Taxonomic Proposal to the ICTV Executive Committee
To create two new species in the genus *Tymovirus*

Code† **2007.063P.04** To designate the following as species in the genus: *Tymovirus*

belonging to the family° : *Tymoviridae*

Anagyris vein yellowing virus

Nemesia ring necrosis virus

† Assigned by ICTV officers

° leave blank if inappropriate or in the case of an unassigned genus

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Old Taxonomic Order

Order: None
Family: *Tymoviridae*
Genus: Tymovirus
Type Species: *Turnip yellow mosaic virus*
Species in the Genus: 23
Tentative Species in the Genus: none
Unassigned Species in the family: None

New Taxonomic Order

Order: None
Family: *Tymoviridae*
Genus: Tymovirus
Type Species: *Turnip yellow mosaic virus*
Species in the Genus: 25
Tentative Species in the Genus: none
Unassigned Species in the family: None

ICTV-EC comments and response of the SG

Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Overall nucleotide sequence identity of less than 80%
- CP sequences less than 90% identical,
- Differential host range
- Differences in the 3'-terminal structure
- Serological specificity

A critical evaluation of these criteria has been published by Koenig et al. 2005 (reference 1). Molecular data seem to be more reliable for species demarcation than the results of serological and host range studies

Argumentation to justify the designation of new species in the genus

NeRNV and AVYV are serologically closely related to Ononis yellow mosaic, Plantago mottle and Scrophularia mottle viruses. They differ from these viruses in host range and especially on the molecular level (see references 1 and 2). The identities of the overall nucleotide and of the coat protein amino acid sequences are far below the 80% and 90%, respectively, required for species demarcation (see annex). AVYV - like many other tymoviruses - has a genomic RNA with a valylatable tRNA-like structure (TLS) on its 3'end. This TLS differs in base composition and in the number of base pairs from those of other tymoviruses (see reference 1). Upstream of the TLS three stem-loops are found which differ in size and base composition from those found with other tymoviral RNAs. The genomic RNA of NeRNV differs from those of all other tymoviruses in having a histidylatable tobamovirus-like 3'end. Details are described in the attached references. A failure of serology to recognize the distinctiveness of new tymoviruses has previously been observed for Calopogonium yellow vein virus which was serologically indistinguishable from Clitoria yellow vein virus although the coat proteins of the two viruses share less than 65% amino acid sequence identity (reference 3).

List of created Species in the genus

Anagyris vein yellowing virus	(AVYV)	AY751780
Nemesia ring necrosis virus	(NeRNV)	AY751778

References

1. Koenig, R., Pleij, C.W.A., Lesemann, D.E., Loss, S. and Vetten H.J. (2005) Molecular characterization of isolates of anagyris vein yellowing virus, plantago mottle virus and scrophularia mottle virus - comparison of various approaches for tymovirus classification. ArchVirol 150: 2325-38
2. Koenig, R., Barends, S., Gultyaev, A.P., Lesemann, D.E Vetten, H.J., Loss, S. and Pleij, C.W.A. (2005) Nemesia ring necrosis virus – a new tymovirus with a genomic RNA having a histidylatable tobamovirus-like 3' end. J. Gen. Virol. 86, 1827-1833
3. Gibbs A., Mackenzie A.M., Abdul-Samad N. (1997) A tymovirus from *Calopogonium mucunoides* in Malaysia is not clitoria yellow vein tymovirus. Arch Virol 142: 1697-1702
4. Rana,G.L, Castellano, M.A. and Koenig,R. (1988) Characterization of a tymovirus isolated from Anagyris foetida as a strain of scrophularia mottle virus. J. Phytopathol. 121, 239-249.

ANNEX 1

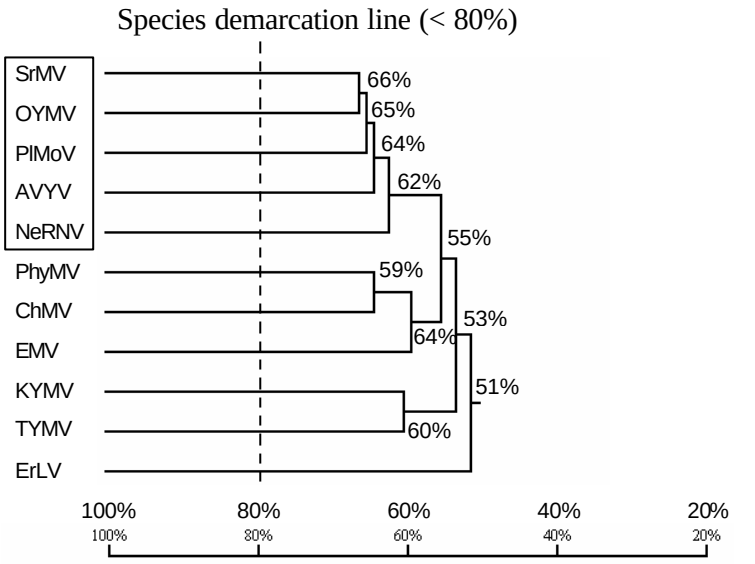

Fig. 1 Tree based on the average percentages of total nucleotide sequence identities between all tymoviruses analysed so far

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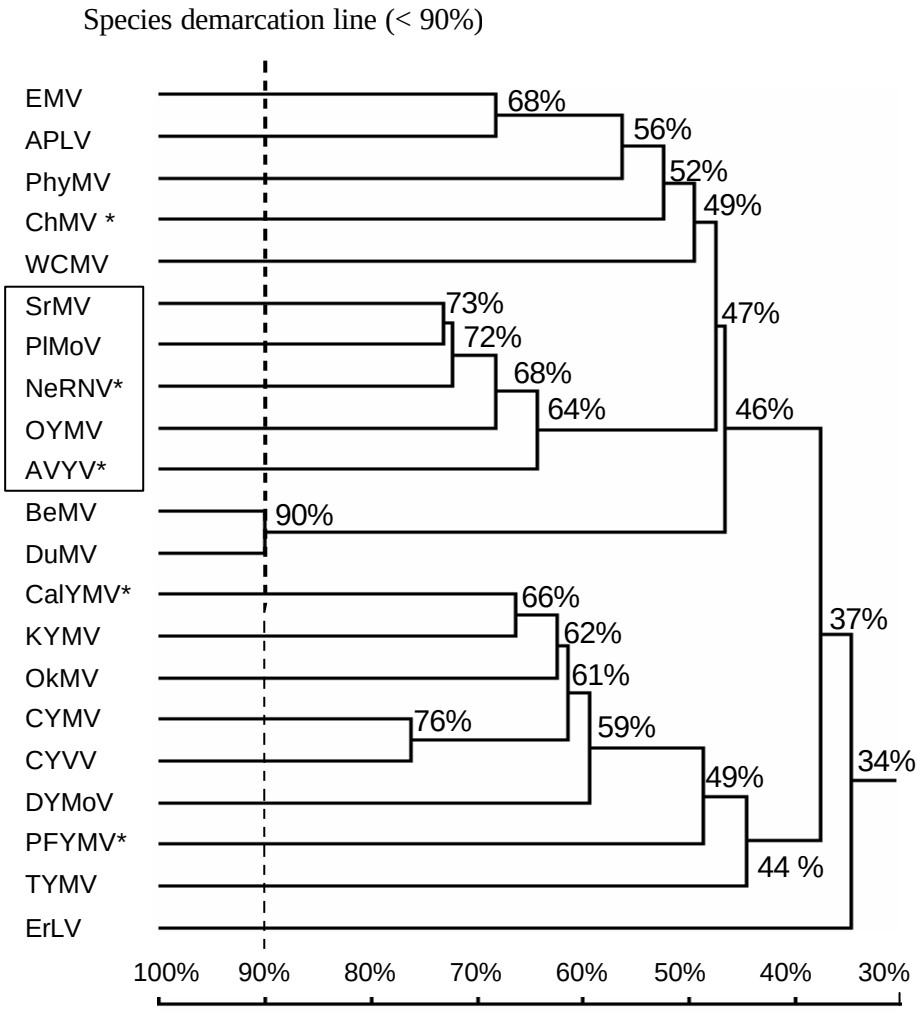

Fig 2. Tree based on the average percentages of amino acid sequence identities between the coat proteins of all tymoviruses analysed so far

ANNEX 2 VIRUS PROPERTIES

Nemesia ring necrosis virus (NeRNV)

NeRNV has isometric particles with a diameter of c. 30 nm and a tymovirus-like morphology. Full particles and 'empty shells' are readily recognized after negative staining. It is widely spread in commercially grown cultivars of various genera belonging to the Scrophulariaceae, i.e. *Nemesia, Diascia, Alonsoa* and *Sutera* and also in *Verbena* spp.. The symptoms produced in naturally infected plants may be very variable. Chlorotic or necrotic flecks or rings are often seen on leaves, sometimes together with flower breaking. Symptomless infections also seem to be common. Type specimen isolated in 1999 from the *Nemesia fruticans* hybrid , 'blue bird' grown commercially in Germany (Bavaria) (Koenig *et al*. 2005a and b).

Biological properties: NeRNV has been transmitted mechanically to *Nicotiana benthamiana, N. occidentalis* P1, *N. hesperis.* In all three species it causes severe systemic infections. A vector has so far not been identified.

Serology: In various serological tests, e.g. the agar gel double diffusion test, ELISA, ISEM and the immunoelectron microscopical decoration test, NeRNV strongly reacts with homologous antisera and also with antisera to some other tymoviruses, in particular Scrophularia mottle virus (SrMV), a virus widely spread in the weed *Scrophularia nodosa.* Because of its strong reactivity with antisera to SrMV, NeRNV was originally thought to be a strain of SrMV. Antisera to NeRNV seem to be better suited to differentiate between these two viruses which have been shown to be quite different on the molecular level (see below). A failure of serology to recognize the distinctiveness of a new virus species has also been observed for another tymovirus, i.e. Calopogonium yellow vein virus which is serologically indistinguishable from Clitorea yellow vein virus although the coat proteins of the two viruses share less than 62% amino acid sequence identity (Gibbs *et al*., 1997).

Molecular properties: The genome of NeRNV (AY751778) consists of a single RNA species comprising 6285 nts. The arrangement of the three ORFs is identical to that found with all other tymoviruses. The most remarkable feature of NeRNV RNA is its histidylatable tobamovirus-like 3'end which is very different from the valine-specific 3'ends of the RNAs of all other tymoviruses. The identities of the overall nucleotide and of the coat protein amino acid sequences with those of all known tymoviruses including SrMV are far below the 80% and 90%, respectively, required for species demarcation (Koenig *et al*. 2005a and b).

Cytopathology: no information

Diagnosis: The virus can be detected in infected plants by means of ELISA, ISEM or RT-PCR. SrMV which cross-reacts in serological tests has not been found in naturally infected ornamental plants so far. For the unequivocal differentiation of NeRNV and SrMV specific PCR primers have been designed.

1. Koenig, R., Pleij, C.W.A., Lesemann, D.E., Loss, S. and Vetten, H.J. (2005a). Molecular characterization of isolates of anagyris vein yellowing virus, plantago mottle virus and scrophularia mottle virus - comparison of various approaches for tymovirus classification. Arch. Virol 150: 2325-38
2. Koenig, R., Barends, S., Gultyaev, A.P., Lesemann, D.E Vetten, H.J., Loss, S. and Pleij, C.W.A. (2005b). Nemesia ring necrosis virus – a new tymovirus with a genomic RNA having a histidylatable tobamovirus-like 3' end. J. Gen. Virol. 86: 1827-1833
3. Gibbs, A., Mackenzie, A.M. and Abdul-Samad, N. (1997). A tymovirus from

Calopogonium mucunoides in Malaysia is not clitoria yellow vein tymovirus. Arch. Virol. 142: 1697-1702

Anagyris vein yellowing virus (AVYV)

AVYV has isometric particles with a diameter of c. 30 nm and a tymovirus-like morphology. Full particles and 'empty shells' are readily recognized after negative staining. It has been isolated from *Anagyris foetida* L., a leguminous perennial weed common in Mediterranean countries. Type specimen isolated in 1983 from *A. foetida* in Southern Italy (Apulia) (Rana *et al.*, 1988)

Biological properties: AVYV has been mechanically transmitted to several plant species belonging to the Leguminosae and the Solanaceae. Systemic infections (sometimes symptomless) were recorded in *Vicia faba, V. narbonensis* and *Vigna sinensis* and in *Nicotiana benthamiana, N. megalosiphon* and *Petunia hybrida*. A vector has not been identified so far.

Serology: In various serological tests, e.g. the agar gel double diffusion test, ELISA, ISEM and the immunoelectron microscopical decoration test AVYV strongly reacts with homologous antisera and also with antisera to Scrophularia mottle, Ononis yellow mosaic and Plantago mottle viruses. For this reason it was originally considered to be a strain of SrMV. These viruses, however, have proved to be quite different on the molecular level (see below). A failure of serology to recognize the distinctiveness of a new virus species has also been observed for another tymovirus, i.e. Calopogonium yellow vein virus which is serologically indistinguishable from Clitorea yellow vein virus although the coat proteins of the two viruses share less than 62% amino acid sequence identity (Gibbs *et al*., 1997).

Molecular properties: The genome of AVYV (AY751780) consists of a single RNA species comprising 6151 nts. The arrangement of the three ORFs is identical to that found with all other tymoviruses. The 3'end of this RNA consists of a valine-specific tRNA-like structure which differs in base composition and in the number of base pairs from those of other tymoviruses. Upstream of the TLS three stem-loops are found which also differ in size and base composition from those found with other tymoviral RNAs. The identities of the overall nucleotide and of the coat protein amino acid sequences with those of all known tymoviruses including SrMV are far below the 80% and 90%, respectively, required for species demarcation (Koenig *et al.*, 2005).

Cytopathology: In ultrathin sections of infected hosts, alterations typically associated with tymovirus infections are found. In particular, numerous flask-shaped double-membrane-bounded vesicles of different sizes are seen at the chloroplast periphery. Nuclei are filled with large numbers of 'empty shells'.

Diagnosis: AVYV can be detected in infected plants by means of serological techniques, such as ELISA or ISEM. RT-PCR with specific primers may be necessary to differentiate the virus from other serologically cross-reacting tymoviruses.

1. Koenig, R., Pleij, C.W.A., Lesemann, D.E., Loss, S. and Vetten, H.J. (2005). Molecular characterization of isolates of anagyris vein yellowing virus, plantago mottle virus and scrophularia mottle virus - comparison of various approaches for tymovirus classification. Arch. Virol. 150: 2325-38
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Virol. 142: 1697-1702
3. Rana, G.L, Castellano, M.A. and Koenig, R. (1988). Characterization of a tymovirus isolated from Anagyris foetida as a strain of scrophularia mottle virus. J. Phytopathol. 121, 239-249.