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:	***2014.003aP***	(to be completed by ICTV officers)

Short title: Create a novel species for *Dahlia latent viroid* in the genus *Hostuviroid*, family *Pospiviroidae*
(e.g. 6 new species in the genus *Zetavirus*)

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:

Ricardo Flores (rflores@ibmcp.upv.es) and J.Th.J. Verhoeven (j.th.j.verhoeven@minlnv.nl)

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 Viroid Study Group

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

This proposal was conditionally approved by the EC. It was suggested that the tree could be rooted (if at all possible) to better visualize the phylogenetic relationships.

ANSWER- Similar clustering (i.e. a clear separation of HSVd and DLVd from members of the genus *Pospiviroid*) was observed in a phylogenetic tree generated using ASSVd (type species of genus *Apscaviroid*, family *Pospiviroidae*) as an outgroup. However, a somewhat different arrangement of the species within the genus *Pospiviroid* was observed in this tree with respect to the tree reported in the previous proposal. To avoid possible misunderstandings, we prefer to maintain the figure as it is in the previous version of the proposal, because it has been already published in the paper in which the creation of a new species for Dahlia latent viroid was proposed (Verhoeven et al., *J. Gen Virol.* 2013).
We have added a specific statement in this respect in the legend to the phylogenetic tree reported in the annex (Please see the last sentence in the legend).

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

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

To create one new species within:

Genus:	***Hostuviroid***
Subfamily:	
Family:	***Pospiviroidae***
Order:	

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)**
Dahlia latent viroid	**4706174 (DLVd)**	**JX263426**

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

Based on the Ninth Report of the ICTV, species demarcation criteria for members of the family *Pospoviroidae* are: (1) less than 90% sequence identity and (2) distinct biological properties (host range and symptoms).

Dahlia latent viroid (DLVd), replicates autonomously, but symptomlessly in dahlia. This 342-nt RNA, which has a sequence identity of less than 56% with other viroids, displays characteristic features of the family *Pospiviroidae* that include a predicted rod-like secondary structure of minimum free energy with a central conserved region (CCR, which plays a major role in replication) identical to that of hop stunt viroid (HSVd), so far the only member of genus *Hostuviroid*. However, DLVd has the terminal conserved region (TCR) present in members of the genus *Pospiviroid*, but absent in HSVd, and lacks the terminal conserved hairpin (TCH) present in HSVd. Therefore, DLVd poses particular questions regarding classification at the genus level; these questions most likely derive from the recombinant nature of DLVd.

Phylogenetic reconstructions (see the annex) indicate that HSVd and pepper chat fruit viroid (PCFVd, genus *Pospiviroid*) are the closest relatives of DLVd, but DLVd differs from these two viroids in its host range, restricted to dahlia so far. Hence, DLVd fulfils molecular and biological criteria to be a novel species of the family *Pospiviroidae.*

Because DLVd has unique biological properties (extremely narrow host range) not shared by typical members of the genus *Pospiviroid*, we propose to allocate it to the genus *Hostuviroid* based on the most important demarcating criterion established for this purpose (a common CCR with the type species of this genus).

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

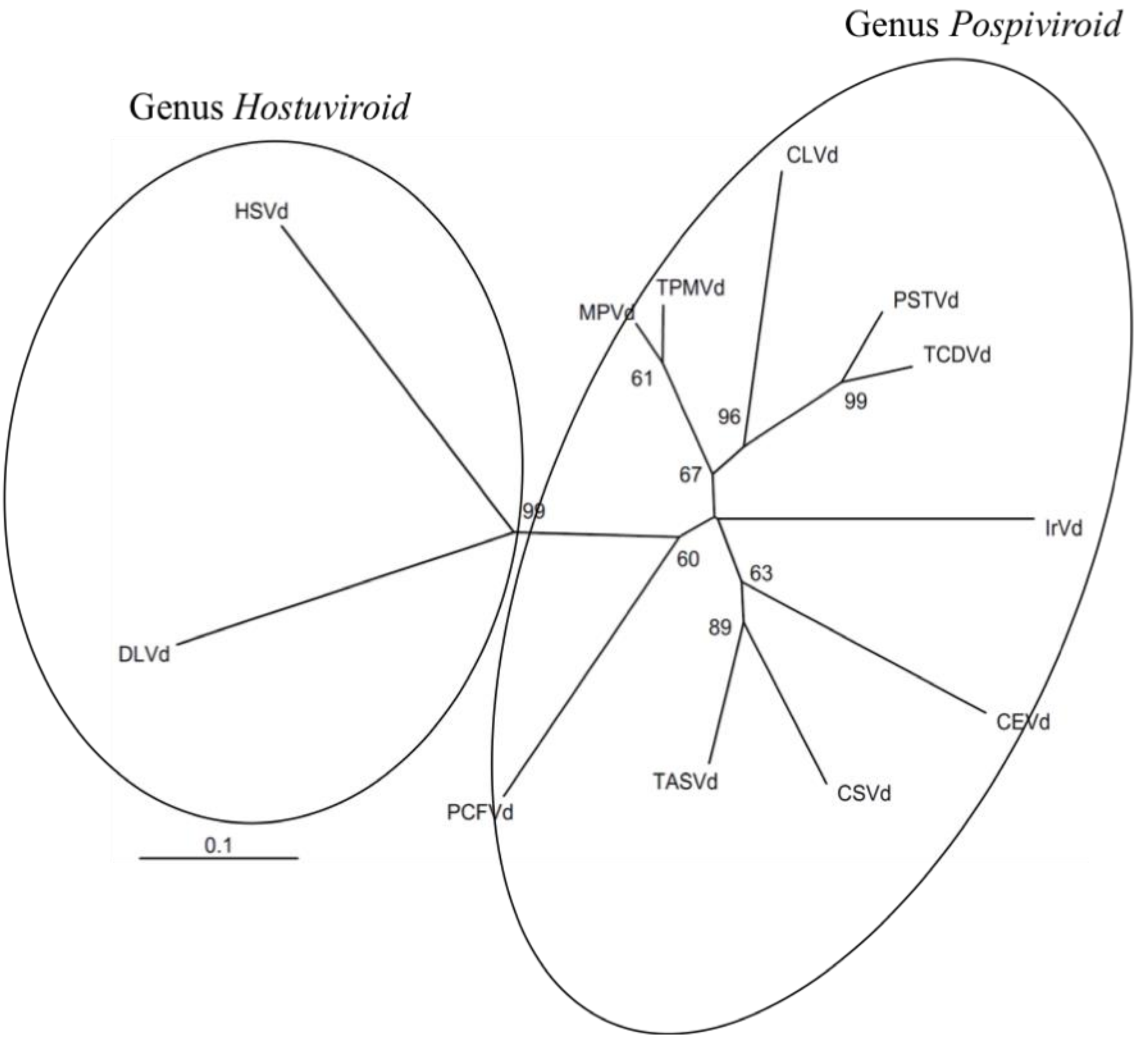
References:

VERHOEVEN, J.TH.J., MEEKES, E.T.M., ROENHORST, J.W., FLORES, R. & SERRA, P. (2013). Dahlia latent viroid: a recombinant new species of the family *Pospiviroidae* posing intriguing questions about its origin and classification. *Journal of General Virology* 94: 711-719.

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.

The nucleotide sequence of DLVd has been deposited in the NCBI GenBank (Accession No JX263426)



Phylogenetic tree showing closer evolutionary relationships of dahlia latent viroid (DLVd) (JX263426) with hop stunt viroid (HSVd) (EU872277) than all known pospiviroids: pepper chat fruit viroid (PCFVd) (FJ409044), chrysanthemum stunt viroid (CSVd) (X16408), tomato apical stunt viroid (TASVd) (NC001553), citrus exocortis viroid (CEVd) (M34917), Iresine viroid (IrVd) (X95734), potato spindle tuber viroid (PSTVd) (V01465), tomato chlorotic dwarf viroid (TCDVd) (AF162131), columnea latent viroid (CLVd) (X15663), Mexican papita viroid (MPVd) (L78454) and tomato planta macho viroid (TPMVd) (K00817). All members of genera Pospiviroid and Hostuviroid (including DLVd) are reported within circles. The phylogenetic reconstruction was performed with the neighbour-joining method (Saitou & Nei, 1987). The percentage of 5000 replicate trees in which the groups clustered together in the bootstrap test (Felsenstein, 1985) is shown next to the branches. Branch lengths, which were estimated with the maximum composite likelihood method (Tamura et al., 2004) and expressed in base substitutions per site, are proportional to the evolutionary distances used to infer the phylogenetic tree. Phylogenetic analyses were conducted with MEGA4 (Tamura et al., 2007). (From Verhoeven et al., *J. Gen Virol.* 2013). Similar results, showing a clear separation of HSVd and DLVd from members of the genus *Pospiviroid* were obtained when the phylogenetic tree was constructed using ASSVd as an outgroup, although a somewhat different arrangement of the species within the genus *Pospiviroid* was observed.