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:	***2015.007aM***	(to be completed by ICTV officers)

Short title: Create one (1) new species, *Alfalfa dwarf cytorhabdovirus*, in the genus *Cytorhabdovirus*, family *Rhabdoviridae*
(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** ☒

Author(s):

N. Bejerman, S. Lenardon and R.G. Dietzgen

Corresponding author with e-mail address:

Nicolas Bejerman; nicobejerman@gmail.com

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* Study Group

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

Date first submitted to ICTV:	June 15, 2015
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		(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Cytorhabdovirus***
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)**
Alfalfa dwarf cytorhabdovirus	alfalfa dwarf virus (ADV)	KP205452

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

This proposal suggests that *Alfalfa dwarf cytorhabdovirus* should be considered a distinct species in the genus *Cytorhabdovirus*, family *Rhabdoviridae.*

The two genera of plant-infecting rhabdoviruses are primarily distinguished on the basis of the sites of member virus replication and morphogenesis. Moreover, genus classification based on sequence diversity has thus far correlated 100% with classification by intracellular virus maturation [1]. Those members within the genus *Cytorhabdovirus* replicate in the cytoplasm of infected cells in association with masses of thread like structures (viroplasms). Virions bud in association with the endoplasmic reticulum (ER) and accumulate in ER-derived vesicles [1]. Within genera, members of distinct species are primarily differentiated by host range and vector specificity. Because the complete nucleotide sequence is available for only a few members this criterion is not presently sufficient for discrimination of species.

Biological data and the complete genome sequence of ADV support its assignment to a new species within the genus *Cytorhabdovirus*.

- Bullet shaped particles (390 x 90nm)
- Virions associated with the endoplasmic reticulum of phloem cells [2]
- Vectored by aphid *Aphis craccivora*
- Genome sequence distinct from that of other plant and animal rhabdoviruses: the complete sequence of 14,491 nucleotides negative-sense RNA genome is available (KP205452) and shows seven ORFs in anti-genomic strand in the order of putative proteins N, P, P3, M, G, P6, L [3], which is similar to the organization reported for the

cytorhabdovirus strawberry crinkle virus. Amino acid (aa) sequence comparisons between the deduced ADV proteins (except P protein) and the corresponding sequences of other plant rhabdoviruses (Table 3) revealed the closest relationships to cytorhabdoviruses, in particular persimmon virus A (PeVA) (the full genome data for strawberry crinkle virus (SCV) is not accessible) [3 and Annex, Table 1); however sequence identity to other rhabdoviruses at amino acid level is <50% for each protein [3 and Annex, Table 1).

- Phylogenetic analysis of the N protein (Annex, Figure1A) and the domain III of the L protein (Annex, Figure 1B) grouped ADV in a clade with all other sequenced cytorhabdoviruses, separate from the nucleorhabdovirus clade and closest to Persimmon virus A (PeVA) (Annex, Figure 1A) and SCV and raspberry vein chlorosis virus (RVCV) (Annex, Figure 1B). Furthermore, in both phylogenetic trees there are two clades in cytorhabdoviruses, one including all dicot-infecting viruses and another, which included two monocot-infecting viruses (Annex, Figure 1). In the genome of the later group (BYSMV and NCMV), multiple transcriptional units are present between the P and M genes, which are distinct from that of ADV and other known dicot cytorhabdoviruses.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. Dietzgen, R.G., Calisher, C.H., Kurath, G., Kuzmin, I.V., Rodriguez, L.L., Stone. D.M., Tesh, R.B., Tordo, N., Walker, P.J., Wetzel, T., Whitfield, A.E., 2011. Family *Rhabdoviridae*, in: King, A.M.Q, Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds), Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, Oxford, pp. 686-714.
2. Bejerman, N., Nome, C., Giolitti, F., de Breuil, S., Kitajima, E., Pérez Férnandez, J., Basigalup, D., Cornacchione, M., Lenardon, S., 2011. First report of a rhabdovirus infecting alfalfa in Argentina. Plant Disease. 95, 771.
3. Bejerman, N., Giolitti, F., de Breuil, S., Trucco, V., Nome, C., Lenardon, S., Dietzgen, R.G., 2015. Complete genome sequence and integrated protein localization and interaction map for alfalfa dwarf virus, which combines properties of both cytoplasmic and nuclear plant rhabdoviruses. Virology. 483, 275-283.

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.

Table 1. Amino acid sequence identities (%) of alfalfa dwarf virus proteins compared with those of other cytorhabdoviruses (**bold**) and selected nucleorhabdoviruses (taken from Table 3 in Bejerman *et al*., 2015)

	N	P	P3	M	G	P6	L
PeVA	31.4	19.7	18.5	17.4	29.5	-	43.1
LNYV	28.1	19.0	15.4	19.0	27.8	-	38.6
LYMoV	30.4	16.4	14.7	22.7	26.4	-	40.4
NCMV	20.7	14.7	16.0	18.5	20.3	-	29.1
BYSMV	19.9	19.1	15.0	20.6	19.4	-	28.6
RYSV	16.2	18.1	8.7	12.9	15.4	6.9	23.1
SYNV	16.9	17.5	11.9	9.0	16.8	-	24.1
PYDV	16.4	16.9	10.8	10.8	14.2	-	22.2

For virus acronyms and GenBank accession numbers see legend to Fig.1.

Figure 1 (taken from Figure 2 in Bejerman *et al*., 2015)

Neighbor-joining phylogenetic trees showing relationship of the amino acid sequences of the nucleocapsid protein (A) and domain III of the polymerase protein (B) of ADV and corresponding sequences of other plant rhabdoviruses. The viruses and their accession numbers are: barley yellow striate mosaic virus (BYSMV; KM213865), eggplant mottled dwarf virus (EDMV; NC_025389), lettuce yellow mottle virus (LYMoV; EF687738), lettuce necrotic yellows virus (LNYV; NC_007642); maize fine streak virus (MFSV; AY618417), maize Iranian mosaic virus (MIMV; DQ186554), maize mosaic virus (MMV; AY618418), northern cereal mosaic virus (NCMV; NC_002251), persimmon virus A (PeVA; NC_018381), potato yellow dwarf virus (PYDV; GU734660), raspberry vein chlorosis virus (RVCV; FN812699), rice yellow stunt virus (RYSV; NC_003746); sonchus yellow net virus (SYNV; L32603), strawberry crinkle virus (SCV; AY250986), taro vein chlorosis virus (TaVCV; AY674964). Bootstrap values of 1000 replicates are indicated at the branch points. The scale bar indicates substitutions per amino acid site.