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:	***2016.004aM***	(to be completed by ICTV officers)

Short title: 3 new species in the genus *Ephemerovirus*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)	**1** ☒	**2** ☒	**3** ☐	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☒

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List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at http://www.ictvonline.org/subcommittees.asp . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	ICTV *Rhabdoviridae* SG

ICTV Study Group comments (if any) and response of the proposer:

10 members have advised support for the proposal; 2 members have not responded.

Date first submitted to ICTV: June 2016
Date of this revision (if different to above):

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	***2016.004aM***	(assigned by ICTV officers)

To create 3 new species within:

Genus:	***Ephemerovirus***
Subfamily:	
Family:	***Rhabdoviridae***
Order:	***Mononegavirales***

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Kimberley ephemerovirus	Kimberley virus (CS368)	JQ941664
Koolpinyah ephemerovirus	Koolpinyah virus (DPP833)	KM085029
Yata ephemerovirus	Yata virus (DakAr B2181)	KM085030

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

Species demarcation criteria have previously been defined as follows:
Ephemeroviruses belonging to separate species "cross-react in complement-fixation and/or indirect immunofluorescence tests but exhibit low to no cross-neutralization. They exhibit similar but distinct genome organizations with the common feature of a non-structural glycoprotein (G_{NS}) gene but vary in the number of accessory protein genes and the location of transcriptional control sequences." Members of "different species may share up to 91% identity in N protein amino acid sequence."

a. *Kimberley ephemerovirus*
Kimberley virus (KIMV) strain CS368 was isolated in 1980 from a healthy sentinel bovid in the Northern Territory of Australia[1]. The virus has also been isolated in Australia from mosquitoes (*Culex annulirostris*) and biting midges (*Culicoides brevitarsis*)[2, 3]. KIMV cross-reacts with bovine ephemeral fever virus (BEFV) and Berrimah virus (BRMV) in indirect immunofluorescence and complement-fixation tests[1]. However, it does not cross-react in neutralisation tests with the three ephemeroviruses to which it is most closely related phylogenetically: BEFV (species *Bovine fever ephemerovirus*), Berrimah virus (species *Berrimah ephemerovirus*) or Adelaide River virus (species *Adelaide River ephemerovirus*)[1, 4, 5]. Unlike BEFV, KIMV has not been associated with disease in cattle or other ruminants. The KIMV genome organisation is similar to those of BEFV and other ephemeroviruses, containing a non-structural glycoprotein gene (G_{NS}) followed by a viroporin gene (α1), and several other accessory genes (α2, β and γ) (**Figure 1**)[6]. In a phylogenetic analysis based on the full-length L gene of most available animal rhabdoviruses, KIMV clusters with ephemeroviruses (**Figure 2**)[6]. N protein amino acid sequence identity (as estimated in MEGA6 by p-distance; **Figure 3**) is less than 91% compared to other ephemeroviruses, except for Malakal virus (98.8%). Malakal virus (isolated from *Mansonia uniformis* mosquitoes in Sudan, Africa) is considered to be a strain of KIMV[7].

b. *Koolpinyah ephemerovirus*
Koolpinyah virus (KOOLV) strain DPP819 was isolated in 1985 from a healthy sentinel bovid in the Northern Territory of Australia[8]. KOOLV cross-reacts in indirect immunofluorescence tests with kotonkan virus (species *Kotonkan ephemerovirus*) and Obodhiang virus (species *Obodhiang ephemerovirus*)[8]. In neutralisation tests, KOOLV cross-reacts very weakly with kotonkan virus[8]. The 16,133-nt KOOLV genome is similar to that of BEFV and other ephemeroviruses, containing a non-structural glycoprotein gene (G_{NS}) followed by a viroporin gene (α1), and several other accessory genes (α2, β, γ, and δ) (**Figure 1**)[6,9]. It differs in length from the kotonkan virus genome (15,870 nt) due primarily to a very long 3' non-coding region (438 nt) in the γ gene. In a phylogenetic analysis based on the full-length L gene of most available animal rhabdoviruses, KOOLV clusters with ephemeroviruses and is most closely related to kotonkan virus (**Figure 2**)[6,9]. N protein amino acid sequence identity (as estimated in MEGA6 by p-distance; **Figure 3**) is less than 91% compared to all other ephemeroviruses, except for kotonkan virus (92.7%). However, there is a similar level of amino acid sequence identity between the N proteins of BEFV and Berrimah virus (92.5%), which are already assigned to separate ephemerovirus species (Figure 1). All BEFV isolates from Australia and Asia (more than 120 sequenced to date) cluster separately from Berrimah virus in phylogenetic analyses[7]. In view of this, and the very low level of cross-neutralisation between KOOLV and kotonkan virus, we regard the level of differentiation sufficient for separate species assignment.

c. Yata ephemerovirus

Yata virus (YATV) strain DakAr B2181 was isolated in 1969 from mosquitoes (*Mansonia uniformis*) in the Central African Republic[10]. YATV failed to cross-react with a large set of other viruses in complement-fixation and indirect immunofluorescence tests, including kotonkan virus and several other ephemeroviruses[10, 11]. The 14,497 nt YATV genome is similar to BEFV and other ephemeroviruses, containing a non-structural glycoprotein gene (G_{NS}) followed by a viroporin gene ($\alpha 1$), and several other accessory genes that show low but identifiable homology with the ephemerovirus $\alpha 2$, β, and γ genes (**Figure 1**)[6, 9]. In a phylogenetic analysis based on the full-length L gene of most available animal rhabdoviruses, YATV clusters with ephemeroviruses and is most closely related to kotonkan virus and Koolpinyah virus (**Figure 2**)[6, 9]. N protein amino acid sequence identity (as estimated in MEGA6 by p-distance; **Figure 3**) is less than 91% compared to all other ephemeroviruses.

MODULE 10: **APPENDIX**: supporting material

References:

1. Cybinski DH, Zakrzewski H, 1983. The isolation and preliminary characterization of a rhabdovirus in Australia related to bovine ephemeral fever virus. Veterinary Microbiology 8: 221-235.
2. 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.
3. Zakrzewski H, Cybinski DH, 1984. Isolation of Kimberley virus, a rhabdovirus, from *Culicoides brevitarsis*. Australian Journal of Experimental Biology and Medical Science 62: 779-780.
4. Gard GP, Cybinski DH, Zakrzewski H, 1984. The isolation of a fourth bovine ephemeral fever group virus. Australian Veterinary Journal 61: 332.
5. Gard GP, Cybinski DH, St. George TD, 1983. The isolation in Australia of a new virus related to bovine ephemeral fever virus. Australian Veterinary Journal 60: 89-90.
6. 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.
7. Blasdell KR, Voysey R, Bulach DM, Trinidad L, Tesh RB, Boyle DB, Walker PJ, 2012. Malakal virus from Africa and Kimberley virus from Australia are geographic variants of a widely distributed ephemerovirus. Virology 433: 236-244.
8. Gard GP, Melville LF, Calisher CH, Karabatsos N, 1992. Koolpinyah: a virus related to kotonkan from cattle in northern Australia. Intervirology 34: 142-145.
9. Blasdell KR, Widen SG, Diviney SM, Firth C, Wood TG, Guzman H, Holmes EC, Tesh RB, Vasilakis N, Walker PJ*. (2014). Koolpinyah and Yata viruses: two newly recognised ephemeroviruses from tropical regions of Australia and Africa. Veterinary Microbiology 174: 547-553.
10. Karabatsos N, 1985. International Catalogue of Arboviruses Including Certain other Viruses of Vertebrates. San Antonio: American Society for Tropical Medicine and Hygiene.

References:

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.

Annex:

Figure 1. Genome organisations of ephemeroviruses.

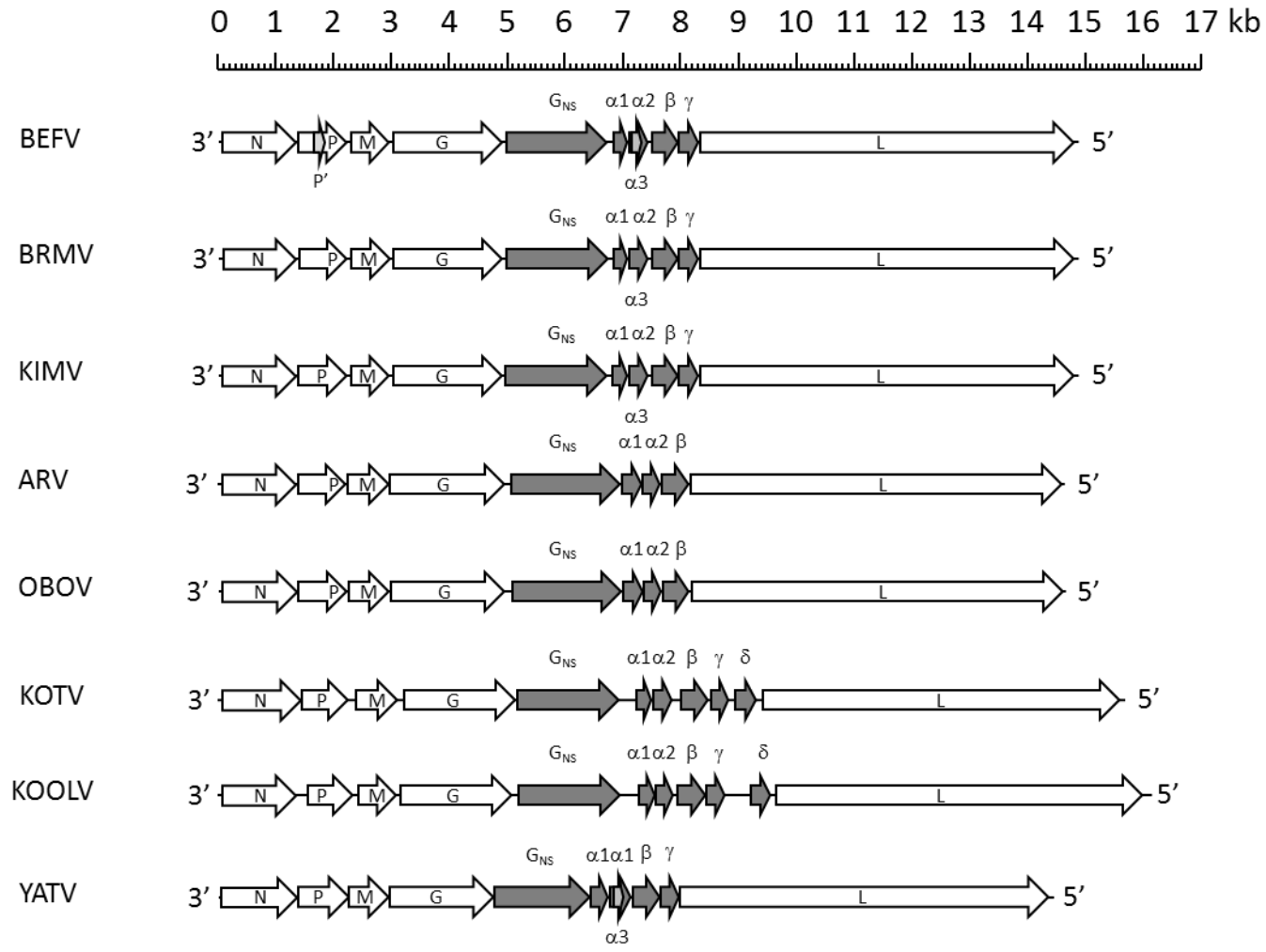

Figure 2. ML phylogenetic tree of 100 animal rhabdovirus L protein sequences (including ephemeroviruses). Branches are colour-coded according to known vector species, while the principal animal host species are shown by indicated symbols. Horizontal branch lengths are drawn to a scale of amino acid substitutions/site, and all bootstrap support values (BSP) ≥ 85% are shown by the * symbol. Newly proposed genera are indicated by a † 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 [21].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 (proposed new genera *Almendravirus* and *Bahiavirus*) that comprise viruses isolated from mosquitoes. Reproduced from Walker *et al*. (2015) PLoS Pathogens 11 (2): e1004664[6].

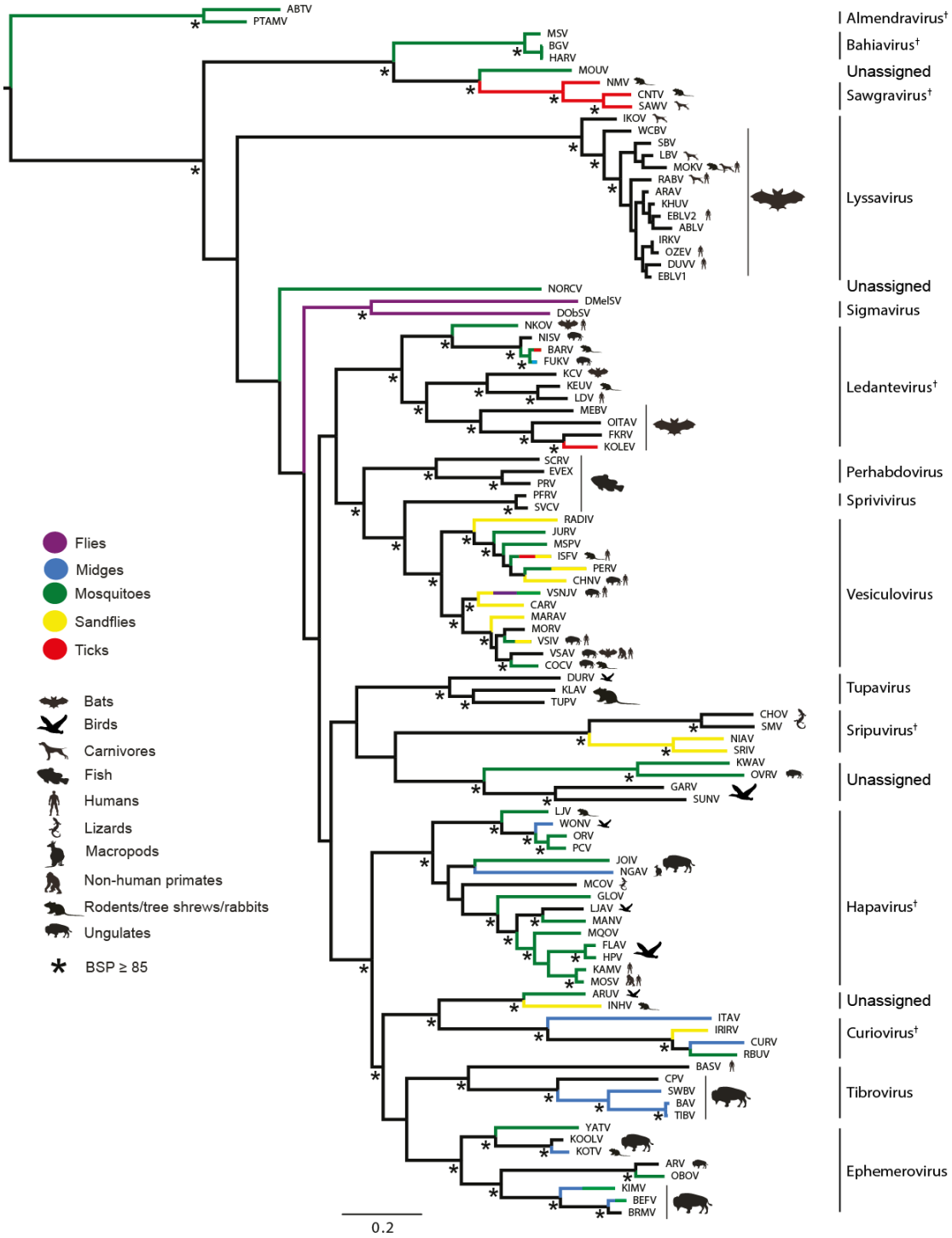

Figure 3. Ephemerovirus N protein amino acid sequence identity (as estimated in MEGA6 by p-distance).

	BEFV	BRMV	KIMV	MALV	ARV	OBOV	YATV	KOOLV	KOTV
BEFV	100								
BRMV	92.5	100							
KIMV	77.6	77.8	100						
MALV	77.4	78.1	98.8	100					
ARV	48.6	48.8	50.5	50.9	100				
OBOV	50.0	49.8	51.4	51.9	86.8	100			
YATV	45.5	46.2	47.2	47.2	41.3	38.7	100		
KOOLV	51.7	52.1	52.1	52.1	46.9	46.7	51.2	100	
KOTV	51.2	52.1	52.6	52.4	47.6	46.5	51.7	92.7	100