

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

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| **Code assigned:** | ***2022.021B*** |  |
| **Short title:** Create one new genus (*Certevirus*) including one new species (*Caudoviricetes*)  |
|  |

**Author(s) and email address(es)**

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**Corresponding author**

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| Emna Grami |

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

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| ICTV Caudovirales Study Group, Bacterial and Archaeal Viruses Subcommittee |

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

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| Direct submission to the SC Chair.Comments: Format proposal according to guidelines. Choose names for species and genus that are compliant with the Code. Genus should be placed directly in class *Caudoviricetes* because family *Myoviridae* is being abolished*.*  |

**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)** |  |

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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 | January 2022 |
| Date of this revision (if different to above) | October 2022 |

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

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| Typo in Excel file fixed.  |

**Part 2:** **NON-TAXONOMIC PROPOSAL**

**Text of proposal**

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

**Name of accompanying Excel module**

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| 2022.021B.A.v2.Certevirus\_ng.xlsx |

**Abstract**

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| Create a genus for the isolated phage Pd\_C23 infecting *Pantoea dispesa* (OL396571.1).(No phages infecting *P. dispersa* have been reported yet*,* it will be the first *p. dispersa* phage classified within the new Genus proposal). |

**Text of proposal**

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| **Genus demarcation criteria**- Based on Bacterial Virus Subcommittee standards as a cohesive group of viruses sharing a high degree (>70%) of nucleotide identity of the whole genome length [**1**], Pd\_C23 nt % similarity with any other phage genome ranges below 70%. -Monophyletic groupings tested with generated phylogenetic tree.**Species demarcation**There is only one phage in this proposed genus, which will be the exemplar for the new species. New species in the genus will differ in their genome with at least 5% over the entire genome length.  |

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**Supporting evidence**

Pantoea phage Pd\_C23 characteristics:

**Size**:44714 pb / **GC content**: 49.66 % / **tRNAs**:1 / **coding sequences**: 49%

1. A total genome BLASTn [**1**] showed similarities with mostly unclassified partial genome phages, and one complete genome classified Klebsiella phage VB chronis(MN013086.1) Multiple Sequence Alignment showed no significant similarity hits apart from the beginning part (6500pb) of the genome for all best hits (**Fig. 1**).

Pairwise alignment between Pd\_C23 (OL396571.1) and Best Hits of the Query Sequence is generated [**2**], Neighbor joining tree generated by NCBI was reconstructed using Mega11 [**3**] Molecular Evolutionary Genetic analysis program (MEGA11), Max Seq Difference (0.5), scale: 0.02, it shows that the phage shares no branches with any existing sequence. (**Fig. 2**),



**Fig 1**. Graphic summary of the whole phage genome Pd\_C23 with best hits according (NCBI new MSA Viewer)



**Fig 2.** Whole genome Neighbor joining tree representation of BLAST pairwise alignment of Pd\_C23 OL396571.1 and blasted best hits by Mega11 of Pd\_C23 (OL396571.1).

(Numbers on the Branches indicates the lengths Max Seq Difference (0.5), scale: 0.02)

1. Thus, based on BLASTn sequence homologies PD\_C23 shares several regions of nucleotide similarity with Klebsiella phage vB\_Kpn\_Chronis, in total the first 23 ORFs among 75 ORFs share similarities with thetotally sequenced genome of Klebsiella phage vB\_Kpn\_Chronis (MN013086.1) that infects the bacteria *Klebsiella pneumoniae*.

*P. dispersa* phage Pd\_C23 consensus genome is 50.6% similar to that of Kpn-Chronis. Pantoea phage Pd\_C23 complete genome is 44,715 pb (GC content % 49.66), Klebsiella phage vB\_Kpn\_Chronis is 45,702 pb (GC content % 52.2).

Genetic distances between Pd\_C23 and Kpn vB chronis phage between the two phages is verified, pairwise sequence identities and similarities are calculated using the Webserver SIAS: ([http://imed.med.ucm.es/Tools ) **[4**](http://imed.med.ucm.es/Tools%20%29%20%5B4)**]**. Along with four other blasted best hits phages. (**Fig.3**)



**Fig 3.** Photo representing the Pairwise sequence identities and similarities values of whole genome of Pd\_C23 calculated along with five complete genomes phages blasted best hits phages using the Webserver SIAS: (http://imed.med.ucm.es/Tools)

1. Nucleotide and amino acid identity heat map between Pd\_C23 whole genome, best hits and with gene markers is calculated (**Fig. 4)** using a Software package (Clustal Omega (RRID:SCR\_001591) as multiple sequence alignment tool between multiple sequences**.**

URL: <http://www.ebi.ac.uk/Tools/msa/clustalo/>

Although, a connection is found between eleven caudovirales phages, they remain far relatives since the Nucleotide Identity between genus is described as a cohesive group of viruses sharing a high degree (>70%) of nucleotide identity of the whole genome length [**5**]. Along with Pd\_C23 embody a distant branch on the neighbor joining tree (**Fig. 2**).



**Fig 4**. Nucleotide and amino acid identity heat map between Pd\_C23 whole genome and eleven best hits (NA: not available)

1. Viral proteomic tree using VipTree based on maximum likelihood method of whole genomic nucleotide sequences of 11 selected dsDNA viruses, among Myoviridae, siphoviridae, podoviridae, autographiviridae [**6**], has been generated (ViPTree - The Viral Proteomic Tree Server), it revealed further information about the phylogenetic positions of Pd\_C23 (**Fig. 5**).

We consider that Pd\_C23 cannot be classified to any genus currently recognized by ICTV considering that the phylogenetic relationship analysis showed no significant closeness with any existing genus and that it represents a new genus.



**Fig 5.** Viral proteomic tree of Pd\_C23 based on maximum likelihood method of whole genomic nucleotide sequences of elven selected blasted best hits (dsDNA viruses). ViPTree analysis was obtained MLM analysis heuristic search with 12 bootstrap replicates. Branch lengths are logarithmically scaled, the log scaled is represented by the numbers on top representing the SG values ((normalized scores by blastx).

Origin of the new genus name: *Certevirus* (After the institute name: CERTE)

**References**

1. **NCBI 2021** Resource Coordinators. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. PMID: 29140470. DOI: 10.1093/nar/gkx1095
2. **Katoh K, Rozewicki J, Yamada KD (2019)** (Briefings in Bioinformatics 20:1160-1166). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. PMID: 28968734. DOI: 10.1093/bib/bbx108
3. **Tamura K, Stecher G, and Kumar S (2021)** MEGA11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution 38:3022-3027.

https://doi.org/10.1093/molbev/msab120

1. **Cai L, Ma H, (2016)** Using nuclear genes to reconstruct angiosperm phylogeny at the species level: A case study with Brassicaceae species. journal of Systematics and Evolution 54(4). https://doi.org/10.1111/jse.12204.
2. **Turner D, Kropinski A M, Adriaenssens E M (2021)** A roadmap for genome-based phage taxonomy. Viruses 2021, 13, 506. PMID: 33803862. doi: 10.3390/v13030506
3. **Nishimura Y et al (2017)** ViPTree: the viral proteomic tree server.Bioinformatics, 33, 2379–2380, doi:10.1093/bioinformatics/btx157.