



Part 1: TITLE, AUTHORS, APPROVALS, etc

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Short title: Modify the species demarcation criteria (<i>Pospiviroidae</i> and <i>Avsunviroidae</i>)	

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List the ICTV Study Group(s) that have seen this proposal

ICTV *Avsunviroidae* and *Pospiviroidae* Study Group

ICTV study group comments and response of proposer

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Authority to use the name of a living person

Is any taxon name used here derived from that of a living person (Y/N)

N

Taxon name	Person from whom the name is derived	Permission attached (Y/N)

Submission dates

Date first submitted to SC Chair

May 26, 2021

Date of this revision (if different to above)

September 14, 2021

ICTV-EC comments and response of the proposer

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Part 2: NON-TAXONOMIC PROPOSAL

Text of proposal

Viroids are currently classified in 33 species, eight genera and two families: *Pospiviroidae* and *Avsunviroidae* (Di Serio et al. 2018; Di Serio et al. 2021). Members of the family *Avsunviroidae* contain, in both polarity strands, hammerhead ribozymes involved in the viroid replication, which takes place in chloroplasts, wherein these viroids also accumulate. Members of the family *Pospiviroidae* adopt a rod-like or quasi rod-like conformation containing a central conserved region (CCR), lack hammerhead ribozymes and replicate in the nucleus, wherein they also accumulate. In the family *Avsunviroidae*, assignment of species to genera considers the secondary structure of the genomic RNA, the type of hammerhead structure, the G+C content and solubility in 2 M LiCl, together with clustering in phylogenetic trees derived from the whole sequences (Di Serio et al. 2018). Different criteria are used in the family *Pospiviroidae*, where specific structural elements [i.e., the type of CCR and the presence/absence of a terminal conserved hairpin (TCH) or terminal conserved region (TCR)], together with clustering in phylogenetic trees derived from whole sequence alignments, are considered to classify species within genera (Di Serio et al. 2021). Biological features (e.g., host range) are used to resolve a few controversial situations involving viroid species likely resulting from recombination of ancestors with traits typical of two or more genera (Di Serio et al. 2017). Despite the different criteria adopted to allocate species within the genera, the same demarcation criteria are used to create new species in the genera of both families; i.e., viroids with less than 90% sequence identity and distinct biological properties with respect to the most closely-related species in the genus are classified as different species.

These criteria have two major limitations: i) the threshold of less 90% sequence identity is arbitrary; and ii) they may be difficult to apply, especially for viroids that are symptomless in their natural hosts and/or have a restricted host range. These difficulties are more pronounced for viroids infecting woody hosts because bioassays are time consuming. As a consequence, many viroids, potentially representing members of new viroid species, some of which were identified many years ago, remain unclassified because discriminating biological features have not been demonstrated (Di Serio et al. 2021). Finally, many new viroids and viroid-like RNAs have been identified in the last few years by high-throughput sequencing (HTS), mainly from symptomless host plants. For most of these viroids biological features have not been deeply studied, thus making their conclusive classification difficult.

Recently, the criteria used for viroid classification were reassessed, and a classification scheme based on pairwise sequence identity matrices calculated using most viroid sequences available in databases has been proposed (Chiumenti et al. 2021). Based on the matrices calculated for each viroid genus, a pairwise identity score (PWIS) to be used as a species demarcation threshold (threshold identity score, TIS) has been identified. The proposed TISs are different for each genus and correspond to the lowest minimum PWIS between variants of each species classified in that genus. Because all the known pairs of sequence variants generating a PWIS lower than the relevant threshold have been already classified as different species within the same genus, we propose to use these TISs as the species demarcation criterion that must be fulfilled in order to create a new viroid species. Any novel sequence variant, meeting the criteria to be classified in a particular genus whose PWIS is lower than the TIS can now be classified as a novel species without reference to its biological properties.

TISs have the advantage of not being arbitrary. Indeed, they derived from pairwise identity matrices calculated based on the sequences of viroids of each genus available in databases. Chiumenti et al. (2021) also verified that the current ICTV classification scheme would not change if the TISs and the PWIS methodology reported above are applied, thus implicitly indicating that the proposed species demarcation criterion, based exclusively on nucleotide sequence comparisons, is consistent with the biological diversity between members of different species contemplated in the current classification. Therefore, the proposed novel

species demarcation criteria seem also to be consistent with the essential principles of virus nomenclature that, as reported at the point 2.1 of the ICTV code, are: i) to aim for stability; (ii) to avoid or reject the use of names which might cause error or confusion; (iii) to avoid the unnecessary creation of names.

In addition, as shown by Chiumenti et al. (2021), the novel species demarcation criteria would allow the immediate classification of most viroids not classified yet (Fig. 1).

According with the study reported by Chiumenti et al. (2021), the proposed TISs for each virus genus are the following:

Family *Pospiviroidae*:

Genus *Apscaviroid*, TIS: 78%

Genus *Cocadviroid*, TIS: 79%

Genus *Coleviroid*, TIS: 91%

Genus *Hostuviroid*, TIS: 79%

Genus *Pospiviroid*, TIS: 83%

Family *Avsunviroidae*:

Genus *Avsunviroid*, TIS: 92%

Genus *Pelamoviroid*, TIS: 73%

Genus *Elaviroid*, TIS: 83%

The reported TISs are calculated based on the available sequence variants. Therefore, they could change especially in the case of genera consisting of few species with a low number of available sequence variants.

The proposed novel species demarcation criterion based on pairwise identity matrices, will limit the need of providing supporting biological evidence only to some uncertain situations that may arise when the PWIS calculated for the new variant is lower than the minimum PWIS calculated for the closest related species and/or when the PWIS of the novel unclassified variant is very close to the threshold identified at the genus level (Chiumenti et al., 2021).

In the absence of solid evidence confirming the ability of these RNAs to systemically infect their hosts in the absence of co-infecting helper viruses, the proposed method should not be considered sufficient for the classification of viroid-like RNAs containing hammerhead ribozymes in both polarity strands (therefore potentially belonging to the family *Asunviroidae*). Indeed, akin to some viroids, several viroid-like satellite RNAs consist of small circular RNAs containing hammerhead ribozymes in both polarity strands. Therefore, biological data supporting the autonomous replication of the candidate viroid is considered as needed before proceeding to its classification, especially in the case of potential members of the family *Avsunviroidae*.

According with the consideration reported above, the species demarcation criteria for viroids are proposed to be established as follows:

Family *Pospiviroidae*:

Genus *Apscaviroid*:

Viroids with rod-like or quasi rod-like conformation, with the TCR, with the CCR identical to that of members of the other species of the genus and with less than 78% pairwise sequence identity with respect to the members of the genus are classified in different species. For viroids with pairwise identity scores close to 78% evidence of distinct biological properties should be provided.

Genus *Cocadviroid*:

Viroids with rod-like conformation, with the TCH, with the CCR identical to that of members of the species in the genus and with less than 79% sequence identity with respect to the other members of the genus, are classified in different species. For viroids with pairwise identity scores close to 79% evidence of distinct biological properties should be provided.

Genus Coleviroid:

Viroids with a rod-like conformation, with the TCR, with the CCR identical to that of members of the other species of the genus and with less than 91% sequence identity with respect to the other members of the genus are classified in different species. Members of certain species of this genus, including the viroids with the smallest genome, may contain the TCH instead of the TCR. For viroids with pairwise identity scores close to 91% evidence of distinct biological properties should be provided.

Genus Hostuviroid:

Viroids with a rod-like conformation, with the TCH (or with the TCR instead of the TCH), with the CCR identical to that of members of the other species of the genus and with less than 79% sequence identity with respect to the other members of the genus are classified in different species. For viroids with pairwise identity scores close to 79% evidence of distinct biological properties should be provided.

Genus Pospiviroid:

Viroids with a rod-like conformation, with the TCH, with the CCR identical to that of members of the other species of the genus and with less than 83% sequence identity with respect to the other members of the genus, should be classified in different species. For viroids with pairwise identity scores close to 83% evidence of distinct biological properties should be provided.

Family Avsunviroidae:

Genus Avsunviroid:

Avocado sunblotch viroid is the only species of the genus *Avsunviroid* reported so far. Viroids with similar molecular features (low G+C content, a rod-like conformation and thermodynamically unstable hammerhead ribozymes,) but with less than 92% sequence identity should be classified as different species. Evidence of infectivity in the absence of a helper virus should be provided.

Genus Pelamoviroid:

Viroids with similar molecular features[multi-branched conformations stabilized by a kissing-loop interaction in the (+) strands, and thermodynamically stable hammerhead ribozymes] but with less than 73% sequence identity should be classified as different species. Evidence of infectivity in the absence of a helper virus should be provided.

Genus Elaviroid:

Eggplant latent viroid is the only species of the genus *Elaviroid* reported. Viroids with similar molecular features (quasi-rod-like conformation and thermodynamically stable hammerhead ribozymes) but with less than 83% sequence should be classified as different species. Evidence of infectivity in the absence of a helper virus should be provided.

References

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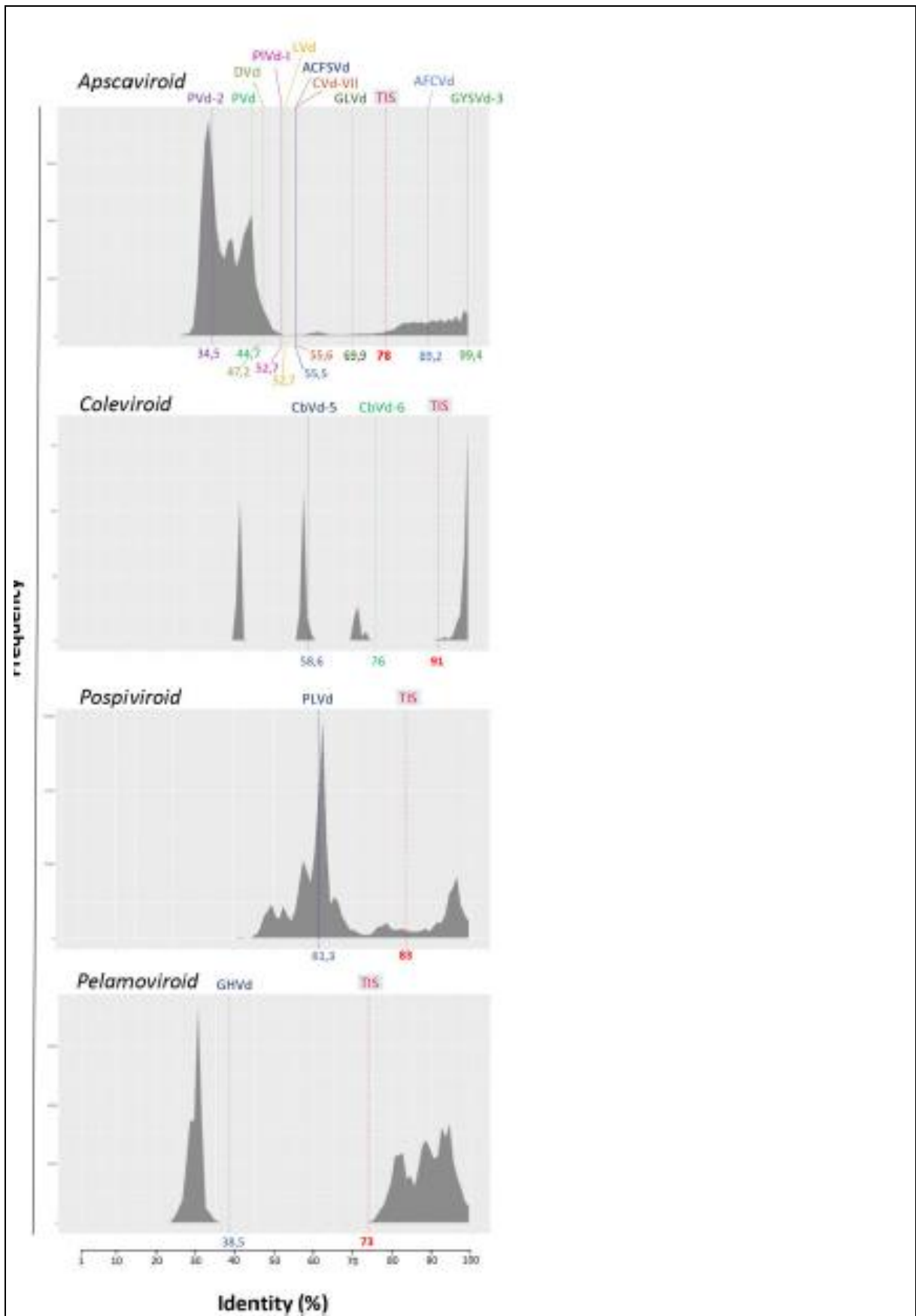


Figure 1. Distribution of PWISs between full-length sequence variants of viroids not classified yet or viroids currently classified in the genera *Apscaviroid*, *Coleviroid* and *Pospiviroid* of family

Pospiviroidae, and in the genus *Pelamoviroid* of family *Avsunviroidae*. The TIS proposed as a species demarcation criterion for each genus is indicated below the graphic and marked by a broken, red, vertical line. The maximum PWIS between variants of each unclassified viroid is marked by a vertical line of different color for each viroid, with the numerical value (identity %) indicated below. According with the proposed novel species demarcation criteria: persimmon viroid 2 (PvD-2), persimmon viroid (PvD), dendrobium viroid (DvD), plum viroid I (PvD-I), lychee viroid (LvD), apple chlorotic fruit spot viroid (ACFSvD), citrus viroid VII (CvD-VII) and grapevine latent viroid (GLvD-) should be classified as belonging to new species in the genus *Apscaviroid*, while apple fruit crinkle viroid and grapevine yellow speckle viroid 3 should not; *Coleus blumei* viroid 5 (CbVd-5) and *Coleus blumei* viroid 6 (CbVd-6) should be classified as new species in the genus *Coleviroid*; portulaca latent viroid (PLvD) should be classified as belonging to new species in the genus *Pospiviroid*. In the case of grapevine latent viroid (GLvD), evidence for its infectivity in the absence of helper virus must be provided to conclusively classify it within the genus *Pelamoviroid* (family *Avsunviroidae*) (Figure from Chiumenti et al. 2021, Virus evolution 2021: veab001).

Part 3: TAXONOMIC PROPOSAL

Name of accompanying Excel module

Abstract

Text of proposal

