This Word module should be used for all taxonomic proposals.

Please complete **Part 1** and:

either **Part 3** for proposals to create new taxa or change existing taxa

or **Part 2** for proposals of a general nature.

Submit the completed Word module, together with the accompanying Excel module named in Part 3, to the appropriate ICTV Subcommittee Chair.

The Word module explains and justifies your proposal. The Excel module is a critical document that will be used to implement the proposed taxonomic changes once they are approved and ratified. If proposals presented in the Word module are not presented accurately in the Excel module, the taxonomic changes cannot proceed.

For guidance, see the notes written in blue, below, and the Help Notes in file Taxonomic\_Proposals\_Help\_2019.

**Part 1:** **TITLE, AUTHORS, etc**

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| --- | --- | --- |
| **Code assigned:** | ***2019.025P*** |  |
| **Short title:** Create one new family *Mayoviridae* and one new genus *Pteridovirus* |
|  |
| **Author(s) and email address(es):**  |
| List authors in a single line *Archives of Virology* citation format (e.g. Smith AB, Huang C-L, Santos, F) | Provide email address for each author in a single line separated by semi-colons |
| Aboughanem-Sabanadzovic N, Valverde RA, DiSerio F, Sabanadzovic S | nja62@msstate.edu; rvalverde@agcenter.lsu.edu; francesco.diserio@ipsp.cnr.it; ss501@msstate.edu; |
| **Author(s) institutional address(es) (optional):**

|  |
| --- |
| Provide institutional addresses, each on a single line followed by author(s) initials (e.g. University of Woolloomooloo [SAB, HCL]) |
| - Mississippi State University, MS 39762, USA **[NAG]**- Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA **[RAV]**- Istituto per la Protezione Sostenibile delle Piante (CNR), 70126 Bari, Italy **[FDS]**- Mississippi State University, MS 39762, USA **[SS]** |

 |
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| **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 (there are six virus subcommittees: animal DNA and retroviruses, animal ssRNA-, animal ssRNA+, fungal and protist, plant, bacterial and archaeal) |  |
| **ICTV Study Group comments (if any) and response of the proposer:** |
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| Date first submitted to ICTV: | June 1, 2019 |
| Date of this revision (if different to above): |       |

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| **ICTV-EC comments and response of the proposer:** |
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**Part 2:** **NON-STANDARD**

Template for any proposal regarding ICTV procedures, rules or policy, not involving the creation of new taxonomy.

| **Text of proposal:** |
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**Part 3:** **PROPOSED TAXONOMY**

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| **Name of accompanying Excel module:** 2019.025P.A.v2.Mayoviridae\_new\_fam |

The taxonomic changes you are proposing should be presented on an accompanying Excel module, 2019\_TP\_Template\_Excel\_module. Please enter the file name of the completed module in this box.

**Supporting material:**

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| **Proposal to create new family *Mayoviridae* that includes currently unassigned genus *Idaeovirus* and a newly established genus *Pteridovirus*** **Summary of the proposal and its impact on current taxonomy** In this multi-layered proposal we seek:* Creation of new genus *Pteridovirus* to include two new species
* Creation of new virus family *Mayoviridae* to classify the new genus *Pteridovirus* and currently unassigned genus *Idaeovirus*

**Current taxonomy:**Realm *Riboviria* * Genus *Idaeovirus* (unassigned to a family; two species)

**Proposed Taxonomy (new taxa proposed in this TP highlighted in red):**Realm *Riboviria** Family *Mayoviridae*
	+ Genus *Idaeovirus* (two species)
	+ Genus *Pteridovirus* (two species)
		- *Japanese holly fern mottle pteridovirus* (type species)
		- *Maize associated pteridovirus*

Etymology of names for newly proposed genus and family*Pteridovirus*: From Ancient Greek ([*πτέρις*](https://en.wiktionary.org/wiki/%CF%80%CF%84%CE%AD%CF%81%CE%B9%CF%82#Ancient_Greek)) ptéris = fern, original host of the virus Japanese holly fern mottle virus, member of proposed type species *Japanese holly fern pteridovirus*. The name for this species was proposed informally a decade ago (Valverde and Sabanadzovic, 2009). *Mayoviridae*: to honor the legacy and contributions of Dr. Michael A. Mayo (1944-2008) to the advancement of virus taxonomy and his service to the ICTV (Plant Virus SC Chair 1993-1999; Joint ICTV Secretary 199-2005; co-editor of ICTV Reports), as well as for his study on understanding the genome organization and replication mechanism of raspberry bushy dwarf virus (RBDV), type member of the genus *Idaeovirus*, one of the two genera classified in the new family (see list of references included in this document). **Justification for creation of the genus *Pteridovirus* to includetwo newly recognized species**A novel virus with a bisegmented RNA genome, named Japanese holly fern mottle virus (JHFMoV), was characterized from diseased Japanese holly ferns (JHF; *Cyrtomium falcatum*) collected in Mississippi and Louisiana, USA (Valverde and Sabanadzovic, 2008; Valverde and Sabanadzovic, 2009). The same virus was later reported from letherleaf fern (LLF), *Rumohra adiantiformis*, showing virus-like symptoms (Valverde and Sabanadzovic, 2011). The virus was experimentally transmitted by grafting, as well as vertically through spores from an infected plant. Partially purified preparations from infected ferns contained quasi-spherical particles that ranged from 30-40 nm in diameter assumed to represent JHFMoV virions. An RT-PCR-based survey suggested a close association between JHFMoV and an emerging disease of JHF in several southern states of the USA (Valverde and Sabanadzovic, 2009). Analyses of complete genome sequences of isolate 'DI' (JHFMoV-DI) from Mississippi, obtained by combination of cloning and Sanger sequencing with 5'/3'RACE, revealed that the genomic organization of JHFMoV RNA1 resembles that of raspberry bushy dwarf virus (RBDV; genus *Idaeovirus*), characterized by the presence of two ORFs (ORFs 1a and 1b; Figure 1). The large ORF 1a encodes a putative polyprotein with three functional domains (methyltransferase, MTR; viral helicase superfamily 1, Hel; RNA-dependent RNA polymerase superfamily 2, RdRP). The three domains encoded by JHFMoV ORF1a share 30-35% identical amino acids with the corresponding products of RBDV and members of the family *Bromoviridae* (Valverde and Sabanadzovic, 2009). Furthermore, an additional ORF (ORF1b), putatively coding for a 12K protein with unknown function, is present at the 3'-end of RNA1. Northern hybridization performed on extracts from JHFMoV-infected plants showed the presence of a possible subgenomic RNA of approximately 400 nt, which may serve as a template for ORF1b expression. JHFMoV RNA2 shows a distinct organization, with the presence of three ORFs separated by intergenic regions versus two ORFs in the case of RBDV RNA2 (Figure 1). Interestingly, ORF2a encodes a putative 32K protein with affinities with movement proteins (MPs) encoded by umbraviruses (capsid-less viruses, currently classified in the family *Tombusviridae*). Products of ORFs 2b and 2c have no significant identities with any protein available in NCBI/GenBank at the time of analyses. Phylogenetic analyses of putative viral-encoded products showed that viral MTR, Hel and RdRP domains formed a sister lineage with RBDV-encoded counterparts, suggesting a common ancestry. On the contrary, the JHFMoV 2a product is closer to movement proteins of umbraviruses and bromovirids than to those encoded by idaeoviruses (see Valverde and Sabanadzovic, 2009).Based upon these results, it is concluded that the virus detected in diseased ferns is an undescribed plant virus referred to as Japanese holly fern mottle virus (JHFMoV). Based upon its distinguishing features it was suggested that JHFMoV represents the type species of a new genus for which the authors informally proposed the name *Pteridovirus*, to reflect the type of host (ferns) (Valverde and Sabanadzovic, 2009). In 2019, during a metaviromics-based study on viruses associated with maize lethal necrosis disease (MLND) in Africa, analyses of RNAseq assembly data showed the presence of two large contigs of 5.8 and 2.7 kb obtained from a single sample collected in the Arusha region (Tanzania), assumed to represent the genome segments of an RNA virus, named maize-associated pteridovirus, isolate '160060' (MaPV-160060) (Read et al., 2019). The larger contig, assumed to represent viral RNA1, contains a single ORF encoding a 1,862 aa-long polyprotein with three putative functional domains (MTR, Hel and RdRP), whereas the smaller contig is tricistronic (Figure 2). This genome organization closely resembles that of JHFMoV (Figure 2). BLASTp comparisons with sequences available in GenBank and phylogenetic analyses revealed relationships between proteins expressed by MaPV-160060 and JHFMoV, and more distantly with those of idaeoviruses (Read et al., 2019). Interestingly, another isolate of this virus, with 99% identity with MaPV-160060, has been discovered independently by another group and deposited in GenBank under the name maize pteridovirus 1 (GenBank MF372912 and MF372913). This virus isolate has been discovered in a maize sample collected from South Sudan and has an unpublished preprint available in BioRxiv (Adams et al., 2017). In summary, * JHFMoV and MaPV have similar genome organization and expression strategy, with a monocistronic RNA1 encoding a replication-associated protein, and a tricistronic RNA2 coding for a putative movement protein, coat protein and a third protein of unknown function.
* Phylogenetic analyses show that functional domains expressed by JHFMoV and MaPV ORF1a always cluster together and belong to a lineage related but distinct to the one of idaeoviruses and bromovirids.
* Proteins encoded by JHFMoV and MaPV ORF 2a share more affinities with movement proteins encoded by umbraviruses, capsidless viruses belonging to family *Tombusviridae*, rather than to those of idaeoviruses or members of the family *Bromoviridae*.

Therefore, considering the above-listed similarities between JHFMoV and MaPV and their distinction from members of any other currently recognized taxon in the ICTV framework of virus classification, we formally propose: * Creation of new genus *Pteridovirus* to classify JHFMoV and MaPV isolates.
* Creation of type species *Japanese holly fern pteridovirus* typified by Japanese holly fern mottle virus isolate DI (JHFMoV-DI), the first and most extensively characterized member of the new genus (Valverde and Sabanadzovic, 2009).
* Creation of new species *Maize associated pteridovirus* to classify recently characterized homonymous virus isolated from maize, with MaPV-160060 as the exemplar isolate.

In addition, we propose a set of initial species demarcation criteria in the newly proposed genus:* differences in amino acid content of viral RdRP domain exceeding 15%
* differences in host range

**Justification for establishment of a new family (proposed name *Mayoviridae*)**Genus *Idaeovirus* is one of the very few genera in the realm *Riboviria* that is unassigned to an immediate higher-rank taxon (<https://talk.ictvonline.org/taxonomy/>). Historically, for more than 25 years it has been a monotypic genus, containing the sole species *Raspberry bushy dwarf virus* represented by the exemplar isolate RBDV-R15 (MacFarlane, 2012). The genome of RBDV-R15 consists of two positive-sense RNA molecules of 5,449 nt (RNA1) and 2,713 nt (RNA2) in size (Ziegler et al., 1992; Natsuaki et al., 1991). Both RNAs are bicistronic. RNA1 contains a major ORF coding for a 188 kDA protein, with three identifiable functional domains (MTR, Hel and RdRP). Some isolates of RBDV potentially encode an additional small ORF towards the 3'-end of RNA1, capable of encoding a product of 12kDa and with limited homology with proteins encoded by other viruses. A recent study carried out in Japan showed that the RBDV 1b gene is a virulence component that facilitates systemic infection of the virus in plants (Isogai et al., 2019). The RNA2 is bicistronic, with ORFs 2a and 2b coding for movement protein and coat protein, respectively (Figure 3). Viral coat protein is expressed from a 3'-co-terminal sgRNA produced in infected plants (RNA3; Mayo et al., 1991). The RBDV genome expression mechanism resembles "genome activation" employed by alfalfa mosaic virus (AMV) and ilarviruses (MacFarlane and McGavin, 2009). The currently known natural host range of RBDV is limited to *Rubus* spp. and grapevines. It is transmitted in association with pollen (horizontally and vertically) (MacFarlane, 2012).A new virus, with similar properties to RBDV, has been recently identified and characterized in leaf blotch-affected privet collected in southern Italy (Navarro et al., 2017). The genome of this virus, named privet leaf blotch-associated virus (PrLBaV), is composed of two segments of +ssRNA with an organization resembling that of RBDV. The only difference is the absence of ORF1b in the 3'-terminal part of RNA1. PrLBaV-encoded proteins share significant levels of amino acid identity with RBDV (ranging from 28% for MP to 47% for RdRP) and have close phylogenetic relationships with its counterparts as they cluster together in analyses including all viral proteins (Navarro et al., 2017). The same study provided the first experimental evidence for the role of 2a protein in virus movement/trafficking within the plant. An official Taxonomic Proposal for creation of a new species, *Privet idaeovirus*, to classify privet leaf blotch-associated virus (2018.021.P.A.v1.Idaeovirus\_sp) has been submitted in 2018 and has been approved in the 2019 ratification vote.Therefore, we believe that the currently available data on idaeoviruses and members of the proposed genus *Pteridovirus* (JHFMoV and MaPV) justify the creation of a new family, named *Mayoviridae*, due to:* similar genome organization (a bi-segmented, +ssRNA genome)
* "alphavirus-like supergroup" replication-associated protein with signature domains of MTR, Hel and RdRP encoded by RNA1
* similar expression strategies involving sgRNA
* close evolutionary relationships.
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**Figure 1.** Comparison of genome organization of Japanese holly fern mottle virus isolate DI (JHFMoV-DI), proposed to represent the type species of the new genus *Pteridovirus*, and a counterpart in the genus *Idaeovirus*, raspberry bushy dwarf virus isolate R15 (RBDV-R15). The same color of putative genome products indicates similar function (MTR = methyltransferase, Hel = helicase, RdRP = RNA-dependent RNA Polymerase, MP = movement protein, CP = coat protein). Difference in font color between MPs of JHMoV-DI and RBDV-R15 indicate their different origins.



**Figure 2.** Genome organization of Japanese holly fern mottle virus isolate DI (JHFMoV-DI) and maize-associated pteridovirus isolate 160060 (MaPV-160060), proposed members of the new genus *Pteridovirus*. JHFMoV-DI is proposed as the exemplar isolate of the type species *Japanese holly fern pteridovirus*. Abbreviations: MTR = methyltransferase, Hel = helicase, RdRp = RNA-dependent RNA polymerase, MP = movement protein, CP = coat protein.



**Figure 3.** Phylogenetic tree based on amino acid sequences of viral RNA-dependent RNA polymerases, as support to the proposal of creation of genus *Pteridovirus* and family *Mayoviridae* (indicated in red font), depicting their evolutionary relationships with viruses classified in currently recognized ICTV taxa. The analysis was implemented in MEGA7 (Kumar et al., 2016). The tree was inferred using the maximum likelihood (ML) method based on the best-fit amino acid substitution model. Percentages of bootstrap support out of 1,000 iterations are reported at the main branching points. Extended names of viruses used to construct trees are: alfalfa mosaic (AMV), apple mosaic virus (ApMV), brome mosaic virus (BMV), beet yellows virus (BYV), cowpea chlorotic mottle virus (CCMV), cucumber mosaic virus (CMV; NP\_049324), grapevine leafroll-associated virus 4 (GLRaV-4), Japanese holy fern mottle virus (JHFMoV), lettuce infectious yellows virus (LIYV), peanut stunt virus (PSV), Pelargonium zonate spot virus (PZSV), potato mop top virus (PMTV), privet leaf blotch-associated virus (PrLBaV), Prunus necrotic ringspot virus (PNRSV), raspberry bushy dwarf virus (RBDV), tobacco mosaic virus (TMV), tobacco rattle virus (TRV), tobacco streak virus (TSV), tomato aspermy virus (TAV).