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:	***2010.002F***	(to be completed by ICTV officers)

Short title: A new mycovirus species, Rosellinia necatrix megabirnavirus 1, in a new family, Megabirnaviridae
(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:

Nobuhiro Suzuki, Ph. D.: nsuzuki@rib.okayama-u.ac.jp

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)	Before submission to the chair of the Fungal Virus Subcommittee, the proposal was shared with and supported by Dr Said A Ghabrial, the chair of the study group on partitiviruses and chrysoviruses.

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

Date first submitted to ICTV: 11-6-09
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.

Code	***2010.002aF***	(assigned by ICTV officers)

To create a new species within:

Genus:	*Megabirnavirus* (new)
Subfamily:	
Family:	*Megabirnaviridae* (new)
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.

And name the new species:

Rosellinia necatrix megabirnavirus 1

Reasons to justify the creation and assignment of the new species:

Rosellinia necatrix megabirnavirus 1 (RnMBV1), isolated from a strain of the phytopathogenic fungus *R. necatrix*, has a bipartite dsRNA genome that is packaged in virions of ~50 nm in diameter. Two genomic segments, dsRNA1 and dsRNA2, are approximately 9 and 7 kb long, each possessing extremely long 5'-UTRs of over 1.6 kb and two open reading frames (ORFs) termed ORFs 1 and 2 for dsRNA1 and ORFS 3 and 4 for dsRNA2. The dsRNA segments have relatively short 3'-UTRs (Fig 1). Although the protein encoded by the 3' proximal ORF2 on dsRNA1 shares sequence identities of 20-30% with RNA-dependent RNA-polymerases from members of the families *Totiviridae* and *Chrysoviridae* (see Table 1), the remaining three virally-encoded proteins lack sequence similarities with any reported mycovirus proteins. Phylogenetic analysis showed that the RnMBV1 belongs to a separate clade distinct from those of other known mycoviruses (Fig 2). Purified virions of ~50 nm in diameter consisted of dsRNA1 and 2, and a single major capsid protein of 135 kDa encoded by dsRNA1 ORF1. The genomic RNA segments are likely to be encapsidated separately.

All these attributes show clearly that RnMBV1 is distinguishable from any other reported mycoviruses.

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 accession numbers for genomic sequences

EMBL/GenBank/DDBJ Accession Nos: AB512282 and AB512283.

- Further material in support of this proposal may be presented in the Appendix, Module 9

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2010.002bF***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	*Megabirnaviridae*
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 family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2010.002cF***	(assigned by ICTV officers)

To name the new genus: *Megabirnavirus*

Assigning the type species and other species to a new genus

Code	***2010.002dF***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Rosellinia necatrix megabirnavirus 1

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain: 1**

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

Only one family member is well characterized. See "Reasons to justify the creation of a new family." A similar case is the family *Barnaviridae* that consists of only one species belonging to the genus *Barnavirus*.

Origin of the new genus name:

Like the name of the proposed family (see module 5), "Megabirna" is from a much greater (mega) size (approximately 16 kbp) of its bisegmented dsRNA genome (birna for bipartite dsRNA genome) than those of members in the family *Birnaviride* (approximately 6 kbp) or *Picobirnaviridae* (approximately 4 kbp).

Reasons to justify the choice of type species:

RnMBV1-strain W779, the prototype of the species *Rosellinia necatrix megabirnavirus 1*, is the only virus fully characterized at the molecular level. Phylogenetic analysis based on RdRp sequences (Fig. 2) indicates that RnMBV1 is closely related to LeV-HKB and PgV-TW2, partially characterized dsRNA viruses from *Lentinula edodes* and *Phlebiopsis gigantea*, respectively. These viruses, when completely characterized, may represent additional species in the new genus or

possibly new genera in the family *Megabirnaviridae*.

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

MODULE 5: **NEW FAMILY**

creating and naming a new family

Code	***2010.002eF***	(assigned by ICTV officers)

To create a new family containing the subfamilies and/or genera listed below within the Order: unassigned

If there is no Order, write "**unassigned**" here.
If the Order has yet to be created (in Module 6) please write **"(new)"** after the proposed name.

Code	***2010.002fF***	(assigned by ICTV officers)

To name the new family: *Megabirnaviridae*

assigning subfamilies, genera and unassigned species to a new family

Code		(assigned by ICTV officers)

To assign the following subfamilies (if any) to the new family:
You may list several subfamilies here. For each subfamily, please state whether it is new or existing.

- If the subfamily is new, it must be created in Module 4
- If the subfamily already exists, please complete Module 7 to 'REMOVE' it from its existing family

Code	***2010.002gF***	(assigned by ICTV officers)

To assign the following genera to the new family:
You may list several genera here. For each genus, please state whether it is new or existing.

- If the genus is new, it must be created in Module 3
- If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family

Megabirnavirus (new)

The new family will also contain any other new species created and assigned to it (Module 3) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of unassigned species that the family will contain (those NOT within any of the genera or subfamilies listed above):**

Reasons to justify the creation of the new family:
The proposed family can be readily differentiated from other known mycovirus families based on the size of its bipartite genome, particle size (~50 nm in diameter), and the length of the 5'-UTRs. Of the four proteins encoded by members of the family, only the RdRp sequence shows low levels (approximately 20-30%) of identities (see Table 1) to those of members of the families *Totiviridae* and *Chrysoviridae*, while the remaining three proteins do not show any significant sequence similarities to other mycovirus proteins.

A phylogenetic tree (Fig 2) generated based on an RdRp sequence alignment is attached in Module 9 that places the prototype (RnMBV1/W779) of the proposed family into a distinct clade from other known dsRNA mycovirus families.

Additional material in support of this proposal may be presented in the Appendix, Module 9

Origin of the new family name:
"Megabirna" is from a much greater (mega) size (approximately 16 kbp) of its bisegmented dsRNA genome (birna for bipartite dsRNA genome) than those of members in the family *Birnaviride* (approximately 6 kbp) or *Picobirnaviridae* (approximately 4 kbp).

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Chiba, S., L Salaipeth, Yu-Hsin Lin, Y.-H., Sasaki, A., Kanematsu, S. and Suzuki, N. A novel bipartite dsRNA mycovirus from the white root rot fungus Rosellinia necatrix: Molecular and biological characterization, taxonomic considerations, and potential for biological control. J. Virol. (in press)

Ghabrial, S. A., and N. Suzuki. 2009. Viruses of plant pathogenic fungi. Annu. Rev. Phytopathol. 47: 353-384.

Ikeda, K., H. Nakamura, and N. Matsumoto. 2005. Comparison between Rosellinia necatrix isolates from soil and diseased roots in terms of hypovirulence. FEMS Microbiol. Ecol. 54: 307-315.

Kozlakidis, Z., C. V. Hacker, D. Bradley, A. Jamal, X. Phoon, J .Webber, C. M. Brasier, K. W. Buck, and R. H. Coutts. 2009. Molecular characterisation of two novel double-stranded RNA elements from Phlebiopsis gigantea. Virus Genes 39: 132-136.

Annex:

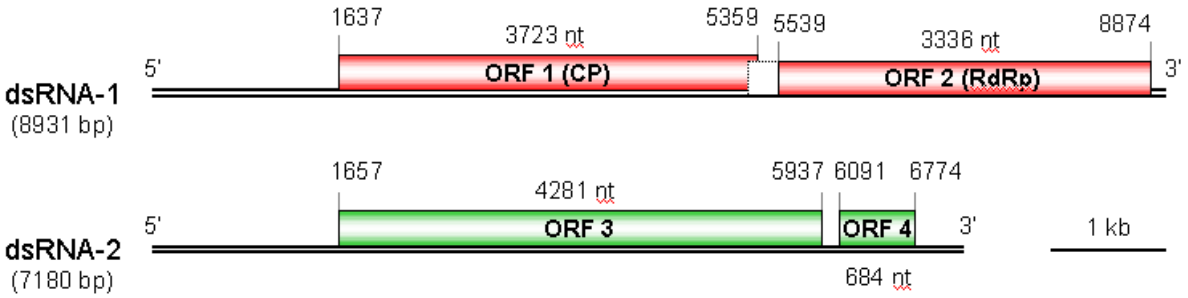
Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate 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.

TABLE 1. Summary of the results of BLASTP search with dsRNA-1 ORF2-coded RNA-dependent RNA polymerase.

	mycovirus [a] (RdRp size in aa)		RdRP_4 conserved region motif I - VIII	(size in aa)	BLAST Identity	(overlap)	Bits score	E-value	Accessions
	RnMBV1	(1111)	378-765	(388)	-	-	-	-	AB512282
	LeV-HKB [c]	(1245)	491-878	(388)	25%	(170/664)	177	4e-42	AB429554
	PgV-TW2 [c] dsRNA1	(1414)	669-1061	(393)	30%	(183/601)	201	4e-49	AM111096
Chrysovirus [e]	PcV	(1117)	453-832	(380)	22%	(120/523)	96.3	2e-17	AF296339
Chrysovirus [e]	HvV145S	(1086)	426-805	(380)	22%	(142/625)	93.2	1e-16	AF297176
Chrysovirus [e]	ACD-CV [b]	(1087)	427-806	(380)	25%	(83/326)	77.8	6e-12	NC_009947
Chrysovirus [e]	CCRS-CV [b]	(1087)	427-806	(380)	25%	(84/326)	80.9	7e-13	AJ781397
Chrysovirus [e]	FoV1 [c]	(858)	360-738	(379)	22%	(76/339)	64.3	6e-08	EF152346
Chrysovirus [e]	AbV1	(1078)	372-785	(414)	23%	(82/356)	62.8	2e-07	X94361
Totivirus	ScV-L-A	(731)	169-520	(352)	24%	(72/297)	67.0	1e-08	J04692
Totivirus	ScV-L-BC	(863)	306-654	(349)	25%	(35/139)	40.0	1.5	U01060
Totivirus	UmV-H1	(1820) [d]	1142-1530 [d]	(389) [d]	22%	(90/400)	72.8	2e-10	NC_003823
Victorivirus	HmV-17	(845)	243-594	(352)	32%	(43/131)	51.2	6e-04	AB085814
Victorivirus	GaRV-L1	(825)	221-573	(353)	29%	(51/171)	50.4	0.001	AF337175
Victorivirus	BfTV1 [e]	(838)	242-585	(344)	24%	(58/235)	43.1	0.16	AM491608
Victorivirus	MoV1	(845)	227-575	(349)	23%	(62/260)	42.0	0.35	AB176964
Victorivirus	HvV190S	(835)	241-585	(345)	27%	(30/111)	37.4	9.3	U41345

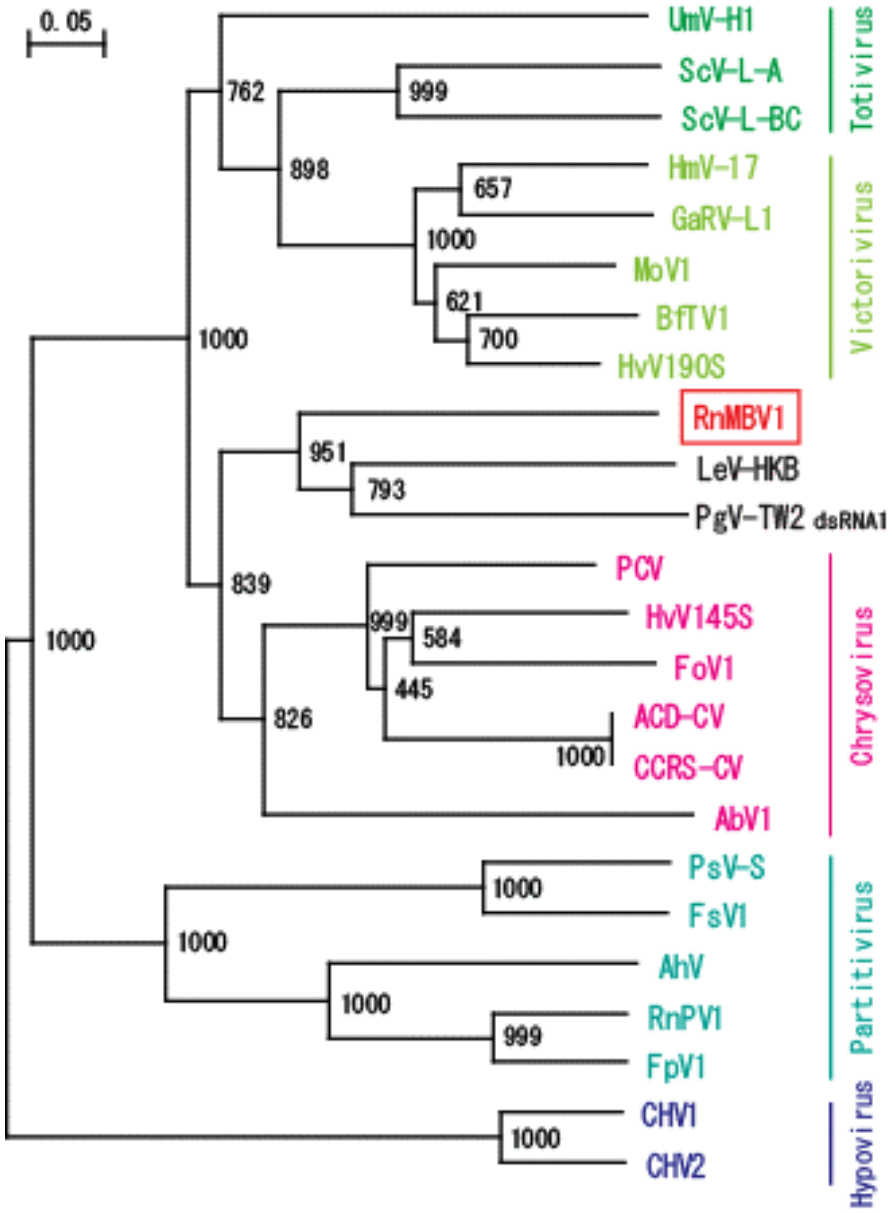
[a]Abbreviated virus names: LeV-HKB, Lentinula edodes mycovirus HKB; mycovirus HKB; PgV1, Phlebiopsis gigantea mycovirus dsRNA1; PcV, Penicillium chrysogenum virus; HvV145S, Helminthosporium victoriae virus 145S; ACD-CV, Amasya cherry disease associated chrysovirus; CCRS-CV, Cherry chlorotic rusty spot associated chrysovirus; FoV1, Fusarium oxysporum chrysovirus 1; AbV1, Agaricus bisporus virus 1; ScV-L-A, Saccharomyces cerevisiae virus L-A; ScV-L-BC, Saccharomyces cerevisiae virus L-BC; UmV-H1, Ustilago maydis virus H1; HmV-17, Helicobasidium mompa No.17 dsRNA virus; GaRV1, Gremmeniella abetina RNA virus L1; BfTV1, Botryotinia fuckeliana totivirus 1; MoV1, Magnaporthe oryzae virus 1; HvV190S, Helminthosporium victoriae virus 190S; FpV1, Fusarium poae virus 1 (AF047013); RnPV1, Rosellinia necatrix partitivirus 1-W8 (NC_007537); AhV, Atkinsonella hypoxylon virus (L39125); FsV1, Fusarium solani virus 1 (D55668); PsV-S, Penicillium stoloniferum virus S (NC_005976); CHV1, Cryphonectria hypovirus 1-EP713 (M57938); CHV2, Cryphonectria hypovirus 2-NB58 (L29010). LeV-HKB and PgV1 are partially characterized and their entire genome sequences are not available.
[b] No evidence demonstrating the fungal origin is provided.
[c] Only partial nucleotide sequences are available.
[d] Data are taken from the cap-pol fusion protein.
[e] Tentative and presumable members are included.

Fig 1 Schematic representation of the genetic organization of RnMBV1/W779



DsRNA-1 and -2 are 8931 nts and 7180 nts in length. DsRNA-1 has a 1636 nt-long 5'-UTR, two ORFs (ORF 1 and ORF 2), and a 57 nt-long 3'-UTR, while dsRNA-2 has a 1656 nt-long 5'-UTR, two ORFs (ORF 3 and ORF 4), and a 3' 406 nt-long UTR. ORFs 1-4 are composed of 1240, 1111, 1427, and 227 codons, respectively. Open boxes drawn using solid lines denote ORFs, while that drawn by dotted lines indicates a possible extension of ORF 2 by frameshifting. Numbers above solid lines refer to map positions of initiation and termination codons of the respective ORFs. A scale bar denotes 1 kb.

Fig 2. Phylogenetic analysis of RnMBV1/W779



A multiple alignment of the conserved motifs and flanking regions of RdRps from 24 related viruses representing established dsRNA mycovirus genera in the families *Chrysoviridae*, *Partitiviridae* and *Totiviridae* as well as dsRNA-like elements from *Phlebiopsis gigantean* and *Lentinula edodes* were used to construct a dendrogram. The neighbor-joining tree was constructed using CLUSTAL X in which hypoviruses with ssRNA genomes were included as an outgroup. Numbers at the nodes denote bootstrap values out of 1000 replicates