Arch Virol 147/9 (2002)

VDN
Virology Division News

The family *Tymoviridae*

G. P. Martelli[1], **S. Sabanadzovic**[2], **N. Abou-Ghanem Sabanadzovic**[2],
M. C. Edwards[3], and **T. Dreher**[4]

[1] Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and
Istituto di Virologia Vegetale del CNR, Bari, Italy
[2] Istituto Agronomico Mediterraneo, Valenzano (Bari), Italy
[3] USDA-ARS Cereal Crop Research Unit, Northern Crop Science Laboratory,
Fargo, North Dakota, U.S.A.
[4] Department of Microbiology, Oregon State University, Corvallis, Oregon, U.S.A.

Summary. The family *Tymoviridae* comprises the genus *Tymovirus*, from which it derives its name, the genus *Marafivirus* and the newly established genus *Maculavirus*. Members of the family share the following characteristics: (i) non-enveloped isometric particles *c*. 30 nm in diameter, with a rounded contour and prominent surface structures, and clustering of coat protein subunits in pentamers and hexamers; (ii) the presence in preparations of purified virus particles of two centrifugal components, made up of non-infectious protein shells (T) that may contain small amounts of RNA (primarily subgenomic coat protein mRNA) and of infectious nucleoproteins (B), that contain the virus genome; (iii) possession of a positive-sense, single-stranded RNA genome with an unusually high cytidine content (32 to *c*. 50%), capped at the 5' terminus and containing a very large ORF encodes replication-related proteins analogous to those of other taxa of the "alpha-like" supergroup of ssRNA viruses; (iv) a replication strategy possibly encompassing post-translational proteolytic cleavage of the polypeptide encoded by ORF1 by a papain-like virus-encoded protease, and coat protein expression via a subgenomic RNA; (v) the presence in infected cells of cytopathic structures, thought to be the sites of RNA replication, originating from severely altered chloroplasts and/or mitochondria, the periphery of which is lined with vesicles produced by the localized invaginations of the bounding membrane. There are 23, 4, and 2 known species in the genera *Tymovirus*, *Marafivirus* and *Maculavirus*, respectively. The genus *Marafivirus* also contains one tentative species.

Introduction

Tymovirus [6], *Marafivirus* [7], and *Maculavirus* [15] are three plant virus genera that have the following traits in common: appearance, structural organization, and physicochemical properties of virus particles, a monopartite single-stranded RNA genome with a very high cytidine content, peripheral vesiculation of chloroplasts and/or mitochondria in infected cells, and similar genome organizations. Most of these properties seem to be specific to these genera, so as to differentiate them from other plant virus taxa, and represent useful characters for defining a higher taxon. Furthermore, phylogenetic analysis of the coat protein (CP) and polymerase (RdRp) genes showed that the three genera are related. In these trees, members of these genera form three distinct clusters [16].

The establishment of a novel family called *Tymoviridae,* after the oldest and the best known of the three genera in question, was therefore proposed and approved by the ICTV in June 2002.

Taxonomic structure of the family

Family *Tymoviridae* ha*s* the following taxonomic structure:

Genus *Tymovirus*
Genus *Marafivirus*
Genus *Maculavirus*

Virion properties

Morphology

Virions are isometric, non enveloped, *c.* 30 nm in diameter, with a rounded contour, and prominent surface structure, with clustering of coat protein subunits in pentamers and hexamers. The capsid of tymoviruses is made up of 20 hexameric and 12 pentameric subunits arranged in a $T = 3$ icosahedron and the RNA appears to be at least partially ordered in an icosahedral arrangement in the centre of the protein shell.

Physicochemical and physical properties

Virus particles sediment as two centrifugal components: T, made up of non-infectious protein shells that contain a small amount of RNA (primarily subgenomic coat protein mRNA) and B, composed of intact nucleoprotein particles. Sedimentation coefficients (S_{w20}) of component T and B range from 42 to 55 and from 109 to 125, respectively. Buoyant densities in CsCl of component T and B are 1.26-1.28 g/cm^3 and 1.40–1.46 g/cm^3 respectively. Virions resist high temperatures (thermal inactivation point is 60–65 °C up to above 80 °C for some tymoviruses) and organic solvents, but are disrupted by SDS [2, 9, 11].

Nucleic acid

Virions contain a single molecule of positive-sense, single-stranded RNA constituting 25 to 35% of the particle weight. The RNA has a very high cytidine content (from 32 to about 50%) and ranges from 6.0 to 7.5 kb in length. The genomic RNAs of seventeen species of the genus *Tymovirus* (see list of species), five of the genus *Marafivirus* (see list of species) including Grapevine asteroid mosaic-associated virus (GAMaV) [1] and two of the genus *Maculavirus* (GFkV, GRGV) have been sequenced completely, or in part. Sequenced tymovirus genomes are capped at the 5' terminus and have a tRNA-like structure at the 3' end which, for TYMV and several other species, accepts valine. The genome of all sequenced marafiviruses is capped at the 5' end, but it may (OBDV, PnMV, GAMaV) [3, 6–8] or may not (MRFV) [10] be polyadenylated at the 3' terminus. Maculavirus genomes so far sequenced (GFkV, GRGV) are capped and polyadenylated [17, and unpublished information]. Infectious RNA transcripts have been generated from cDNA clones of the tymoviruses TYMV, EMV, and OYMV.

Proteins

The coat protein (CP) of virus particles contains either a single protein species with a molecular mass of 20 kDa (tymoviruses), 24.5–25 kDa (maculaviruses), or a major protein of 21–21.5 kDa and a minor protein of 24.4–25 kDa (marafiviruses OBDV and MRFV). The CP of two additional marafiviruses, PnMV and a strain of *Bermuda grass etched line virus* (BELV), is made up of a single protein of c. 21 kDa [3, 8, 10, 12, 17].

Lipids: None reported.

Carbohydrates: None reported.

Genome organization and replication

The genome organization and the position and number of ORFs differ according to the genus or individual viral species. Genomes of tymoviruses do not seem to vary with the species and contain three ORFs, one of which (ORF1) is always very large [6]. The genome of marafiviruses and maculaviruses may differ according to the species. In particular, the genome of marafiviruses is basically structured as a single large ORF with CP genes nested at its 3' end [3, 8]. An exception is MRFV, which has a second putative ORF in a frame overlapping the 5' terminus of the genome [10]. Viral RNA of tymoviruses replicates in the cytoplasm in association with the double membran-bounded vesicles that line the periphery of the chloroplasts [11]. Comparable vesicles occurring at the periphery of mitochondria or chloroplasts in cells infected by maculaviruses or some marafiviruses (PnMV, GAMaV) [4, 13, 14, 16] may have the same function. Genome expression may be through post-translational autocatalytic cleavage of the largest ORF by a papain-like virus-encoded protease, and synthesis and translation of a 3'-co-terminal subgenomic RNA for CP expression [6, 7, 10].

Antigenic properties

Virions are moderately to highly antigenic. Monoclonal antibodies have been produced to GFkV. Tymoviruses cluster roughly into two serological groups with cross-reactivities ranging from strong to weak within each group. Cross-reactivities between members are weak to undetectable. Serological relationships occur between some marafiviruses, but not between individual maculaviruses [7, 11, 16].

Biological properties

Host range

Tymoviruses and maculaviruses infect dicotyledonous plants, whereas marafiviruses preferentially infect Poaceae, with the noteworthy exception of PnMV. Natural and experimental host ranges of individual virus species are restricted, sometimes to a single type of host (e.g., GFkV, GRGV, GAMaV infect only *Vitis*; MRFV only *Zea;* PnMV, only

Euphorbia). Disease symptoms are a bright yellow mosaic or mottling (tymoviruses), chlorotic stripes, white etched lines or dwarfing (marafiviruses), and flecking of the leaves (maculaviruses).

Transmission

Tymoviruses and PnMV, but not other members of the family, are transmissible by mechanical inoculation. In vegetatively propagated crops, long distance virus dissemination is primarily via infected propagating material. Transmission through seeds is rare and has been reported only for some tymoviruses. Natural vectors are coleoptera and leafhoppers which transmit tymoviruses or marafiviruses in a semi-persitent or persistent manner, respectively.

Geographical distribution

Members of the family have been recorded from most parts of the world. Geographical distribution of individual species varies from restricted to widespread.

Cytopathic effects

Most species elicit derangement of the internal structure and alteration of the shape of chloroplasts and/or mitochondria, which also show rows of peripheral vesicles derived from localized invaginations of the limiting membrane.

Genus *Tymovirus*

Type species: *Turnip yellow mosaic virus* (TYMV).

Distinguishing features

The genomic RNA (6.0–6.7 kb in size) contains three ORFs (Fig. 1). ORF1 encodes a 206 kDa protein with the conserved sequence motifs of methytransferase, papain-like protease, helicase, and RNA polymerase. The C termini of these large polypeptides are highly conserved. ORF2 almost entirely overlaps ORF1 and encodes a 69 kDa proline-rich protein that is dispensable for replication but is required for cell-to-cell movement. ORF3 codes for the viral CP (20 kDa), which is expressed via a subgenomic RNA. Genome RNA contains a 16 nt sequence known as the “tymobox”, which functions as subgenomic RNA promoter [5]. All species induce the intracytoplasmic formation of “polyplasts”, i.e. highly characteristic aggregates of swollen and modified chloroplasts, which presumably clump because of the aggregating action of the viral CP that accumulates around them. All main

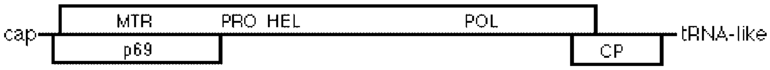

Fig. 1. Genome structure of *Turnip yellow mosaic virus* (TYMV), the type species of the genus *Tymovirus* showing the relative position of the ORFs and their expression products. *MTR*, methyltransferase; *PRO*, papain-like protease; *HEL*, helicase; *POL*, polymerase (RdRp); *CP*, coat protein; *p69*, putative movement protein

tissues of the hosts are invaded. Cells react with cytological modifications that can be used for discriminating group of species or individual species [14]. Empty virions shells accumulate in the nuclei. Virions accumulate in parenchyma cells from where they are acquired by the vectors. These are coleoptera of the families *Chrysomelidae* and *Curculionidae*, which transmit in a semi-persistent manner. All members of the genus are mechanically transmissible and a few (TYMV, EMV, DuMV) are transmitted through seeds.

List of species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Differences in the 3'-terminal structure
- Overall sequence identity of less than 80%
- Capsid protein sequences less than 90% identical
- Serological specificity
- Vector type
- Different extent of seed transmissibility
- Different effects on cell ultrastructure
- Differential host range

List of species in the genus

Official virus species names are in italics. Tentative virus species names, alternative names ( ), strains or serotypes are not italicized. Virus names, CMI/AAB description numbers ( ), genome sequence accession numbers [ ], and assigned abbreviations ( ) are:

Species in the genus

Andean potato latent virus (124)	[AF035402]	(APLV)
Belladonna mottle virus (52)	[X54529]	(BeMV)
Cacao yellow mosaic virus (11)	[X54354]	(CYMV)
Calopogonium yellow vein virus	[U91413]	(CalYVV)
Clitoria yellow vein virus (171)	[M15963]	(CYVV)
Desmodium yellow mottle virus (168)	[AF035201]	(DYMoV)
Dulcamara mottle virus (124)	[AF035634]	(DuMV)
Eggplant mosaic virus	[J04374]	(EMV)
Erysimum latent virus (222)	[AF098523]	(ErLV)
Kennedya yellow mosaic virus	[D00637]	(KYMV)
Melon rugose mosaic virus		(MRMV)
Okra mosaic virus (128)	[AF035202]	(OkMV)
Ononis yellow mosaic virus	[J04375]	(OYMV)
Passion fruit yellow mosaic virus	[AF47107]	(PFYMV)
Peanut yellow mosaic virus		(PeYMV)
Physalis mottle virus	[Y16104]	(PhyMV)
Plantago mottle virus		(PlMoV)
Scrophularia mottle virus (113)		(SrMV)
(Anagyris vein yellowing virus)		
Turnip yellow mosaic virus (2, 230)	[J04373, X16378, X07441]	(TYMV)

Voandzeia necrotic mosaic virus		(VNMV)
Wild cucumber mosaic virus	[AF035633]	(WCMV)

Tentative species in the genus

Chayote mosaic virus	[AF195000]	(ChMV)
Petunia vein banding virus	[AF210709]	(PetVBV)

None reported

Genus *Marafivirus*

Type species: *Maize rayado fino virus* (MRFV).

Distinguishing features

The distinct feature of the marafivirus genome (6.0–6.5 kb in size) is the possession of a single large single ORF encoding a polypeptide of 221–227 kDa (OBDV, Fig. 2) that contains the conserved signature motifs of the replication-associated proteins (methytransferase, helicase, RdRp), a papain-like protease domain, and the "marafibox" [12], a conserved 16 nucleotide stretch comparable to the "tymobox", from which it differs by two or three residue changes. Virions have two CP subunits whose cistrons are located at the extreme 3' end of the polypeptide, of which they are an integral part. The MRFV genome differs from the above by the presence of a second ORF in a frame overlapping the 5' region. This ORF codes for a 43 kDa proline-rich protein showing weak sequence similarity with the putative movement protein of tymoviruses. Another feature that distinguishes MRFV from other members of the genus is the lack of a poly(A) tail at the 3' terminus (MRFV, Fig. 2).

Some species induce peripheral vesiculation of chloroplasts but these vesicles are either double membrane-bound (GAMaV) or single membrane-bound (PnMV), as they derive from localized invaginations of the inner lamella of the organelle's envelope. Most species are strictly confined to the phloem of infected hosts and are not transmissible by inoculation of sap. An exception is PnMV, which is mechanically transmissible, invades most host tissues, and behaves intracellularly like a tymovirus (e.g. intranuclear accumulation of empty shells). None of the species is transmitted through seeds. PnMV and GAMaV have no known vector. Other members of the genus, however, are transmitted by leafhop-

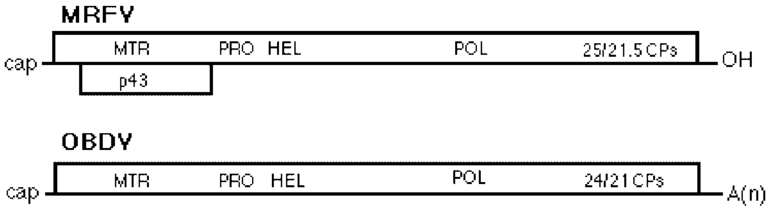

Fig. 2. The two known types of genome structure in the genus *Marafivirus,* exemplified by *Maize rayado fino virus* (MRFV), the type species of the genus, and *Oat blue dwarf virus* (OBDV), showing the relative position of the ORFs and their expression products. *MTR*, methyltransferase; *PRO*, papain-like protease; *HEL*, helicase; *POL*, polymerase (RdRp); *CPs*, coat proteins; *p43*, proline-rich protein

pers, each by a different genus, i.e. MRFV by *Dalbulus*, OBDV by *Macrosteles*, and BELV by *Aconurella*. Transmission is of the persistent-propagative type with viral replication occurring in the insect host. Insect-transmitted species infect primarily Poaceae.

List of species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Differences in the 3'-terminal structure and in the number of ORFs
- Overall sequence identity of less than 70%
- Capsid protein sequences less than 85% identical
- Serological specificity
- Vector specificity
- Different effects on cell ultrastructure
- Differential host range

Species in the genus

Bermuda grass etched-line virus	[AY040531]	(BELV)
Maize rayado fino virus (220)	[U977717, U97730, AF265566]	(MRFV)
Oat blue dwarf virus (123)	[U87832]	(OBDV)

Tentative species in the genus

Grapevine asteroid mosaic-associated virus	[on request]	(GAMaV)
Poinsettia mosaic virus (311)	[AJ271595]	(PnMV)

Genus *Maculavirus*

Type species: *Grapevine fleck virus* (GFkV).

Distinguishing features

The genomic RNA of GFkV (7.5 kb in size) is the largest in the family and consists of four ORFs (Fig. 3). ORF1 encodes a polypepetide of 215 kDa that contains the conserved signature motifs of the replication-associated proteins (methytransferase, helicase, RdRp), a papain-like protease domain. ORF2 codes for the 24 kDa CP. ORF3 and ORF4, which are located at the extreme 3' end of the genome, code for proline-rich proteins of 31 and 16 kDa, respectively, which show a distant relationship with the putative movement proteins of

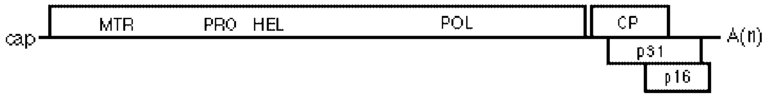

Fig. 3. Genome structure of *Grapevine fleck virus* (GFkV) the type species of the genus *Maculavirus* showing the relative position of the ORFs and their expression products. *MTR*, methyltransferase; *PRO*, papain-like protease; *HEL*, helicase; *POL*, polymerase (RdRp); *CP*, coat protein; *p31* and *p16*, proline-rich proteins

tymoviruses. The RNA does not seem to have a conserved sequence comparable to the “tymobox” or the “marafibox”. There are two known species in the genus (GFkV and GRGV), both of which infect latently only *Vitis* species, with the exception of *V. rupestris,* which reacts to GFkV with translucent spots (flecks) on the leaves. Both species are strictly confined to the phloem of infected hosts and are not transmissibile by sap inoculation. The cytopathology of GFkV infections is characterized by a severe modification of mitochondria into structures called “multivesiculate bodies” [4]. Field spread of GFkV has been reported but the vector is unknown. GFkV is not transmitted through seeds, thus virus dissemination is primarily by distribution of infected propagative material.

List of species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Overall sequence identity of less than 70%
- Capsid protein sequences less than 85%
- Serological specificity

Species in the genus

Grapevine fleck virus	[AJ309022]	(GFkV)

Tentative species in the genus

Grapevine redglobe virus	[AF521977]	(GRGV)

Unassigned species in the family: None reported.

Phylogenetic relationships within the family

See Fig. 4.

Similarity with other taxa

Replication-associated proteins (RdRp, methyltransferase, and helicase) contain signature sequences homologous to those of other taxa of the “alpha like” supergroup of ssRNA viruses, especially those of the genera *Carlavirus* and *Potexvirus*.

Derivation of names

Tymo: sigla from *t*urnip *y*ellow *mo*saic virus; *Marafi*: sigla from *ma*ize *ra*yado *fi*no virus; *Macula*: from *macula*, Latin for fleck.

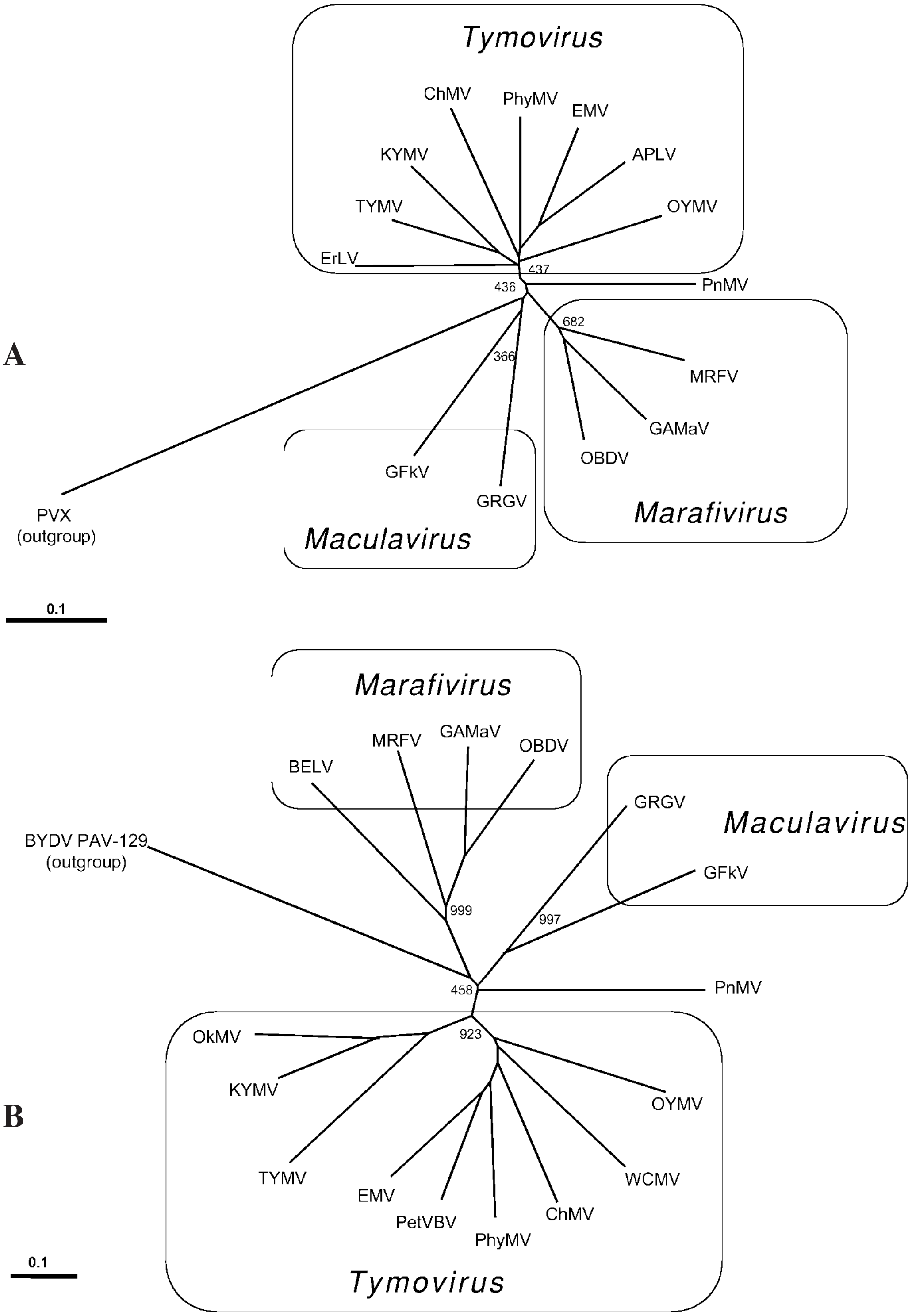

Fig. 4. Phylogenetic tree showing the relationships between the species and genera of the family *Tymoviridae* based on the RdRp (**A**) and CP (**B**) sequences. The tree was produced using CLUSTALW. Branch lengths are proportional to sequence distances

References

1. Boscia D, Sabanadzovic S., Savino V, Kyriakopoulou PE, Martelli GP, Lafortezza R (1994) A non mechanically-transmissibile virus associated with asteroid mosaic of the grapevine. Vitis 33: 101–102
2. Boulila M, Boscia D, Di Terlizzi B, Castellano MA, Minafra A, Savino V, Martelli GP (1990) Some properties of a phloem-limited non mechanically-transmissibile grapevine virus. J Phytopathol 129: 151–158
3. Bradel BG, Preil WX, Jeske H (2000) Sequence analysis and genome organisation of Poinsettia mosaic virus (PnMV) reveal closer relationship to marafiviruses than to tymoviruses. Virology 271: 289–297
4. Castellano MA, Martelli GP (1984) Ultrastructure and nature of the vesiculated bodies associated with isometric virus-like particles in diseased grapevines. J Ultrastruct Res 89: 56–64
5. Ding S, Howe J, Keeese P, Mackenzie A, Meek D, Osorio-Keese M, Skotnicki M, Pattana S, Torronen M, Gibbs A (1990). The tymobox, a sequence shared by most tymoviruses: its use in molecular studies of tymoviruses. Nucleic Acids Res 18: 1181–1187
6. Dreher TW, Haenni AL, Mayo MA (2000) Genus *Tymovirus.* In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens E, Estes MK, Lemon S, Maniloff J, Mayo MA, McGeoch D, Pringle CR, Wickner RB (eds) Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, pp 964–969
7. Edwards MC (2000) Genus *Marafivirus*. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens E, Estes MK, Lemon S, Maniloff J, Mayo MA, McGeoch D, Pringle CR, Wickner RB (eds) Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, pp 969–973
8. Edwards MC, Zhang Z, Weiland JJ (1997) Oat blue dwarf marafivirus resembles the tymoviruses in sequence, genome organization, and expression strategy. Virology 232: 217–229
9. Gamez R, Leon P (1988) Mayze rayado fino and related viruses. In: Koenig R (ed) The plant viruses. Polyhedral virions with monopartite RNA genome. Plenum Press, New York, pp 213–233
10. Hammond RW, Ramirez P (2001) Molecular characterization of the genome of *Maize rayado fino virus*, the type member of the genus *Marafivirus*. Virology 282: 338–347
11. Hirth L, Givord L (1988) Tymoviruses. In: Koenig R (ed) The plant viruses. Polyhedral virions with monopartite genomes. Plenum Press, New York, pp 163–212
12. Izadpanah K, Zhang YP, Daubert S, Rowhani A (2002) Sequence of the coat prtoein gene of bermuda grass etched-line virus and of the adjacent "marafibox" motif. Virus Genes 24: 131–134
13. Koenig R, Lesemann DE, Fulton RW (1986) Poinsettia mosaic virus. AAB Descr Pl Viruses 311
14. Lesemann DE (1977) Virus group-specific and virus-specific cytological alterations induced by members of the tymovirus group. Phytopathol Z 90: 315–336
15. Martelli GP, Sabanadzovic S, Abouh-Ghanem Sabanadzovic N, Saldarelli P (2002) *Maculavirus*, a new genus of plant viruses. Arch Virol 147: 1847–1853
16. Sabanadzovic S, Abou Ghanem-Sabanadzovic N, Castellano MA, Digiaro M, Martelli GP (2000) Grapevine fleck virus-like viruses in *Vitis*. Arch Virol 145: 553–565
17. Sabanadzovic S, Abou Ghanem-Sabanadzovic N, Saldarelli P, Martelli GP (2001) Complete nucleotide sequence and genome oganization of grapevine fleck virus. J Gen Virol 82: 2009–2015

Authors' address: G. P. Martelli, Dipartimento di Protezione delle Piante e Microbiologia Applicata, Via Amendola 165/A, I-70126 Bari, Italy