

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:	2012.003a-fV			(to be completed by ICTV officers)		
Short title: 1 new genus <i>Tupa</i> species (e.g. 6 new species in the genus . Modules attached (modules 1 and 9 are required)		ly <i>Rhabd</i> 1 ⊠ 6 □	oviridae, 2 🔀 7 🔀	containin 3 $\boxed{3}$ 8 $$	g 1 new ar 4 □ 9 ⊠	nd 1 moved

Author(s) with e-mail address(es) of the proposer:

Rhabdoviridae Study Group (contact: Dr. Ralf Dietzgen, e-mail: r.dietzgen@uq.edu.au)

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

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

Rhabdoviridae SG; ICTV EC

ICTV-EC or Study Group comments and response of the proposer:

Supported by the Rhabdoviridae SG.

Comments from the EC are addressed below:

- 1. It seems strange to derive a genus name from the name of a virus that does not form the type species.
- 2.

Answer: We still think that the genus name and type species should not be changed. Tupaia virus was described much earlier than Durham virus, and has been better studied. However, tupaia virus is restricted to cells of tree shrew (*Tupaia belangeri*) whereas Durham virus seems to be more "cosmopolitan". Therefore, we derived genus name from the tupaia virus (yet, we could not use name "Tupaiavirus", as there are other viruses described in the same animal), but designate Durham virus as the type species.

- 3. Species demarcation for the new genus is based upon distribution and host range and does not consider genetic distances, which are very different for each gene.
- 4.

Answer: As we explained in the proposal, at this time we have only two viruses, and it would be immature to establish a genetic distance demarcation based on these examples. As these viruses are clearly distinct ecologically/pathobiologically, we are confident with such demarcation for the moment. When other similar viruses are characterized, genetic distances and other characteristics will be employed for demarcation as well.

3. Inspection of the phylogenetic trees in this proposal led the EC to question whether the Rhabdoviridae SG may need to revisit the taxonomic structure of the entire family in order to make sure that the creation of new genera is justified.

Answer: We are not sure that we completely understand this statement, but we certainly disagree with an option to classify such ancient and diverse pathogens as rhabdoviruses based solely on phylogenetic reconstructions and genetic distances. We collectively agreed to use L gene sequences for rhabdovirus phylogeny on the family level, and therefore replaced the former trees with a new one.

Date first submitted to ICTV:	05/14/2012	
Date of this revision (if different to above):	05/29/2013	

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 20	91	2.003aV	(assigned by IC	TV offic	cers)
To create 1	l ne	ew species within:			
Comu		Tour minute (record)			in all that apply. the higher taxon has yet to be
Genu Subfamil		<i>Tupavirus</i> (new)		cr	eated (in a later module, below) write new) " after its proposed name.
Famil	y:	Rhabdoviridae			no genus is specified, enter
Orde	er:	Mononegavirales			nassigned" in the genus box.
And name	the	e new species:			GenBank sequence accession number(s) of reference isolate:
Durham v	iru	5			FJ952155 (complete genome)

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

- 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.
 - 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

Durham virus (DURV) was isolated from the brain of a moribund American coot (*Fulica americana*) in Durham County, North Carolina, in 2005. The bird was ataxic, unable to stand, and shortly died. Microscopic examination of the brain showed signs of meningitis and encephalitis. The other tissues examined did not have any significant lesions.

The virus was pathogenic for suckling mice via intracranial inoculation route, causing fatal encephalitis. However, peripheral (intraperitoneal) inoculation of adult mice and Syrian hamsters did not cause a disease but modulated serologic response.

In vitro host range analysis demonstrated that quail glial cells of the neuroretina (QNR/K2) and monkey kidney (Vero), cattle (CPAE), and bat (Tb 1 Lu) cells all supported replication of DURV, but duck embryo (PDE), fish (FHM), mosquito (C6/36), snake (VH-2), and turtle (TH-1) cell lines were either refractory or relatively non-permissive to infection.

In ultrathin sections of infected Vero cells, areas of massive virion formation could be observed in the cytoplasm. Rod-like virions 30–35 nm in diameter and 140–160 nm long were observed to be budding from the membranes into the expanded membrane-limited compartments formed by rough endoplasmic reticulum.

The negative-sense RNA genome of DURV is 11,265 nt long, encoding 3784 aa. It encompasses the five structural genes, found in all rhabdoviruses, but demonstrates particular relatedness to the tupaia virus (TUPV) genome, as it contains an additional gene coding for a small hydrophobic (SH) protein between the M and G genes, and an additional C gene within

the P gene ORF: 3'–N–P/C–M–SH–G–L-5' (Figure 1). The putative transcription start and stop/polyadenylation sequences are KUKY and NBACUUUUUUU (NBACU₇), respectively. The deduced intergenic region between each transcription unit is GA, similar to that of vesiculoviruses.

Phylogenetic analysis, performed on several different genome fragments, demonstrated consistent relatedness between DURV and TUPV. Relatedness to other viruses was limited and depended on the genome fragments compared (Figure 2).

MODULE 3: NEW GENUS

creating a new genus

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

Code	201	2.003bV	(assigned by IC	CTV 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 module, helpsu) write "(new)"
Fa	mily:	Rhabdoviridae		(in a later module, below) write "(new)" after its proposed name.
C	Order:	Mononegavirales		 If no family is specified, enter
				"unassigned" in the family box

naming a new genus

Code	2012.003cV	(assigned by ICTV officers)
To name tl	ne new genus: <i>Tupavirus</i>	

Assigning the type species and other species to a new genus

Code	2012.003dV	(assigned by ICTV officers)	
To designa	ate the following as the type sp	oecies of	the new genus
Durham vi	rus		Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered
•			created and assigned to it (Module 2) and any that

are being moved from elsewhere (Module 7b). Please enter here the TOTAL number of sp (including the type species) that the genus will contain: 2

Reasons to justify the creation of a new genus:

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

Tupaia virus (TUPV) was first described in 1984, and characterized in detail during subsequent years, with the complete genome sequenced and described in 2005. The unique characteristics of tupaia virus were the presence of an additional gene between the M and G genes, coding for a small hydrophobic (SH) protein, not known in other rhabdoviruses (Figure 1), an additional C gene within the P gene ORF and phylogenetic separation from other rhabdoviruses, which did not allow to include TUPV in any recognized genus. Based on these and other characteristics, the species *Tupaia virus* (not assigned to a genus) was created within the family *Rhabdoviridae* in 2009.

However, Durham virus (DURV), isolated in 2005 and extensively characterized in 2011, demonstrated the same genome organization asTUPV, including the presence of an SH gene between M and G genes, and an additional C gene within the P gene ORF (Figure 1). Furthermore, TUPV and DURV constitute a monophyletic cluster in phylogenetic trees (Figure 2).

Therefore, we propose the creation of a new genus, *Tupavirus*, which at present will include the newly proposed species *Durham virus* (see Module 2), and the unassigned *Tupaia virus*, moved to this genus (see Module 7).

Based on the N gene phylogeny, TUPV and DURV are mostly related to unclassified African viruses Kolongo (KOLV) and Sandjimba (SJAV), both isolated from birds. In other analysis, where fragments of the L gene were compared, TUPV and DURV demonstrated relatedness to

China fish rhabdovirus (CFRV), also known as Scophthalmus maximus rhabdovirus (SMRV). But these latter viruses need additional characterization before taxonomic assignment, and it is unclear whether any of them may be included in the future in the proposed genus *Tupavirus*.

Origin of the new genus name:

From the first described and characterized tupaia virus.

Reasons to justify the choice of type species:

DURV is better characterized than TUPV, and seems to be more "cosmopolitan", infecting a broader range of cell types *in vitro*. In contrast, TUPV appears restricted to the tree shrew (*Tupaia belangeri*) cell lines.

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.

To date, the only 2 available members of the proposed genus do not allow delineation of rigorous quantitative demarcation criteria (such as genetic distances and serologic cross-reactivity), as these viruses are quite divergent (identity values 57%, 16%, 8%, 20%, 25%, 25% and 51% for the N, P, C, M, SH, G and L proteins, respectively). The obvious demarcation between TUPV and DURV is their distribution and host ranges. The first was isolated from a tree shrew (*Tupaia belangeri*), imported from south-east Asia, and demonstrated ability to replicate in cells of this mammal only. In contrast, DURV was isolated in North America from an American coot (*Fulica americana*), and demonstrated ability to replicate in cells of a primate, cattle and bat origin, and obviously in the avian host [yet, except duck embryo cell line]).

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	2.003eV	(assigned by ICTV officers)		
To remo	To remove the following taxon (or taxa) from their present position:				
Tupaia v	v iru s				
The pres	sent ta	axonomic position of the	ese taxon/taxa:		
G	enus:	(unassigned)			
Subfa	mily:	(unassigned)	Fill in all that apply		
Fa	mily:	Rhabdoviridae	Fill in all that apply.		
C	Order:	Mononegavirales			
If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right					

Reasons to justify the removal:

Explain why the taxon (or taxa) should be removed

Based on specific characteristics of tupaia virus (TUPV), not present in other rhabdoviruses (see Module 3), the species *Tupaia virus* was created in the family *Rhabdoviridae* during 2009, without assignment to any specific genus.

However another virus, Durham virus (DURV), characterized in 2011, demonstrated the same genome organization and phylogenetic relatedness to TUPV. Therefore, we propose to create a new species for DURV, *Durham virus*, and assign both *Tupaia virus* and *Durham virus* into the newly proposed genus, *Tupavirus* (see Modules 2 and 3 above).

Part (b) re-assign to a higher taxon

2012.003fV (assigned by ICTV officers)		
the taxon (or taxa) listed	in Part (a) as follows:	
	Fill in all that apply.	
: Tupavirus (new)	 If the higher taxon has yet to be 	
:	created write " (new) " after its proposed name and complete	
: Rhabdoviridae	relevant module to create it.	
: Mononegavirales	If no genus is specified, enter " unassigned " in the genus box.	
	the taxon (or taxa) listed : Tupavirus (new) : Rhabdoviridae	

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.
 - 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

The proposed re-assignment to the newly proposed genus *Tupavirus* is based on genomic and phylogenetic similarities between TUPV and the other proposed member, DURV (see Part A above for justification). GenBank accession numbers for TUPV: NC_007020; AY840978 (complete genomes); for DURV: FJ952155 (complete genome).

MODULE 9: APPENDIX: supporting material

additional material in support of this proposal

References:

Kurz, W., H. Gelderblom, R. M. Flugel, and G. Darai. (1986). Isolation and characterization of a tupaia rhabdovirus. Intervirology. 25:88–96.

Springfeld, C., Darai, G., Cattaneo, R. (2005). Characterization of the tupaia rhabdovirus genome reveals a long open reading frame overlapping with P and a Novel gene encoding a small hydrophobic protein. J. Virol. 79, 6781–6790.

Allison AB, Palacios G, Travassos da Rosa A, Popov VL, Lu L, Xiao SY, DeToy K, Briese T, Lipkin WI, Keel MK, Stallknecht DE, Bishop GR, Tesh RB. (2011). Characterization of Durham virus, a novel rhabdovirus that encodes both a C and SH protein. Virus Res. 155(1):112-22.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.



Figure 1. Genome organization of TUPV and DURV.

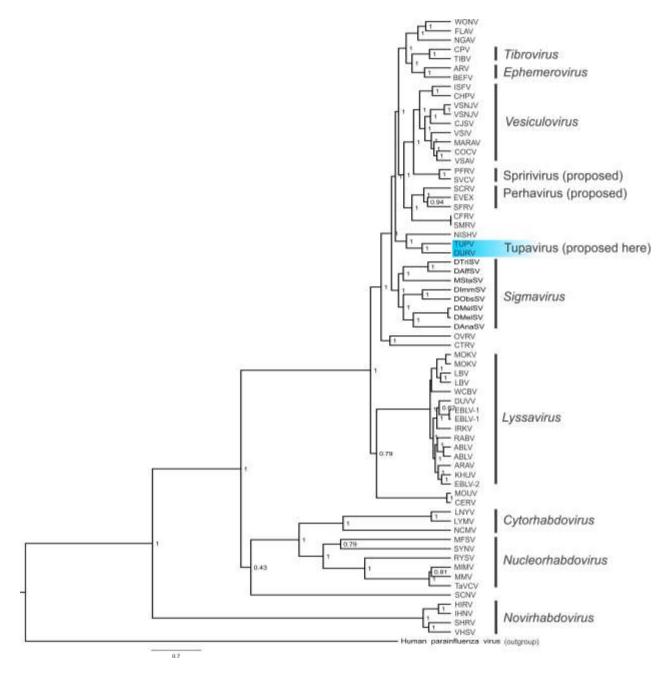


Figure 2. Bayesian tree of rhabdoviruses, based on an alignment of partial L gene sequences (\sim 1000 bp). Posterior probabilities are shown for the key nodes.