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:	***2014.003a-dV***	(to be completed by ICTV officers)

Short title: Create 2 new species, *Orchid fleck dichorhavirus* and *Coffee ringspot dichorhavirus*, in a new genus, *Dichorhavirus*, in the family *Rhabdoviridae*

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

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

The ICTV *Rhabdoviridae* Study Group:

Walker, Peter	Chair	Australia	Peter.Walker@csiro.au
Blasdell, Kim	Member	Australia	kim.blasdell@csiro.au
Calisher, Charlie H.	Member	USA	calisher@cybersafe.net
Dietzgen, Ralf G.	Member	Australia	r.dietzgen@uq.edu.au
Kondo, Hideki	Member	Japan	hkondo@rib.okayama-u.ac.jp
Kurath, Gael	Member	USA	gael_kurath@usgs.gov
Longdon, Ben	Member	UK	b.longdon@ed.ac.uk
Stone, David	Member	UK	david.stone@cefas.co.uk
Tesh, Robert B.	Member	USA	rtesh@UTMB.EDU
Tordo, Noël	Member	France	ntordo@pasteur.fr
Vasilakis, Nikos	Member	USA	nivasila@utmb.edu
Whitfield, Anna	Member	USA	aewtospo@ksu.edu

And the ICTV *Mononegavirales* Study Group:

Kuhn, Jens H.	Chair	USA	kuhnjens@mail.nih.gov
Dietzgen, Ralf G.	Member	Australia	r.dietzgen@uq.edu.au
Easton, Andrew J.	Member	UK	A.J.Easton@warwick.ac.uk
Kurath, Gael	Member	USA	gael_kurath@usgs.gov
Nowotny, Norbert	Member	Austria	NorbertNowotny@gmx.at
Rima, Bertus K.	Member	Northern Ireland, UK	b.rima@qub.ac.uk
Rubbenstroth, Dennis	Member	Germany	Dennis.Rubbenstroth@uniklinik-freiburg.de

Vasilakis, Nikos	Member	USA	nivasila@utmb.edu
Walker, Peter	Member	Australia	Peter.Walker@csiro.au

and:
Anna N. Clawson, logosconsult@gmail.com
Juliana Freitas-Astúa, juliana.astua@embrapa.br
Michael M. Goodin, mgoodin@uky.edu
Elliott W. Kitajima, ewkitaji@usp.br
Thierry Wetzel, thierry.wetzel@agroscience.rlp.de
Anna E. Whitfield, aewtospo@k-state.edu

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 *Bornaviridae*, *Filoviridae*, *Nyamiviridae*, and *Paramyxoviridae* Study Groups

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

Date first submitted to ICTV:	June 2014
Date of this revision (if different to above):	June 15, 2015

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	***2014.003aV***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Dichorhavirus* (new)**
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:**	**GenBank sequence accession number(s)**
Orchid fleck dichorhavirus	orchid fleck virus (OFV) isolate So	AB244417 (RNA1) AB244418 (RNA2)
Coffee ringspot dichorhavirus	coffee ringspot virus (CoRSV) isolate Lavras	KF812525 (RNA1) KF812526 (RNA2)

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

Orchid fleck virus (OFV) and coffee ringspot virus (CoRSV) are unassigned negative-sense, single-stranded (−)ssRNA plant viruses that were previously suggested to be included in the family *Rhabdoviridae*, order *Mononegavirales* [1]. Although both viruses share biological characteristics, including nuclear cytopathological effects, gene order, and significant sequence similarities, with nucleorhabdoviruses, their taxonomic status was unclear because unlike all mononegaviruses so far, OFV and CoRSV have segmented, bipartite genomes and their particles are generally not enveloped.

This proposal provides available biological, physico-chemical, and nucleotide sequence evidence that indicates that OFV and CoRSV are related, but distinct, *Brevipalpus* mite-transmitted short bacilliform (−)ssRNA viruses and should be classified taxonomically in novel species in a new genus *Dichorhavirus* [1]. On the other hand, the closely related citrus leprosis virus nuclear type (CiLV-N) and citrus necrotic spot virus (CNSV) appear to be strains of OFV (see Appendix, Module 9).

The new species and genus were initially proposed in 2014 and the ICTV EC supported this classification, but asked for the ICTV *Rhabdoviridae* SG to consider placing the genus *Dichorhavirus* in the family *Rhabdoviridae* (initially a free-floating genus was proposed). The SG, together with the ICTV *Mononegavirales*, agrees with the EC and supports including the bipartite genome dichorhaviruses as members of the family *Rhabdoviridae*.
This proposal is based on growing evidence that segmentation and particle morphology are relatively flexible characteristics in evolutionary terms, and the clear phylogenetic relationship with rhabdoviruses, and the similar gene order and replication strategies that provide a logical fit with this family.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2014.003bV***	(assigned by ICTV officers)

To create a new genus within:

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 family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2014.003cV***	(assigned by ICTV officers)

To name the new genus: ***Dichorhavirus***

Assigning the type species and other species to a new genus

Code	***2014.003dV***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Orchid fleck dichorhavirus

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

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:**

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

Dichorhavirions differ from bona fide rhabdovirions by being smaller in size and not usually being enveloped. In addition, the genomes of dichorhaviruses are composed of bipartite, negative-sense, single-stranded RNAs rather than of monopartitite, negative-sense, single-stranded RNAs.

Origin of the new genus name:

Rhabdo-like viruses (*rha*) whose genomes are split in two (*Dicho*).

Reasons to justify the choice of type species:

OFV is the best studied and best-known of the dichorhaviruses and the first to have its complete genome sequenced and detailed molecular and genetic analysis completed.

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.

Genome sequence difference of >25% in the L gene nucleotide sequence (Dietzgen et al. 2014).

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

[1] Dietzgen RG, Kuhn JH, Clawson AN, Freitas-Astúa J, Goodin MM, Kitajima EW, Kondo H, Wetzel T, Whitfield AE (2014) *Dichorhavirus*: a proposed new genus for *Brevipalpus* mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA plant viruses. *Arch Virol* 159:607-619 (available from http://link.springer.com/article/10.1007/s00705-013-1834-0**)**

[2] Kondo H, Maruyama K, Chiba S, Andika IB, Suzuki N (2014) Transcriptional mapping of the messenger and leader RNAs of orchid fleck virus, a bisegmented negative-strand RNA virus. *Virology* 452: 166-174.

[3] Kondo H, Chiba S, Andika IB, Maruyama K, Tamada T, Suzuki N (2013) Orchid fleck virus structural proteins N and P form intranuclear viroplasm-like structures in the absence of viral infection. *J Virol* 87:7423-7434.

[4] Peng DW, Zheng GH, Zheng ZZ, Tong QX, Ming YL (2013) Orchid fleck virus: an unclassified bipartite, negative-sense RNA plant virus. *Arch Virol* 158:313-323.

[5] Kondo H, Maeda T, Shirako Y, Tamada T (2006) Orchid fleck virus is a rhabdovirus with an unusual bipartite genome. *J Gen Virol* 87:2413-2421.

[6] Boari AJ, Freitas-Astúa J, Ferreira PTO, Neder DG, Nogueira NL, Rossi ML, Kitajima EW (2004) Purification and serology of the Coffee ringspot virus. *Summa Phytopathologica* 30:453-458

[7] Roy A, Stone A, Otero-Colina G, Wei G, Choudhary N, Achor D, Shao J, Levy L, Nakhla MK, Hollingsworth CR, Hartung JS, Schneider WL, Brlansky RH (2013) Genome assembly of citrus leprosis virus nuclear type reveals a close association with orchid fleck virus. *Genome Announc* 1(4). Doi.10.1128/genomeA.

[8] Roy A, et al. (2015) Identification and molecular characterization of nuclear *Citrus leprosis virus*, a member of the proposed *Dichorhavirus* genus infecting multiple *Citrus* species in Mexico. *Phytopathology* 105: 564-575.

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.

Appendix 1: Comparison of the genome organization of OFV-So, a member of the proposed genus "*Dichorhavirus*", and representative members of other genera harboring plant-infecting viruses, such as *Nucleorhabdovirus* and *Cytorhabdovirus* (both family *Rhabdoviridae*) and *Varicosavirus* (separate proposal to move genus into *Rhabdoviridae*).

Appendix 2: Characteristics of dichorhaviruses in comparison to nucleorhabdoviruses.

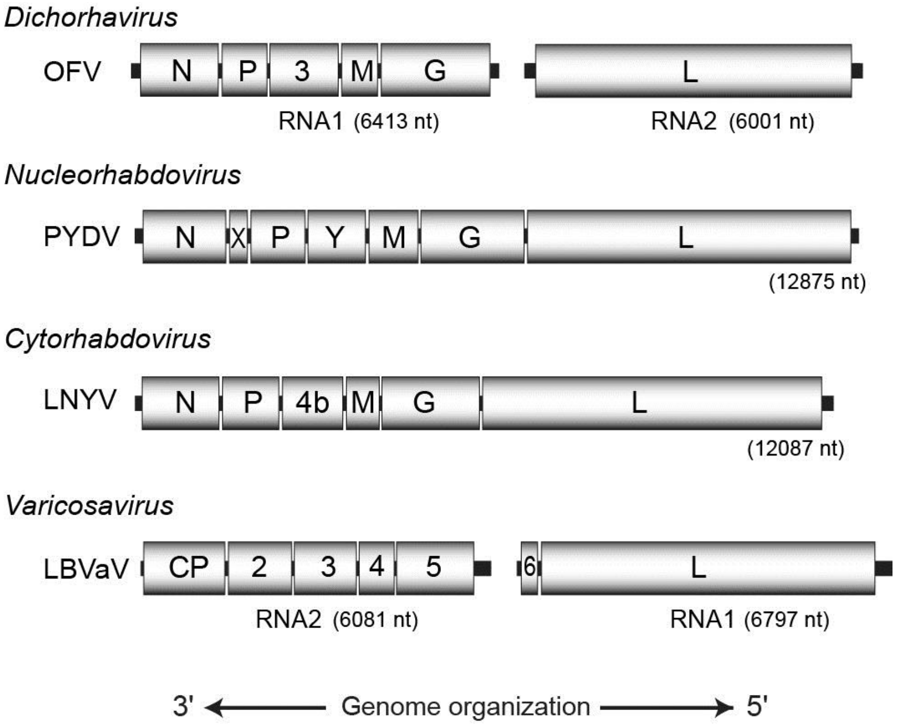
Appendix 3: Comparison of genome size and encoded proteins of dichorhaviruses and selected plant (-) ssRNA viruses.

Appendix 4: Amino acid sequence similarity comparison between OFV-So, CoRSV and plant rhabdoviruses.

Appendix 5: Phylogenetic relationship of OFV and CoRSV to selected mononegaviruses and a varicosavirus.

Appendix 1

Comparison of the genome organization of OFV-So, a member of the proposed genus "*Dichorhavirus*", OFV-So and representative members of other genera harboring of plant-infecting viruses, such as *Nucleorhabdovirus* and *Cytorhabdovirus* (both family *Rhabdoviridae*) and unassigned genus *Varicosavirus* (free-floating). OFV RNA1 encodes 49 kDa (ORF1: nucleocapsid protein N), 26 kDa (ORF2: putative phosphoprotein P), 38 kDa (ORF3: putative movement protein), 20 kDa (ORF4: putative matrix protein M) and 61 kDa (ORF5: putative glycoprotein G) proteins, and RNA2 encodes a single protein of 212 kDa (ORF6: large polymerase protein L). Virus names and GenBank/Refseq accession numbers are as follows: proposed genus "*Dichorhavirus*" - orchid fleck virus (OFV; RNA1: NC_009608, RNA2: NC_009609), *Nucleorhabdovirus* - potato yellow dwarf virus (PYDV; NC_016136), *Cytorhabdovirus* - lettuce necrotic yellows virus (LNYV; NC_007642), *Varicosavirus* - lettuce big-vein associated virus (LBVaV: NC_011558, RNA2: NC_011568). The genomic locations in the 3'-5' negative-sense arrangement are shown, as well as the relative sizes of the viral genes.



Appendix 2: Characteristics of proposed dichorhaviruses in comparison to nucleorhabdoviruses

	orchid fleck virus	**coffee ringspot virus**	**citrus leprosis virus nuclear type,** **syn. citrus necrotic spot virus** (OFV strain)	**potato yellow dwarf virus (a nucleorhabdovirus)**
Virus abbreviation	OFV	CoRSV	CiLV-N, CNSV	PYDV
Virion size (leaf dip)	40 x 100-150 nm (short bacilliform or bullet-shaped)	40 x 100-110 nm (short bacilliform)	40-50 x 100-110 nm (short bacilliform)	75 x 380 nm (long bacilliform)
Presence of lipid envelope	No [a]	No	No	Yes
Viroplasms (replication) in	Nucleus	Nucleus	Nucleus	Nucleus
Symptoms	Chlorotic or necrotic ringspots and 'systemic' flecks	Ringspots on leaves, twigs and berries	Lesions on fruits, leaves, and twigs	Vein clearing, stunting, leaf malformation, mosaic
Genome type	Bipartite, (-)ssRNA	Bipartite, (-)ssRNA	Bipartite, (-)ssRNA	Monopartite (-)ssRNA
Genome sequence availability and GenBank accession number	RNA1 (≈6.4 kb): AB244417 (So); AB516442 (NHHS1) RNA2 (≈6.0 kb): AB244418 (So); AB516441 (NHHS1)	RNA1: ≈6.4 kb KF812525 (Lavras) RNA2: ≈6.0 kb KF812526 (Lavras)	RNA1: ≈6.4 kb KF209275 (CiLV-N) KF198064 (CNSV Jal-1) RNA2: ≈6.0 kb KF209276 (CiLV-N) KF198065 (CNSV Jal-1)	12,875 nt (full length genome): NC_016136
Sequence similarity with	Nucleorhabdoviruses, CiLV-N, CoRSV	Nucleorhabdoviruses, OFV, CiLV-N	Strain of OFV (>90% nt sequence identity)	Nucleorhabdoviruses

Arthropod vector	False spider mite *Brevipalpus californicus* Banks, 1904, *B. phoenicis* Geijskes, 1939	False spider mite *B. phoenicis* Geijskes, 1939	False spider mite *B. californicus* Banks, 1904	Leafhopper *Aceratagallia sanguinolenta* Provancher, 1872
Transmission mode	Propagative & persistent [b]	Propagative & persistent [b]	Propagative & persistent [b]	Propagative & persistent

[a] OFV virions do not appear to acquire a lipid envelope. However, some enveloped virions are found in the cytoplasm

[b] Transmission electron microscopy (presence of viroplasms in the mite) and RT-qPCR evidence suggest replication in the mite vector

Appendix 3: Comparison of genome size and encoded proteins of dichorhaviruses and nucleorhabdoviruses

Genus	Virus name (abbreviation)	Genome size (nt)	Proteins (kDa) ORF # (putative function/denomination)						
			1 (N/CP)*	X*	2 (P)	3 (MP)	4 (M)	5 (G)	6 (L)
Nucleorhabdovirus	potato yellow dwarf virus (PYDV)	Non-segmented 12,875	52	9.7	31	33	29	70	220
“*Dichorhavirus*”	orchid fleck virus (OFV)	RNA 1: 6413 RNA 2: 6001	49	-	26	38	20	61	212
	coffee ringspot virus (CoRSV)	RNA 1: 6552 RNA 2: 5595	49	-	27	36	20	60	212

* A protein of unknown function; N/CP indicates the different naming of ORF #1 in the listed genera; -: not present.

Appendix 4: Amino acid sequence similarity (%) of OFV-So with CoRSV and plant rhabdoviruses

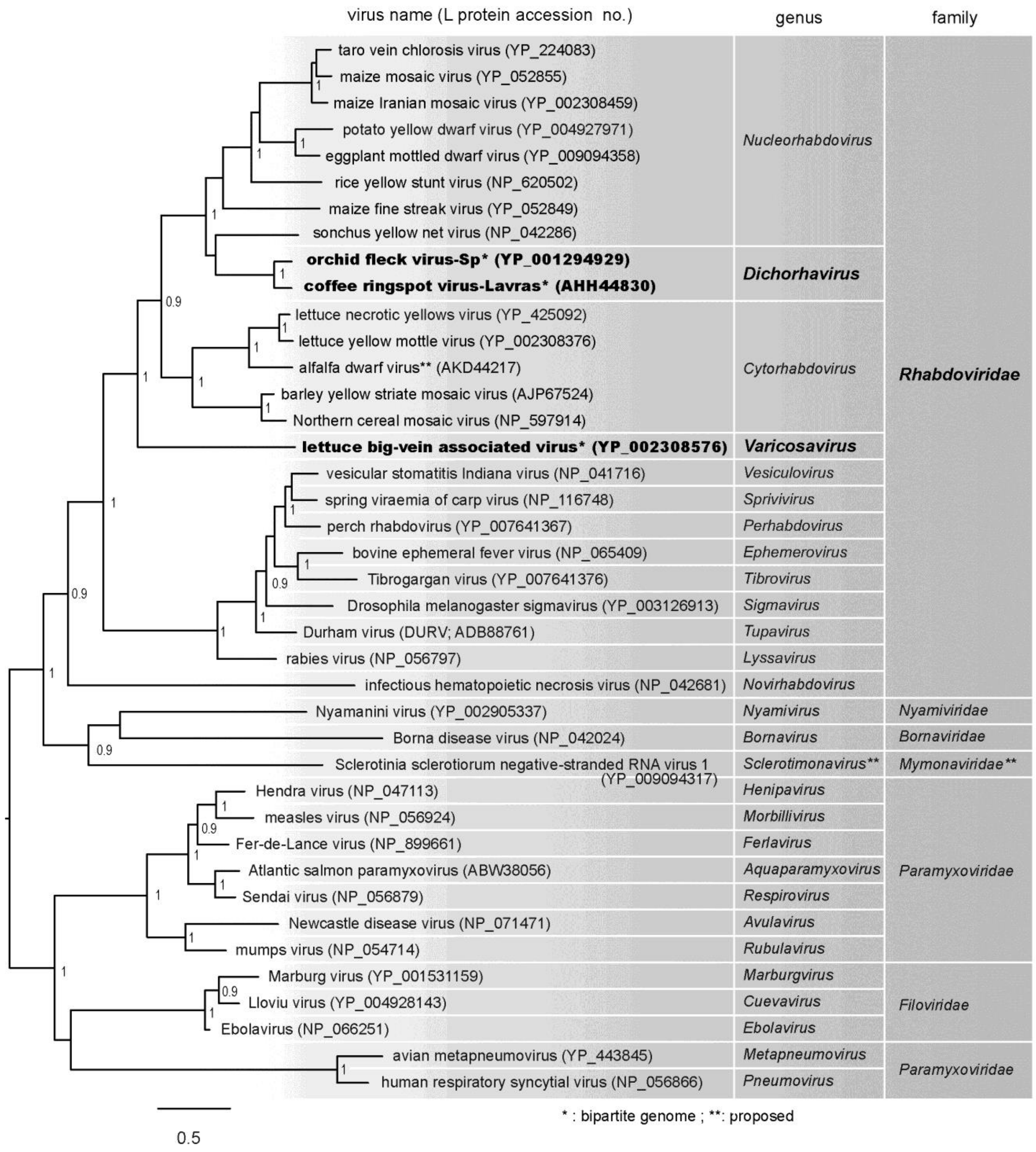
Genus	**Virus name (accession number)**	**Virus abbreviation**	**N protein**	**G protein**	**L protein**
"*Dichorhavirus*"	citrus leprosis virus nuclear type (OFV strain)	CiLV-N	98 (0.0)	93 (0.0)	98 (0.0)
	coffee ringspot virus	CoRSV	51 ($4e^{-148}$)	32 ($2e^{-88}$)	58 (0.0)
Cytorhabdovirus	lettuce necrotic yellows virus (AJ867584)	LNYV	24 ($2e^{-8}$)[a]	23 (7.7)	31 ($3e^{-155}$)
	northern cereal mosaic virus (AB030277)	NCMV	ND[b]	ND	29 ($2e^{-120}$)
Nucleorhabdovirus	maize fine streak virus (AY618417)	MFSV	27 ($8e^{-25}$)	ND	38 (0.0)
	maize mosaic virus (AY618418)	MMV	27 ($1e^{-23}$)	21 ($8e^{-6}$)	38 (0.0)
	potato yellow dwarf virus (GU734660)	PYDV	27 ($3e^{-23}$)	23 ($5e^{-9}$)	38 (0.0)
	rice yellow stunt virus (AB011257)	RYSV	27 ($9e^{-25}$)	25 ($7e^{-16}$)	36 (0.0)
	sonchus yellow net virus (L32603)	SYNV	24 ($5e^{-17}$)	21 (3.3)	34 (0.0)
	maize Iranian mosaic virus (DQ186554)	MIMV	27 ($3e^{-21}$)	25 (0.003)	35 (0.0)
	taro vein chlorosis virus (AY674964)	TaVCV	29 ($5e^{-27}$)	21 ($1e^{-4}$)	34 (0.0)

[a] % identity (E-value); BLAST N searches were limited to virus (taxid 10239) entries

[b] Not detectable

Appendix 5

Phylogenetic relationship of OFV and CoRSV to selected mononegaviruses and a varicosavirus. A maximum-likelihood tree was constructed using PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/) based on a multiple amino acid sequence alignment of the entire L polymerase. Ambiguously aligned regions were removed using Gblocks 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) with the all options of less stringent selection. The best-fit model LG+I+G+F was selected using ProtTest ver. 2.4 (http://darwin.uvigo.es/software/prottest2_server.html). Virus names and GenBank/Refseq accession numbers of the L polymerase sequences are shown. The members of the proposed genus "*Dichorhavirus*" and the genus *Varicosavirus* are indicated by black bold letters. Numbers at the nodes represent aLRT values derived using an SH like calculation (only values greater than 0.9 are shown).



* : bipartite genome ; **: proposed