

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

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| **Code assigned:** | **2022.001F** |  |
| **Short title:** Create a new family (*Alternaviridae*) accommodating one new genus (*Alternavirus*) and five new species |
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**Author(s) and email address(es)**

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**List the ICTV Study Group(s) that have seen this proposal**

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**ICTV Study Group comments and response of proposer**

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**ICTV Study Group votes on proposal**

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| **Study Group** | **Number of members** |
| **Votes support** | **Votes against** | **No vote** |
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**Authority to use the name of a living person**

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| **Is any taxon name used here derived from that of a living person (Y/N)** | N |

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| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
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**Submission dates**

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| Date first submitted to SC Chair | May 21,2022 |
| Date of this revision (if different to above) | September 21, 2022 |

**ICTV-EC comments and response of the proposer**

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| EC Comments: 1. Can you please justify use of “homology of dsRNA4” is one of demarcation criteria when there are three-segmented members?
2. Can you please explain in the Legend how numerical values in Fig 2 relate to % homology? For example: 0 = no detectable homology 1= identical

Response: The text concerning demarcation criterion #4 has now been amended to read “presence and homology of dsRNA 4”. The original figure generated with MEGAX has been replaced with a new one done in SDT v.1.2 software as it directly shows percentages of pairwise identities reported as color code.  |

**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

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| 2022.001F.N.v1.Alternaviridae\_newfam.xlsx |

**Abstract**

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| Here we propose the establishment of a new family *Alternaviridae*, a new genus *Alternavirus* and new five species to accommodate ten recently described mycoviruses. |

**Text of proposal**

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| The first member of the proposed family *Alternaviridae* was isolated from the ascomycete *Alternaria alternata* by Aoki *et al*. in 2009 and since then a total of ten related viruses have been reported. These recent discoveries have inspired the proposal for creation of novel taxa to classify these viruses (Table 1):1. Creation of the family *Alternaviridae* (from *Alternaria alternata*, the name of the original fungal host, which also contains the acronym RNA, the type of nucleic acid comprising the genome of these viruses).
2. Creation of the genus *Alternavirus* within the proposed family *Alternaviridae*.
3. Creation and assignment of five new species to the proposed genus *Alternavirus*.

Members of the family “Alternaviridae” have isometric virus particles (33 nm in diameter) and genome composed of 3-4 molecules of double stranded (ds) RNA; the overall size of the genome ranges from 8.4 to 10.7 kbp excluding polyA tails. In all currently known alternaviruses, the largest dsRNA encodes the RNA-directed RNA polymerase (RdRP); dsRNA 2 encodes a conserved protein of unknown function; dsRNA 3 encodes the major capsid protein (CP; Wu *et al.*, 2021); and dsRNA 4, when present, encodes a non-conserved protein of unknown function which is often non homologous among alternaviruses. The buoyant density alternavirus virions in CsCl was 1.35–1.40 g/cm3 depending on the size of the packaged dsRNAs (Aoki *et al.*, 2009). To date, alternaviruses have been found exclusively in ascomycetes. Nevertheless, a study on the virome of sisal using high-throughput sequencing revealed short fragments of sequences with significant similarity to alternaviruses (Quintanilha-Peixoto *et al.*, 2021).As evidenced by the phylogenetic analysis (Fig. 1), putative members of the proposed family *Alternaviridae* form a group together. Members of the *Chrysoviridae*, *Megabirnaviridae*, *Quadriviridae*, and *Totiviridae* – established families within the order *Ghabrivirales* whose RdRP contains an RDRP\_4 domain (PF02123, Pfam database) similar to alternaviruses – were used as the outgroup. The proposed classification is also supported by the pairwise distance matrix (Fig. 2), illustrating that each virus has an evolutionary distance smaller than 1.05 compared to all putative members of the genus. The genomic organization of Alternaria alternata virus 1 (AaV1), the first member of this group (Aoki *et al.*, 2009), is depicted in Fig. 3. Information on the exemplars and additional isolates within the five proposed species in the genus *Alternavirus* can be found in Table 2. The species demarcation criteria within the genus *Alternavirus* are:* amino acid sequence data (≤ 70% aa sequence identity in the RdRP)
* host of isolation
* size and number of dsRNA segments
* presence and homology of dsRNA 4

All proposed species are represented by fully sequenced viruses with publicly available accession numbers and with an evolutionary distance larger than 0.3.  |

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**Supporting evidence**

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**Figure 1:** Maximum likelihood phylogenetic tree created based on the RdRP sequences of alternaviruses and related viruses. The sequences were aligned with MUSCLE as implemented by MEGA X (Kumar *et al*., 2018), all positions with less than 30% site coverage were eliminated and the WAG+G+I+F substitution model was used. The number in brackets after the virus name indicates the number of dsRNA genomic segments. Red circles at the tip of branches indicate members of the proposed family “Alternaviridae”. Full red circles indicate exemplar viruses of the proposed species. Squares indicate members of related, established families: blue for *Totiviridae*, teal for *Quadriviridae*, green for *Megabirnaviridae* and gold for *Chrysoviridae*.

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**Figure 2:** Pairwise distance matrix created based on the RdRP sequences of alternaviruses (black font) and selected related viruses (red font). The sequences were aligned with MUSCLE as implemented by SDT v.1.2 (Kumar *et al*., 2018). Percentage identity is color-coded.

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**Figure 3:** Schematic representation of the genomic organization of Alternaria alternata virus 1, exemplar virus for the proposed species “Alternavirus alternariae”, genus “Alternavirus”, family “Alternaviridae”. The genome consists of four monocistronic dsRNA segments, with a 7-methylguanosine (m7G) capping structure and a poly(A) tail at respectively the 5’ and 3’ terminus (Wu *et al.*, 2021). The proteins encoded are represented by rectangular boxes, flanked by 5’- and 3’-UTRs represented by double lines. The light-coloured box in dsRNA 1 represents the RdRP\_4 domain (PF02123, Pfam database).

**Table 1:** Proposed organization of the family *Alternaviridae*.

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| **FAMILY: *Alternaviridae*** |
| **GENUS: *Alternavirus*** |
| **SPECIES:** *Alternavirus alternariae* |
| *Alternavirus aspergilli* |
| *Alternavirus cordycipitae* |
| *Alternavirus foxyspori* |
| *Alternavirus fusarii* |

**Table 2:** Exemplar and additional isolates of the proposed species in genus “Alternavirus”, family “Alternaviridae”.

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| **Virus name & abbreviation** | **Accession number & size** | **Reference** |
| Alternaria alternata virus 1 (AaV1)  | dsRNA 1: AB368492 (3567 bp) | Aoki *et al*., 2009 |
| dsRNA 2: AB438027 (2744 bp) |
| dsRNA 3: AB438028 (2540 bp) |
| dsRNA 4: AB438029 (1288 bp) |
| Aspergillus foetidus dsRNA mycovirus (AfV) | dsRNA 1: HE588144 (3571 bp) | Kozlakidis *et al.*, 2013 |
| dsRNA 2: HE588145 (2734 bp) |
| dsRNA 3: HE588146 (2418 bp) |
| dsRNA 4: HE647818 (1961 bp) |
| Aspergillus heteromorphus alternavirus 1(AheAV1) | dsRNA 1: MK279437 (3559 bp) | Gilbert *et al*., 2019 |
| dsRNA 2: MK279438 (2734 bp) |
| dsRNA 3: MK279439 (2417 bp) |
| Aspergillus mycovirus 341 (AsV341) | dsRNA 1: EU289897 (3571 bp) | Hammond *et al*., 2008 |
| Cordyceps chanhua alternavirus 1 (CcAV1) | dsRNA 1: OK481552 (3488 bp) | Zhang *et al*., 2022 |
| dsRNA 2: OK481553 (2632 bp) |
| dsRNA 3: OK481554 (2369 bp) |
| Fusarium graminearum alternavirus 1(FgAV1)  | dsRNA 1: MG254901 (3524 bp) | He *et al.*, 2018 |
| dsRNA 2: MG254902 (2470 bp) |
| dsRNA 3: MG697236 (2460 bp) |
| Fusarium incarnatum alternavirus 1(FiAV1) | dsRNA 1: MH899114 (3525 bp) | Zhang *et al*., 2019 |
| dsRNA 2: MH899115 (2469 bp) |
| dsRNA 3: MH899116 (2451 bp) |
| Fusarium oxysporum alternavirus 1(FoAV1) | dsRNA 1: MT659125 (3424 bp) | Wen *et al*., 2021 |
| dsRNA 2: MT659126 (2620 bp) |
| dsRNA 3: MT659127 (2335 bp) |
| dsRNA 4: MT659128 (1815 bp) |
| Fusarium poae alternavirus 1 (FpAV1) | dsRNA 1: LC150613 (3559 bp) | Osaki *et al.*, 2016 |
| dsRNA 2: LC150614 (2473 bp) |
| dsRNA 3: LC150615 (2462 bp) |
| Stemphylium lycopersici mycovirus 1(SlMV1) | dsRNA 1: MH040331 (3564 bp)  | unpublished |
| dsRNA 2: MH040332 (2750 bp) |
| dsRNA 3: MH040333 (2543 bp) |
| dsRNA 4: MH040334 (1398 bp) |

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