in Australia<sup>1</sup>. The complete JOIV genome (13,155 nt) has been sequenced (**Figure 1A**)<sup>2</sup>. The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L), and four other long ORFs. ORF U1 occurs in an independent transcriptional unit between the P and M genes; it encodes a protein that shares no identifiable homology with other known proteins, including hapavirus PMIPs<sup>2</sup>. ORF Gx overlaps the 3´ end of the G gene and is predicted to encode a class 1a viroporin<sup>2</sup>. ORFs U2 and U3 lie in consecutive independent transcriptional units between the G and L genes; they encode proteins that share identifiable amino acid sequence homology but are unrelated to any other known protein; they appear to have arisen by gene duplication<sup>2</sup>. Phylogenetic analysis of L protein sequences (**Figure 2**) and amino acid sequence identity in the G protein (**Figure 3**) indicate LJV is most closely related to NGAV<sup>2</sup>.

# Ngaingan hapavirus

We propose the reassignment of the unassigned species *Ngaingan virus* as the species *Ngaingan hapavirus* in the new genus *Hapavirus*. The rationale for the reassignment is provided in **Module 7**.

Ngaingan virus (NGAV) was isolated from a pool of biting midges collected at Kowanyama in northern Queensland, Australia, in 1970<sup>8</sup>. The midge pool was thought to consist of only

Culicoides brevitarsis but later studies suggested that C. actoni may also have been present<sup>9</sup>. Neutralizing antibodies have been detected in marsupials, cattle and buffaloes<sup>8, 10</sup>. It has been shown to cross-react in IFA tests with tibroviruses<sup>11</sup>. The complete NGAV genome (15,764 nt) has been sequenced<sup>10</sup>. The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L), and eight other long ORFs (Figure 1A). Three of these additional ORF located between the P and M genes; ORF U1 and overlapping ORF U1x (also called U2) lie in the first transcriptional unit and U2 (also called U3) lies in the second transcriptional unit. There is no identifiable sequence identity between U1, U1x and U2 or between these proteins and other hapavirus PMIPs. ORF U3 (also called U4) lies in a transcriptional unit between the M and G genes and encodes a unique protein of unknown function. Four long ORFs lie between the G and L genes: ORF G<sub>NS</sub> lies in the first transcriptional unit and encodes a class I transmembrane protein that is related in sequence to the NGAV G protein and the G proteins of other rhabdoviruses; ORF U4 (also called U5) and ORF U4x (also called U6) occur consecutively in a single transcriptional unit and encode, respectively, a unique protein of unknown function and a viroporin-like protein; ORF U5 (also called U7) lies in an independent transcriptional unit and encodes a unique protein of unknown function. There are also alternative ORFs in the P gene (Px) and M gene (Mx) encoding small proteins (7.7 kDa and 10.9 kDa, respectively); the initiation codons are each in moderate Kozak context for translation and are located proximal to the start of the transcriptional units and so they may be expressed. Phylogenetic analysis of L protein sequences indicates that NGAV sits centrally in the hapavirus clade (Figure 2) and amino acid sequence identities indicate that the NGAV N protein and G protein (**Figure 3**) suggest it is most closely related to JOIV<sup>2</sup>.

# Marco hapavirus

Marco virus (MCOV) was isolated from lizards (Ameiva ameiva ameiva) collected in 1962 in Brazil<sup>12</sup>. Complete coding regions (13,294 nt) of the MCOV genome have been sequenced with only the 3' and 5' ends incomplete (**Figure 1A**)<sup>2</sup>. The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L), and two other long ORFs each located in independent transcriptional units between the G and L genes<sup>2</sup>. ORF U1 encodes a predicted class 1 transmembrane glycoprotein with a Cterminal signal peptide, N-terminal transmembrane domain and two N-linked glycosylation sites; unlike NGAV and ephemerovirus G<sub>NS</sub> glycoproteins, the MCOV U1 glycoprotein shares no identifiable homology with rhabdovirus G proteins. ORF U2 appears to encode a unique protein. There are also 2 long alternative ORFs (Nx and Ny) in different reading frames in the MCOV N gene: ORF Nx (7.6 kDa protein) is in unfavourable Kozak context and located centrally in the transcriptional unit, and so is unlikely to be expressed; ORF Ny (7.7 kDa protein) overlaps the end of the N ORF and has a favourable Kozak context, and so may be expressed. Phylogenetic analysis of L protein sequences indicates that MCOV sits centrally in the hapavirus clade (Figure 2) but amino acid sequence identities indicate that the MCOV N protein and G protein (**Figure 3**) are marginally the most distant amongst hapaviruses<sup>2</sup>.

#### Gray Lodge hapavirus

Gray Lodge virus (GLOV) was isolated from mosquitoes (*Culex tarsalis*) collected in 1971 in California, USA<sup>1</sup>. It was shown to cross-react in CF tests with Hart Park virus<sup>1</sup>. The complete GLOV genome (12,403 nt) has been sequenced (**Figure 1A**)<sup>2</sup>. The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L), and three other long ORFs. ORF U1 and ORF U2 are located in independent transcriptional units between the P and M genes; they demonstrate significant amino acid sequence homology with each other and with other hapavirus PMIPs<sup>2</sup>. ORF Gx lies consecutively following the G ORF within the G gene transcriptional unit and is predicted to encode a class 1a viroporin<sup>2</sup>. There is also an alternative long ORFs (Px) in a different reading

frame in the GLOV P gene; ORF Px (11.5 kDa protein) it is in moderately favourable Kozak context and is located proximally to the start of the transcriptional unit, and a so it may be expressed. Phylogenetic analysis of L protein sequences indicates that GLOV sits centrally in the hapavirus clade (**Figure 2**). Amino acid sequence identities indicate that the GLOV L protein is most closely related to those of HPV, FLAV, MQOV, KAMV and MOSV whereas the GLOV G protein is most closely related to those of WONV, PCV and ORV (**Figure 3**) <sup>2</sup>.

# Landjia hapavirus

Landjia virus (LJAV) was isolated from a bird (*Riparia paludicola*) collected in 1970 in the Central African Republic<sup>1</sup>. Complete coding regions (13,695 nt) of the LJAV genome have been sequenced with only the 3' and 5' ends incomplete (Figure 1A)<sup>2</sup>. The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M. G and L), and five other long ORFs<sup>2</sup>. U1, U2 and U3 are located in independent transcriptional units between the P and M genes; they demonstrate significant amino acid sequence homology with each other and with other hapavirus PMIPs<sup>2</sup>. ORF Gx lies consecutively following the G ORF within the G gene transcriptional unit and is predicted to encode a class 1a viroporin<sup>2</sup>. ORF U4 lies in an independent transcriptional unit between the G and L genes and appears to encode a unique protein. There are also alternative long ORFs in different reading frames in the P gene (Px) and in the U1 gene (U1x): ORF Px (9.3 kDa protein) is in highly favourable Kozak context and is located proximally in the transcriptional unit, and so may be expressed; ORF U1x (6.9 kDa protein) in in poor Kozak context and is located centrally, and so may not be expressed. Phylogenetic analysis of L protein sequences (Figure 2) and amino acid sequence identities in the N, L and G proteins indicate that LJAV sits centrally in the hapavirus clade and is most closely related to MANV (**Figure 3**)<sup>2</sup>.

#### Manitoba hapavirus

Manitoba virus (MANV) was isolated from mosquitoes (Culex tarsalis) collected in 1977 in Canada<sup>13</sup>. Complete coding regions (13,784 nt) of the MANV genome have been sequenced with only the 3' and 5' ends incomplete (**Figure 1A**)<sup>2</sup>. The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L), and five other long ORFs<sup>2</sup>. ORFs (U1, U1x, U2 and U3 are located between the P and M genes. ORFs U1, U2 and U3 are in independent transcriptional units and U1x occurs within the U1 transcriptional unit as an overlapping ORF<sup>2</sup>. All four putative protein demonstrate significant amino acid sequence homology with each other and with other hapavirus PMIPs<sup>2</sup>. ORF Gx lies within the G gene transcriptional unit, consecutively following the G ORF, and is predicted to encode a class 1a viroporin<sup>2</sup>. There are also alternative long ORFs in different reading frames in the N gene (Nx and Ny), the P gene (Px) and in the U1x gene (U1y). ORF Nx (7.9 kDa) is in moderately favourable Kozak context and is located centrally; ORF Ny overlaps the end of the N ORF, is in highly favourable Kozak context and encodes a 9.3 kDa protein with a predicted transmembrane domain, suggesting it is likely to be expressed; ORF Px (10.5) kDa) in favourable Kozak context and is located proximally, and so may also be expressed; ORF U1y (8.6 kDa) is in favourable Kozak context but located distally but so may not be expressed. Phylogenetic analysis of L protein sequences (Figure 2) and amino acid sequence identities in the N, L and G proteins indicate that MANV sits centrally in the hapavirus clade and is most closely related to LJAV (**Figure 3**) $^2$ .

#### Mosqueiro hapavirus

Mosqueiro virus (MQOV) was isolated from mosquitoes (*Culex portesi*) collected in 1970 in Brazil<sup>14</sup>. MQOV cross-reacts strongly indirect immunofluorescence (IFA) tests and weakly in complement-fixation (CF) tests with HPV, FLAV, MOSV and KAMV, but there is low to no cross-neutralisation<sup>11, 15</sup>. The complete MOOV genome (12,957 nt) has been sequenced

(**Figure 1A**)<sup>2</sup>. The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L), and four other long ORFs<sup>2</sup>. ORFs U1, U2 and U3 are located in independent transcriptional units between the P and M genes; all three putative proteins demonstrate significant amino acid sequence homology with each other and with other hapavirus PMIPs<sup>2</sup>. ORF Gy lies within the G gene transcriptional unit, consecutively following the G ORF, and is predicted to encode a class 1a viroporin<sup>2</sup>. There are also alternative long ORFs in different reading frames in the P gene (Px), the U1 gene (U1x) and G gene (Gx). ORF Px (9.1 kDa) is in favourable Kozak context and located proximally; ORF U1x (8.6 kDa) is in favourable Kozak context and located centrally; and ORF Gy (7.5 kDa) is in moderate Kozak and located proximally; each may be expressed. Phylogenetic analysis of L protein sequences (**Figure 2**) and amino acid sequence identities in the N, L and G proteins indicate that MQOV is most closely related to FLAV, HPV, MOSV and KAMV (**Figure 3**)<sup>2</sup>.

#### Hart Park hapavirus

Hart Park virus (HPV) was first isolated from mosquitoes (Culex tarsalis) in 1955 in California, USA<sup>1</sup>. It has subsequently been isolated from various species of culicine mosquitoes and birds in the USA. In CF and IFA tests, HPV cross-reacts strongly with FLAV, and more weakly with MQOV, MOSV and KAMV <sup>11, 15</sup>. In neutralisation tests, HPV cross-reacts partially with FLAV<sup>11</sup>. The complete HPV genome (13,104 nt) has been sequenced (**Figure**  $(1A)^2$ . The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M, G and L), and four other long ORFs<sup>2</sup>. U1, U2 and U3 are located in independent transcriptional units between the P and M genes; all three putative proteins demonstrate significant amino acid sequence homology with each other and with other hapavirus PMIPs<sup>2</sup>. ORF Gx lies within the G gene transcriptional unit, consecutively following the G ORF, and is predicted to encode a class 1a viroporin<sup>2</sup>. There are also alternative long ORFs in different reading frames in the N gene (Nx), U3 gene (U3x) and L gene (Lx). ORF Nx (8.9 kDa protein) also occurs in FLAV and so may encode a functional protein. ORF U3x (7.2 kDa protein) and ORF Lx (7.8 kDa protein) are each in poor Kozak context and located distally in their transcriptional units, and so may not be expressed. Phylogenetic analysis of L protein sequences (Figure 2) and amino acid sequence identities in the N, L and G proteins indicate that HPV is most closely related to FLAV, MQOV, MOSV and KAMV (Figure 3)<sup>2</sup>.

# Flanders hapavirus

We propose the reassignment of the unassigned rhabdovirus species *Flanders virus* as the species *Flanders hapavirus* in the new genus *Hapavirus*. The rationale for the reassignment is provided in **Module 7**.

Flanders virus (FLAV) was first isolated from mosquitoes (*Culiseti melanura*) in 1961 in New York, USA<sup>16</sup>. It was subsequently isolated from various species of culicine mosquitoes and birds in the USA and Canada<sup>17</sup>. In CF and IFA tests, FLAV cross-reacts strongly with HPV and more weakly with MQOV, MOSV and KAMV <sup>11, 15</sup>. In neutralisation tests, FLAV cross-reacts partially with HPV<sup>11</sup>. Complete coding regions (13,308 nt) of the FLAV genome have been sequenced with only the 3′ and 5′ ends incomplete (**Figure 1A**)<sup>2</sup>. The genome organisation is very similar to that of HPV including ORFs U1, U2 and U3 located in independent transcriptional units between the P and M genes (PMIPs), ORF Gy encoding a viroporin-like protein within the G gene transcriptional unit and ORF Nx in an alternative long ORFs in different reading frame in the N gene. Unlike HPV, FLAV lacks alternative ORFs in the U3 gene (U3x) and L gene (Lx) but contains an additional alternative small ORF (Gx) in the G gene which is unique to FLAV. ORF Gx (8.5 kDa protein) is in moderately favourable Kozak context but is located centrally in the transcriptional unit and so may not be expressed as a functional protein. Phylogenetic analysis of L protein sequences (**Figure 2**) and amino acid

sequence identities in the N, L and G proteins indicate that FLAV is most closely related to HPV, MQOV, MOSV and KAMV (**Figure 3**)<sup>2</sup>.

FLAV and HPV cross-react weakly in neutralisation tests<sup>18</sup>. Amino acid sequence identity between HPV and FLAV is high, particularly in the N gene (95.4%) (**Figure 3**), and they have similar genome organisations, differing only in small ORFs in the U3, G and L genes that may not be expressed (**Figure 1**). They appear to have similar ecology (transmitted by culicine mosquitoes) and each occurs in the Americas<sup>17</sup>. However, phylogenetic analysis of U1 gene sequences indicates that the single available HPV isolate (California/1955) is distinct from all 91 available isolates of FLAV from USA and Brazil, spanning the time period 1961 to 2010 (**Figure 4**). Although the HPV isolate precedes all FLAV isolates, the evolutionary rate of FLAV is slow and the phylogeny does not indicate that HPV is the progenitor of the two extant FLAV lineages. We therefore propose that HPV and FLAV should be assigned as different species.

# Mossuril hapavirus

Mossuril virus (MOSV) was isolated from mosquitoes (Culex sitiens) collected in 1959 in Mozambique<sup>19</sup>. It has also been isolated from other culicine mosquitoes and birds (*Andropadis* virens; Coliuspasser macrouris) in Africa. Neutralising antibodies have been detected in humans and baboons 19. In CF and IFA tests, MOSV cross-reacts strongly with KAMV and more weakly with MOOV, HPV and FLAV <sup>11, 15</sup>. In neutralisation tests, MOSV cross-reacts partially with KAMV<sup>11</sup>. Complete coding regions (13,106 nt) of the MOSV genome have been sequenced with only the 3' and 5' ends incomplete (Figure 1A)<sup>2</sup>. The genome is large and complex, comprising five genes encoding the canonical rhabdovirus structural proteins (N, P, M. G and L), and four other long ORFs<sup>2</sup>, ORFs U1, U2 and U3 are located in independent transcriptional units between the P and M genes; all three putative proteins demonstrate significant amino acid sequence homology with each other and with other hapavirus PMIPs<sup>2</sup>. ORF Gy lies within the G gene transcriptional unit, consecutively following the G ORF, and is predicted to encode a class 1a viroporin<sup>2</sup>. There are also alternative long ORFs in different reading frames in the U2 gene (U2x), G gene (Gx) and L gene (Lx): ORF U2x (11.6 kDa protein) also occurs in KAMV, is located proximally to the start of the transcriptional unit, is in favourable Kozak context and so is highly likely to be expressed; ORF Gx (9.3 kDa protein) and ORF Lx (7.3 kDa) are located distally in the transcriptional units, are in moderately favourable Kozak context and so may not be expressed. Phylogenetic analysis of L protein sequences (Figure 2) and amino acid sequence identities in the N, L and G proteins indicate that MOSV is most closely related to KAMV, MQOV, HPV and FLAV (Figure 3)<sup>2</sup>.

#### Kamese hapavirus

Kamese virus (KAMV) was isolated from mosquitoes (*Culex annulioris*) collected in 1967 in Uganda<sup>20</sup>. It cross reacts strongly in with MOSV in CF tests but is clearly distinguishable from MOSV in virus neutralisation tests<sup>20</sup>. Neutralising antibodies to KAMV have been detected in humans<sup>1, 20</sup>. The complete KAMV genome (13,209 nt) has been sequenced (**Figure 1A**)<sup>2</sup>. The genome organisation is very similar to that of MOSV including ORF U1, U2 and U3 located in independent transcriptional units between the P and M genes, ORF Gy (viroporin-like protein) within the G gene transcriptional unit, and alternative ORF U2x within the U2 transcriptional unit. Alternative ORFs within the G gene (Gx) and L gene (Lx and Ly) are unique to KAMV: ORF Gx (8.1 kDa protein) is located centrally in the transcriptional unit and is in poor Kozak context; ORF Lx (7.8 kDa protein) is located centrally in the transcriptional unit and is in moderate Kozak context; ORF Ly (7.2 kDa protein) is located distally in the transcriptional unit and is in poor Kozak context; each may not be expressed as functional proteins. Phylogenetic analysis of L protein sequences (**Figure 2**) and amino acid sequence identities in the N, L and

G proteins indicate that KAMV is most closely related to MOSV, MQOV, HPV and FLAV (**Figure 3**)<sup>2</sup>.

KAMV and MOSV cross-react only weakly in neutralisation tests<sup>11</sup>. Although amino acid sequence identity between KAMV and MOSV is very high in the N protein (99.2%), it is relatively low in the G protein (80.4%), possibly explaining the neutralisation test data (**Figure 3**). They have similar genome organisations, differing only in small ORFs in the G and L genes that may not be expressed (**Figure 1**). They appear to have similar ecology (transmitted by culicine mosquitoes and infecting humans) and each occurs in sub-Saharan Africa. Based on neutralisation test data and relatively low identity of G protein sequences, we propose that MOSV and KAMV should be assigned to different species.

### **MODULE 3: NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	201	16.005bM	(assigned by ICTV officers)							
To create	a new	genus within:		Fill in all that apply.						
Subfa	mily:			If the higher taxon has yet to be created  (in a later was duly below) write "frame"						
Fa	mily:	Rhabdoviridae		(in a later module, below) write "(new)" after its proposed name.						
C	Order:	Mononegavirales		<ul> <li>If no family is specified, enter</li> <li>"unassigned" in the family box</li> </ul>						

naming a new genus

Code	2016.005cM	(assigned by ICTV officers)
To name th	he new genus: Hapavirus	

Assigning the type species and other species to a new genus

Code	2016.005dM	(assigned by ICTV officers)
To design	nate the following as the type	species of the new genus
Flanders	hapavirus	Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered
		and any that

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). Please enter here the TOTAL number of species (including the type species) that the genus will contain:

15 Species:

La Joya hapavirus

Wongabel hapavirus (reassigned and renamed)

Parry Creek hapavirus

Ord River hapavirus

Joinjakaka hapavirus

Ngaingan hapavirus (reassigned and renamed)

Marco hapavirus

Gray Lodge hapavirus

Landjia hapavirus

Manitoba hapavirus

Mosqueiro hapavirus

Hart Park hapavirus

Flanders hapavirus (reassigned and renamed – type species)

Mossuril hapavirus

Kamese hapavirus

### Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

The *Rhabdoviridae* is a large and diverse family of viruses. There are currently 11 approved genera in the *Rhabdoviridae*. However, many rhabdoviruses remain unclassified. Here, we propose

the establishment of a new genus (Hapavirus) that will comprise 15 new species, two of which are currently unassigned species in the Rhabdoviridae. Several viruses to be assigned to the proposed genus infect birds, reptiles or mammals. Most have been isolated from culicine mosquitoes. The viruses form a distinct clade in a well-supported ( $BSP \ge 85$ ) tree based on full length L protein (RdRp) sequences ( $Figure\ 2$ ). The clade is linked phylogenetically to the approved genera Ephemerovirus and Tibrovirus (arthropod-borne rhabdoviruses infecting ruminants), and more distantly to the genus Vesiculovirus (arthropod-borne rhabdoviruses infecting various mammals). Complete or near-complete genome sequences are known for each of the viruses to be assigned to the genus.

Hapavirus genomes are all large and complex with multiple accessory genes and display considerable diversity across the clade (**Figure 1A**). All but one of the viruses (MCOV) feature an ORF encoding a viroporin-like protein between the G and L genes and one or more additional long ORFs between the P and M genes. In most cases, the proteins encoded in the P-M intergenic region (PMIPs) display identifiable sequence homology with each other and with the PMIPs of other hapaviruses; they appear to have arisen by gene duplication. Although the MCOV genome does not share these specific features, it lies centrally in the hapavirus clade based on phylogenetic analysis of L protein sequences, suggesting the relevant genes have been lost during its evolution<sup>2</sup>. Like viruses in the genus *Ephemerovirus*, the NGAV genome encodes two consecutive class I transmembrane glycoproteins (G and G<sub>NS</sub>) that are structurally related and also appear to have arisen by gene duplication. However, NGAV is centrally located in the hapavirus clade in the L protein tree and is most closely related to hapaviruses in other genes. It is likely that the NGAV G<sub>NS</sub> gene has arisen either through an independent duplication of the G gene or by recombination with an ancestral ephemerovirus.

Serological cross-reactions (CF, indirect fluorescence antibody or ELISA) have been reported between some members of the genus and these viruses (HPV, FLAV, MQOV, MOSV and KAMV) have been assigned to the Hart Park serogroup.

### Origin of the new genus name:

The name is derived as a siglum from <u>Hart Park</u> serogroup which is the well-established antigenic designation of FLAV, HPV and several other members of the new genus.

## Reasons to justify the choice of type species:

Flanders virus (type species *Flanders hapavirus*) is the best characterized virus in the new genus in terms of ecology, virus molecular and generic structure, and antigenic and evolutionary analyses <sup>17, 18, 21</sup>

#### **Species demarcation criteria in the new genus:**

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

Viruses assigned to different species within the genus *Hapavirus* display several of the following characteristics: A) minimum amino acid sequence divergence of 5% in N proteins; B) minimum sequence divergence of 10% in the L proteins; C) minimum amino acid sequence divergence of 15% in G proteins; D) significant differences in genome organization as evidenced by numbers and locations of ORFs; E) can be distinguished in serological tests; and F) occupy different ecological niches as evidenced by differences in hosts and or arthropod vectors.

### MODULE 7: REMOVE and MOVE

Use this module whenever an existing taxon needs to be removed:

- Either to abolish a taxon entirely (when only part (a) needs to be completed)
- Or to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	201	16.005eM	(assigned by ICTV officers)
To remo	ove the	e following taxon (or tax	a) from their present position:
Wongab	el viru	us	
Ngainga	ın viru	us .	
Flander	s virus	1	
The pre	sent ta	axonomic position of the	se taxon/taxa:
G	lenus:	unassigned	
Subfa	mily:		Fill in all that apply
Fa	mily:	Rhabdoviridae	Fill in all that apply.
(	Order:	Mononegavirales	
If the tax			t reassigned to another taxon) write "yes"
	•	stify the removal: taxon (or taxa) should be re	amoved
Explain	viry the	taxon (or taxa) should be re	emoved

#### **Part (b)** re-assign to a higher taxon

Code	201	6.005fM	(assigned by ICTV officers)										
To re-assign the taxon (or taxa) listed in Part (a) as follows:													
				Fill in all that apply.									
Ge	nus:	Hapavirus (new)		If the higher taxon has yet to be									
Subfan	nily:			created write "(new)" after its proposed name and complete									
Fan	nily:	Rhabdoviridae		relevant module to create it.									
Oı	rder:	Mononegavirales		If no genus is specified, enter									
				"unassigned" in the genus box.									

#### **Reasons to justify the re-assignment:**

- If it is proposed to re-assign species to an existing genus, please explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
  - o If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

# Wongabel hapavirus

WONV (currently unassigned species *Wongabel virus*) falls within the clade of viruses (Figure 2) to be assigned to the new genus *Hapavirus*. Like other viruses assigned to the genus, WONV has a large and complex genome featuring: (i) ORFs encoding three proteins in the P-M intergenic region (PMIPs) that display identifiable sequence homology with each other and with the PMIPs of other hapaviruses; and (ii) an ORF encoding a viroporin-like protein (Gy) that lies within the G transcriptional unit and overlaps the end of the ORF encoding the G protein. In genome organisation and amino acid sequence identity in the N, L and G proteins, WONV is closely related to PCV and ORV (assigned to the species *Parry Creek hapavirus* and *Ord River hapavirus* in the genus *Hapavirus*).

#### Ngaingan hapavirus

NGAV (currently unassigned species *Ngaingan virus*) falls centrally in the clade of viruses (**Figure 2**) to be assigned to the new genus *Hapavirus*. Like other viruses assigned to the genus, NGAV has a large and complex genome. NGAV features ORFs encoding three proteins in the P-M intergenic region but they do not display identifiable sequence homology with each other and with the PMIPs of other hapaviruses. NGAV also features an ORF encoding a viroporin-like protein (U4x) that lies consecutively within the U4 transcriptional unit. In genome organisation and amino acid sequence identity in the N and G proteins, NGAV is most closely related to JOIV (assigned to the species *Joinjakaka hapavirus* in the genus *Hapavirus*).

## Flanders hapavirus

FLAV (currently unassigned species *Flanders virus*) falls centrally in the clade of viruses (**Figure 2**) to be assigned to the new genus *Hapavirus*. Like other viruses assigned to the genus, FLAV has a large and complex genome featuring: (i) ORFs encoding three proteins in the P-M intergenic region (PMIPs) that display identifiable sequence homology with each other and with the PMIPs of other hapaviruses; and (ii) an ORF encoding a viroporin-like protein (Gy) that lies within the G transcriptional unit and overlaps the end of the ORF encoding the G protein. In genome organisation and amino acid sequence identity in the N, L and G proteins, FLAV is closely related to HPV.

## MODULE 8: RENAME

Use this module to change the name of one or more existing taxa (but note that stability of nomenclature is encouraged wherever possible). Insert extra lines in the table if needed.

٠

Renaming one or more taxa

Code	2016.005gM	(assigned by ICTV officers)
To rena	me the following taxon (or tax	a):
Wongab		
Ngainga		
Flander	s virus	
Current	t name	Proposed name
Wongab	el virus	Wongabel hapavirus
Ngainga	ın virus	Ngaingan hapavirus
Flander	s virus	Flanders hapavirus
	·	

# **Reasons to justify the renaming:**

Explain why the taxon (or taxa) should be renamed

The species *Wongabel virus* will be renamed *Wongabel hapavirus*, *Ngaingan virus* will be renamed *Ngaingan hapavirus* and *Flanders virus* will be renamed *Flanders hapavirus* to conform with the binomial nomenclature recently introduced for all rhabdovirus species.

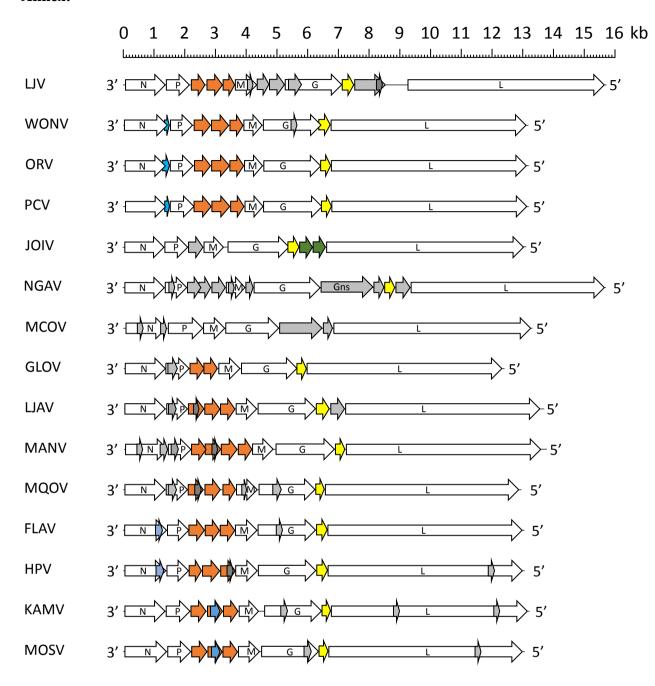
#### References:

- 1. Karabatsos N, 1985. International Catalogue of Arboviruses Including Certain other Viruses of Vertebrates. San Antonio: American Society for Tropical Medicine and Hygiene.
- 2. Walker PJ, Firth C, Widen SG, Blasdell KR, Guzman H, Wood TG, Paradkar PN, Holmes EC, Tesh RB, Vasilakis N, 2015. Evolution of genome size and complexity in the *Rhabdoviridae*. PLoS Pathogens 11: e1004664.
- 3. Gubala AJ, Proll DF, Barnard RT, Cowled CJ, Crameri SG, Hyatt AD, Boyle DB, 2008. Genomic characterisation of Wongabel virus reveals novel genes within the *Rhabdoviridae*. Virology 376: 13-23.
- 4. Humphrey-Smith I, Cybinski DH, Byrne KA, St George TD, 1991. Seroepidemiology of arboviruses among seabirds and island residents of the Great Barrier Reef and Coral Sea. Epidemiology and Infection 107: 435-440.
- 5. Joubert DA, Rodriguez-Andres J, Monaghan P, Cummins M, McKinstry WJ, Paradkar PN, Moseley GW, Walker PJ, 2015. Wongabel rhabdovirus accessory protein U3 targets the SWI/SNF chromatin remodeling complex. Journal of Virology 89: 1377-1388.
- 6. Liehne CG, Leivers S, Stanley NF, Alpers MP, Paul S, Liehne PFS, Chan KH, 1976. Ord River arboviruses Isolations from mosquitos. Australian Journal of Experimental Biology and Medical Science 54: 499-504.
- 7. Liehne PFS, Anderson S, Stanley NF, Liehne CG, Wright AE, Chan KH, Leivers S, Britten DK, Hamilton NP, 1981. Isolation of Murray Valley encephalitis virus and other arboviruses in the Ord River Valley 1972-1976. Australian Journal of Experimental Biology and Medical Science 59: 347-356.
- 8. Doherty RL, Carley JG, Standfast HA, Dyce AL, Kay BH, Snowdon WA, 1973. Isolation of arboviruses from mosquitoes, biting midges, sandflies and vertebrates collected in Queensland, 1969 and 1970. Transactions of the Royal Society for Tropical Medicine Hygiene 67: 536-543.
- 9. Kay BH, Boreham PFL, Dyce AL, Standfast HA, 1978. Blood feeding of biting midges (Diptera Ceratopogonidae) at Kowanyama, Cape-York Peninsula, North Queensland. Journal of the Australian Entomological Society 17: 145-149.
- 10. Gubala A, Davis S, Weir R, Melville L, Cowled C, Walker P, Boyle D, 2010. Ngaingan virus, a macropod-associated rhabdovirus, contains a second glycoprotein gene and seven novel open reading frames. Virology 399: 98-108.
- 11. Calisher CH, Karabatsos N, Zeller H, Digoutte J-P, Tesh RB, Shope RE, Travassos da Rosa APA, St. George TD, 1989. Antigenic relationships among rhabdoviruses from vertebrates and hematophagous arthropods. Intervirology 30: 241-257.
- 12. Causey OR, Shope RE, Bensabath G, 1966. Marco, Timbo, and Chaco, newly recognized arboviruses from lizards of Brazil. American Journal Tropical Medicine and Hygiene 15: 239-243.
- 13. Artsob H, Doane F, Sekla L, Stackiw W, Brust R, 1991. Manitoba virus, a new rhabdovirus isolated from *Culex tarsalis* mosquitoes collected in Manitoba, Canada. Canadian Journal of Microbiology 37: 329-332.
- 14. Davies JB, Corbet PS, Gillies MT, Mccrae AWR, 1971. Parous rates in some Amazonian mosquitoes collected by three different methods. Bulletin of Entomological Research 6: 125-132.

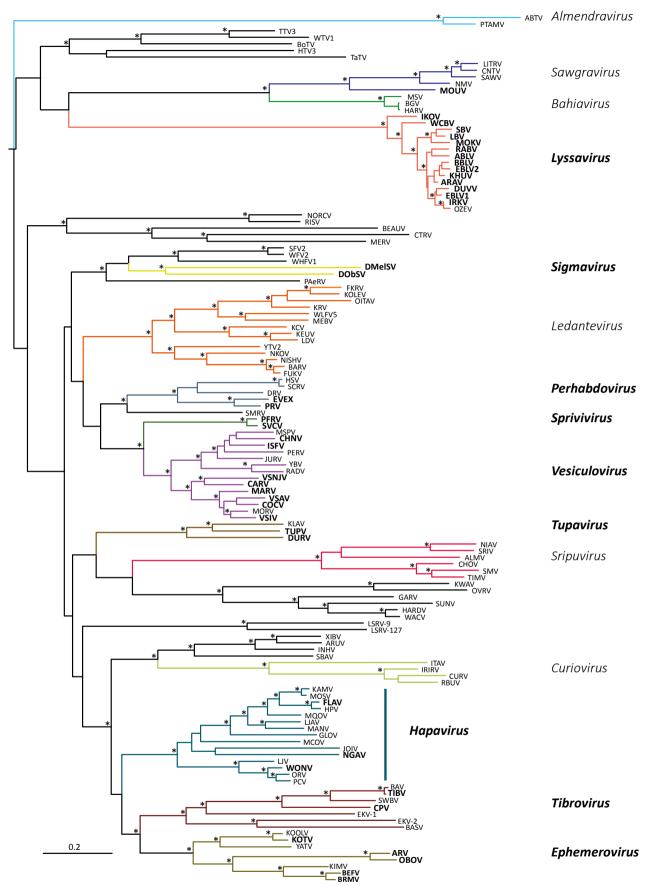
### **References:**

- 15. Tesh RB, Travassos da Rosa APA, Travassos da Rosa JS, 1983. Antigenic relationship among rhabdoviruses infecting terrestrial vertebrates. Journal of General Virology 64: 169-176.
- 16. Whitney E, 1964. Flanders strain, an arbovirus newly isolated from mosquitoes and birds of New York State. American Journal of Tropical Medicine and Hygiene 13: 123-131.
- 17. Allison AB, Mead DG, Palacios GF, Tesh RB, Holmes EC, 2014. Gene duplication and phylogeography of North American members of the Hart Park serogroup of avian rhabdoviruses. Virology 448: 284-292.
- 18. Boyd KR, 1972. Serological comparisons among Hart Park virus and strains of Flanders virus. Infection and Immunity 5: 933-937.
- 19. Kokernot RH, Mc IB, Worth CB, De Sousa J, 1962. Isolation of viruses from mosquitoes collected at Lumbo, Mozambique. II. Mossuril virus, a new virus isolated from the *Culex* (*culex*) *sitiens* Wiedemann group. American Journal of Tropical Medicine and Hygiene 11: 683-684.
- 20. Henderson BE, Tukei PM, Lule M, West R, Mujomba E, 1967. Arbovirus identification studies. Isolations from mosquitoes. . East African Virus Research Report 17: 22-25.
- 21. Boyd KR, Whitaker-Dowling P, 1988. Flanders virus replication and protein synthesis. Virology 163: 349-358.

### Annex:



**Figure 1.** Genome organisations of 15 hapaviruses. ORFs are indicated as block arrows. PMIP ORFs are coloured in red; class 1a viroporin-like protein ORFs are coloured in yellow; other colours indicate ORFs encoding homologous proteins.



**Figure 2.** ML phylogenetic tree of 132 animal rhabdovirus L protein sequences. Branches are colour-coded according to existing genera (named in bold italics) or proposed new genera (named light italics) Walker *et al.* (2015) PLoS Pathogens 11 (2): e1004664<sup>2</sup>. The clade representing the proposed new genus *Hapavirus* is highlighted. Horizontal branch lengths are drawn to a scale of

amino acid substitutions/site, and all bootstrap support values (BSP)  $\geq$  75% are shown by the \* symbol. The tree is rooted based on the position observed in a broader analysis that included more distant members of the *Rhabdoviridae* (i.e., including members of the genera *Novirhabdovirus*, *Cytorhabdovirus* and *Nucleorhabdovirus*) and in other publications. Cytorhabdovirus, novirhabdovirus and nucleorhabdovirus outgroup sequences were excluded from the tree as they were too divergent to establish a reliable rooting. The tree is therefore rooted arbitrarily on one of two basal clades (potential new genera *Almendravirus* and *Bahiavirus*) that comprise viruses isolated from mosquitoes. The ML tree was generated as described in Walker *et al.* (2015) PLoS Pathogens 11 (2): e1004664<sup>2</sup>. Virus abbreviations and Genbank accession numbers are as listed in **Table 1**.

**Figure 3A.** Hapavirus N protein amino acid sequence identity (%; as estimated in MEGA6 by p-distance).

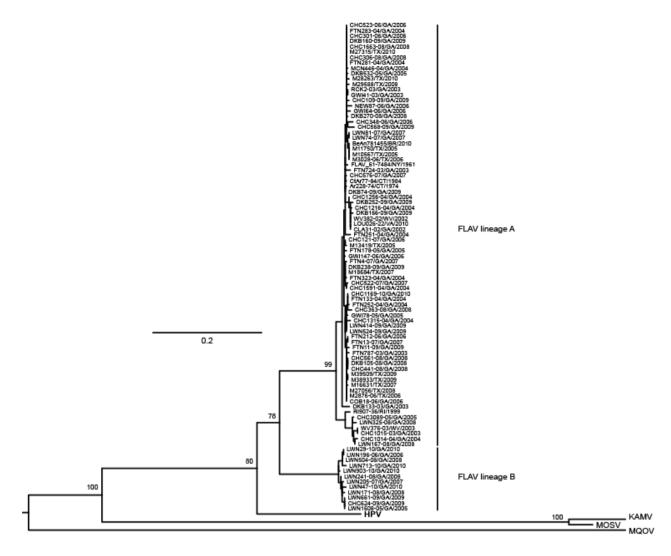
	LJV	WONV	ORV	PCV	JOIV	NGAV	MCOV	GLOV	LJAV	MANV	MQOV	FLAV	HPV	KAMV	MOSV
LJV	100														
WONV	64.9	100													
ORV	64.9	94.7	100												
PCV	64.1	91.1	91.3	100											
JOIV	45.3	45.0	45.5	46.8	100										
NGAV	42.2	41.5	41.7	42.2	45.3	100									
MCOV	33.1	32.6	33.1	32.1	35.6	30.5	100								
GLOV	48.1	50.4	50.6	56.0	49.6	44.0	36.1	100							
LJAV	45.0	42.0	42.7	43.3	45.8	41.0	34.4	52.9	100						
MANV	42.7	42.5	43.0	44.0	44.3	40.2	33.6	55.7	72.5	100					
MQOV	45.8	46.6	46.1	45.5	47.1	42.0	35.1	57.3	56.2	54.7	100				
FLAV	44.5	44.5	44.5	44.0	45.8	41.2	34.1	54.5	53.7	54.7	72.5	100			
HPV	43.3	44.5	45.0	44.0	45.0	41.5	34.4	54.5	53.2	55.0	71.5	95.4	100		
KAMV	45.3	44.3	44.3	43.8	43.8	40.7	34.1	55.0	53.2	56.5	75.3	74.0	74.0	100	
MOSV	45.0	44.5	44.5	44.0	43.8	40.7	34.1	54.7	52.9	56.0	75.3	74.0	74.0	99.2	100

**Figure 3B.** Hapavirus L protein amino acid sequence identity (%; as estimated in MEGA6 by p-distance).

		<i>,</i> .													
	LJV	WONV	ORV	PCV	JOIV	NGAV	MCOV	GLOV	LJAV	MANV	MQOV	FLAV	HPV	KAMV	MOSV
LJV	100														
WONV	67.7	100													
ORV	67.2	84.3	100												
PCV	66.1	84.5	86.5	100											
JOIV	49.4	50.2	49.9	50.4	100										
NGAV	49.2	49.3	49.1	49.7	50.0	100									
MCOV	52.4	52.7	52.6	52.3	49.3	49.5	100								
GLOV	51.8	52.5	52.1	52.1	50.4	49.6	51.8	100							
LJAV	52.3	52.2	52.0	51.6	51.7	50.9	52.9	60.2	100						
MANV	52.9	53.4	53.1	53.0	52.0	52.0	53.2	59.8	71.7	100					
MQOV	52.6	53.4	53.2	52.9	51.4	51.0	53.3	60.4	64.8	64.7	100				
FLAV	51.7	52.1	51.7	51.9	50.1	50.5	52.1	59.9	64.7	64.8	69.2	100			
HPV	51.5	52.6	51.9	52.1	50.6	50.8	52.1	60.6	65.4	65.7	69.8	89.7	100		
KAMV	51.8	53.3	52.9	52.2	50.2	50.9	54.0	61.1	65.7	65.5	70.4	73.2	74.1	100	
MOSV	52.0	53.7	53.1	52.3	50.8	51.5	54.0	61.2	66.0	66.1	70.7	73.0	74.0	91.6	100

**Figure 3C.** Hapavirus G protein amino acid sequence identity (%; as estimated in MEGA6 by p-distance).

	LJV	WONV	ORV	PCV	JOIV	NGAV	MCOV	GLOV	LJAV	MANV	MQOV	FLAV	HPV	KAMV	MOSV
LJV	100														
WONV	43.2	100													
ORV	41.4	71.4	100												
PCV	43.6	70.4	74.7	100											
JOIV	26.3	29.6	30.4	30.7	100										
NGAV	28.2	26.7	27.6	28.4	34.8	100									
MCOV	25.5	24.3	25.1	24.5	22.2	24.1	100								
GLOV	30.0	33.5	33.9	33.3	25.5	27.2	24.3	100							
LJAV	29.2	29.4	30.4	28.4	25.7	27.2	26.1	39.1	100						
MANV	28.4	29.8	30.7	29.4	27.8	28.6	24.3	41.4	54.7	100					
MQOV	27.8	29.2	28.0	29.4	27.8	26.1	23.5	38.5	45.1	42.4	100				
FLAV	29.6	302	29.8	30.4	27.0	26.1	24.1	38.3	45.7	44.0	51.0	100			
HPV	29.4	30.4	29.4	29.0	27.6	26.5	25.1	38.1	46.9	46.3	52.3	85.2	100		
KAMV	26.7	28.4	28.4	28.0	27.2	25.9	25.3	37.5	43.0	39.3	51.2	54.9	55.4	100	
MOSV	28.6	29.0	28.8	27.6	25.1	26.5	24.5	38.7	43.0	40.1	49.8	54.3	55.6	80.4	100



**Figure 4.** ML phylogenetic tree of nucleotide sequences of HPV, MOSV, KAMV, MQOV and 91 isolates of FLAV (1961-2010) from the USA and Brazil. Bootstrap values (100 replicates) at significant nodes are shown. Two sympatric lineages of FLAV (A and B) are resolved and HPV is shown to be distinct from each lineage.

**Table 1.** Rhabdoviruses for which genome sequences have been used in this proposal.

Virus	Abbrev.	Strain	Rhabdovirus	Species	Genome	GenBank
Virus	Abbiev.	Strain	genus	Species	size (nt)	accession
Arboretum virus	ABTV	LO-121	not classified		11492	KC994644
Puerto Almendras virus	PTAMV	LO-39	not classified		11876	KF534749
Tacheng tick virus 3	TTV3	TC255 (seq)	not classified		partial	KM817640
Wuhan tick virus 1	WTV1	X78-2 (seq)	not classified		10306+	KM817660
Bole tick virus 2	BoTV2	BL076	not classified		11843	KM817629
Huangpi tick virus 3	HTV3	H124-2 (seq)	not classified		13169+	KM817630
Taishun_Tick_virus	TaTV	BL198 (seq)	not classified		11280+	KM817643
Long Island tick rhabdovirus	LITRV	LS1	not classified		11176	KJ396935
Connecticut virus	CNTV	Ar1152-78	not classified		11169+	KM205020
Sawgrass virus	SAWV	64A-1247	not classified		11216	KM205013
New Minto virus	NMV	579	not classified		11156+	KM205009
Moussa virus	MOUV	D24	unassigned sp.	Moussa virus	11526	FJ985749
Muir Springs virus	MSV	76V-23524	not classified		12580	KM204990
Bahia Grande virus	BGV	TB4-1054	not classified		12639	KM205018
Harlingen virus	HARV	PV01-3828	not classified		12626	KM205003
Ikoma virus	IKOV	RV2508	Lyssavirus	Ikoma lyssavirus	11902	JX193798
West Caucasian bat virus	WCBV	NZ86	Lyssavirus	West Caucasian bat lyssavirus	12278	EF614258
Shimoni bat virus	SBV	N613	Lyssavirus	Shimoni bat lyssavirus	12045	GU170201 EU293108
Lagos bat virus	LBV	0406SEN	Lyssavirus	Lagos bat lyssavirus Mokola lyssavirus	12016	
Mokola virus	MOKV	RV1035	Lyssavirus		11939	KF155006
rabies virus Australian bat lyssavirus	RABV ABLV	HN10 96-1256	Lyssavirus Lyssavirus	Rabies lyssavirus	11932 11918	EU643590 AF081020
Bokeloh bat lyssavirus	BBLV	21961	Lyssavirus	Australian bat lyssavirus  Bokeloh bat lyssavirus	11918	JF311903
-	EBLV2	RV1333	Lyssavirus	· · · · · · · · · · · · · · · · · · ·	11900	EF157977
European bat lyssavirus 2  Khujand virus	KHUV	KV1555	Lyssavirus	European bat lyssavirus 2 Khujand lyssavirus	11930	EF614261
Arayan virus	ARAV		Lyssavirus	Aravan lyssavirus	11903	EF614259
Duvenhage virus	DUVV	86132SA	Lyssavirus	Duvenhage lyssavirus	11918	EU293119
European bat lyssavirus 1	EBLV1	RV9	Lyssavirus	European bat lyssavirus 1	11966	EF157976
Irkut virus	IRKV	J426	Lyssavirus	Irkut lyssavirus	11980	EF614260
Ozernoe virus	OZEV	OI56	not classified	Trai tyssuvirus	11980	FJ905105
North Creek virus #	NORCV	954	not classified		partial	KF360973
Riverside virus	RISV	Drava-1	not classified		11713	KU248085
Beaumont virus	BEAUV	6	not classified		partial	KF310911
Culex tritaeniorhynchus rhabdovirus	CTRV	GHK	not classified		11190	LC026102
Merida virus	MERDV	Mex-07	not classified		11798	KU194360
Shayang fly virus 2	SFV2	SYY1-8	not classified		12291+	KM817635
Wuhan fly virus 2	WFV2	SYY1-3	not classified		12247+	KM817646
Wuhan house fly virus 1	WHFV1	SYY2-4	not classified		12651+	KM817648
Drosophila melanogaster sigmavirus	DMelSV	HAP23	Sigmavirus	Drosophila melanogaster sigmavirus	12390+	GQ375258
Drosophila obscura sigmavirus	DObSV	10A	Sigmavirus	Drosophila obscura sigmavirus	12676+	NC022580
Pararge aegeria rhabdovirus	PAeRV		not classified		13062	KR822826
Fikirini virus	FKRV	KEN352	not classified		11139+	KC676792
Kolente virus	KOLEV	DakArK7292	not classified		11120	KC984953
Oita virus	OITAV	296-1972	not classified		11355+	KM204998
Kumasi rhabdovirus	KRV	M35	not classified		11072	KJ179955
Wuhan louse fly virus 5	WLFV5	BFJSC-5	not classified		11103+	KM817654
Mount Elgon bat virus	MEBV	BP846	not classified		10941+	KM205026
Kern Canyon virus	KCV	M03790	not classified		11528+	KM204992
Le Dantec virus	LDV	DakHD763	not classified		11450+	KM205006
Yongjia tick virus 2	YTV2	YJ1-2	not classified		10833	KM817662
Nkolbisson virus	NKOV	YM 31-65	not classified		10942+	KM205017
Nishimuro virus	NISV	6225	not classified	+	10881+	AB609604
Barur virus	BARV	6235	not classified	+	10853+	KM204983
Fukuoka virus	FUKV	FUK-11	not classified not classified	+	10863	KM205001
Keuraliba virus	KEUV HSV	DakAnD5314 C1207		+	11457+ 11545	KM205021
hybrid snakehead virus Siniperca chuatsi rhabdovirus	SCRV	C1207	not classified not classified	+	11545	KC519324 DQ399789
dolphin rhabdovirus	DRV	pxV1	not classified	+	11141	KF958252
eel virus European X	EVEX	153311	Perhabdovirus	Anguillid perhabdovirus	11141	FN557213
perch rhabdovirus	PRV	J424	Perhabdovirus	Perch perhabdovirus	11487+	JX679246
Scophthalmus maximus rhabdovirus	SMRV	J727	not classified	1 степ ретионочниз	11492	HQ003891
pike fry rhabdovirus	PFRV	F4	Sprivivirus	Pike fry sprivivirus	11097	FJ872827
spring viremia of carp virus	SVCV	VR-1390	Sprivivirus	Carp sprivivirus	11097	AJ318079
Malpais Spring virus	MSPV	85-488NM	not classified	Carp sprivivius	11019	KC412247
Chandipura virus	CHNV	CIN0451	Vesiculovirus	Chandipura vesiculovirus	11120	GU212856
Isfahan virus	ISFV	91026-167	Vesiculovirus	Isfahan vesiculovirus	11088	AJ810084
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Perinet virus	PERV	DakArMg802	not classified	I	11103+	HM566195
Jurona virus	JURV	BeAr40578	not classified		11121+	KM204996
Yug Bogdanovac virus	YBV	Yu4-76	not classified		11202	JF911700
Radi virus	RADV	ISS Phl-166	not classified		11068+	KM205024
vesicular stomatitis New Jersey virus	VSNJV	NJ89GAS	Vesiculovirus	New Jersey vesiculovirus	11123	JX121110
Carajas virus	CARV	BeAr411391	Vesiculovirus	Carajas vesiculovirus	10716+	KM205015
Maraba virus	MARV	BeAr411459	Vesiculovirus	Maraba vesiculovirus	11135	HQ660076
vesicular stomatitis Alagoas virus	VSAV	Indiana 3	Vesiculovirus	Alagoas vesiculovirus	11070	EU373658
Cocal virus	COCV	TRVL40233	Vesiculovirus	Cocal vesiculovirus	11003	EU373657
Morreton virus	MORV	CoAr191048	not classified		11181+	KM205007
vesicular stomatitis Indiana virus	VSIV	98COE	Vesiculovirus	Indiana vesiculovirus	11161	AF473864
Klamath virus	KLAV	M-1056	not classified		11478+	KM204999
tupaia rhabdovirus	TUPV		Tupavirus	Tupaia tupavirus	11440	AY840978
Durham virus	DURV	CC228-C5	Tupavirus	Durham tupavirus	11092+	FJ952155
Niakha virus	NIAV	DakArD88909	not classified		11124	KC585008
Sripur virus	SRIV	733646	not classified		11290+	KM205023
Almpiwar virus	ALMV	MRM4059	not classified		11156	KJ399977
Chaco virus	CHOV	BeAn42217	not classified		11397+	KM205000
Sena Madureira virus	SMV	BeAn303197	not classified		11422+	KM205004
Timbo virus	TIMV	BeAn41787	not classified		partial	na
Kwatta virus	KWAV	A-57	not classified		11211+	KM204985
Oak Vale virus	OVRV	K13965	not classified		11220	JF705877
Garba virus	GARV	DakAnB439a	not classified		10821+	KM204982
Sunguru virus	SUNV	UG#41	not classified		11056	KF395226
Harrison Dam virus	HARDV	CS75	not classified		11284+	KJ432573
Walkabout Creek virus	WACV	CS1056	not classified		11214	KJ432572
Lepeophtheirus salmonis rhabdovirus 9	LSRV-9	C51030	not classified		11681+	KJ958535
Lepeophtheirus salmonis rhabdovirus 127	LSRV-127		not classified		11519+	KJ958536
Xiburema virus	XIBV	BeAr362159	not classified		12240	KJ636781
Aruac virus	ARUV	TRVL9223	not classified		11906+	KJ030781 KM204987
Inhangapi virus	INHV	BeAr177325	not classified		12026	KM204987 KM204991
Santa Barbara virus	SBAV	Ar775619	not classified		12026	
	ITAV				12536+	KM350503
Itacaiunas virus		BeAr427036	not classified			KM204984
Iriri virus	IRIRV	BeAr408005	not classified		13070	KM204995
Curionopolis virus	CURV	BeAr440009	not classified		13170	KM204994
Rochambeau virus	RBUV	CaAr16102	not classified		13593	KM205012
Kamese virus	KAMV	MP6186	not classified	14 11 1	13209	KM204989
Mossuril virus	MOSV	SAAr1995	Hapavirus*	Mossuril hapavirus	13106+	KM204993
Flanders virus	FLAV	61-7484	Hapavirus*	Flanders hapavirus	13038	KM205002
Hart Park virus	HPV	AR7C	Hapavirus*	Hart Park hapavirus	13104	KM205011
Mosqueiro virus	MQOV	BeAr185559	Hapavirus*	Mosqueiro hapavirus	12957	KM205014
Landjia virus	LJAV	DakAnB769d	Hapavirus*	Landjia hapavirus	13695+	KM205010
Manitoba virus	MANV	Mn936-77	Hapavirus*	Manitoba hapavirus	13784+	KM205008
Gray Lodge virus	GLOV	BFN3187	Hapavirus*	Gray Lodge hapavirus	12403	KM205022
Marco virus	MCOV	BeAn40290	Hapavirus*	Marco hapavirus	13294+	KM205005
Joinjakaka virus	JOIV	AusMK7937	Hapavirus*	Joinjakaka hapavirus	13155	KM205016
Ngaingan virus	NGAV	MRM14556	Hapavirus*	Ngaingan hapavirus	15764	NC013955
La Joya virus	LJV	J-134	Hapavirus*	La Joya hapavirus	15721	KM204986
Wongabel virus	WONV	CS264	Hapavirus*	Wongabel hapavirus	13196	NC011639
Ord River virus	ORV	OR1023	Hapavirus*	Ord River hapavirus	13189+	KM205025
Parry Creek virus	PCV	OR189	Hapavirus*	Parry Creek hapavirus	13205+	KM204988
Bivens Arm virus	BAV	UF-10	not classified		13288+	KM205019
Tibrogargan virus	TIBV	CS132	Tibrovirus	Tibrogargan tibrovirus	13298	GQ294472
Sweetwater Branch virus	SWBV	UF-11	not classified		13141+	KM204997
Coastal Plains virus	CPV	DPP53	Tibrovirus	Coastal Plains tibrovirus	13203	GQ294473
Ekpoma-1 virus	EKV-1		not classified		12,659+	KP324827
Ekpoma-2 virus	EKV-2		not classified		12,674+	KP324828
Bas Congo virus	BASV	BASV-1	not classified		11892+	JX297815
Koolpinyah virus	KOOLV	DPP833/819	not classified		16133	KM085029
Kotonkan virus	KOTV	IbAr23380	Ephemerovirus	Kotonkan ephemerovirus	15870	HM474855
Yata virus	YATV	DakArB2181	not classified		14479	KM085030
Adelaide River virus	ARV	DPP61	Ephemerovirus	Adelaide River ephemerovirus	14627	JN935380
Obodhiang virus	OBOV	SudAr1154-64	Ephemerovirus	Obodhiang ephemerovirus	14717	HM856902
Kimberley virus	KIMV	CS368	not classified	1	15442	JQ941664
bovine ephemeral fever virus	BEFV	BB7721	Ephemerovirus	Bovine fever ephemerovirus	14900	AF234533
Berrimah virus	BRMV	DPP63	Ephemerovirus	Berrimah ephemerovirus	15024	HM461974
* Toyonomia assignments proposed	1		• •			

<sup>\*</sup> Taxonomic assignments proposed here. + Complete coding sequences only.