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Dichorhavirus: a proposed new genus for *Brevipalpus* mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA plant viruses

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Abstract Orchid fleck virus (OFV) is an unassigned negative-sense, single-stranded (−)ssRNA plant virus that was previously suggested to be included in the family *Rhabdoviridae,* order *Mononegavirales*. Although OFV shares some biological characteristics, including nuclear cytopathological effects, gene order, and sequence similarities, with nucleorhabdoviruses, its taxonomic status is unclear because unlike all mononegaviruses, OFV has a segmented genome and its particles are not enveloped. This article analyses the available biological, physico-chemical, and nucleotide sequence evidence that seems to indicate that OFV and several other *Brevipalpus* mite-transmitted short bacilliform (−)ssRNA viruses are likely related and may be classified taxonomically in novel species in a new free-floating genus *Dichorhavirus*.

Abbreviations

adj.	*adjectivum* (adjective)
fam. nov.	*familia nova* (new family)
fem.	*femininum*
gen. nov.	*genus novum* (new genus)
geo.	geographic
IPA	International Phonetic Alphabet
Lat.	Latin
n.	*nomen substantivum* (noun)
Neo-Lat.	Neo-Latin
neut.	*neutrum*
pl.	*numerus pluralis* (plural)
sg.	*numerus singularis* (singular)
sp. nov.	*species nova* (new species)
suff.	suffix

Introduction

There are more than 60 large bacilliform or bullet-shaped plant viruses that have not been characterized much beyond

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electron microscopic visualization of their virions in infected plant tissues [19]. Due to their characteristic virion shapes, which at least superficially resemble rhabdoviruses, they had been considered potential rhabdoviruses [19]. At least three of these 60 viruses, citrus leprosis virus (CiLV), coffee ringspot virus (CoRSV), and orchid fleck virus (OFV), are transmitted by false spider mites of the genus *Brevipalpus* Donnadieu, 1875 (Arthropoda: Arachnida: Acari: Trombidiformes: Tenuipalpidae). The 1st and 2nd Reports of the International Committee on Taxonomy of Viruses (ICTV) did not list any of these three viruses [15, 55]. The 3rd Report listed OFV as an ungrouped plant rhabdovirus [38]. In the 4th Report, CiLV, CoRSV, and OFV were recognized as ungrouped rhabdoviruses ("*Citrus* leprosis 'rhabdovirus'", "coffee ringsport 'rhabdovirus'", and "orchid fleck 'rhabdovirus'", respectively) [39]. In the 5th Report, CiLV and OFV were considered "possible" members, and CoRSV a "probable" member, of the "plant rhabdovirus group" [17]. The 6th Report considered all three viruses "possible" members of the family *Rhabdoviridae* and listed them as "unassigned plant rhabdoviruses" [56]. In the 7th Report, CiLV and CoRSV remained listed as "unassigned plant rhabdoviruses" [53], but OFV was removed from the rhabdovirus section and classified as an "unassigned plant virus" [5]. This classification was upheld for all three viruses in the 8th Report [40, 49]. Further studies on citrus leprosis disease revealed that CiLV can be differentiated into two different viruses: the cytoplasmic type CiLV-C and the nuclear type CiLV-N [2]. In the latest, 9th ICTV Report, CiLV-C is listed as a member of the novel genus *Cilevirus* [37]; CiLV-N and CoRSV are no longer listed [21], and OFV is listed as an unclassified virus [1].

As a group, CiLV-N, CoRSV, and OFV produce virions that have similar particle morphology clearly different from bona fide rhabdovirions. In addition, the genomes of CiLV-N, CoRSV, and OFV are all composed of bipartite negative-sense, single-stranded (−)ssRNAs. This contradicts the current member classification criteria for the order *Mononegavirales*, which exclusively groups viruses with single-stranded monopartite negative-strand RNA genomes [14, 42, 43]. If the order demarcation criteria were modified to "have predominantly non-segmented genomes", then these CiLV-N, CoRSV, and OFV viruses could be considered as possible order members. However, a second characteristic of mononegavirions is that they have lipid envelopes – a feature which CiLV-N, CoRSV, and OFV particles lack, although virions appear to at least associate with cellular membranes. Based on sequence similarities of nucleocapsid protein, putative glycoprotein (G) and RNA-dependent RNA polymerase (L) genes with those of nucleorhabdoviruses (genus *Nucleorhabdovirus*), OFV has been suggested as a member of a new species to be included in a new rhabdoviral genus (*Dichorhavirus*) [29] or as a member of an unnamed free-floating genus outside of the order *Mononegavirales* [41]. This article proposes the taxonomic classification of OFV and similar plant viruses as members of novel species in the new free-floating genus *Dichorhavirus*, and considers options for future higher classification.

Bacilliform 'nuclear-type' viruses transmitted by false spider mites

Depending on their site of replication and accumulation in plant cells, Brevipalpus mite-transmitted viruses have been classified into nuclear (BrTV-N) and cytoplasmic (BrTV-C) types [25]. CiLV-N, CoRSV, OFV, and another similar virus that has never been listed in ICTV Reports, *Clerodendrum* chlorotic spot virus (ClCSV), are considered nuclear types that potentially replicate in the nuclei of infected plant cells [6, 23, 24, 28, 44], in which they cause similar characteristic cytopathic effects (electron-lucent viroplasms) [22, 31]. Another virus not listed in ICTV Reports, *Viola* ringspot virus (ViRSV), is a virus with properties similar to those of CiLV-N, ClCSV, CoRSV, and OFV, but its vector is currently unknown [18].

Brevipalpus mites are capable of feeding on diverse tissues of host plants, including leaves, flowers, fruits, and branches. Feeding of these mites causes direct damage to plants and may induce symptoms such as chlorosis, necrosis, gall formation, and malformation of fruit [9]. Mites belonging to at least three distinct species can persistently transmit bacilliform 'nuclear-type' viruses, including *B. californicus* Banks, 1904; *B. phoenicis* Geijskes, 1939; and *B. obovatus* Donnadieu, 1875 (reviewed in ref. [10]). A minimum inoculation access period of 30 min is required for transmission of OFV by *B. californicus* mites, and mites are capable of transmitting OFV after a three-week latent period on a virus-resistant host [28]. OFV was transmitted after mites had molted, which suggests that the virus circulates throughout the body of the mite, providing additional evidence for persistent transmission [28]. *Brevipalpus*-transmitted bacilliform 'nuclear-type' viruses are not transovarially transmitted [34]. Two lines of initial

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evidence currently suggest that CoRSV, CiLV-N, and ClCSV replicate in their mite vectors: transmission electron microscopy shows the presence of viroplasms in viruliferous mites and RT-qPCR shows increased virus titers ([24, 25]; Kitajima, Bastianel, Freitas-Astúa, unpublished data).

Citrus leprosis virus, nuclear type (CiLV-N)

Recently, CiLV-N has been identified as the likely cause of a mild form of citrus leprosis disease in Florida, USA [27]. CiLV-N produces bacilliform or rod-shaped particles (40-50 × 100-110 nm) that can be found in the nucleus and cytoplasm and are often associated with membranes (Table 1). The nucleus of infected cells features an electron-lucent viroplasm. The complete bipartite genome of a Mexican isolate of CiLV-N has recently been sequenced [45]. The CiLV-N genome, composed of RNA 1 (≈6.4 kb) and RNA 2 (≈6.0 kb) closely resembles that of OFV, with 92-95 % sequence similarity at the nucleotide level and 96-98 % at the amino acid level. This suggests that this Mexican isolate of CiLV-N may be considered a citrus-infecting strain of OFV rather than a distinct virus [45]. Sequencing of other isolates from different countries (there are known isolates in Panamá and Brazil) and from various citrus species will help to determine whether or not the name CiLV-N should be abandoned.

Clerodendrum chlorotic spot virus (ClCSV)

Chlorotic spots on the ornamental plant *Clerodendrum x speciosum* Dombrain (Lamiales: Lamiaceae) linked to *Brevipalpus* mite infestations were observed in Brazil in 2000 [24]. Electron-microscopic analysis of thin sections revealed electron-translucent viroplasms in the nuclei of infected leaf cells. Short bullet-shaped virions (40 × 100-110 nm) were observed in the nuclei and cytoplasm. ClCSV was transmitted by *B. phoenicis* mites feeding on symptomatic and then healthy *C. x speciosum* plants and plants of several other species, causing chlorotic spots on inoculated leaves (Table 1) [24]. The virus was also mechanically transmissible in leaf extracts to several indicator plants. ClCSV was purified, and a rabbit polyclonal antiserum was prepared that reacted strongly with ClCSV in ELISA. Antisera against OFV and CoRSV reacted weakly with purified ClCSV, indicating some shared antigenic sites [24]. RT-PCR was used to amplify a 311-bp fragment of the putative polymerase gene of ClCSV, and another primer set was used to detect the virus in infected mites [34]. A BLAST P search showed that the deduced amino acid sequence of this L gene fragment (HQ853700) was 68 % identical to the corresponding fragment of the L protein of OFV, with an E value of $3e^{-40}$ and 48 % or less sequence identity to the L proteins of nucleorhabdoviruses.

Coffee ringspot virus (CoRSV)

Coffee ringspot was first described in Brazil in 1938 and has been generally considered as a minor disease of low economic importance, except for several disease epidemics in Brazil in the 1990s [3]. CoRSV produces short bacilliform particles (45 × 100-140 nm) that are transmitted by *Brevipalpus phoenicis* mites (Table 1). Virions can be found in the nucleus and cytoplasm of infected cells, often associated with membranes [6]. Characteristic electron translucent nuclear inclusions are indicative of virus replication in the nucleus. The complete genome sequence of CoRSV is not currently available, but as for OFV, agarose gel electrophoresis of RNA extracted from purified CoRSV preparations (using an OFV purification protocol) yielded two bands of ≈6,000 and ≈6,400 nucleotides, respectively [4]. The partial deduced amino acid sequence of the L protein from a Brazilian isolate of CoRSV showed high similarity across 305 amino acids with OFV L protein (61 % identity, E-value $3e^{-124}$) and 30-35 % identity with that of nucleorhabdoviruses (Locali-Fabris, Freitas-Astúa and Machado, upublished data). A short fragment of that L gene has been deposited in GenBank (Table 1; [27]). A BLAST N search against this sequence revealed the L gene sequences on RNA 2 of both sequenced OFV isolates as the closest match, with 71 % nucleotide sequence identity across 77 % of the 394-nt CoRSV sequence and an E-value of $1e^{-32}$. This sequence was used to design a pair of CoRSV-specific primers used for virus detection by RT-PCR in plants of several *Coffea* species [26]. The apparent nucleotide sequence similarity between CoRSV and OFV was also supported by unpublished RT-PCR results, showing that the N gene of CoRSV could be weakly amplified using primers designed from the OFV N gene sequence (cited in ref. [22]). However, tissues infected with CoRSV did not react strongly with OFV antiserum in indirect ELISA or immunocytochemical assays [4, 22], nor did tissues infected with OFV give clear positive reactions with CoRSV antiserum in indirect ELISA [4]. Nevertheless, there were slight, yet consistently higher ELISA readings in heterologous reactions of infected samples compared to those in healthy controls. Similarly, only slight labelling of tissue sections was seen by *in situ* immunogold experiments in heterologous reactions using anti-CoRSV and anti-OFV sera, while the homologous reactions were strong [4]. Altogether, these data indicate a

Table 1 Characteristics of *Brevipalpus* mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA viruses in comparison to a nucleorhabdovirus

	citrus leprosis virus N	*Clerodendrum* chlorotic spot virus	coffee ringspot virus	orchid fleck virus	*Viola* ringspot virus	potato yellow dwarf virus (a nucleorhabdovirus)
Virus abbreviation	CiLV-N	ClCSV	CoRSV	OFV	ViRSV	PYDV
Virion size	40-50 × 100-110 nm	40 × 100-110 nm	40 × 100-110 nm	40 × 100-150 nm	40 × 98 nm	75 × 380 nm
(leaf dip)	(short bacilliform)	(short bacilliform)	(short bacilliform)	(short bacilliform or bullet-shaped)	(short bacilliform)	(long bacilliform)
Presence of lipid envelope	No	No	No	No[a]	No	Yes
Viroplasms (replication) in	Nucleus	Nucleus	Nucleus	Nucleus	Nucleus	Nucleus
Symptoms	Lesions on fruits, leaves, and twigs	Chlorotic spots on leaves; brownish spots on petals	Ringspots on leaves, twigs and berries	Chlorotic or necrotic ringspots and 'systemic' flecks	Chlorotic spots	Vein clearing, stunting, leaf malformation, mosaic
Genome type	Bipartite, (−)ssRNA	Unknown	Bipartite, (−)ssRNA	Bipartite, (−)ssRNA	Unknown	Monopartite (−)ssRNA
Genome sequence availability and GenBank accession number	Mexican isolate: RNA1: ≈6.4 kb RNA2: ≈6.0 kb	311 nt (*L* gene): HQ853700	RNA1: ≈6.4 kb RNA2: ≈6.0 kb 394 nt (*L* gene): GQ979998	RNA1: AB244417 (So) ≈6.4 kb; AB516442 (NHHS1) ≈6.4 kb RNA2: AB244418 (So) ≈6.0 kb; AB516441 (NHHS1) ≈6.0 kb	Unknown	12,875 nt (full-length genome): NC_016136
Sequence similarity with	Mexican isolate (OFV strain): 92-95 % nt sequence identity with OFV	OVF RNA2	OFV RNA2	Nucleorhabdoviruses, CiLV-N, CoRSV	N/A	Nucleorhabdoviruses
Arthropod vector	False spider mite *B. californicus* Banks, 1904[c]	False spider mite *B. phoenicis* Geijskes, 1939	False spider mite *B. phoenicis* Geijskes, 1939	False spider mite *B. californicus* Banks, 1904; false spider mite *B. phoenicis* Geijskes, 1939	Unknown	Leafhopper *Aceratagallia sanguinolenta* Provancher, 1872
Transmission mode	Propagative & persistent[b]	Propagative & persistent[b]	Propagative & persistent[b]	Propagative & persistent[b]	Unknown	Propagative & persistent

[a] OFV virions do not appear to acquire a lipid envelope. However, some enveloped virions are found in the cytoplasm [6]

[b] Transmission electron microscopy (presence of viroplasms in the mite) and RT-qPCR evidence suggest replication in the mite vector ([24, 25]; Kitajima and Alberti, unpublished data)

[c] Otero-Colina and Kitajima, personal communication

clear but distant relationship between CoRSV and OFV [4, 22].

Orchid fleck virus (OFV)

The history, etiology, serology, epidemiology, molecular biology, and detection of OFV and the pathology and prevention methods of orchid fleck disease were recently reviewed in detail [41]. Briefly, OFV was discovered in Japan in 1969 [12]. It is an important viral pathogen of orchids because it causes chlorotic or necrotic spots in orchids of many species and has a global distribution [22]. The particles of OFV superficially resemble those of plant rhabdoviruses, but they are significantly smaller (40 × 100-150 nm) and have no clearly distinguishable lipid envelope [7, 22, 28] (Table 1). Outer lipid membranes were not observed in purified OFV particles, but in rare cases, enveloped virions were seen in infected plant cells [4]. In thin sections of infected plant tissue, some virions were arranged radially, surrounded by a membrane. This so-called “spoke wheels” configuration is a typical feature of OFV and the other viruses discussed here. Unlike mononegaviruses, all of which produce enveloped particles, purified orchid fleck virions remain structurally intact and infectious after treatment with non-ionic detergents such as Triton X-100 under high-salt conditions [13, 30]. Cytopathic effects caused by OFV resemble those of nucleorhabdoviruses by developing an electron-lucent inclusion (viroplasm) in the nuclei of infected cells [31].

The (−)ssRNA genome of OFV isolates So and NHHS1 consists of two molecules, RNA 1 (6,413 nt) and RNA 2 (6,001 nt), with a total coding capacity of ≈12.4 kb [20, 29]. The genome sequences of both sequenced isolates (Table 1) are more than 98.5 % identical [20]. RNA 1 encodes, in the complementary sense, five proteins representing the nucleocapsid protein (ORF1/N), putative phosphoprotein (ORF2/P), putative movement protein (ORF3/3), putative matrix protein (ORF4/M), and putative glycoprotein (ORF5/G). RNA 2 encodes an RNA-dependent RNA polymerase (ORF6/L). Therefore, despite having a divided genome, the gene order of OFV resembles that of plant rhabdoviruses, all of which have a monopartite genome of 11-13 kb and the general gene order 3’-N-P-3-M-G-L-5’ (Fig. 1; Table 2) [11]. The N and L, and to a lesser degree G, proteins of OFV have low but clear sequence similarity to cognate proteins of nucleorhabdoviruses, whereas the other gene products have no significant similarities to other known proteins [20, 29] (Table 3). Phylogenetic analysis of the L protein core module suggests that OFV is most closely related to nucleorhabdoviruses (Fig. 2). As far as this has been investigated, similarities between OFV and rhabdoviruses also extend to the 3’- and 5’-terminal complementary sequences of the genomic RNA and the conserved intergenic sequences [29].

Two groups of OFV isolates could be distinguished from sequence analysis of a 660-bp RT-PCR-amplified fragment of the N gene. Sequences between groups differed by at least 15.6 % and 1.8 % at the nucleotide and amino acid level, respectively, whereas within-group nucleotide sequence differences were less than 2 % [3]. A recent study of a 328-bp N gene fragment from OFV isolates collected in Brazil, Costa Rica and Australia confirmed the two groups and showed similar sequence diversity between and within the groups [33]. OFV N gene fragment sequences of isolates from Japan, Germany, Korea and China are closely related to each other and distantly related to N gene regions of plant rhabdoviruses, with nucleorhabdoviruses being the closest relatives [3, 20, 29]. In SDS-PAGE and western blot analyses of purified particles, the structural proteins N, P, and M, and also small amounts of L protein, were detected, but not the G protein

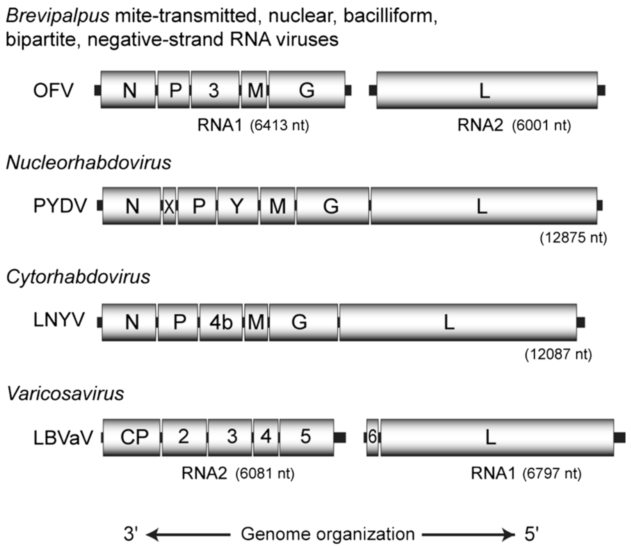

Fig. 1 Comparative genome organization of OFV-So and representative members of the genera of plant-infecting viruses (*Nucleorhabdovirus* and *Cytorhabdovirus*) of the family *Rhabdoviridae* and the unassigned genus *Varicosavirus*. OFV RNA1 encodes 49 kDa (ORF1: nucleocapsid protein N), 26 kDa (ORF2: 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) [29, 30]. Virus names and GenBank/Refseq accession numbers are as follows: *Brevipalpus* mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA viruses - 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. The gene junction sequences for OFV are not yet clearly defined

Table 2 Comparison of genome size and encoded proteins of plant **(−)ssRNA viruses**

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)	Nonsegmented 12,875	52	9.7	31	33	29	70	220
Brevipalpus mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA viruses	orchid fleck virus (OFV)	RNA 1: 6413 RNA 2: 6001	49	-	26	38	20	61	212
Varicosavirus	lettuce big vein-associated virus (LBVaV)	RNA 1: 6797 RNA 2: 6081	44	-	36	32	19	41	232

* A protein of unknown function and N/CP indicates the different naming of ORF #1 in the listed genera

Table 3 Amino acid sequence similarity (%) comparison between OFV-So and plant rhabdoviruses

Genus	Virus name (accession number)	Virus abbreviation	N protein	G protein	L protein
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[b]	29 (2e^{-120})
Nucleorhabdovirus	maize fine streak virus (AY618417)	MFSV	27 (8e^{-25})	ND[b]	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

[30]. Unlike the transmembrane glycoprotein spikes of enveloped rhabdovirions, the OFV G protein may exist in a soluble form with an unknown function, similar to the secreted glycoproteins of filoviruses [46, 52], although this has not been shown experimentally.

Viola ringspot virus (ViRSV)

Common violets (*Viola odorata* L.) with chlorotic spots and containing "small rhabdovirus-like particles" were first identified in 1981 in Australia [18], and the associated virus was named *Viola* ringspot virus (ViRSV). ViRSV produces bacilliform particles resembling those of OFV [18]. Virions are found in the nucleus and cytoplasm of infected cells in close association with cellular membranes. As with OFV and CoRSV infections, characteristic electron-lucent nuclear inclusions are indicative of ViRSV replication in the nucleus [18]. Its genome sequence and organization are unknown. However, the N gene of ViRSV was amplified by RT-PCR using primers designed from the OFV N gene sequence (cited in ref. [22]), indicating that these viruses may be related. Due to the limited data, it would be premature to suggest a taxonomic classification for this virus.

Other plant viruses with segmented (−)ssRNA genomes and sequence similarities to rhabdoviruses

Lettuce big-vein associated virus (LBVaV), the type member of the free-floating genus *Varicosavirus*, produces non-enveloped, rod-shaped particles of 18 × 320-360 nm and is transmitted by the soil-borne fungus *Olpidium brassicae* Dang, 1886 [47, 54]. It differs considerably in virion morphology and vector from those of *Brevipalpus* mite-transmitted, nuclear, bacilliform, bipartite, (−)ssRNA viruses. The LBVaV bipartite (−)ssRNA genome is composed of two segments of 6.8 kb and 6.1 kb in length, respectively. Similar to rhabdoviruses, its genome has conserved intergenic regions and genes that are transcribed

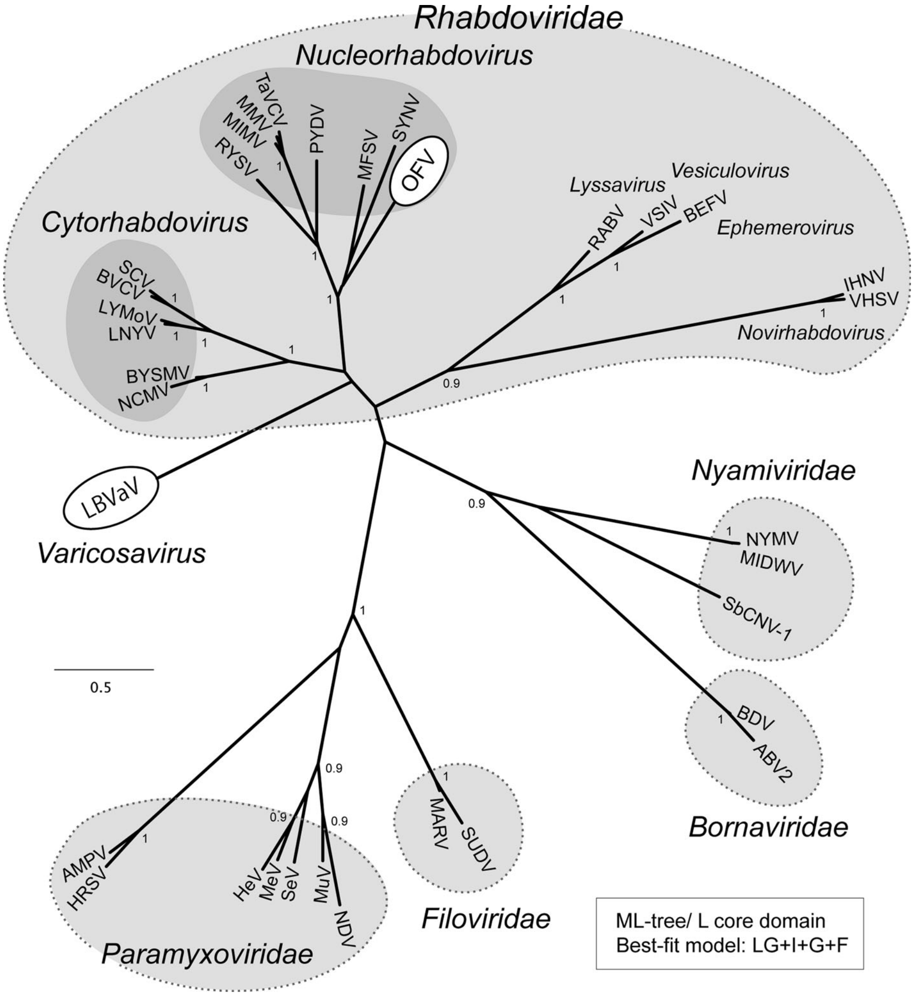

Fig. 2 Phylogenetic relationship of OFV to mononegaviruses and varicosaviruses. A maximum-likelihood tree was constructed using PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/) based on a multiple amino acid sequence alignment of the RdRp (L) polymerase core module as described previously [32]. The 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 are as follows: bornaviruses - Borna disease virus (BDV; NP_042024), avian bornavirus genotype 2 (ABV2; ADU05398); filoviruses - Sudan virus (SUDV; YP_138527), Marburg virus (MARV; YP_001531159); paramyxoviruses - Newcastle disease virus (NDV; NP_071471), Hendra virus (HeV; NP_047113), measles virus (MeV; NP_056924), Sendai virus (SeV; NP_056879), mumps virus (MuV; NP_054714), avian metapneumovirus (AMPV; YP_443845), human respiratory syncytial virus (HRSV; NP_056866); nyamiviruses (newly proposed family [36]) - Nyamanini virus (NYMV; YP_002905337), Midway virus (MIDWV; YP_002905331), soybean cyst nematode virus 1 (SbCNV-1; AEF56729). rhabdoviruses: lyssavirus - rabies virus (RABV; NP_056797), ephemerovirus - bovine ephemeral fever virus (BEFV; NP_065409), vesiculovirus - vesicular stomatitis Indiana virus (VSIV; NP_041716), novirhabdoviruses - infectious hematopoietic necrosis virus (IHNV; NP_042681), viral hemorrhagic septicemia virus (VHSV; NP_049550); cytorhabdovirus – LNYV (YP_425092), lettuce yellow mottle virus (LYMoV; YP_002308376), northern cereal mosaic virus (NCMV, NP_597914), strawberry crinkle virus (SCV; AAP03645), barley yellow striate mosaic virus (BYSMV; ACT21686); nucleorhabdovirus – PYDV (YP_004927971), maize fine streak virus (PYDV; YP_052849), maize mosaic virus (MMV; YP_052855), rice yellow stunt virus (RYSV; NP_620502), sonchus yellow net virus (SYNV; NP_042286), taro vein chlorosis virus (TVCV; YP_224083), maize Iranian mosaic virus (MIMV; YP_002308459); unclassified plant rhabdovirus - raspberry vein chlorosis virus (RVCV; CBL76312); varicosavirus - LBVaV (YP_002308576), *Brevipalpus* mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA viruses - OFV (YP_001294929). Dashed circles: the members of the *Mononegavirales*; solid circles: *Brevipalpus* mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA viruses and members of the genus *Varicosavirus*. Numbers at the nodes represent aLRT values derived using an SH-like calculation (only values greater than 0.9 are shown)

as monocistronic mRNAs (Table 2; Fig. 1) [51]. As is the case for other (−)ssRNA viruses, the amino acid sequence of the conserved L protein polymerase motif of LBVaV has significant similarities to other L proteins, especially those of plant rhabdoviruses, but is more distantly related to that of OFV (Fig. 2) [29, 47].

Table 4 Proposed new taxonomy for *Brevipalpus* mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA viruses

Proposed genus	Proposed species	Member virus (abbreviation)
Dichorhavirus		
	Clerodendrum chlorotic spot dichorhavirus	*Clerodendrum* chlorotic spot virus (ClCSV)
	Coffee ringspot dichorhavirus	coffee ringspot virus (CoRSV)
	<u>*Orchid fleck dichorhavirus*</u>	orchid fleck virus (OFV)

Proposed type species is underlined

Taxonomic placement of *Brevipalpus* mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA viruses

OFV is currently the only completely sequenced short bacilliform bipartite (−)ssRNA virus with significant nucleocapsid protein and polymerase sequence similarities to nucleorhabdoviruses in the family *Rhabdoviridae*, order *Mononegavirales*. Several other putative plant (−)ssRNA viruses share biological, physico-chemical, and sequence similarities with OFV. It therefore seems reasonable to create a new free-floating genus, tentatively named *Dichorhavirus* (roughly meaning rhabdo-like viruses whose genomes are split in two), containing *Orchid fleck dichorhavirus* as the type species and two other species, *Coffee ringspot dichorhavirus* and *Clerodendrum chlorotic spot dichorhavirus* (Table 4) [50]. Because the only sequenced isolate of CiLV-N appears to be a strain of OFV, we do not propose the inclusion of CiLV-N in a new species at this stage. Until more detailed information becomes available for ViRSV, it should be considered as a possible member of the new genus.

Template descriptions of taxa and viruses

Description of *Dichorhavirus* gen. nov. Dietzgen et al. 2013 (tentative)

Etymology of *Dichorhavirus*: sigil of dich(o)-, from Greek *διχα* - meaning “in two, apart or asunder”; *rhabdovirus*; and -*virus* – ending denoting a virus genus → Neo-Lat. n. neut. sg. *Dichorhavirus* – the genus of rhabdo-like viruses that are split in two.

- Valid taxon name (fulfills ICVCN Article 3 Rule II-3.8): yes, because name is compliant with ICVCN Article 3 Rules, in particular Rules IV-3.26, IV-3.27, IV-3.28, and IX-3.39 [19]; because it has been published [this article]; and because it is associated with descriptive material
- Accepted name (fulfills ICVCN Article 3 Rule II-3.8): no, because this name has yet to go through the ICTV approval process
- Use of the taxon:
  - Style: capitalized, italicized, zero article
  - Suggested pronunciation: [daɪˌkɒrəˈvaɪrəs] (IPA); dahy-ko-r*uh*-**vahy**-r*uh*s (English phonetic notation)
  - Abbreviation: none

- Use of taxon vernaculars:
  - n. sg.: dichorhavirus (-virus: ending denoting a physical member of a virus genus [19]). Suggested pronunciation: [daɪˌkɒrəˈvaɪrəs] (IPA); dahy-ko-r*uh*-**vahy**-r*uh*s (English phonetic notation)
  - n. pl. dichorhaviruses. Suggested pronunciation: [daɪˌkɒrəˈvaɪrəsɨz] (IPA); dahy-ko-r*uh*-**vahy**-r*uh*s-iz (English phonetic notation)
  - adj.: dichorhavirus/dichorhaviral. Suggested pronunciation: [daɪˌkɒrəˈvaɪrəs]/[daɪˌkɒrəˈvaɪrəl] (IPA); dahy-ko-r*uh*-**vahy**-r*uh*s/dahy-ko-r*uh*-**vahy**-r*uh*l (English phonetic notation)
  - Style: lower case, not italicized, one word, zero article
  - Abbreviation: none

- Type species: *Orchid fleck dichorhavirus* (tentative, see below)
- Genus members: species *Clerodendrum chlorotic spot dichorhavirus* (tentative, see below), *Coffee ringspot dichorhavirus* (tentative, see below), *Orchid fleck dichorhavirus* (tentative, see below)

Description of *Clerodendrum chlorotic spot dichorhavirus* sp. nov. Dietzgen et al. 2013 (tentative)

Etymology of *Clerodendrum chlorotic spot dichorhavirus*: derived from *Clerodendrum* chlorotic spot disease (the disease caused by members of this species); and *Dichorhavirus* – the genus of dichorhaviruses → the clerodendrum chlorotic spot-causing species of dichorhaviruses.

- Valid taxon name (fulfills ICVCN Article 3 Rule II-3.8): yes, because name is compliant with ICVCN Article 3 Rules, in particular Rule IX-3.40 [19]; because it has been published [this article]; and because it is associated with descriptive material
- Accepted name (fulfills ICVCN Article 3 Rule II-3.8): no, because this name has yet to go through the ICTV approval process

- Use of the taxon:
  - Style: capitalized, italicized, zero article
  - Suggested pronunciation: [ˌklɛrəˈdɛndrəm klɔˈrɒtɪk spɒt daɪˌkɒrəˈvaɪrəs[1]], (IPA); kler-*uh*-**den**-dr*uh*m klaw-**rot**-ik spot dahy-ko-r*uh*-**vahy**-r*uh*s (English phonetic notation)
  - Abbreviation: none

- Use of taxon vernaculars:
  - n. sg.: *Clerodendrum* chlorotic spot dichorhavirus. Suggested pronunciation: [klɛrəˈdɛndrəm klɔˈrɒtɪk spɒt daɪˌkɒrəˈvaɪrəs], (IPA); kler-*uh*-**den**-dr*uh*m klaw-**rot**-ik spot dahy-ko-r*uh*-**vahy**-r*uh*s (English phonetic notation)
  - n. pl. *Clerodendrum* chlorotic spot dichorhaviruses. Suggested pronunciation: [klɛrəˈdɛndrəm klɔˈrɒtɪk spɒt daɪˌkɒrəˈvaɪrəsɨz], (IPA); kler-*uh*-**den**-dr*uh*m klaw-**rot**-ik spot dahy-ko-r*uh*-**vahy**-r*uh*s-iz (English phonetic notation)
  - adj.: *Clerodendrum* chlorotic spot dichorhavirus/*Clerodendrum* chlorotic spot dichorhaviral. Suggested pronunciation: [ˌklɛrəˈdɛndrəm klɔˈrɒtɪk spɒt daɪˌkɒrəˈvaɪrəs]/[ˌklɛrəˈdɛndrəm klɔˈrɒtɪk spɒt daɪ ˌkɒrəˈvaɪrəl] (IPA); kler-*uh*-**den**-dr*uh*m klaw-**rot**-ik spot dahy-ko-r*uh*-**vahy**-r*uh*s/kler-*uh*-**den**-dr*uh*m klaw-**rot**-ik spot dahy-ko-r*uh*-**vahy**-r*uh*l (English phonetic notation)
  - Style: lower case, not italicized, one word, zero article
  - Abbreviation: none
  - Species members are characterized by having the properties of dichorhaviruses; having an L gene polymerase motif nucleotide sequence different from the type virus of the type species of the genus *Dichorhavirus* (orchid fleck virus) by >25 %; and infecting *Clerodendrum* spp.

- Suggested type virus: *Clerodendrum* chlorotic spot virus (ClCSV)
- Species members: *Clerodendrum* chlorotic spot virus (ClCSV)

Description of *Coffee ringspot dichorhavirus* sp. nov. Dietzgen et al. 2013 (tentative)

Etymology of *Coffee ringspot dichorhavirus*: derived from coffee ringspot disease (the disease caused by members of this species); and *Dichorhavirus* – the genus of dichorhaviruses → the coffee ringspot disease-causing species of dichorhaviruses.

- Valid taxon name (fulfills ICVCN Article 3 Rule II-3.8): yes, because name is compliant with ICVCN Article 3 Rules, in particular Rule IX-3.40; because it has been published [this article]; and because it is associated with descriptive material
- Accepted name (fulfills ICVCN Article 3 Rule II-3.8): no, because this name has yet to go through the ICTV approval process
- Use of the taxon:
  - Style: capitalized, italicized, zero article
  - Suggested pronunciation: [ˈkɔfi ˈrɪŋspɒt daɪˌkɒrə ˈvaɪrəs[2]]; (IPA); **kaw**-fee **ring**-spot dahy-ko-r*uh*-**vahy**-r*uh*s (English phonetic notation)
  - Abbreviation: none

- Use of taxon vernaculars:
  - n. sg.: coffee ringspot dichorhavirus. Suggested pronunciation: [ˈkɔfi ˈrɪŋspɒt daɪˌkɒrəˈvaɪrəs] (IPA); **kaw**-fee **ring**-spot dahy-ko-r*uh*-**vahy**-r*uh*s (English phonetic notation)
  - n. pl. coffee ringspot dichorhaviruses. Suggested pronunciation: [ˈkɔfi ˈrɪŋspɒt daɪˌkɒrəˈvaɪrəsɨz] (IPA); **kaw**-fee **ring**-spot dahy-ko-r*uh* -**vahy**-r*uh*s-iz (English phonetic notation)
  - adj.: coffee ringspot dichorhavirus/coffee ringspot dichorhaviral. Suggested pronunciation: [ˈkɔfi ˈrɪŋspɒt daɪˌkɒrəˈvaɪrəs]/[ˈkɔfi ˈrɪŋspɒt daɪˌkɒrə ˈvaɪrəl] (IPA); **kaw**-fee **ring**-spot dahy-ko-r*uh*-**vahy**-r*uh*s/**kaw**-fee **ring**-spot dahy-ko-r*uh*-**vahy**-r*uh*l (English phonetic notation)
  - Style: lower case, not italicized, one word, zero article
  - Abbreviation: none
  - Species members are characterized by having the properties of dichorhaviruses; having an L gene polymerase motif nucleotide sequence different from the type virus of the type species of the genus *Dichorhavirus* (orchid fleck virus) by >25 %; and infecting *Coffea arabica*

- Suggested type virus: coffee ringspot virus (CoRSV)
- Species members: coffee ringspot virus (CoRSV)

Description of *Orchid fleck dichorhavirus* sp. nov. Dietzgen et al. 2013 (tentative)

Etymology of *Orchid fleck dichorhavirus*: derived from orchid fleck disease (the disease caused by members of this

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[1] Pronunciation variant for *Clerodendrum*: [□kl□ərə□d□ndrəm] (IPA); kleer-*uh*-**den**-dr*uh*m (English phonetic notation).

[2] Pronunciation variant: coffee [’kɒfi] (IPA); kof-ee (English phonetic notation).

species); and *Dichorhavirus* – the genus of dichorhaviruses → the orchid fleck disease-causing species of dichorhaviruses.

- Valid taxon name (fulfills ICVCN Article 3 Rule II-3.8): yes, because name is compliant with ICVCN Article 3 Rules, in particular Rule IX-3.40; because it has been published [this article]; and because it is associated with descriptive material
- Accepted name (fulfills ICVCN Article 3 Rule II-3.8): no, because this name has yet to go through the ICTV approval process
- Use of the taxon:
  - Style: capitalized, italicized, zero article
  - Suggested pronunciation: [ˈɔrkɪd flɛk daɪˌkɒrə ˈvaɪrəs] (IPA); **awr**-kid flek dahy-ko-r*uh*-**vahy**-r*uh*s (English phonetic notation)
  - Abbreviation: none

- Use of taxon vernaculars:
  - n. sg.: orchid fleck dichorhavirus. Suggested pronunciation: [ˈɔrkɪd flɛk daɪˌkɒrəˈvaɪrəs] (IPA); **awr**-kid flek dahy-ko-r*uh*-**vahy**-r*uh*s (English phonetic notation)
  - n. pl. orchid fleck dichorhaviruses. Suggested pronunciation: [ˈɔrkɪd flɛk daɪˌkɒrəˈvaɪrəsɨz] (IPA); **awr**-kid flek dahy-ko-r*uh*-**vahy**-r*uh*s-iz (English phonetic notation)
  - adj.: orchid fleck dichorhavirus/orchid fleck dichorhaviral. Suggested pronunciation: [ˈɔrkɪd flɛk daɪˌkɒrəˈvaɪrəs]/[ˈɔrkɪd flɛk daɪˌkɒrə'vaɪrəl] (IPA); **awr**-kid flek dahy-ko-r*uh*-**vahy**-r*uh*s/**awr**-kid flek dahy-ko-r*uh*-**vahy**-r*uh*l (English phonetic notation)
  - Style: lower case, not italicized, one word, zero article
  - Abbreviation: none
  - Species members are characterized by having the properties of dichorhaviruses; having a full-length genomic sequence different from the type virus of the type species of the genus *Dichorhavirus* (orchid fleck virus) by <10 %; and infecting orchid species

- Suggested type virus: orchid fleck virus (OFV)
- Species members: orchid fleck virus (OFV); citrus leprosis virus nuclear type (CiLV-N) 'Florida', a citrus-infecting strain of OFV

Description of *Clerodendrum* chlorotic spot virus vir. Kitajima et al. 2008 (as described in ref. [24])

Etymology of *Clerodendrum* chlorotic spot virus: derived from *Clerodendrum* chlorotic spot disease (the disease caused by this virus); and Lat. n. neut. sg. *virus* – poison, slime, venom.

- Use of the name:
  - Style: first word capitalized (because proper noun), not italicized, all types of articles
  - Suggested pronunciation: [ˌklɛrəˈdɛndrəm klɔˈrɒtɪk spɒt ˈvaɪrəs] (IPA); kler-*uh*-**den**-dr*uh*m klaw-**rot**-ik spot **vahy**-r*uh*s (English phonetic notation)
  - Abbreviation: ClCSV

Description of coffee ringspot virus vir. Boari et al. 2004 (as described in ref. [4])

Etymology of coffee ringspot virus: derived from coffee ringspot disease (the disease caused by this virus); and Lat. n. neut. sg. *virus* – poison, slime, venom.

- Use of the name:
  - Style: first word capitalized (because proper noun), not italicized, all types of articles
  - Suggested pronunciation: [ˈkɔfi ˈrɪŋspɒt ˈvaɪrəs] (IPA); **kaw**-fee **ring**-spot **vahy**-r*uh*s (English phonetic notation)
  - Abbreviation: CoRSV

Description of orchid fleck virus vir. Doi et al. 1977 (as described in ref. [4])

Etymology of orchid fleck virus: derived from orchid fleck disease (the disease caused by this virus); and Lat. n. neut. sg. *virus* – poison, slime, venom.

- Use of the name:
  - Style: first word capitalized (because proper noun), not italicized, all types of articles
  - Suggested pronunciation: [ˈɔrkɪd flɛk ˈvaɪrəs] (IPA); **awr**-kid flek **vahy**-r*uh*s (English phonetic notation)
  - Abbreviation: OFV

Description of *Viola* ringspot virus vir. Gowanlock and Dietzgen 1995 (as described in ref. [18])

Etymology of *Viola* ringspot virus: derived from viola ringspot disease (the disease caused by this virus); and Lat. n. neut. sg. *virus* – poison, slime, venom.

- Use of the name:
  - Style: first word capitalized (because proper noun), not italicized, all types of articles
  - Suggested pronunciation: [ˈvaɪələ ˈrɪŋspɒtˈvaɪrəs] (IPA); **vahy**-*uh-luh* **ring**-spot **vahy**-r*uh*s (English phonetic notation)
  - Abbreviation: ViRSV

Proposal for nomenclature below the species level

We propose to adapt the recently published nomenclature scheme for filovirus variants for dichorhavirus variants [35]. Accordingly, dichorhaviruses would be named <virus name> <strain>/<isolation host-suffix>/<country of sampling>/<year of sampling>/<genetic variant designation>-<isolate designation>. Instructions on how to fill the individual fields can be found in ref. [35].

Future prospects

Functional and sequence similarities among members of non-segmented (−)ssRNA virus families (order *Mononegavirales*) and bisegmented viruses such as OFV and LBVaV suggest that they may share a common ancestor [48]. However, higher-level classification will require additional evolutionary data and/or 'fossil' sequences [16] to support or deny significant changes to the taxonomic system to accommodate such relationships. In this context it should be noted that rhabdovirus and varicosavirus N (CP)-like sequences have been found endogenized in several plant genomes [8]. If the virology community accepts the generation of a new genus with OFV as a member of its type species, then there are several options of future higher taxonomic placement within or outside of the family *Rhabdoviridae* and/or the order *Mononegavirales* that should be discussed for their merit. 1. The current definition of mononegaviruses would need to be revised to "predominantly nonsegmented" to allow inclusion of viruses with bipartite genomes in the *Rhabdoviridae*. 2. Taxonomic placement in a new family outside of the *Rhabdoviridae*, but within the order *Mononegavirales*, would also require a more relaxed definition of what encompasses this order. 3. Classification could also be in a newly created order that may be named "(Poly)negavirales", including a new family, maybe including the genus *Varicosavirus*. The mono- and polynegaviruses could then come together in a new class "Negarnaviropsida". 4. Alternatively, a new order "Rhabavirales" could be created for all rhabdovirus-like viruses (rhabdoviruses + dichorhaviruses + varicosaviruses), and the four families *Bornaviridae*, *Filoviridae*, *Nyamiviridae*, and *Paramyxoviridae* could be elevated to order status; this would involve abandoning *Mononegavirales* and establishing a class for all those orders. Instead of new orders, suborders could also be established.

Note added in proof The recently sequenced citrus necrotic spot virus, strain Jal-1 from Mexico (GenBank accession numbers KF198064, KF198065) also appears to be a strain of ORF. The nucleotide sequences of its RNA 1 and RNA 2 are 90% identical to OFV and 96% identical to CiLV-N, respectively.

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