This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections).

Code(s) assigned:	***2008.008-011P***	(to be completed by ICTV officers)

Short title: Creation of genus Lolavirus in the family Alphaflexiviridae for Lolium latent virus
(e.g. 6 new species in the genus *Zetavirus*; re-classification of the family *Zetaviridae* etc.)

Modules attached (please check all that apply)**:** **1** ☐ **2** ☐ **3** ☐ **4** ☒ **5** ☒ **6** ☐ **7** ☐

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

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Anna Maria Vaira (a.vaira@ivv.cnr.it)
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ICTV-EC or Study Group comments and response of the proposer:

The EC requested a phylogenetic tree that provided information on genetic distances between the sequences. This has been added to the proposal as Figure 3 by the chair of the plant virus subcommittee to further support the proposal.

MODULE 4: **NEW GENUS** (if more than one genus is to be created, please complete additional copies of this section)

Code	***2008.008P***	(assigned by ICTV officers)

To create a new genus assigned as follows:

Subfamily:	
Family:	***Alphaflexiviridae***
Order:	***Tymovirales***

Fill in all that apply. Ideally, a genus should be placed within a higher taxon, but if not put "unassigned" here.

Code	***2008.009P***	(assigned by ICTV officers)

To name the new genus: *Lolavirus*

Code	***2008.010P***	(assigned by ICTV officers)

To assign the following as species in the new genus:

Lolium latent virus **(new species)**

Code	***2008.011P***	(assigned by ICTV officers)

Note: every genus must have a type species

To designate the following as the type species in the new genus:
Lolium latent virus

Argument to justify the creation of a new genus:

See below on the creation of the species

Origin of the new genus name:

From the (only) species **Lo**lium **la**tent virus

Argument to justify the choice of type species:

It is the only species

Species demarcation criteria in the 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.

Not applicable: only one species

MODULE 5: **NEW SPECIES**

Code	***2008.011bP***	(assigned by ICTV officers)

To create 1 new species assigned as follows:

Genus:	***Lolavirus***
Subfamily:	
Family:	***Alphaflexiviridae***
Order:	***Tymovirales***

Fill in all that apply. Ideally, species should be placed within a genus, but it is acceptable to propose a species that is within a Subfamily or Family but not assigned to an existing genus (in which case put "unassigned" in the genus box)

Name(s) of proposed new species:

Lolium latent virus

Argument to justify the creation of the new species:
If the species are to be assigned to an existing genus, list the criteria for species demarcation and explain how the proposed members meet these criteria.

Analysis of the full sequence of Lolium latent virus (LoLV) demonstrates that the genome organization is typical of the flexiviruses, having five ORFs with homologies to the replicase (ORF 1), triple gene block (ORFs 2-4), and coat protein (ORF 5) of the genera *Potexvirus*, *Allexivirus*, *Mandarivirus*, *Carlavirus*, and *Foveavirus* (Vaira et al., 2008). A putative sixth ORF (ORF 6) potentially encodes a small, highly basic 45 residue peptide that is a possible

Argument to justify the creation of the new species:

nucleic acid binding protein, with no significant homology to any characterized proteins in the database. Different portions of the LoLV genome are most closely related to viruses in various genera of flexiviruses (particularly in the *Alphaflexiviridae*), without clear affinity to any single one of the existing genera (See Annex Table 1). The replicase (ORF 1) is most similar (35-42% identity) to those of several potexviruses, and includes an AlkB domain; TGB 1 has the highest degree of amino acid identity (29-34%) to the RdRps of a potexvirus and two allexiviruses; TGB 2 to those of three carlaviruses (50-52%); TGB 3 to an unassigned flexivirus and two carlaviruses (29-33%); and the CP to those of an unassigned flexivirus, a potexvirus, and a carlavirus (32-37%). A phylogenetic analysis of the full replicase places LoLV within the cluster of Potex-like viruses (*Alphaflexiviridae*) but distinct from the Potexvirus and Allexivirus clades or the monophyletic Mandarivirus branch; LoLV is also clearly distinguished from several unassigned flexiviruses (see Annex Figure 1a and also Figure 3); analysis of the CP does not clearly distinguish LoLV from these unassigned flexiviruses, in a large cluster also containing all genera except *Trichovirus* and *Vitivirus* (see Annex Figure 1b).

PASC analysis of the full LoLV genome showed the closest global match to *Indian citrus ringspot virus* (genus *Mandarivirus*, showing 55.24% identity), followed by six potexviruses and a vitivirus. Of 36 full genome comparisons with similar levels of identity (55-55.5%), 23 pairs (about 64%) were between isolates of different genera or unassigned species, and only 13 pairs were between viruses assigned to the same genus.

Species in different genera of flexiviruses typically share less than 40% amino acid identity within both the CP and replicase proteins (Martelli et al., 2007). The highest identity score for the LoLV CP was 35% to the unassigned Banana mild mosaic virus, and 37% to the potexvirus Potato virus X; the highest identity score of the replicase was 36% to Clover yellow mosaic virus, and 42% to Opuntia virus X (both potexviruses). The LoLV replicase is clearly distinguished from those of any currently unassigned flexiviruses. The presence of the putative ORF 6 is unique to LoLV, and no comparable protein has been identified in the database; however, viruses in several flexivirus genera encode a presumed nucleic acid binding protein (albeit significantly larger) in a similar genomic position. Taken together with the PASC results and the highest identities of each LoLV ORF to those of other flexiviruses, these data support the conclusion that LoLV belongs to a new and distinct genus within the *Alphaflexiviridae*.

A further unusual characteristic of LoLV, not previously reported for any flexivirus, is that there are two forms of CP produced within infected plants, which are found both in extracts of infected tissue and in purified virions at an approximately equimolar ratio (see Annex Figure 2). This unusual characteristic further supports the proposal to assign LoLV to a separate genus, for which we propose the name *Lolavirus*, from Lolium latent virus.

References:

Martelli GP, Adams MJ, Kreuze JF, Dolja VV. 2007. Family *Flexiviridae*: a case study in virion and genome plasticity. Ann Rev Phytopathol 45:73-100.

Rozanov MN, Koonin EV, Gorbalenya AE (1992) Conservation of the putative methyltransferase domain: a hallmark of the 'Sindbis-like' supergroup of positive-strand RNA

References:

viruses. J Gen Virol 73:2129-34

Vaira AM, Maroon-Lango CJ, Hammond J. 2008. Molecular characterization of Lolium latent virus, proposed type member of a new genus in the family *Flexiviridae*. Arch. Virol. 153:1263-1270. (DOI 10.1007/s00705-008-0108-8)

Annexes:

Include as much information as necessary to support the proposal. The use of Figures and Tables is strongly recommended.

Table 1. Summary of characteristics of LoLV genome ORFs, their predicted protein products, highest amino acid identities to other viral species using BLAST-P search (the three best matches are presented in order of BLAST-P score) and identified domains.

ORF/nt coordinates /length	Predicted product	Identities	Domain motifs/aa coordinates, [E value]
ORF 1 nt 88-5277 5190 nt	196 KDa, replicase	36% ClYMV (*Potexvirus*) 35% ZVX (*Potexvirus*) 42% OVX (*Potexvirus*)	*MT*, Methyl transferase[1] aa 66-234
			AlkB, (COG3145) Alkylated DNA repair protein aa 700-831, [2e-6]
			Viral helicase 1 (Superfamily 1) (pfam01443) Viral RNA helicase, aa 982-1212, [2e-33]
			RNA-dep-RNApol2 (pfam00978), RNA dependent RNA polymerase aa 1373-1589, [3e-10]
ORF 2 5349-6155 807 nt	30.5 KDa TGBp1	29% PapMV (*Potexvirus*) 34% GarVB (*Allexivirus*) 32% GarVA (*Allexivirus*)	*Viral helicase 1* (Superfamily 1) (pfam01443) Viral RNA helicase, aa 27-236, [1e-17]
ORF 3 6037-6399 363 nt	13 KDa TGBp2	50% NSV (*Carlavirus*) 52% AcLV (*Carlavirus*) 50% ShLV (*Carlavirus*)	*Plant-vir-prot* (pfam01307) plant viral movement protein, aa 11-108, [2e-19] 2 predicted transmembrane regions[2]
ORF 4 6314-6532 219 nt	7.5 KDa TGBp3	33% BanMMV (*Flexiviridae*) 29% PVM (*Carlavirus*) 32% CVB (*Carlavirus*)	1 predicted transmembrane region[2]
ORF 5 6611-7492 882 nt	31.6 KDa CP	35% BanMMV (*Flexiviridae*) 37% PVX (*Potexvirus*) 32% ShLV (*Carlavirus*)	*Flexi-CP* (pfam00286) viral coat protein from Potex- and Carlavirus, aa 112-248, [3e-27]
ORF 6 7440-7577 138 nt	5.1 KDa NABP		

[1] The methyl transferase domain was identified by visual inspection of the sequence and comparison with the alignment and motifs identified by Rozanov et al. [1992].
[2] Prediction of transmembrane regions was performed through DAS-TMfilter Server.

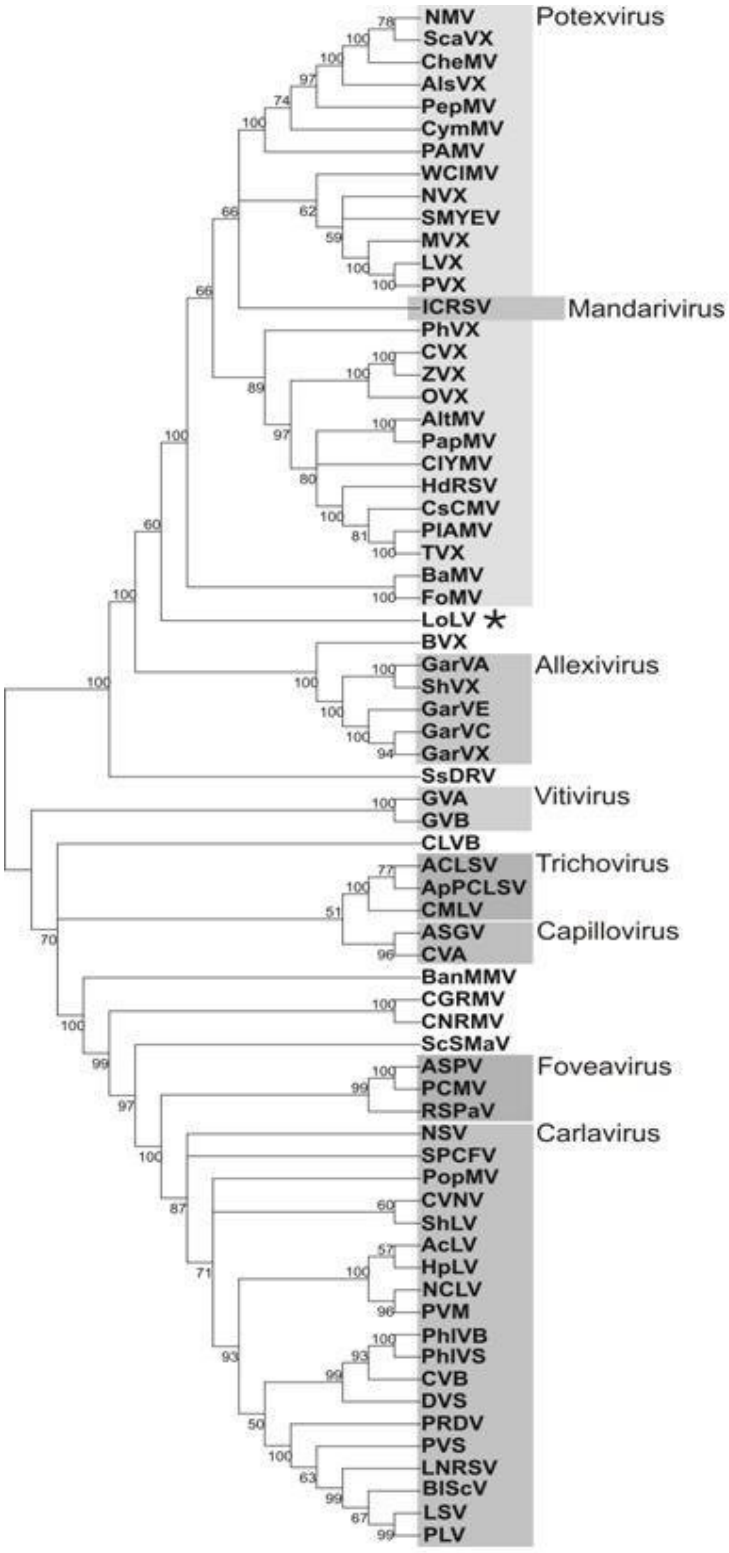


Figure 1a, Phylogenetic analysis of flexiviruses using the amino acid sequences of the full viral replicase. The position of LoLV is marked with an asterisk. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4. Selected bootstrap percentage values (1000 replicates) are shown.

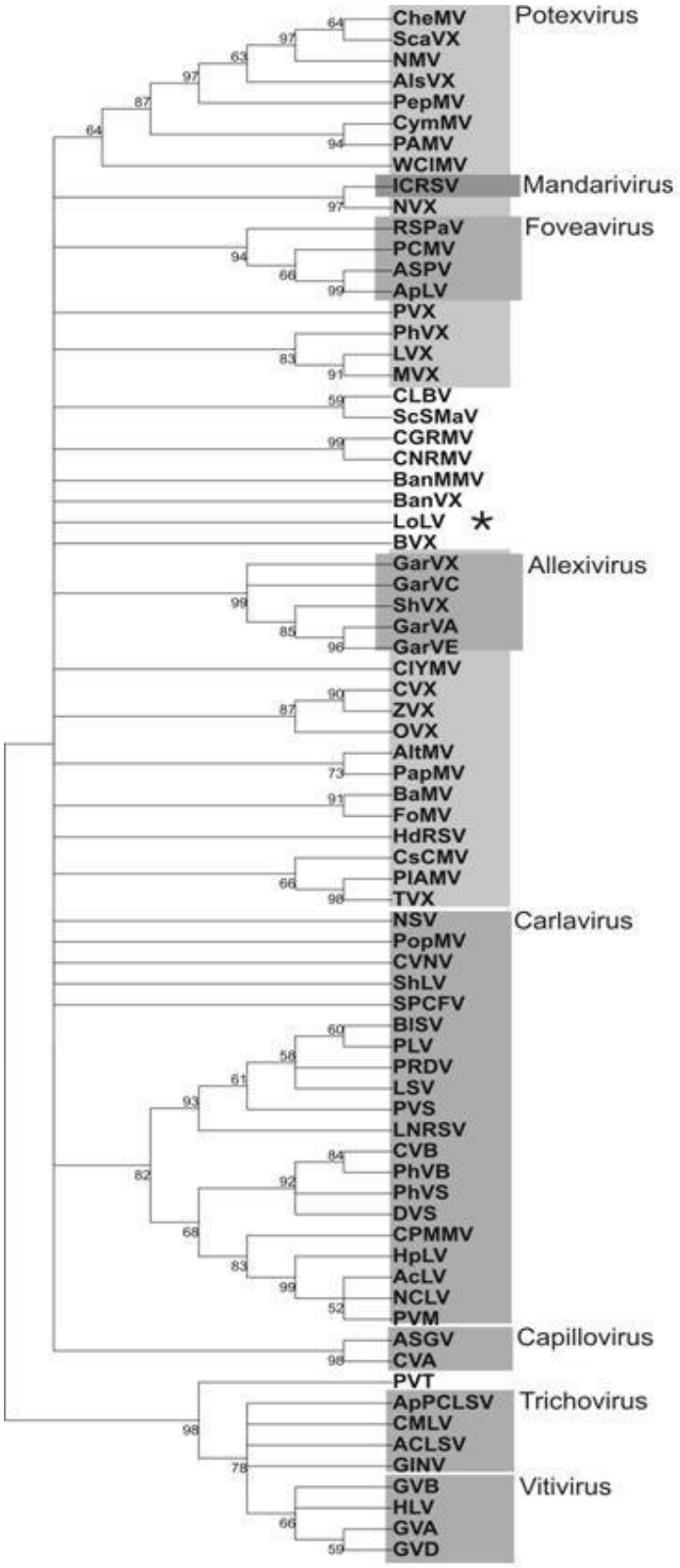


Figure 1b. Phylogenetic analysis of flexiviruses using the amino acid sequences of the coat protein. The position of LoLV is marked with an asterisk. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4. Selected bootstrap percentage values (1000 replicates) are shown.

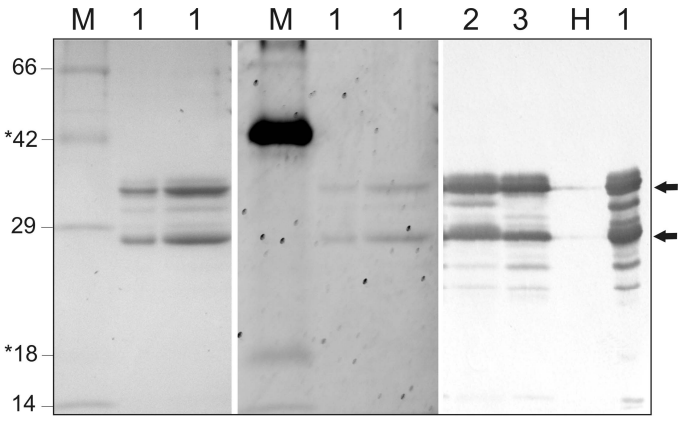
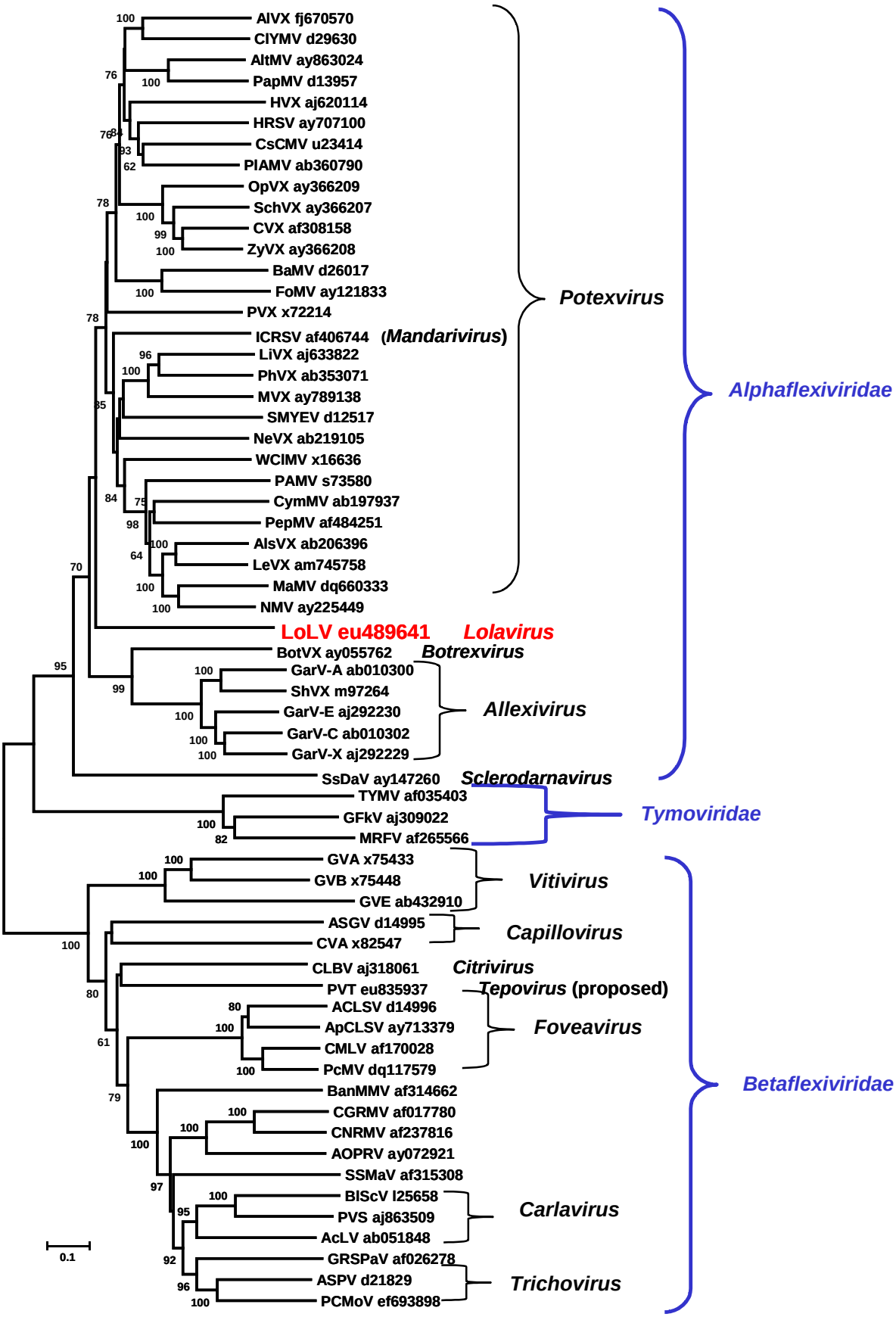


Figure 2. Protein analysis: **a**) Total protein stain and **b**) Glycoprotein-specific stain; in lanes 1 total protein extraction at two concentrations from purified LoLV preparation; M, *CandyCane* molecular marker (glycosylated proteins are marked by an asterisk).
c) Western blotting; lane 1, total protein extract from purified LoLV preparation, 2, total protein extract from LoLV-infected *N. benthamiana* leaves, 3, total protein extract from LoLV-infected ryegrass, H, total protein extract from healthy *N. benthamiana* leaves as negative control. LoLV antiserum was used at 1:2000 dilution. Arrows indicate the two proteins of about 28 and 33 kDa.

Figure 3 (next page). Phylogenetic analysis of the codon-aligned nucleotide sequences of the replication protein of flexiviruses to show the position of the proposed new species LoLV and genus *Lolavirus*. The tree was generated in MEGA 4 using maximum composite likelihood distances and 10,000 bootstrap replicates. Bootstrap percentage values are shown when >60%. Abbreviations are: ACLSV, Apple chlorotic leaf spot virus; AcLV, Aconitum latent virus; AlsVX, Alstroemeria virus X; AltMV, Alternanthera mosaic virus; AlVX, Allium virus X; AOPRV, African oil palm ringspot virus; ApCLSV, Apricot pseudo-chlorotic leaf spot virus; ASGV, Apple stem grooving virus; ASPV, Apple stem pitting virus; BaMV, Bamboo mosaic virus; BanMMV, Banana mild mosaic virus; BlScV, Blueberry scorch virus; BotVX, Botrytis virus X; CGRMV, Cherry green ring mottle virus; CLBV, Citrus leaf blotch virus; ClYMV, Clover yellow mosaic virus; CMLV, Cherry mottle leaf virus; CNRMV, Cherry necrotic rusty mottle virus; CsCMV, Cassava common mosaic virus; CVA, Cherry virus A; CVX, Cactus virus X; CymMV, Cymbidium mosaic virus; FoMV, Foxtail mosaic virus; GarV-A, Garlic virus A; GarV-C, Garlic virus C; GarV-E, Garlic virus E; GarV-X, Garlic virus X; GFkV, Grapevine fleck virus; GRSPaV, Grapevine rupestris stem pitting-associated virus; GVA, Grapevine virus A; GVB, Grapevine virus B; GVE, Grapevine virus E; HRSV, Hydrangea ringspot virus; HVX, Hosta virus X; ICRSV, Indian citrus ringspot virus; LeVX, Lettuce virus X; LiVX, Lily virus X; LoLV, Lolium latent virus; MaMV, Malva mosaic virus; MRFV, Maize rayado fino virus; MVX, Mint virus X; NeVX, Nerine virus X; NMV, Narcissus mosaic virus; OpVX, Opuntia virus X; PAMV, Potato aucuba mosaic virus; PapMV, Papaya mosaic virus; PCMoV, Peach chlorotic mottle virus; PcMV, Peach mosaic virus; PepMV, Pepino mosaic virus; PhVX, Phaius virus X; PlAMV, Plantago asiatica mosaic virus; PVS, Potato virus S; PVT, Potato virus T; PVX, Potato virus X; SchVX, Schlumbergera virus X; ShVX, Shallot virus X; SMYEV, Strawberry mild yellow edge virus; SsDaV, Sclerotinia sclerotiorum debilitation-associated RNA virus; SSMaV, Sugarcane striate mosaic-associated virus; TYMV, Turnip yellow mosaic virus; WClMV, White clover mosaic virus; ZyVX, Zygocactus virus X.


AlVX fj670570
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AltMV ay863024
PapMV d13957
HVX aj620114
HRSV ay707100
CsCMV u23414
PlAMV ab360790
OpVX ay366209
SchVX ay366207
CVX af308158
ZyVX ay366208
BaMV d26017
FoMV ay121833
PVX x72214
ICRSV af406744 (Mandarivirus)
LiVX aj633822
PhVX ab353071
MVX ay789138
SMYEV d12517
NeVX ab219105
WClMV x16636
PAMV s73580
CymMV ab197937
PepMV af484251
AlsVX ab206396
LeVX am745758
MaMV dq660333
NMV ay225449
Potexvirus
LoLV eu489641
Lolavirus
BotVX ay055762
Botrexvirus
GarV-A ab010300
ShVX m97264
GarV-E aj292230
GarV-C ab010302
GarV-X aj292229
Allexivirus
SsDaV ay147260
Sclerodarnavirus
Alphaflexiviridae
TYMV af035403
GFkV aj309022
MRFV af265566
Tymoviridae
GVA x75433
GVB x75448
GVE ab432910
Vitivirus
ASGV d14995
CVA x82547
Capillovirus
CLBV aj318061
Citrivirus
PVT eu835937
Tepovirus (proposed)
ACLSV d14996
ApCLSV ay713379
CMLV af170028
PcMV dq117579
Foveavirus
BanMMV af314662
CGRMV af017780
CNRMV af237816
AOPRV ay072921
SSMaV af315308
BlScV l25658
PVS aj863509
AcLV ab051848
Carlavirus
GRSPaV af026278
ASPV d21829
PCMoV ef693898
Trichovirus
Betaflexiviridae
0.1