

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

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| **Code assigned:** | **2020.130B** |  |
| **Short title:** Create seven new genera and thirteen new species of *Ralstonia* phages (*Caudovirales*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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

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

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**Authority to use the name of a living person**

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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 | June 2020 |
| Date of this revision (if different to above) | November 2020 |

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

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

**Name of accompanying Excel module**

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| 2020.130B.R.Ralstonia\_phages |

**Abstract**

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| The aim of this proposal is to provide with taxonomic evidence to support the creation of new genera and species of diverse *Ralstonia solanacearum* phages sampled in the South West Indian Ocean islands of Mauritius and Reunion. |

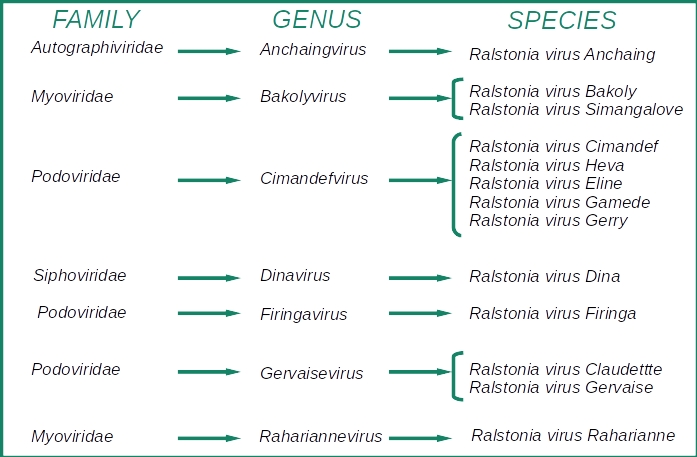
**Text of proposal**

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| |  | | --- | | Twenty-three new phages of the phytopathogenic bacterium *R. solanacearum* were isolated from agricultural settings in Mauritius (thirteen phages) and Reunion (ten phages) islands. The phages were purified and their DNA extracted, sequenced and subsequent genomic comparative analysis made. According to our analysis, they represent fourteen new species and seven new genera of *Ralstonia* phages. Five of the new genera include exclusively our new phage species and, for two genera, other phages are described in the literature but lack complete taxonomic classification. We provide with genetic evidence such as phylogenetic trees, genetic distances and BLASTN searches. We have also produced TEM micrographs for the reference phages belonging to the five completely new genera. Assembled and annotated genomes of these Ralstonia phages were uploaded to GenBank and publicly available, and we provide with the respective accession numbers.  The 23 phages of our study represent 13 new species, within 7 new genera. We have chosen 95% DNA sequence identity as the criterion for demarcation of species and 70% for new genus (Adriaenssens & Rodney Brister, 2017). Each of the proposed species or genera differs from the others with more than 5% or 30% at the DNA level, respectively, as confirmed with the VIRIDIC genetic distance algorithms genera (Moraru et al., 2020). Four genera (*Anchaingvirus*, *Dinavirus*, *Firingavirus* and *Rahariannevirus*) include only 1 phage species. The genus *Bakolyvirus* and *Gervaisevirus* include 2 new phage species, and 5 new *Ralstonia* phage species belong to the *Cimandefvirus* genus. We searched for homologous phages using the BLASTN algorithm and found only 2 already published *Ralstonia* phages belonging to the genera *Gervaisevirus* and 1 to *Firingavirus*. As these 3 phages have not been completely described, we suggest to include them in the newly created genera. We constructed phylogenetic trees using the full genome of our 23 phages and including 9 closest relatives. The closest viruses are 4 *Ralstonia* phages, 4 *Burkholderia* phages and 1 *Halomonas* phage isolated from China, Japan and the USA (Fu et al., 2017; Gill et al., 2011; Summer et al., 2006; Wang et al., 2019)⁠. The branch lengths are scaled in terms of the Genome BLAST Distance Phylogeny (GBDP) intergenomic distance formula d0 for nucleotide based VICTOR phylogenetic trees (Meier-Kolthoff & Göker, 2017)⁠. The tree branches at the genera taxon level are in agreement with the genera classification by VIRIDIC (Virus Intergenomic Distance Calculator) and highly consistent (higher or equal to 96 bootstrap replicates). The electron micrographs confirm the Virfam genetic-based predictions of morphological phage families, that is, *Podoviridae*, *Siphoviridae* and *Myoviridae* (Lopes et al., 2014)⁠. Virfam automatically identifies proteins of the phage head-neck-tail module and assign phages to the most closely related cluster of phages (Lopes et al., 2014)⁠.  The bacterium *R. solanacearum* causes bacterial wilt in a wide range of agriculturally important plants. This disease is specially dramatic in tropical areas such as Mauritius and Reunion, where it produces yearly economic losses (Yahiaoui et al., 2017)⁠. The current phages are part of a biocontrol project and represent a promising hope to limit the damage caused by this phytopathogen. We propose to name the phages after historical cyclones for the Mauritius ones, and anti-colonialist slaves for the Reunion ones, as suggested by our local collaborators. Intense weather phenomena are a well-known feature of the South West Indian Ocean region. Historically, cyclones were named after women common names but we have included some recent men cyclone names to designate Mauritian phages too, for a gender balance purpose. The slave trade history and the role played by rebel slaves in Reunion island are a local historical event worth to be underlined. We have chosen men and women names of anti-colonialist slaves to name Reunion phages. In addition, some of the local mountains are named after these legendary slaves.  In summary, we hope we provide with sufficient evidence to create thirteen new species of *R. solanacearum* phages and seven new genera, based on a highly diverse phage collection from the tropical region of the South West Indian Ocean. | |

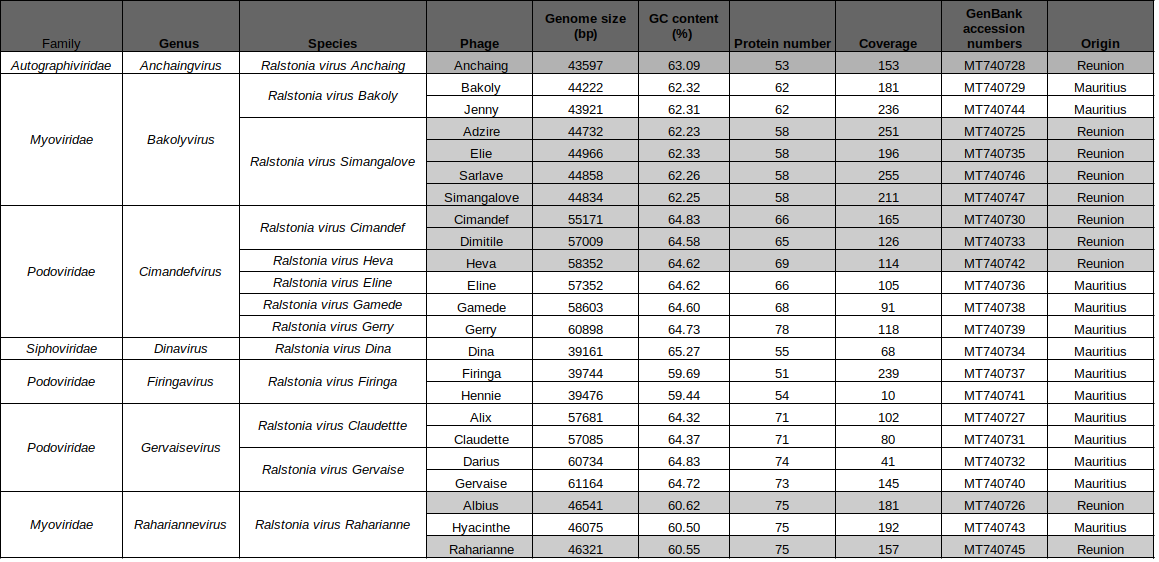
**Supporting evidence**

**Specific Reference:** Angelina Trotereau, Claudine Boyer, Isabelle Bornard, Max Jean Bernard Pécheur, Catherine Schouler and Clara Torres-Barceló. “High genomic diversity of novel phages infecting the plant pathogen *Ralstonia solanacearum*, isolated in Mauritius and Reunion islands”. (submittedto *Scientific Reports, October 2020*).

**Figure 1.** **Summary of phage taxonomical proposition**

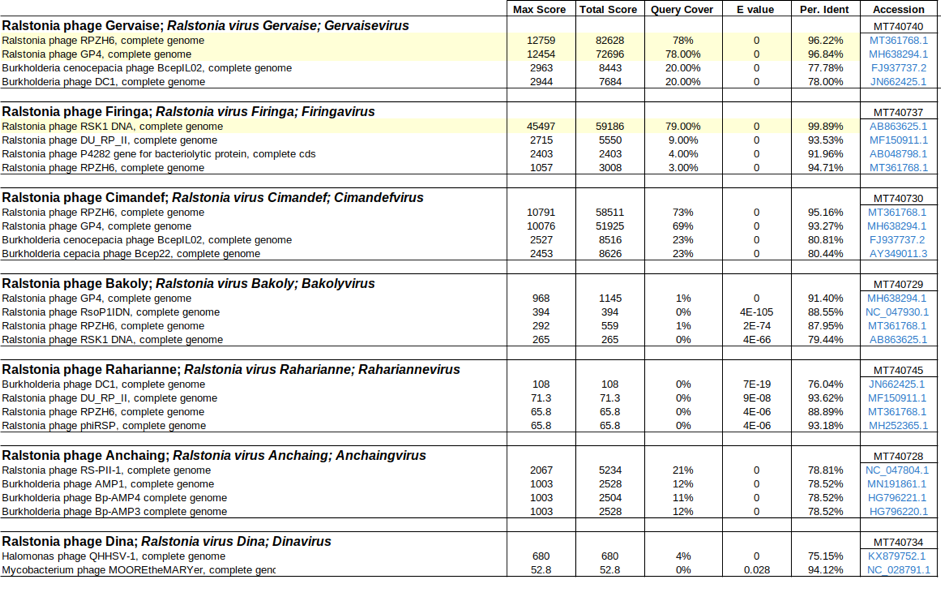
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**Table 1. Summary of phage genomes characteristics, taxonomy and GenBank accession numbers of 23 newly isolated *Ralstonia* phages.** Shaded lines indicate phage clones from Reunion and white ones phages from Mauritius.

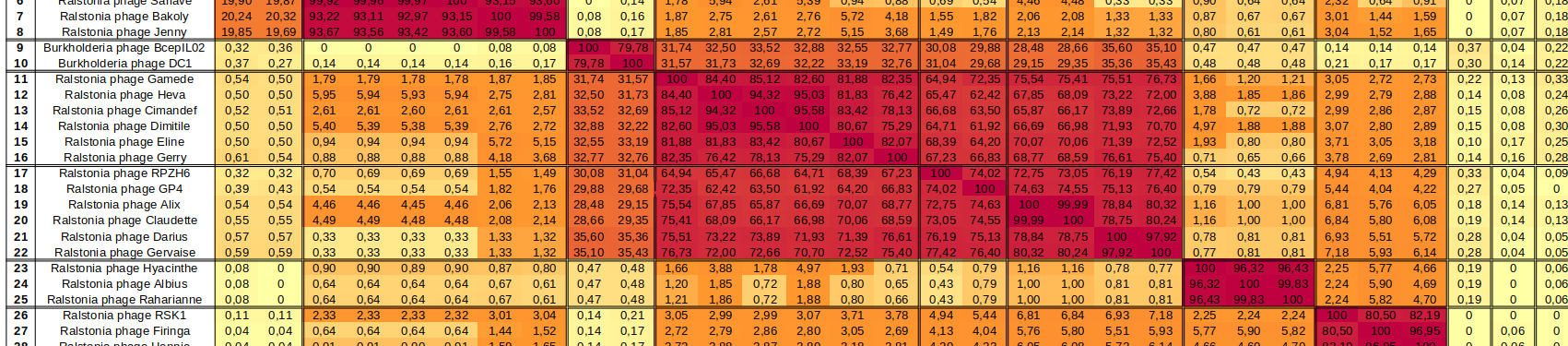


*GC content determined using VICTOR (Meier-Kolthoff & Göker, 2017)⁠. Protein number determined using Prokka (Seemann, 2014)⁠. Species and Genus determined with VIRIDIC (Moraru et al., 2020)⁠. Family determined with Virfam and TEM (Lopes et al., 2014)⁠.*

**Table 2.** **BLASTN homologs:** The next most closely related virus to the representative species of the seven *Ralstonia* phage genera proposed are displayed in the following table. Phage genomes presenting DNA sequence identity above 70% are highlighted in yellow. These unclassified homologous viruses should be included in the new genera proposed.



**Table 3. VIRIDIC analysis:** The VIRIDIC pairwise intergenomic distances/similarities amongst the 23 *Ralstonia* phage genomes isolated in our study and the 9 closest phages as determined by BLASTN analysis (Moraru et al., 2020)⁠. Phages belonging to the same genera are differentiated by double black lines, with sequence homology over 70% and orange-red colour code in the heatmap. The darker red colour code is associated with the species taxon level.

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**Figure 2. Electron TEM micrographs** of the representative *Ralstonia* phage species of five new genera with unique viruses (non described before). a) Ralstonia phage Cimandef (*Cimandefvirus, Podoviridae*); b) Ralstonia phage Anchaing (*Anchaingvirus, Autographiviridae*); c) Ralstonia phage Raharianne (*Rahariannevirus, Myoviridae*); d) Ralstonia phage Bakoly (*Bakolyvirus, Myoviridae*); e) Ralstonia phage Dina (*Dinavirus, Siphoviridae*).



**Figure 3. Phylogeny:** Phylogenetic tree based on nucleotides using the full genome of our 23 *Ralstonia* phages (in red those from Reunion island and in blue from Mauritius) and including the 9 closest relatives (in black). The 7 proposed genera are indicated by colours on the right side of the tree. The branch lengths are scaled in terms of the GBDP distance formula d0, as used by VICTOR phylogenetic trees calculations (Meier-Kolthoff and Göker, 2017).

**References**

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