

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

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| **Code assigned:** | **2020.012D** |  |
| **Short title:** Create one new realm (*Ribozyviria*) including one new family (*Kolmioviridae*) including genus *Deltavirus* and seven new genera for a total of 15 species | | |
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**Author(s) and email address(es)**

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| Hepojoki J, Hetzel U, Paraskevopoulou S, Drosten C, Harrach B, Zerbini M, Koonin EV, Krupovic M, Dolja V, Kuhn JH | jussi.hepojoki@helsinki.fi; jussi.hepojoki@uzh.ch; udo.hetzel@uzh.ch; sofia.paraskevopoulou@charite.de; christian.drosten@charite.de; balazs.harrach@gmail.com; zerbini@ufv.br; koonin@ncbi.nlm.nih.gov; krupovic@pasteur.fr; doljav@oregonstate.edu; kuhnjens@mail.nih.gov |

**Corresponding author**

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| Hepojoki J |

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

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| The ICTV *Hepadnaviridae* and Hepatitis Delta Virus Study Group was contacted for input, but only two responses were received by the time of initial proposal submission |

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

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| Balázs Harrach, ICTV Animal DNA Viruses and Retroviruses Subcommittee Chair, supports this TaxoProp with the exception of the step to establish a novel realm.  One member of the ICTV *Hepadnaviridae* and Hepatitis Delta Virus Study Group commented:  “I’d like to stress that, to my knowledge, most of the newly discovered HDV-like RNAs have not been proven to be, or to be part of, transmissible agents. Transmission was just suggested, but not proven, for snake HDV RNA. HDV itself does not fulfill the criteria for the definition of a virus – but it is admitted to be referred to as hepatitis delta virus -. As far as we know, the new HDV RNAs fulfill the virus definition criteria to a lesser degree. It might thus be too early to give a virus name to the new HDV RNAs.”  Our responses are appended below:   * The ICTV Code doesn’t define “virus” in its Code and hence a discussion about whether HDV and/or its direct relatives are viruses or not is only philosophical in nature but doesn’t really have bearing on the TaxoProp, in particular because ICTV, despite the absence of a virus definition, does accept HDV as a virus in its current taxonomic framework. * Hence the question becomes whether the new sequences are sufficiently *similar* to the classified HDV to also consider them viruses and HDV-like viruses at that. The question is therefore how HDV is defined (say, circular single-stranded RNA, encoding one protein with this kind of structure, ribozyme etc.) and whether the -like viruses fulfill most of these properties. Since there is no codified virus definition, the capability of transmission is not a prerequisite of classifying something as a virus. In fact, the vast majority of novel viruses that are being classified in all realms right now come from metagenomic datasets in the absence of isolates – and no transmission is therefore proven for any of them and such proof has not been required in recent years (in fact, there are many non-transmissible viruses that are accepted by the ICTV, such as pseudoviruses, metaviruses, and many mycoviruses). Classification of such entities is clearly permitted by the ICTV, which means that the only question is whether the metagenomic HDV-like sequences fulfill the other requirements for sequence-based classification, i.e., whether the genome sequences are coding-complete (yes) and whether it is known where they come from in terms of sample/potential host material (yes).   We therefore argue that classification of all these novel HDV-like viruses is clearly permissible (and one could argue encouraged by the Code). In addition, several papers have already been published demonstrating that, for instance, the ”snake deltavirus” forms infectious particles in presence of replicating reptarenaviruses or hartmaniviruses *in vitro* and could be isolated from a brain sample of an infected snake; classical HDV can form infectious particles in the absence of HBV as long as in presence of other viruses; these novel HDV-like agents are not found in all individuals of a particular host species (thereby strongly suggesting that they are indeed transmissible); and four representatives of the ICTV *Avsunviroidae* and *Pospiviroidae* Study Group weighed in, arguing, like we do, that HDV are to be considered viruses, and not, for instance, viroids or virusoids for a variety of reasons.  A second response was received right at the TaxoProp submission time and hence wasn’t addressed for the ICTV EC discussion:  ” I recommend a more conservative nomenclature approach, at least for the present.  First, for the various human HDVs that have been divided (solely) using their nucleotide sequences, into 8 genotypes, I would still call them no more than "HDV". It can also be made clearer that such HDV can naturally and experimentally be found with different envelope proteins. The natural situations include all the known genotypes of human HBV. Experimentally, the envelope proteins can be replaced by those of non-human hepadnaviridae, such as from the woodchuck, woolly monkey, and a bat species. Also, experimentally, non-hepadnaviridae can be substituted, such as hepatitis C virus and an arenavirus.  Second, in terms of the new HDV-like RNAs and viruses, I would leave them (at least for now) just as "HDV-like RNAs and viruses". Currently they have been reported as present in many non-human species. Also, the extent to which they are characterized remains highly varied. Some of the HDV-like RNAs have only been found via computer-based assembly from RNA sequencing data, to exist as RNA circles with some features reminiscent of human HDV.  Others have been much further characterized. For example, some undergo replication and experimentally, can be assembled using separately provided envelope proteins, to release viral particles that are able to infect susceptible cells.  Third, I would be more careful about referring to the RNA-protein complex in HDV particles. It is deceptive to call it a nucleocapsid. The antigen is a highly basic protein. It forms multimers of the delta antigen, most likely octamers. And, somehow genomic HDV RNA, and not antigenomic RNA, forms "ribonucleoprotein complexes" that can be released from infected cells using provided envelope proteins.” |

**Submission dates**

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| Date first submitted to SC Chair | July 31, 2020 |
| Date of this revision (if different to above) | December 6, 2020 |

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

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| The TaxoProp was favorable reviewed by the ICTV Executive Committee (EC) during EC52. The following minor revisions were requested:   1. in the accompanying Excel file, an error needs to be corrected: genus *Deltavirus* should be formally moved from ”unassigned” into the newly proposed family; 2. taxon demarcation criteria should be included in the TaxoProp text; 3. the comments on the TaxoProp received directly prior to TaxoProp submission should be formalled addressed and any additional Study Group comments, if made, should be addressed as well; and 4. finally, indpendent of this TaxoProp, a new Study Group should be formed in the Animal DNA Viruses and Retroviruses Subcommittee for the newly proposed family/realm.   We therefore implemented the following changes in this revised TaxoProp:   1. the Excel file was corrected; 2. taxon demarcation criteria are now included; 3. a new Study Group will be established under the Animal dsRNA and ssRNA- Viruses Subcommittee; and 4. our responses to the TaxoProp comments received directly prior to TaxoProp submission are appended below (no other comments were received from the Study Group previously responsible for ”HDV”):  * We agree with the reviewer that viruses other than hepadnavirids can support the replication of HDV-like entities (see response to first reviewer above). However, the type of helper virus needed for replication is not a necessary taxonomic marker for a particular virus—instead, we base our proposed classification on measurable genome or genome-encoded protein differences. Indeed, “HDV” isolates show great sequence variation even at the delta antigen amino acid level. We use the same demarcation criteria used for decades to differentiate HDV genotypes and simply officialize them as species (i.e., comparison at nucleotide level for the entire genome), recognizing that all “HDV” genotypes/species are more closely related to each other than to the newly discovered HDV-like entities. This similarity justifies the grouping of these species into the established genus *Deltavirus* and by extrapolation establishment of novel genera that are sisters to *Deltavirus*, and thereby the establishment of a family. * We appreciate the concerns regarding the novel HDV-like viruses. However, certain HDV-like sequences were already demonstrated to be replication-competent in suitable cell lines, and there are is currently no scientific evidence indicating that the other HDV-like sequences would not be replication-competent. * We agree with the reviewer: ribonucleoprotein (RNP) describes the complex better and we therefore replaced the term “nucleocapsid” throughout accordingly. |

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

**Name of accompanying Excel module**

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| 2020.012D.R.Ribozyviria.xlsx |

**Abstract**

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| We propose to create a new realm including a single otherwise unassigned family for hepatitis D virus (HDV) and its immediate relatives in a total of eight genera. |

**Text of proposal**

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| |  | | --- | | The unassigned genus *Deltavirus* currently includes a single species, *Hepatitis D virus*, for a single agent, hepatitis D virus (HDV), represented by eight genotypes (HDV-1 to HDV-8). HDV has a ribozyme-containing negative-sense single-stranded circular RNA genome reminiscent of those of viroids but encodes a protein that binds the genomic RNA forming a ribonucleoprotein complex. HDV is not related to any other classified negative-sense RNA virus (*Riboviria: Negarnaviricota)* or any other classified virus.  Here, we propose to   * create a realm, *Ribozyviria*, specific for HDV and its relatives based on the uniqueness of these agents; * to establish a novel family, *Kolmioviridae*, and to include it in this new realm; and * to assign the 8 HDV genotypes virus status (HDV-1 to HDV-8) and assigned them to individual species (one renamed, seven new).   Furthermore, an HDV-like nucleic acid (“avHDV”) was identified by next-generation sequencing in a combined sample taken from the cloacas of Pacific black ducks (*Anas superciliosa* Gmelin, 1789), chestnut teals (*A. castanea* Eyton, 1838), and grey teals (*A. gracilis* Buller, 1869) sampled in Melbourne Water Western Treatment Plant, Victoria, Australia [[8](#_ENREF_8)][. These findings were followed by identification of an HDV-like nucleic acid (“snake HDV/sHDV”) during metatranscriptomic analysis of a boa constrictor (](#_ENREF_7)*Boa constrictor* Linnaeus, 1758) and a water python/white-eyed python (*Liasis mackloti savuensis* Brongersma, 1956) from a Swiss snake colony [[2](#_ENREF_2)][. Then, similar agents were detected in samples collected in China from various fish (“fish HDV/fiHDV”), Chusan Island toads (](#_ENREF_2)*Bufo gargarizans* Cantor, 1842) (“tfHDV”), Chinese fire belly newts (*Cynops orientalis* (David, 1873) (“amphibian HDV/amHDV”), and termites (*Schedorhinotermes intermedius* (Brauer, 1866)) (“termite HDV/tHDV”) [[1](#_ENREF_1)][. Finally, a similar agent (“rodent deltavirus/RDeV”) was detected in Tome’s spiny-rats (](#_ENREF_1)*Proechimys semispinosus* (Tomes, 1860)) sampled at the Barro Colorado Nature Monument, Panama [[4](#_ENREF_4)][.](#_ENREF_3)  It is well established that HDV-1–8 rely on hepatitis B virus (HBV; *Hepadnaviridae*) as their helper for infectious particle formation. However, all of the novel nonhuman viruses were found in the absence of co-infecting hepadnavirids, or at least in the absence of actively replicating hepadnavirids. Recent evidence indicates that infectious HDV-1–8 particle generation is not restricted to HBV co-infection, and that, e.g., vesiculo-, flavi- and hepacivirus glycoproteins can serve as surrogates [[5](#_ENREF_5)][. The HDV-1–8-like virus that was initially identified by metatranscriptomic analysis of snake brain samples was found with accompanying arenavirids (genera](#_ENREF_4) *Hartmanivirus* and *Reptarenavirus*), and RT-PCR and immunohistology confirmed systemic spread of the HDV-1-8–like virus [[2](#_ENREF_2)][. Subsequently, the agent was shown to use the co-infecting arenavirids as packaging helpers](#_ENREF_2) [[7](#_ENREF_7)][.](#_ENREF_6) A systemic spread of the HDV-1–8-like viruses was also observed in the available organs of the neotropical rodents [[4](#_ENREF_4)][. These findings have drastically changed the view on the evolution of HDV-1–8, which was suggested to possibly represent an escaped human](#_ENREF_3) gene [[6](#_ENREF_6)][,](#_ENREF_5) a hypothesis that cannot be supported anymore.  All thus far identified HDV-1–8-like viruses share the open reading frame (ORF) for a defining protein referred to as the delta antigen (DAg) in the case of HDV-1–8 [[1](#_ENREF_1), [2](#_ENREF_2), [4](#_ENREF_4), [8](#_ENREF_8)][. In addition, avian](#_ENREF_7), [snake](#_ENREF_7), and rodent HDV-1–8-like agents appear to comprise genomic and antigenomic ribozymes as suggested by predicted structural similarity [[2](#_ENREF_2), [4](#_ENREF_4), [8](#_ENREF_8)][. We propose that HDV-1–8-like agent classification at genus and species ranks should be based on DAg similarity as analyzed primarily at amino acid level for more distantly related viruses, and at the nucleotide level for comparison of more closely related viruses. Species rank-classification could include whole genome comparisons.](#_ENREF_7)  Proposed taxon classification criteria:   * + HDV-like entities encoding DAg with >60% amino acid sequence similarity should be assigned to the same genus   + The species demarcation criterion is identical to that chosen previously for “HDV genotype” differentiation: 80% complete genome nucleotide identity [[3](#_ENREF_3)].   The identification and analysis of the novel HDV-1–8-like agents from amphibian, birds, fish, insects, reptiles, and rodents (see Supporting evidence) indicates the need for additional *Deltavirus*-like taxa. Hence, we propose to   * rename “avHDV” → dabbling duck virus 1 (DabDV-1); * rename “sHDV” → Swiss snake colony virus 1 (SwSCV-1); * rename “fHDV” → ray-finned fish virus 1 (RFFV-1); * rename “tfHDV” → Chusan Island toad virus 1 (CITV-1); * rename “amHDV” → Chinese fire belly newt virus 1 (CFBNV-1); * rename “tHDV” → rhinotermitid virus 1 (RTV-1); and * rename “RDeV” → Tome’s spiny-rat virus 1 (TSRV-1);   and the establishment of seven genera each including a single species to accommodate these viruses:   * genus *Dalvirus* including species *Dalvirus* *anatis* for DaDV-1; * genus *Daletvirus* including species *Daletvirus* *boae* for SwSCV-1; * genus *Deevirus* including species *Deevirus* a*ctinopterygii* for RFFV-1; * genus *Dobrovirus* including species *Dobrovirus* *bufonis* for CITV-1; * genus *Daazvirus* including species *Daazvirus* *cynopis* for CFBNV-1; * genus *Dagazvirus* including species *Dagazvirus* *schedorhinotermitis* for RTV-1; and * genus *Thurisazvirus* including species *Thurisazvirus* *myis* for TSRV-1.   Etymology of new taxon names:   * *Ribozyviria*: from ribozyme, a unique and defining component of viruses of the realm * *Kolmioviridae*: from Finnish kolmio, meaning triangle – a tongue-in-cheek reference to the Greek letter Δ spelled out in genus *Deltavirus* * *Dalvirus*: from Arabic letter Ḏāl (ذ) – a predecessor of Greek letter Δ spelled out in genus *Deltavirus* * *Daletvirus*: from Hebrew letter 'Dālet (ד) – a predecessor of Greek letter Δ spelled out in genus *Deltavirus* * *Deevirus*: from American letter Dee (D) – a successor of Greek letter Δ spelled out in genus *Deltavirus* * *Dobrovirus*: from Early Cyrillic letter Dobro (Д) – a successor of Greek letter Δ spelled out in genus *Deltavirus* * *Daazvirus*: from Gothic letter Daaz (𐌳) – a successor of Greek letter Δ spelled out in genus *Deltavirus* * *Dagazvirus*: from Runic letter Dagaz (ᛞ), a possible descendant of Old Italic D – a successor of Greek letter Δ spelled out in genus *Deltavirus* * *Thurisazvirus*: from Runic letter Thurisaz (ᚦ), a possible descendant of Old Italic D – a successor of Greek letter Δ spelled out in genus *Deltavirus* * Species epithet *anatis*: referring to anas (duck) * Species epithet *boae*: referring to snake genus *Boa* * Species epithet *actinopterygii*: referring to ray-finned fish (Actinopterygii) * Species epithet *bufonis*: referring to bufo (toad) * Species epithet *cynopis*: referring to newt genus *Cynops* * Species epithet *schedorhinotermitis*: referring to termite genus *Schedorhinotermes* * Species epithet *myis*: referring to mus (mouse) * Species epithets *italiense*, *japanense*, *peruense*, *taiwanense*, *togense*, *carense*, *cameroonense*, *senegalense*: referring to countries where first exemplars of these HDVs were first discovered.   **Supporting evidence**  **Table 1.** Percentage amino acid identity among HDAgs of eight HDV “genotypes” (HDV-1–8), and nonhuman putative HDAgs. | | **Figure 1.** Maximum-likelihood phylogeny based on a full-genome nucleotide alignment of HDV representatives from the eight different genotype groups and the nonhuman deltavirus-like agents. Bootstrap support values are shown for all nodes. Tree tips correspond to GenBank accession numbers. The tree is rooted to the branch leading to the nonhuman deltavirus-like agents [[4](#_ENREF_4)].  **References**  1. Chang W-S, Pettersson JH-O, Le Lay C, Shi M, Lo N, Wille M, Eden J-S, Holmes EC (2019) Novel hepatitis D-like agents in vertebrates and invertebrates. Virus Evol 5:vez021. PMID: 31321078. PMCID: PMC6628682. doi: 10.1093/ve/vez021.  2. Hetzel U, Szirovicza L, Smura T, Prähauser B, Vapalahti O, Kipar A, Hepojoki J (2019) Identification of a novel deltavirus in boa constrictors. mBio 10:e00014-19. PMID: 30940697. PMCID: PMC6445931. doi: 10.1128/mBio.00014-19.  3. Le Gal F, Gault E, Ripault M-P, Serpaggi J, Trinchet J-C, Gordien E, Dény P (2006) Eighth major clade for hepatitis delta virus. Emerg Infect Dis 12:1447-50. PMID: 17073101. PMCID: PMC3294742. doi: 10.3201/eid1209.060112.  4. Paraskevopoulou S, Pirzer F, Goldmann N, Schmid J, Corman VM, Gottula LT, Schroeder S, Rasche A, Muth D, Drexler JF, Heni AC, Eibner GJ, Page RA, Jones TC, Muller MA, Sommer S, Glebe D, Drosten C (2020) Mammalian deltavirus without hepadnavirus coinfection in the neotropical rodent Proechimys semispinosus. Proc Natl Acad Sci U S A. 117:17977-83. PMID: 32651267. doi: 10.1073/pnas.2006750117.  5. Perez-Vargas J, Amirache F, Boson B, Mialon C, Freitas N, Sureau C, Fusil F, Cosset FL (2019) Enveloped viruses distinct from HBV induce dissemination of hepatitis D virus in vivo. Nat Commun 10:2098. PMID: 31068585. PMCID: PMC6506506. doi: 10.1038/s41467-019-10117-z.  6. Salehi-Ashtiani K, Luptak A, Litovchick A, Szostak JW (2006) A genomewide search for ribozymes reveals an HDV-like sequence in the human CPEB3 gene. Science 313:1788-92. PMID: 16990549. doi: 10.1126/science.1129308.  7. Szirovicza L, Hetzel U, Kipar A, Martinez-Sobrido L, Vapalahti O, Hepojoki J (2020) Snake deltavirus utilizes envelope proteins of different viruses to generate infectious particles. mBio 11:e03250-19. PMID: 32184255. PMCID: PMC7078484. doi: 10.1128/mBio.03250-19.  8. Wille M, Netter HJ, Littlejohn M, Yuen L, Shi M, Eden J-S, Klaassen M, Holmes EC, Hurt AC (2018) A divergent hepatitis D-like agent in birds. Viruses 10:720. PMID: 30562970. PMCID: PMC6315422. doi: 10.3390/v10120720. | |