

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

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| **Code assigned:** | **2020.028M** |  |
| **Short title:** Abolish the family *Reoviridae* and promote subfamilies *Sedoreovirinae* and *Spinareovirinae* to the rank of family (*Sedoreoviridae* and *Spinareoviridae*, respectively) (*Reovirales*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| ICTV *Reoviridae/Reovirales* Study Group |

**ICTV study group comments and response of proposer**

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| N/A |

**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 |

**Submission dates**

|  |  |
| --- | --- |
| Date first submitted to SC Chair | July 31, 2020 |
| Date of first revision (if different to above) | May 28, 2021 |
| Date of second revision (if different to above) | September 17, 2021 |

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

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| Original submission and first revision: The EC voted Ud on the previous version of this proposal, asking for scientific evidence that the current reoviral subfamilies, proposed to be reoviral families, indeed should be grouped together in one higher taxon. This new version of the proposal is completely rewritten.  Second revision:  The EC asked for a minor revision and for the authors to work with Peter Simmonds, who had voiced minor concerns. As a result of these discussions with Dr. Simmonds and his recommendation, the trees were removed from this proposal as ideally they would contain RdRPs from all dsRNA viruses, which however is highly challenging because many dsRNA viruses have permutated RdRPs that cannot easily be aligned with reoviral RdRPs. |

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

**Name of accompanying Excel module**

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| 2020.028M.R.Reovirales\_2nfam |

**Abstract**

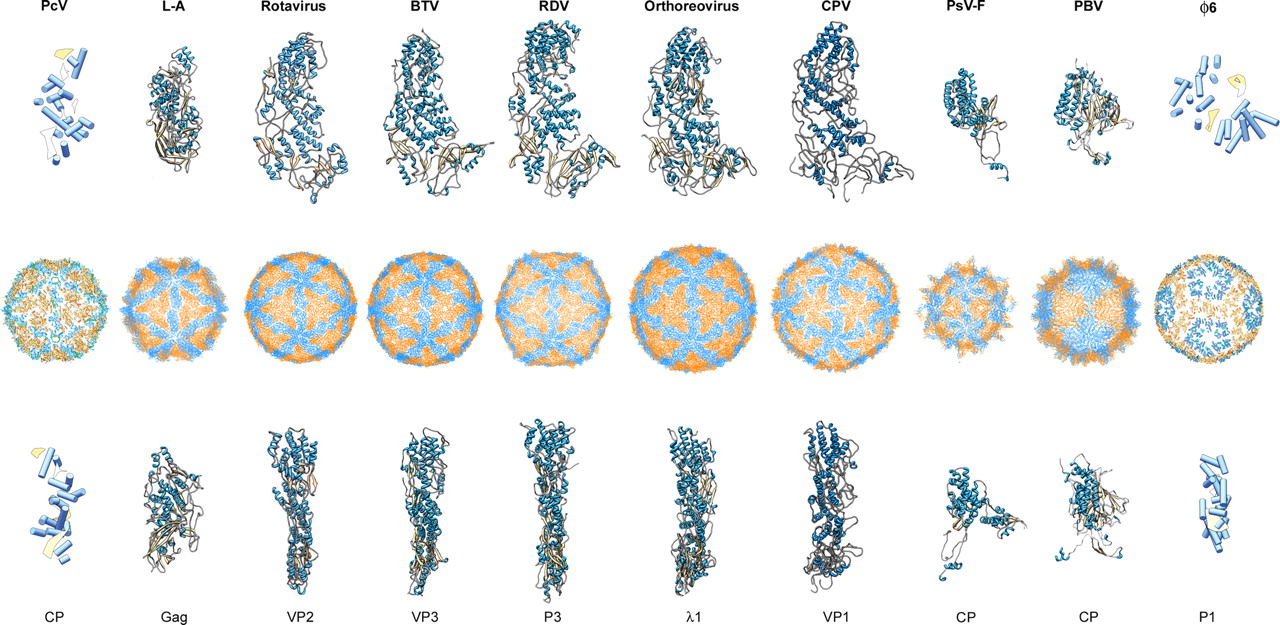
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| The very high levels of sequence diversity that are observed in the majority of reovirid proteins and RNAs across the family as a whole, along with major differences in their coding strategies, host ranges, virus particle structures, transmission mechanisms and clinical signs of disease, reflect a very large and diverse group of viruses that rationally should be reclassified as two families (*Sedoreoviridae* and *Spinareoviridae*), within the order *Reovirales*. |

**Text of proposal**

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| |  | | --- | | The family *Reoviridae* currently includes 15 virus genera, distributed within two subfamilies, *Sedoreovirinae* and *Spinareovirinae*. The subfamily *Sedoreovirinae* includes 6 genera of viruses with icosahedral core particles that have a relatively smooth outer-surface, hence the prefix ‘sedo’ which signifies ‘smooth’. The subfamily *Spinareovirinae* includes 9 genera of viruses with core particles that have ‘turrets’ or ‘spikes’ located on their surface at the 12 icosahedral five-fold vertices, hence the prefix ‘spina’ which signifies ‘spikes’.  Viruses belonging to family *Reoviridae* (reovirids) have evolved over relatively long periods of time so that it has become difficult to detect significant amino acid identities between many of their more variable genes, even if they encode homologous proteins. However, all reovirids have a more highly conserved and fully conservative, core-associated RNA-directed RNA polymerase (RdRp), enabling relatively reliable sequence comparisons across the entire family. Within individual genera, the amino acid (aa) identities of the RdRp are higher than 30%, except for rotaviruses, where aa values as low as 20-22% can exist between viruses of certain species.  The levels of amino acid identity detected in the RdRps between viruses of different reovirid genera are broadly similar within either ofthe two subfamilies, *Sedoreovirinae* or *Spinareovirinae,* ranging from ~7 to 13%. However, there are two exceptions within the subfamily *Spinareovirinae,* with higher aa identities between RdRps of coltiviruses and mycoreoviruses (26-27%) and those between aquareoviruses and orthoreoviruses (37-43%), indicating closer relationships between these genera, and suggesting more recent evolutionary links.  Structural analyses have identified the presence of an inner-core shell among different reovirids, with T1 or “pseudo T2” symmetry, constructed from 120 copies of a single protein, with a related structure, indicating a common ancestry. However, variations in virus particle structure have also helped to identify closer relationships between viruses of certain genera. Phylogenetic analyses (including those of the inner-core shell ‘T2’ protein) have identified groupings of individual genera within each subfamily, which correlate with morphological differences observed in the core structure. All sedoreovirins have a core with a relatively smooth surface. In contrast all spinareovirins have a core with external ‘turrets’ or ‘spikes’ located at the 12 vertices of the icosahedron.    The turrets of the spinareovirin particles consist of pentamers of the capping enzyme, whereas this enzyme is encapsidated together with other components of the replication complexes (including the RdRp and helicase) inside the core structure of sedoreovirin particles. These structural differences inevitably result in some differences in the mechanisms of mRNA of transcription/capping during virus replication.  Their overall replication strategies, the properties of the RdRp, the structural findings (including the presence of the T1 / “pseudo T2” inner-core shell), the polysegmented nature of their dsRNA genomes and sequence analyses, all indicate a close relationship of the viruses within each subfamily in particular and within the family as a whole. However, the ultrastructure studies, subtle transcriptional differences and closer phylogenetic relationships, all reflect specificities within each subfamily. Together with the very high level of sequence diversity that is observed across reovirids as a whole, particularly in the outer capsid proteins these observations warrant the elevation of the two current subfamilies to the rank of family. These families will be designated *Sedoreoviridae* and *Spinareoviridae* within the order *Reovirales.*  The reason that the *Spinareovirinae* and *Sedoreovirinae* (order *Reovirales*) belong in a single order are because they share the following features, some of which are not found in other families of viruses and collectively show a common and unique replication strategy, as follows:   * Multi segmented dsRNA genomes (9-12 segments), a greater number than found in any other known viruses. * They have an RNA selection and packaging mechanism which ensures a single copy of each genome segment in each progeny particle. Recent studies indicate that this is mediated by complementary base-pairing between short highly conserved and specific sequences on individual viral mRNAs. Other dsRNA viruses have fewer genome segments and different selection and packaging mechanisms. * They have conserved virus-species specific, terminal sequence common to all of their genome segments (4-8 base pairs). * Their genome segments have capped 5’ terminal +ve RNAs. * They do not have 3’ poly-A tails on their mRNAs. * The majority of their genome segment are mono-cistronic, although some secondary overlapping ORFs also exist. * They share icosahedral symmetry, with a sub-core layer that can be described as T=1 or pseudo T=2 structure * The T2 sub-core shell protein structure itself, although different in sequences, is to some extent superimposable between spina- and sedoreovirins (examples are the structure of bluetongue virus ‘orbivirus’ and mammalian orthoreovirus) ‘orthoreovirus’) but is not directly comparable with proteins of other dsRNA virus families (Figure 1). * The subcore and outer-core layers interact via a match of T2 and T13 symmetries that involves a series of 13 different and unique interactions. * The *Reovirales* polymerase is fully conservative (table 1). * The virus particle has proteins with pol, hel and capping enzyme activities. * In the ‘Sedo-‘ family these transcriptase complexes are packaged at the icosahedral 5 fold vertices within the core particle structure, while in the ‘Spina-‘ family they are also packaged as part of the core structure, at the icosahedral 5 fold vertices, but the capping enzyme is situated on the outer surface of the core particle.   Because of these similarities in function, structure and a shared replication-strategy, it is logical to retain the grouping of all of the viruses currently included with the order *Reovirales*.  However, in order for the taxonomy of these viruses to remain useful and rational, It is also essential to exclude the other dsRNA viruses from the order *Reovirales*. These other dsRNA viruses have one or more properties (usually several) that clearly distinguish them from members of the order *Reovirales* (Table 1).  These may include:   * Much smaller polymerase sequences (endorna and toti: almost 50% shorter), therefore lacking regions or domains found in the *Reovirales* PdRP. totiviruses do not use a capping enzymes, but rather an IRES to initiate translation of their mRNAs. * The birnaviruses pol is semiconservative, functions as a VPg polymerase, is self-guanylating and has a reverse transcriptase activity. Shwed et al (2002) state that *“ VP1 proteins of birnaviruses form a defined subgroup of polymerases that either are lacking the conserved RdRp motif VI (GDD), or have repositioned this motif to different structural regions*” (Figure 2). This suggests that any phylogenetic comparisons of the whole RdRp protein of the birnaviruses, toti viruses or endornaviruses with those of the members of the *Reovirales*, will be difficult and prone to errors in alignments.   A phylogenetic tree constructed with the sequences of all members of the current subfamilies *Sedoreovirinae* and *Spinareovirinae* suggest that viruses belonging to each subfamily cluster together.  Our previously published work has indicated that members of the genera *Aquareovirus* and *Coltivirus* have strong genetic links including non-structural proteins, indicating a common ancestry and are both grouped within the *Spinareovirinae* (Figure 3 - Mohd Jaafar et al., 2008).Our previously published data also indicate that members of the *Rotavirus* and the *Seadornavirus* have strong genetic links based both on sequence relatedness (Figures 4 and 5, Mohd Jaafar et al.,2005a and 2005b) having a superimposable morphology, as well as other shared structural features, collectively indicating an strong evolutionary link between them.  We therefore argue that there are convincing structural grounds and sequence relatedness for elevating the two subfamilies currently classified within family *Reoviridae* to the rank of families designated *Sedoreoviridae* and *Spinareoviridae* within order *Reovirales*.  We further comment, related to Identification of individual species within the different genera of the *Reovirales*:   * Although these viruses all have conserved polymerase, core and RNA structure/functions, many of their individual species characteristics (e.g. host and / arthropod vector range, antigenic properties, clinical signs of disease, transmission routes, etc.) are determined by the more variable ‘outer’ structural proteins and/non-structural proteins of the virus. * A further comparison of the conserved ‘T2’ sub-core protein, common across the entire *Reovirales* and other outer-capsid proteins (figure 1) may provide a better indication of virus species within each genus. * In addition, many of the specific characteristics of individual virus species appear to be determined by proteins that are unique to those genera and cannot therefore be simply compared across different virus genera.   Therefore, we feel very strongly that it is still of prime importance to retain the polythetic nature of species definition.  Previously a primary charter to define *Reovirales* species was the ability to reassortment / exchange genome segments creating new reassortant viruses with the same species. Recent studies with the orbiviruses have indicated that selection and packaging of viral mRNA segments during replication and assembly, involve specific short sequences that can base pair between different pairs of viral mRNAs during their selection for packaging and -ve RNA strand synthesis, to form progeny virus genome-segments (Boyce et al., 2016). This becomes an important character of these viruses that may be used in future to indicate the reassortment compatibility of viruses and therefore their species. However, we do not yet have sufficient information to identify these sequences within most of the different genome segments and species of the *Reovirales*. Combined with a better characterization of each genus, as expanded by newly discovered (yet unclassified) viruses, this may provide a way forward to enhance species-vs-virus nomenclature. We recognize that some aspects of this discussion were originally included in the plan for this year – but we have only recently taken over the chair position for the ICTV *Reovirales* Study Group and have not yet had time to pursue this line of investigation. | |

**Supporting evidence**

**Figure 1 : A T1 (T2) protein (sub-core) layer made of 120 copies of a single protein arranged as 60 dimers or 12 decamers**



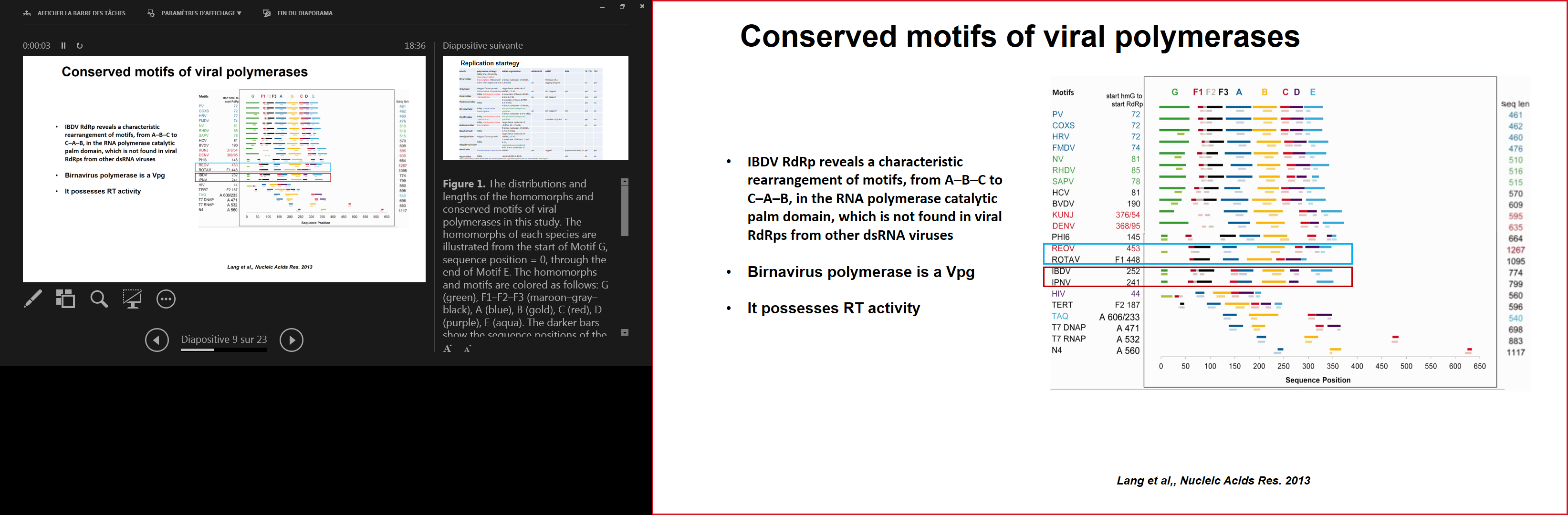
***Chrysoviridae***

***Totiviridae***

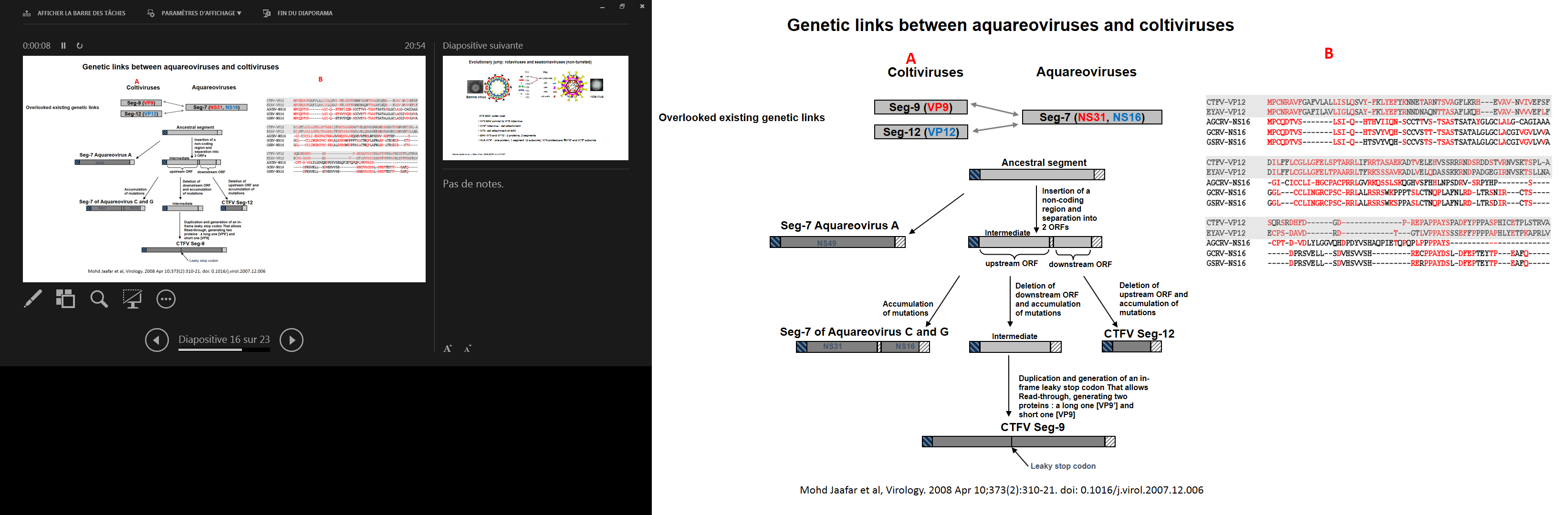
***Partitiviridae***

***Picobirnaviridae***

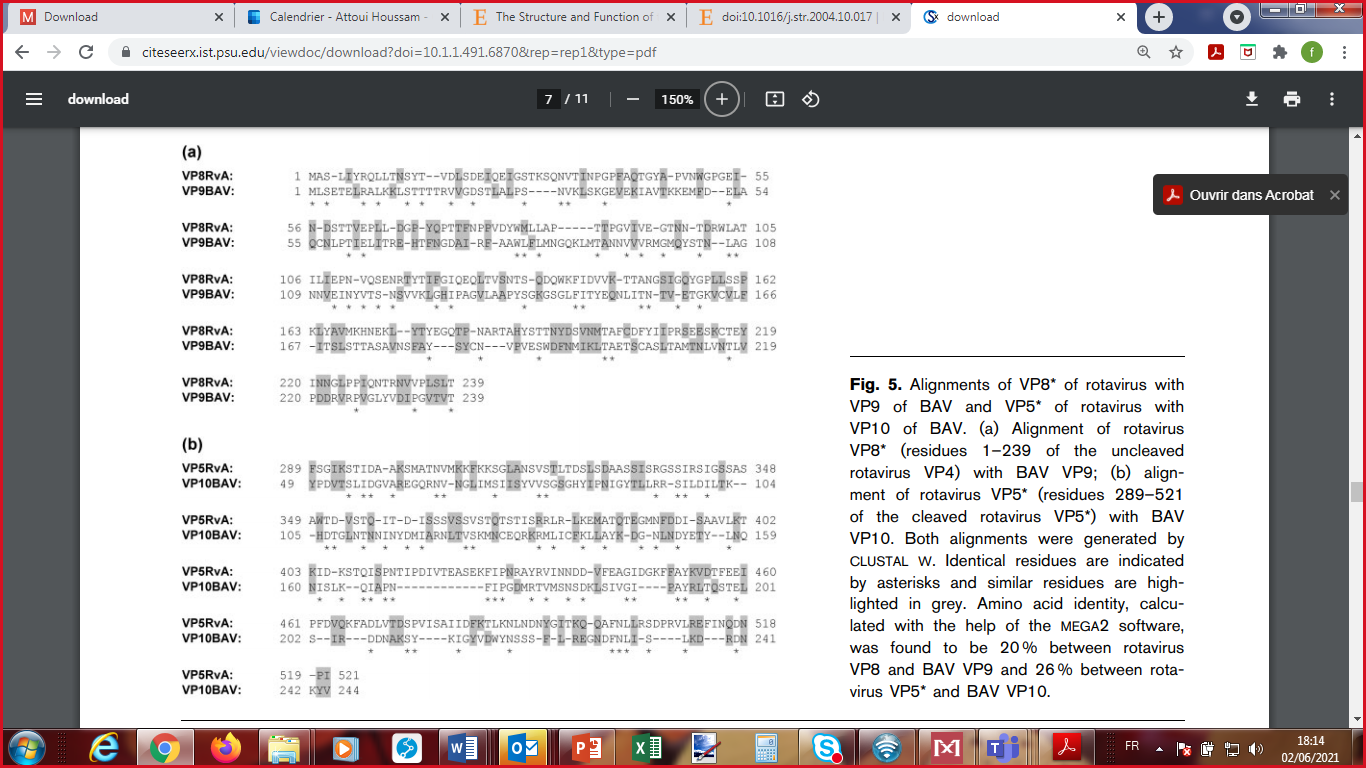
***Cystoviridae***



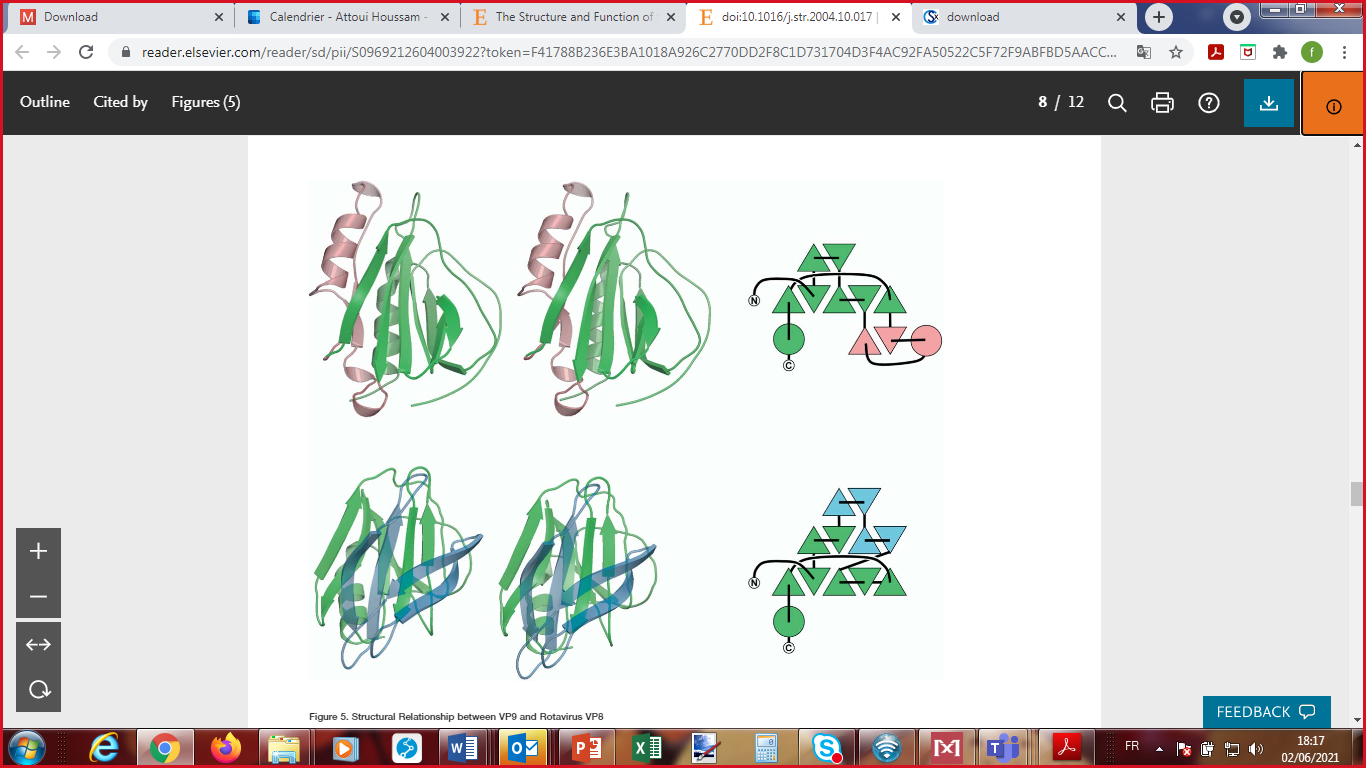
**Figure 2:** A schematic representation showing the differences in terms of rearrangements of the catalytic palm domain of the birnavirids RdRP. Hence using this RdRP in the alignment of polymerases will skew the alignment.



**Figure 3:** A) Diagrammatic representation of the evolutionary model that the ancestral segment 7 followed to give rise to segment 7 of CHSRV (AQRV-A), GCRV (AQRV-C), AGCRV (AQRV-G), MRV and segments 9 and 12 of CTFV. The higher levels of [sequence homology](https://www.sciencedirect.com/topics/medicine-and-dentistry/sequence-homology) detected between the translation products of segment 9 and segment 12 from the [coltiviruses](https://www.sciencedirect.com/topics/medicine-and-dentistry/coltivirus" \o "Learn more about Coltivirus from ScienceDirect's AI-generated Topic Pages), with those from the two ORFs of AQRV-G segment 7, than with the product of the single ORF from AQRV-A, support the hypothesis of insertion of a non-coding region and separation of the long ORF in the ancestral segment into 2 ORFs. The previously published co-speciation hypothesis ([Attoui et al., 2002a](https://www.sciencedirect.com/science/article/pii/S0042682207008136?via%3Dihub" \l "bib11)) suggested that the ancestral virus is a marine virus, from which the mammalian viruses (presented in this diagram) possibly evolved. https://ars.els-cdn.com/content/image/1-s2.0-S0042682207008136-fx1.jpg: non-coding regions. B) Alignment and [hydrophobicity](https://www.sciencedirect.com/topics/immunology-and-microbiology/hydrophobicity) profiles of the VP12 of [coltiviruses](https://www.sciencedirect.com/topics/medicine-and-dentistry/coltivirus" \o "Learn more about Coltivirus from ScienceDirect's AI-generated Topic Pages) and the NS16 of aquareoviruses (identical or highly similar residues are shown in red). (A) Alignment of the full-length [amino acid sequence](https://www.sciencedirect.com/topics/medicine-and-dentistry/peptide-sequence) of the VP12 (accession number [U53227](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=search&db=nucleotide&doptcmdl=genbank&term=U53227)) protein of CTFV (Colorado tick fever virus) and EYAV (Eyach virus), and a comparison to that of NS16 of AGCRV (American [grass carp](https://www.sciencedirect.com/topics/medicine-and-dentistry/grass-carp) reovirus), GCRV (grass carp reovirus) and GSRV (golden shiner reovirus). Identical or similar residues (between proteins of aquareoviruses and coltiviruses) in the alignments are shown in red. Similar residues are defined as those that preserve the chemical character or the structural character of the amino acid. (B) The grey plot represents the hydrophobicity profile of VP12 of CTFV. The black plot represents the hydrophobicity profile of NS16 of AGCRV. The blue plot represents the hydrophobicity profile of the [p14 protein](https://www.sciencedirect.com/topics/medicine-and-dentistry/protein-p14) of reptilian [orthoreovirus](https://www.sciencedirect.com/topics/medicine-and-dentistry/orthoreovirus" \o "Learn more about Orthoreovirus from ScienceDirect's AI-generated Topic Pages). The thick red bar indicates the trans-membrane domain (residues 36–68) of NS16 (48 to 76) of the VP12, were predicted by Winpep software program ([Hennig, 2001](https://www.sciencedirect.com/science/article/pii/S0042682207008136?via%3Dihub" \l "bib32)). The p14 of reptilian orthoreovirus also contains a trans-membrane domain (positions 39–57). The aquareovirus NS16, CTFV VP12 and reptilian orthoreovirus P14 all contain a proline-rich motif towards the [COOH terminus](https://www.sciencedirect.com/topics/medicine-and-dentistry/carboxy-terminal-sequence) of the proteins.



**Figure 4:** Alignments of VP8\* of rotavirus A (*Rotavirus*) with VP9 of BAV (*Seadornavirus*) and VP5\* of rotavirus with VP10 of BAV. (a) Alignment of rotavirus VP8\* (residues 1–239 of the uncleaved rotavirus VP4) with BAV VP9; (b) alignment of rotavirus VP5\* (residues 289–521 of the cleaved rotavirus VP5\*) with BAV VP10. Both alignments were generated by CLUSTAL W. Identical residues are indicated by asterisks and similar residues are highlighted in grey. Amino acid identity of 20% was found between rotavirus VP8 and BAV VP9 and 26% between rotavirus VP5\* and BAV VP10.



**Figure 5:** Structural Relationship between VP9 and Rotavirus VP8Comparison of VP9 with rotavirus VP8 (Dormitzer et al., 2002). The two molecules are drawn separately for clarity (top: residues 125–283 of VP9; bottom: residues 108–224 of VP8 bottom). The relative orientation is that determined by SHP (Stuart et al., 1979), which matches 79 Cαatoms with an rms deviation of 3.5 Å. The topologically similar core region is drawn in green and represented as a solid object. The insertion in VP9 relative to the conserved core is drawn in semi-transparent red, while the insertion in VP8 is drawn in semi-transparent blue. For clarity, a topological diagram of each molecule is also drawn, coloured as drawn in the stereo cartoons, with the conserved core in green and insertions in red and blue for VP9 and VP8, respectively.

**Table 1: A comparison of the replication strategies / properties of the dsRNA viruses**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***Family*** | **polymerase strategy** | **dsRNA organisation** | **dsRNA CAP** | **mRNA** | **IRES** | **T1 (T2)** | **T13** |
| ***Birnaviridae*** | RdRp-VPg, RT activity,  semi-conservative transcription. Palm motif A-B-C rearranged to C-A-B | 2 linear molecules of dsRNA 2.8-3.6Kb | no | Presence of a VPG |  | no | yes |
| ***Totiviridae*** | Gag-pol fusion protein. Conservative transcription | single linear molecule of dsRNA, 7.5 Kb | no | not capped, | yes | yes | no |
| ***Cystoviridae*** | RdRp, semi-conservative  transcription | 3 molecules of linear dsRNA 2.6 to 6.7 Kb | no | not capped |  | yes | yes |
| ***Picobirnaviridae*** | RdRp | 2 molcules of linera dsRNA 1.5-2.6 Kb |  |  |  | yes | no |
| ***Chrysoviridae*** | RdRp, conservative transcription | 4 linear molecules of dsRNA,  encapsidated in separate particles | no | not capped | yes | yes | no |
| ***Partitiviridae*** | RdRp, semi-conservative  mechanism | 2 linear molecules  1.4–2.4 kbp  encapsidated in separate particles |  | stretches of polyA | no | yes | no |
| ***Endornaviridae*** | RdRp, semi-conservative transcription | single linear molecule of dsRNA, 14- 17,6 Kb |  |  |  | no | no |
| ***Quadriviriade*** | RdRp | 4 linear molecules of dsRNA, 3.7 to 4.9 kbp |  |  |  |  |  |
| ***Amalgaviridae*** | Gag-pol fusion protein | single linear molecule of dsRNA, 3.5 Kb |  |  |  |  |  |
| ***Megabirnaviridae*** | RdRp | 2 molecules of dsRNA, 7 and 9 Kb, separately encapsidated |  |  |  |  |  |
| ***Reoviridae*** | Conservative transcription | 9-12 linear molecules of dsRNA | yes | capped | (mycoreoviruses)  no | yes | yes |
|  |  |  |  |  |  |  |  |
| ***Hypoviridae*** | RdRp | Linear dsRNA 9-13Kb |  |  | yes | no | no |
| Semi-conservative, which means that the newly synthesized (+)strand displaces the old one from the RNA duplex | | | | | | | |

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