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:	*2009.001a,bP*	(to be completed by ICTV officers)

Short title: 1 new species in the genus Ampelovirus
(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:

G.P. Martelli on behalf of the Study Group Closteroviridae martelli@agr.uniba.it

Has this proposal has been seen and agreed by the relevant study group(s)? Please select answer in the box on the right	Yes

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

Date first submitted to ICTV: May 2009
Date of this revision (if different to above):

MODULE 2: **NEW SPECIES**

Part (a) to create and name one or more new species.

If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	***2009.001aP***	(assigned by ICTV officers)

To create 1 new species with the name(s):

Pineapple mealybug wilt-associated virus 3

Part (b) assigning new species to higher taxa

All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

Code	***2009.001bP***	(assigned by ICTV officers)

To assign the species listed in section 2(a) as follows:

Genus:	***Ampelovirus***
Subfamily:	
Family:	***Closteroviridae***
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.

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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Pineapple mealybug wilt-associated virus 3 (PMWaV-3) is one of the viruses of the pineapple mealybug wilt virus complex, first reported by Sether *et al.* (2005). It has been found in a wide range of *Ananas* species as PMWaV-1 and PMWaV-2 and, like these viruses, is transmitted by mealybugs (Sether *et al*., 2005). Genome organization and phylogenetic relationships with other members of the family *Closteroviridae* have recently been described (Sether *et al*., 2009).

PMWaV-3 properties

- **(i)** Virus particles: filamentous, closterovirus-like, size undetermined
- **(ii)** dsRNA: *ca.* 13.5 kb in size (estimated from sequence comparison with PMWaV-1)
- **(iii)** CP: 29 kDa (determined by deduced sequence data)
- **(iv)** Nucleic acid: single molecule of ssRNA, *ca.* 13,500 nt in size (estimated)
- **(v)** Genome: monopartite, nearly completely sequenced (11891 nt, accession No. DQ399259): there are 7 ORFs and no CPm gene. Genome structure similar to that of a distinct lineage comprising most members of the genus *Ampelovirus* (a small-sized genome and lack of the CPm ORF). See Appendix, Fig. 1.
- **(vi)** Phylogenetic relationships: PMWaV-3 clusters in a clade of the genus *Ampelovirus* comprising PMWaV-1 and a number of Grapevine leafroll-associated viruses (i.e. GLRaV-4, -5, and -9), which is distinct from a second clade comprising PMWaV-2, GLRaV-1 and GLRaV-3 (See Appendix, Fig. 2). None of the proteins encoded by relevant genes (polymerase, HSP70, CP) has an identity at the amino acid level higher than 80% with comparable proteins of other ampelovirus species
- **(vii)** Serological relationships: PMWaV-3 is serologically unrelated to PMWaV-1 and PMWaV-2.
- **(viii)** Transmission by vectors: transmitted by the mealybug species *Dysmicoccus previpes* and *D. neobrevipes.*
- **(ix)** Cytopathology: no data

Positive mealybug transmission is a clear indication that PMWaV-3 is an ampelovirus, a notion strongly supported by molecular data (genome sequence and organization, and phylogenetic relationships).

`The criteria demarcating species in the genus *Ampelovirus* are:

- Particle size,
- Size of CP, as determined by deduced aa sequence data,
- Serological specificity using discriminatory monoclonal or polyclonal antibodies,
- Genome structure and organization (number and relative location of the ORFs),
- Amino acid sequence of relevant gene products (CP, CPm, HSP70h) differing by more than 10%, (see Appendix, Table 1)
- Vector species and specificity,
- Magnitude and specificity of natural and experimental host range,
- Cytopathological features (i.e., aspect of inclusion bodies and origin of cytoplasmic vesicles).

Serological and molecular properties differentiate PMWaV-3 enough from PMWaV-1 and PMWaV-2, to justify its classification as a separate definitive species in the genus *Ampelovirus*.

additional material in support of this proposal

References:

Sether D.M., Melzer M.J., Busto J., Zee F., Hu J.S., 2005. Diversity and mealybug transmissibility of ampleviruses in pineapple. Plant Disease 89: 450-456.

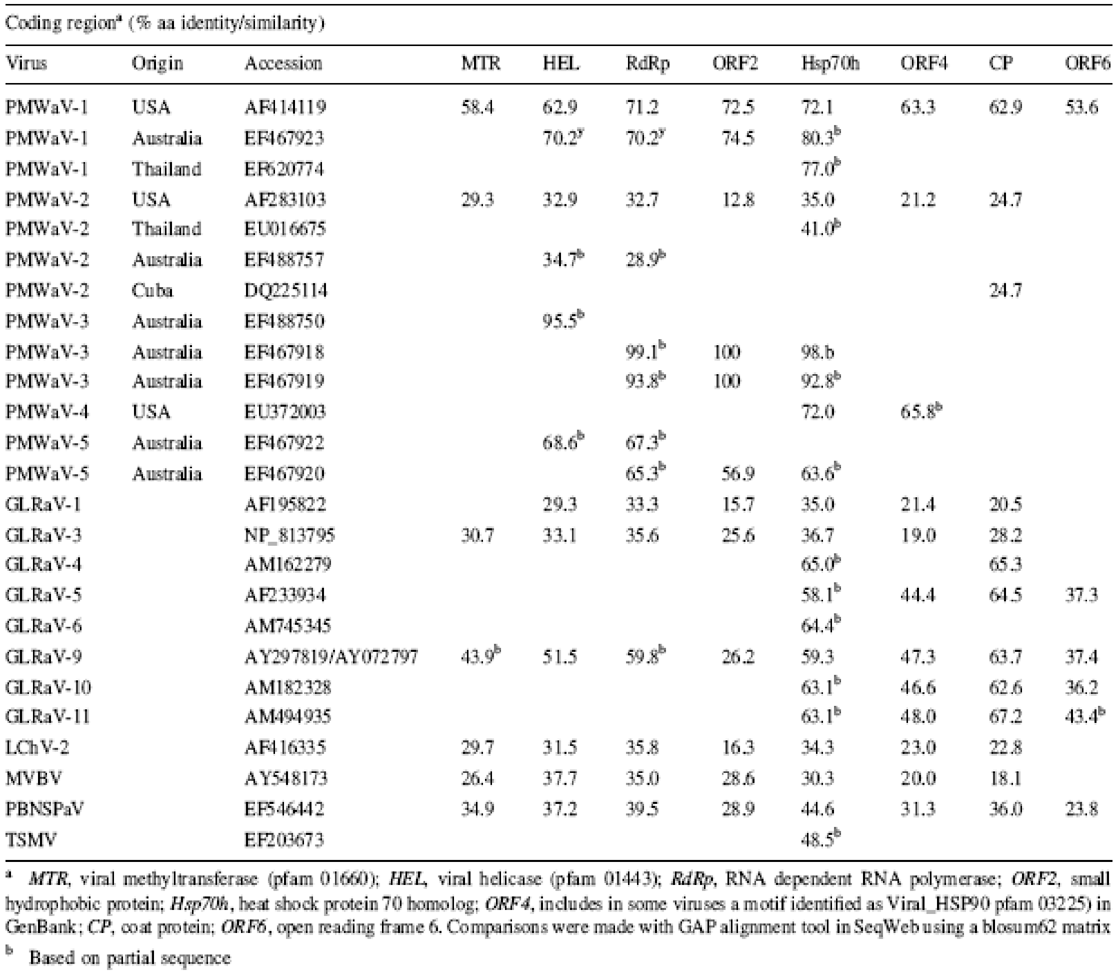
Sether D.M., Melzer M.J., Borth W.M., Hu J.S., 2009. Genome organization and phylogenetic analysis of Pineapple mealybug wilt associated virus-3 with family Closteroviridae members. Virus Genes 38: 414-420.

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.

Table 1. Percentage amino acid (aa) identity of Pineapple mealybug wilt-associated virus 3 (PMWaV-3) and other representative PMWaV and genus *Ampelovirus* accessions for various domains or gene products (from Sether et al., 2009).



Coding region[a] (% aa identity/similarity)										
Virus	Origin	Accession	MTR	HEL	RdRp	ORF2	Hsp70h	ORF4	CP	ORF6
PMWaV-1	USA	AF414119	58.4	62.9	71.2	72.5	72.1	63.3	62.9	53.6
PMWaV-1	Australia	EF467923		70.2[y]	70.2[y]	74.5	80.3[b]			
PMWaV-1	Thailand	EF620774					77.0[b]			
PMWaV-2	USA	AF283103	29.3	32.9	32.7	12.8	35.0	21.2	24.7	
PMWaV-2	Thailand	EU016675					41.0[b]			
PMWaV-2	Australia	EF488757		34.7[b]	28.9[b]					
PMWaV-2	Cuba	DQ225114							24.7	
PMWaV-3	Australia	EF488750		95.5[b]						
PMWaV-3	Australia	EF467918			99.1[b]	100	98.b			
PMWaV-3	Australia	EF467919			93.8[b]	100	92.8[b]			
PMWaV-4	USA	EU372003					72.0	65.8[b]		
PMWaV-5	Australia	EF467922		68.6[b]	67.3[b]					
PMWaV-5	Australia	EF467920			65.3[b]	56.9	63.6[b]			
GLRaV-1		AF195822		29.3	33.3	15.7	35.0	21.4	20.5	
GLRaV-3		NP_813795	30.7	33.1	35.6	25.6	36.7	19.0	28.2	
GLRaV-4		AM162279					65.0[b]		65.3	
GLRaV-5		AF233934					58.1[b]	44.4	64.5	37.3
GLRaV-6		AM745345					64.4[b]			
GLRaV-9		AY297819/AY072797	43.9[b]	51.5	59.8[b]	26.2	59.3	47.3	63.7	37.4
GLRaV-10		AM182328					63.1[b]	46.6	62.6	36.2
GLRaV-11		AM494935					63.1[b]	48.0	67.2	43.4[b]
LChV-2		AF416335	29.7	31.5	35.8	16.3	34.3	23.0	22.8	
MVBV		AY548173	26.4	37.7	35.0	28.6	30.3	20.0	18.1	
PBNSPaV		EF546442	34.9	37.2	39.5	28.9	44.6	31.3	36.0	23.8
TSMV		EF203673					48.5[b]			

[a] *MTR*, viral methyltransferase (pfam 01660); *HEL*, viral helicase (pfam 01443); *RdRp*, RNA dependent RNA polymerase; *ORF2*, small hydrophobic protein; *Hsp70h*, heat shock protein 70 homolog; *ORF4*, includes in some viruses a motif identified as Viral_HSP90 pfam 03225) in GenBank; *CP*, coat protein; *ORF6*, open reading frame 6. Comparisons were made with GAP alignment tool in SeqWeb using a blosum62 matrix

[b] Based on partial sequence

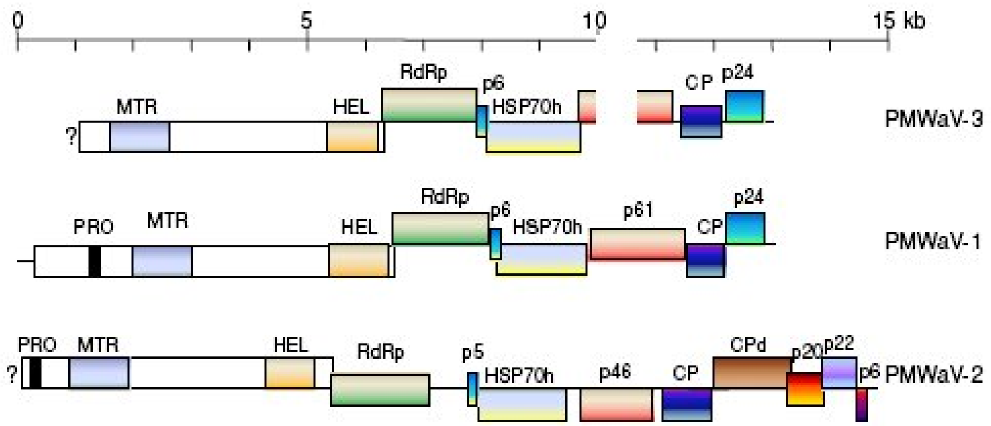
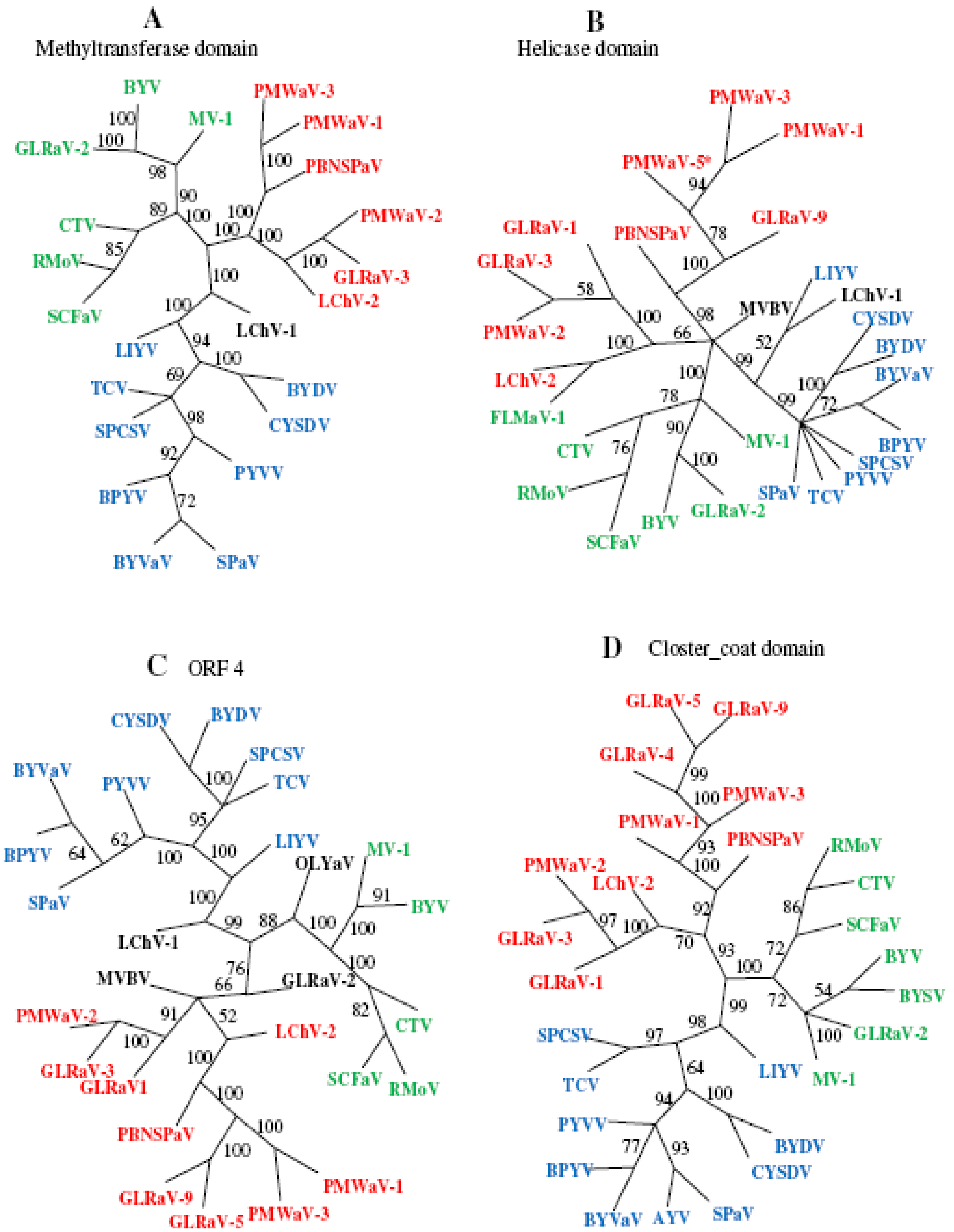


Fig. 1 Genomic organization and approximate size of PMWaV-3, PMWaV-1, and PMWaV-2. Shared color indicates similar protein products. ORF1a: MTR, viral methyltransferase region; HEL, viral helicase region; ORF1b: RdRp, RNA dependent RNA polymerase; ORF2, small hydrophobic protein; ORF3 Hsp70h, heat shock protein 70 homolog; ORF4: p61 or p46 proteins; ORF5: CP, coat protein; ORF6, p24 or CPd, coat protein duplicate; ORF7, p20; ORF8, p22;ORF9, p6 (from Sether et al., 2009).

Fig. 2 (next page). Phylogenetic analyses of the A methyltransferase, B helicase, C ORF4, and D Closter_coat region of nonredundant sequences from members of the family *Closteroviridae*. * denotes partial sequence. Values along branches of neighbor joined trees are bootstrap values based on 1000 replications (from Sether et al., 2009). Sequences used were:
Ampelovirus (red): Grapevine leafrollassociated virus-1 (GLRaV-1) AF195822, GLRaV-3 NP_813795, GLRaV-4 AM162279, GLRaV-5 AF233934, GLRaV-6 AM745345, GLRaV-9 AY297819/AY072797, GLRaV-10 AM182328,GLRaV-11 AM494935, Little cherry virus-2 (LChV-2) AF416335, Plum bark necrosis stem pitting-associated virus (PBNSPaV) EF546442, PMWaV-1 NC_010178, PMWaV-2 AF283103, PMWaV-3 DQ399259, PMWaV-4 EU372003, PMWaV-5 EF467920, and Tulip severe mosaic virus (TSMV) EF203673.
Closterovirus (green): Beet yellow stunt virus (BYSV) U51931, Beet yellows virus (BYV) X73476, Citrus tristeza virus (CTV) isolate T36 AY170468, Fig leaf mottle-associated virus (FLMaV) AM279677, FLMaV-2 AM286422, FLMaV-3 EF654103, GLRaV-2 EF012721, Mint virus-1 (MV-1) AY792620, Olive leaf yellowing-associated virus (OLYaV) AJ440010, Raspberry mottle virus (RMoV) DQ357218, and Strawberry chlorotic fleck-associated virus (SCFaV) DQ860839.
Crinivirus (blue): Abutilon yellows virus (AYV) AY422070, Bean yellow disorder virus (BYDV) NC_010560/NC_010561, Beet Pseudo-yellows virus (BPYV) AY330919/AY330919, Blackberry yellow vein associated virus (BYVaV) AY77335, Cucurbit yellow stunting disorder virus (CYSDV) AJ243000, Lettuce infectious yellows virus (LIYV) NP_733943, Potato yellow vein virus (PYVV) AJ557129, Strawberry pallidosis associated virus (SPaV) AY262159, Sweet potato chlorotic stunt virus (SPCSV) AJ428555, and Tomato chlorosis virus (TCV) AY903448.
Unassigned members in the family (black): LChV-1 NP_045006 and Mint vein banding virus (MVBV) AY548173.


A
Methyltransferase domain
BYV
100
MV-1
GLRaV-2
100
98
90
CTV
89
100
100
100
100
100
RMoV
85
SCFaV
PMWaV-3
PMWaV-1
100
PBNSPaV
PMWaV-2
100
GLRaV-3
LChV-2
100
100
LChV-1
LIYV
94
100
TCV
69
BYDV
SPCSV
98
CYSDV
92
PYVV
BPYV
72
BYVaV
SPaV
B
Helicase domain
PMWaV-3
PMWaV-1
PMWaV-5*
94
GLRaV-1
PBNSPaV
78
GLRaV-9
GLRaV-3
100
LIYV
58
LChV-1
100
98
MVBV
CYSDV
PMWaV-2
100
66
52
BYDV
LChV-2
99
100
BYVaV
100
99
72
FLMaV-1
78
CTV
90
MV-1
BPYV
76
100
SPCSV
PYVV
RMoV
SPaV
TCV
GLRaV-2
BYV
SCFaV
C
ORF 4
CYSDV
BYDV
SPCSV
BYVaV
PYVV
100
TCV
95
BPYV
64
62
100
100
LIYV
MV-1
OLYaV
SPaV
100
91
BYV
99
88
100
100
LChV-1
100
MVBV
76
66
GLRaV-2
PMWaV-2
91
52
LChV-2
82
CTV
100
100
GLRaV-3
GLRaV1
SCFaV
RMoV
100
PBNSPaV
100
100
PMWaV-1
GLRaV-9
GLRaV-5
PMWaV-3
D
Closter_coat domain
GLRaV-5
GLRaV-9
GLRaV-4
99
100
PMWaV-3
PMWaV-1
93
PBNSPaV
100
RMoV
PMWaV-2
LChV-2
CTV
92
86
97
100
SCFaV
GLRaV-3
70
93
72
BYV
100
GLRaV-1
54
BYSV
98
99
72
SPCSV
97
100
GLRaV-2
LIYV
MV-1
TCV
64
100
94
PYVV
BYDV
77
93
CYSDV
BPYV
BYVaV
AYV
SPaV