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:	***2016.017aM***	(to be completed by ICTV officers)

Short title: One (1) new species in the genus *Cytorhabdovirus*, family *Rhabdoviridae*

(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 11 are required)	**2** ☒ **3** ☐ **4** ☐ **5** ☐ **6** ☐ **7** ☐ **8** ☐ **9** ☐ **10** ☐

Author(s):

Colleen M. Higgins, Nicolas Bejerman, Ming Li, Anthony P. James, Ralf G. Dietzgen, Michael N. Pearson, Peter A. Revill, Robert M. Harding

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Colleen Higgins; colleen.higgins@aut.ac.nz

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:

11 members have advised support for the proposal; 1 member has not responded.

Date first submitted to ICTV: 18 July, 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	***2016.017aM***	(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)**
Colocasia bobone disease-associated cytorhabdovirus	Colocasia bobone disease-associated virus (CBDaV)	KT381973

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 11

This proposal suggests that Colocasia bobone disease-associated virus (CBDaV) should be assigned to a distinct new species in the genus *Cytorhabdovirus,* family *Rhabdoviridae.*

The primary differentiation between cytorhabdoviruses and nucleorhabodviruses is the site within the host cell where virus replication and morphogenesis takes place. Classification of viruses in these genera has shown a 100% correlation between virus sequence and intracellular location of virus maturation (Dietzgen et al., 2011). Viruses assigned to the genus *Cytorhabdovirus* undergo cytoplasmic replication with budding of virions associated with the endoplasmic reticulum (ER); virions accumulate within the ER (Dietzgen et al., 2011). There are few coding-complete or complete genome sequences for plant rhabdoviruses. Species are generally distinguished on the basis of host range, vector specificity, and phylogeny.

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

- Colocasia bobone disease is found in taro (*Colocasia esculenta*) from Solomon Islands and Papua New Guinea. Thin section electron microscopy of infected leaves showed bacilliform virions (James et al., 1973, Pearson et al., 1999). Virions were present in samples from Solomon Islands but not from Fiji or Vanuatu (Pearson et al, 1999).
- Bullet shaped particles (269 nm (SD=31) x 73 nm (SD=6)) were associated with severe stunting and gall formation, symptoms associated with bobone disease. These particles are the larger rhabdovirion-like particles found in plants infected with bobone or alomae

diseases, whereas smaller particles were identified to stem from infection with taro vein chlorosis virus (TaVCV, a nucleorhabdovirus). The putative virus producing the larger particles was named Colocasia bobone disease virus (CBDV) (Pearson et al., 1999).

- NOTE: CBDaV is likely the causative agent of Bobone disease, but Koch's postulates have not been fulfilled and therefore the name Colocasia bobone disease-associated virus (CBDaV) was chosen. Until the causative link can be established, this virus will be known as CBDaV. Thus, EM evidence, virion particles and serological evidence associated with CBDV are assumed to be that of the sequenced CBDaV.
- CBDaV virion particles are distinguishable in size from TaVCV.
- Virus particles were observed in cytoplasm of phloem sieve tubes, in companion cells, and in the mesophyll. Particles were not found within cell nuclei but occasionally in the perinuclear region. Virus particles were associated with cytoplasmic membranes. Although viral aggregates occurred in the perinuclear space, there is no evidence that particles bud through the inner nuclear membrane (James et al., 1973).
- The virus is serologically distinct from another rhabdovirus that infects taro, TaVCV (Pearson et al., 1999).
- The genome sequence of this virus was determined during RNA-seq analysis of Solomon Island taro plants known to have had bobone disease and that were also infected with the potyvirus Dasheen mosaic virus (DsMV). This sequence was not found in taro plants infected with DsMV that were known never to have had bobone disease (Higgins et al., 2016).
- CBDV is transmitted by the taro planthopper *Tarophagus proserpina.*
- The genome sequence of CBDaV is distinct from that of other sequenced plant and animal rhabdoviruses: the complete sequence of 12,193 nt negative-sense RNA genome is available (KT381973) and has six ORFs in the anti-genomic strand equivalent to putative (3′ to 5′) N, P, P3, M, G, and L genes. Figure 1 shows the gene junction regions of CBDaV compared with those of other rhabdoviruses; an example of the distinctiveness of the CBDaV genome. Table 1 shows the differences in genome and ORF lengths between CBDaV and other monopartite plant rhabdoviruses (Higgins et al., 2016).
- Based on deduced amino acid sequence identity, CBDaV is most closely related to the respective proteins of the cytorhabdovirus barley yellow striate mosaic virus (BYSMV). Nucleotide identities with BYSMV ORFs are: N: 50.3%, P: 46.5%, P3: 48.2%, M: 46.9%, G: 44.5%, L: 47.0%). Amino acid identities with BYSMV are: N: 33.5%, P: 23.5%, P3: 29.3%, M: 24.1%, G: 26.3%, L: 53.5%) (Table 2) (Higgins et al., 2016).
- Phylogenetic analysis of the whole genome and each ORF grouped CBDaV in a clade with all other sequenced cytohabdoviruses, separate from the nucleohabdovirus clade. Its closest relatives appear to be BYSMV and NCMV, which however feature additional accessory genes in their genomes. A ML tree of L ORF nucleotide sequences is shown in Figure 2 (Higgins et al., 2016).

MODULE 11: **APPENDIX**: supporting material

additional material in support of this proposal

References:

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. 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.* Oxford: Elsevier.

HEIM, F., LOT, H., DELECOLLE, B., BASSLER, A., KRCZAL, G. & WETZEL, T. 2008. Complete nucleotide sequence of a putative new cytorhabdovirus infecting lettuce. *Archives of Virology,* 153**,** 81-92.

HIGGINS, C. M., BEJERMAN, N., LI, M., JAMES, A. P., DIETZGEN, R. G., PEARSON, M. N., REVILL, P. A. & HARDING, R. M. 2016. Complete genome sequence of Colocasia bobone disease-associated virus, a putative cytorhabdovirus infecting taro. *Archives of Virology,* 161**,** 745-748.

JAMES, M., KENTEN, R. H. & WOODS, R. D. 1973. Virus like particles associated with two diseases of Colocasia esculenta (L.) Schott in the Solomon Islands. *Journal of General Virology,* 21**,** 145-153.

PEARSON, M. N., JACKSON, G. V. H., SAELEA, J. & MORAR, S. G. 1999. Evidence for two rhabdoviruses in taro (Colocasia esculenta) in the Pacific region. *Australasian Plant Pathology,* 28**,** 248-253.

REVILL, P., TRINH, X., DALE, J. & HARDING, R. 2005. Taro vein chlorosis virus: Characterization and variability of a new nucleorhabdovirus. *Journal of General Virology,* 86**,** 491-499.

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.

CBDaV	AUUCUUUUU	GGN_n	CUC
BYSMV	AUUAUUUUU	GA	CUC
NCMV	AUUCUUUUU	GACU	CUA
LYMoV	AUUCUUUU	GN_n	CUN
LNYV	AUUCUUUUU	GN_n	CUA
TaVCV	AUUCUUUUU	GG	GUU
MMV	AUUCUUUUU	GG	GUU
RYSV	AUUAUUUUU	GG	UUG
SYNV	AUUCUUUUU	GG	UUG
MFSV	UUUAUUU	GUAG	UUG
	3′end	IS	5′end

Figure 1: Consensus sequences of the gene junction regions of CBDaV compared with other monopartite plant rhabdoviruses (Heim et al., 2008, Revill et al., 2005). (Figure taken from Higgins et al. (2016)).

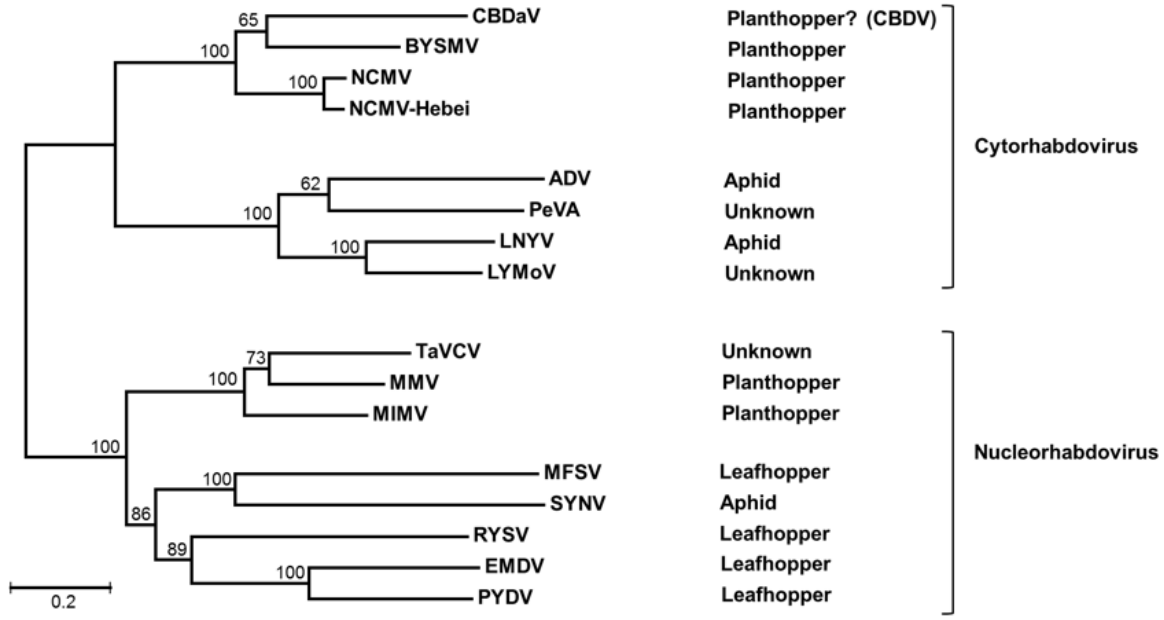

Figure 2: Maximum likelihood analysis using the GTR+G+I model of monopartite plant rhabdovirus L polymerase open reading frame nucleotide sequences. The tree is rooted at the midpoint, the insect vectors for each virus are shown while nucleorhabdovirus and cytorhabdovirus clades are indicated by brackets. Bootstrap values greater than 50 are shown for the major nodes and the scale indicates the number of substitutions per site (Figure (revised) taken from Higgins et al. (2016)).

Table 1. Comparison of the nucleotide sequence lengths of the CBDaV genome and ORFs with the equivalent regions of other cytorhabdoviruses and example nucleorhabdoviruses (Table taken from Higgins et al., 2016).

Virus	Whole genome	3' Leader[1]	Nucleo capsid protein	Phospho protein	P3 [P4, P5, P6]	Matrix protein	Glyco protein	P6/P9	L Polymerase	5' Trailer[2]
CBDaV	**12193**	***177***	**1269**	**843**	**579**	**513**	**1512**	**N/A**	**6207**	***260***
LNYV	12807	*168*	1379	902	908	533	1655	N/A	6206	*244*
LYMoV	12926	*163*	1358	920	935	527	1646	N/A	6206	*259*
PeVA	13467	*128*	1352	938	668	566	1682	N/A	6269	*233*
ADV	14491	*174*	1446	936	723	573	1695	198	6258	*335*
BYSMV	12706	*157*	1284	888	516 [375, 240, 303]	501	1437	156	6171	*360*
NCMV	13222	*141*	1295	860	518 [344,377, 368]	524	1451	N/A	6176	*306*
NCMV-Hebei	13221	*141*	1295	860	518 [344, 377, 368]	524	1451	N/A	6176	*305*
TaVCV	12020	140	1509	816	864	708	1767	N/A	5787	61
PYDV	12875	149	1419	843	858	898	1892	N/A	5796	97

[1] Numbers in italics may include 5'UTR of N gene and gene junction sequences
[2] Numbers in italics may include 3'UTR of L gene.

Table 2. Comparison of nucleotide and amino acid sequences of the CBDaV genome and ORFs with equivalent regions of other cytorhabdoviruses and example nucleorhabdoviruses (Table taken from Higgins et al., 2016).

Virus	Nucleotide sequence % identity									Amino acid sequence % identity					
	Whole genome	3' Leader	Nucleo capsid protein	Phospho protein	P3	Matrix protein	Glyco protein	L Polymerase	5' Trailer	Nucleo capsid protein	Phospho protein	P3	Matrix protein	Glyco protein	L Polymerase
LNYV	44.4	39.8	44.6	42.8	35.2	42.7	43.1	46.1	38.2	22.0	17.2	12.6	19.6	19.1	28.8
LYMoV	44.6	41.4	43.7	43.3	34.4	43.2	41.2	46.9	46.2	22.5	12.8	12.8	15.6	18.0	28.2
PeVA	44.1	40.8	43.1	43.1	42.1	36.4	42.9	46.9	46.6	20.7	15.6	14.7	15.0	18.9	29.0
ADV	42.3	42.2	39.9	43.8	41.6	44.4	42.7	47.0	46.6	20.0	19.3	14.0	14.7	18.9	28.6
BYSMV	50.3	46.5	48.2	46.9	44.5	47.0	45.7	57.3	44.3	33.5	23.5	29.3	24.1	26.3	53.5
NCMV	48.9	44.5	48.7	49.0	42.3	47.1	45.4	56.5	47.9	33.7	25.6	24.2	21.2	26.1	52.2
NCMV-Hebei	48.9	45.3	49.2	48.1	42.3	47.6	44.4	56.5	45.7	33.0	25.3	24.2	21.2	27.1	52.2
TaVCV	42.6	42.0	40.8	38.5	38.0	38.3	39.4	44.0	23.4	19.3	14.9	12.2	15.8	16.3	23.4
PYDV	41.6	40.7	42.0	42.9	38.3	36.2	40.6	43.7	30.2	18.1	16.2	11.7	15.0	14.9	22.5