

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

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| **Code assigned:** | **2021.005F** |  |
| **Short title:** Create one new order (*Yadokarivirales*), one new family (*Yadokariviridae*), two new genera (*Alphayadokarivirus* and *Betayadokarivirus*) and 10 new species in the phylum *Pisuviricota* | | |
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**Author(s) and email address(es)**

|  |  |
| --- | --- |
| Sato Y, Das S, Velasco L, Turina M, Osaki H, Kotta-Loizou I, Coutts RHA, Kondo H, Sabanadzovic S, Suzuki N | [y-sato@okayama-u.ac.jp](mailto:y-sato@okayama-u.ac.jp);  [subhadas1981@gmail.com](mailto:subhadas1981@gmail.com);  leonardo.velasco@juntadeandalucia.es;  [massimo.turina@ipsp.cnr.it](mailto:massimo.turina@ipsp.cnr.it);  [hideki@affrc.go.jp](mailto:hideki@affrc.go.jp);  i.kotta-loizou13@imperial.ac.uk;  r.coutts@herts.ac.uk;  hkondo@okayama-u.ac.jp;  ssabanadzovic@entomology.msstate.edu;  [nsuzuki@okayama-u.ac.jp](mailto:nsuzuki@okayama-u.ac.jp) |

**Author(s) institutional address(es) (optional)**

|  |
| --- |
| Okayama University [YS, SD, NS]  Instituto Andaluz de Investigación y Formación Agraria [LV]  University of Hertfordshire [RHAC]  Institute for Sustainable Plant Protection, CNR [MT]  Imperial College London [IKL]  Mississippi State University [SS]  National Agricultural Research Organization [HO] |

**Corresponding author**

|  |
| --- |
| Nobuhiro Suzuki [nsuzuki@okayama-u.ac.jp](mailto:nsuzuki@okayama-u.ac.jp) |

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

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| **EC Comments:** Please address few errors present in the Excel file, consider minor style improvements in the Word file. Please also place Figure 2 with a correct version.  **Response:** All suggestions incorporated in the present version. Errors from Excel file eliminated. Figure 2 replaced with an updated version. |

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

**Name of accompanying Excel module**

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| 2021.005F.R.Yadokarivirales\_neworder.xlsx |

**Abstract**

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| This TaxoProp proposes the creation of a new order *Yadokarivirales* comprising of a new family named *Yadokariviridae* with two genera *Alphayadokarivirus* and *Betayadokarivirus* which accommodate six and four species respectively. Members of the proposed family are all fungal viruses with positive-sense RNA genomes, whose sizes range from 3.6 to 6.3 kb, and encoding either one or two open reading frames. Yadokarivirids appear to be hetero-encapsidated by double-stranded virus partners. Yadokarivirids are distantly related to animal-infecting calicivirids in the order *Picornavirales*, though they are distinct in genome organization. The highest sequence identity of RNA-directed RNA polymerase (RdRP) between yadokarivirids and the most closely related classified viruses (viruses in the class *Pisoniviricetes)* is lower than 20%. These low-level of amino acid sequence identities justifiy the creation of the new family. Inter-genus and inter-species RdRP sequence identities in the family are < 25.4% and 27.3-67.5%, respectively. Phylogentic analyses of yadokarivirids and related viruses strongly support our proposal. |

**Text of proposal**

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This virus was isolated from a strain (W1032) of the phytopathogenic fungus *Rosellinia necatrix* in 2013 [15]*.* Since then,many members of the proposed family *Yadokariviridae* have been reported from filamentous fungi such as *Penicillium aurantiogriseum* and *Fusarium poae* (see Table 1) [1, 8, 10, 12, 14].  **Biological and molecular properties:**  Yadokarivirids, including AfV-S2 and YkV1, have an undivided positive-sense, single stranded genome ranging from 3.6 kb for AfV-S2 to 6.3 kb for YkV1 (Fig. 1). Yadokarivirid genomes have one or two open reading frames with or without poly(A) tails at the 3’-end, and long 5’-untranslated regions (UTR) of over 478 nt in the case of completely sequenced members.  YkV1, the best-studied yadokarivirus, is hetero-encapsidated by a taxonomically unrelated, double-stranded RNA virus, termed yadonushi virus 1 (YnV1) into spherical particles of ~45 nm in diameter. YnV1 capsid protein is encoded by the 5’-proximal open reading frame (ORF). YkV1 was hypothesized to replicate in the hetero-capsid as it were a dsRNA virus. Such trans-encapsidation is plausible to suspect for other yadokarivirids too, because they were always discovered in co-infections with dsRNA viruses. However, no experimental substantiation has been achieved yet, except for YkV1.  The YkV1 genomic RNA is approximately 6.3 kb long, possessing a long 5’-UTR of approximately 0.8 kb and a single open reading frame (ORF) that encodes two functional domains for RNA-directed RNA polymerase (RdRP) and self-cleaving 2A-like peptide. These two domains are essential for the viability of YkV1 in the presence of YnV1 [3]. The 2A-like peptide, which was first identified in an animal aphthovirus, and later was shown to be conserved across diverse RNA viruses [1, 9, 11], is encoded by all yadokarivirids with the monocistronic genome organization (Fig. 1) [4]. Purified virion fractions of ~45 nm in diameter from *R. necatrix* strain W1032 consisted of YkV1 dsRNA regarded as a replicative form, YnV1 genomic dsRNA, and a single major capsid protein of 120 kDa encoded by YnV1. Whether the two RNA elements are encapsidated separately remains elusive. Particles encasing only YkV1 dsRNA with YkV1 RdRP are present in infected mycelia [3].  Based on BLAST search, YkV1 RdRP showed homology only to those of animal-infecting sapoviruses in the family *Caliciviridae* as well as of the other yadokarivirids (Table 2). However, phylogenetic analysis showed that YkV1 and related viruses belong to a separate clade distinct from caliciviruses and other viruses in the phylum *Pisuviricota* (Figs. 2-5). The family *Yadokariviridae* is proposed to be classified into the kingdom *Orthornavirae* in the realm *Riboviria* since they have RNA genome encoding RdRP. Yadokarivirids show phylogenetic affinity to, but are distinct from members in the class *Pisoniviricetes* in the phylum *Pisuviricota* (Figs. 3-5). The tree and sequence identity shown in Figs. 3 & 5 suggest that yadokariviruses belong to a new order.  **Justification for the creation of the new family, two genera and 10 species:**  The proposed family can be readily differentiated from other known mycovirus families based on the genome size and organization of member viruses, presumed trans-encapsidation (yadokari) nature, the length of the 5’-UTRs, original hosts (fungi) and distinct evolutionary history of their RdRPs. The majority of yadokariviruses have a monocistronic genome that encodes a polyprotein with RdRP and 2A-like self-cleaving peptide, while a member of one species has a bicistronic genome with an additional ORF instead of 2A-like cleavage site. Of the two assumed mature proteins encoded by members of the family, only the RdRP sequence shows low levels (less than 20%) of identities to those of caliciviruses, barnaviruses, and solemoviruses (Fig. 6), while the remaining protein does not show any significant sequence similarities to other known proteins.    Proposed taxonomy of yadokarivirids:   |  | | --- | | Realm: *Riboviria* | | Kingdom: *Orthornavirae* | | Phylum: *Pisuviricota* | | Class: Unassigned | | Order: *Yadokarivirales* | | Family: *Yadokariviridae* | | Genus: *Alphayadokarivirus* | | Genus: *Betayadokarivirus* |  |  |  |  | | --- | --- | --- | | **New species** | **Exemplar isolate** | **GenBank sequence accession number(s)** | | **genus *Alphayadokarivirus*** | | | | *Alphayadokarivirus ichibani* | yadokari virus 1 | LC006253 | | *Alphayadokarivirus nibani* | Aspergillus foetidus slow virus 2 | HE588148 | | *Alphayadokarivirus sanbani* | Penicillium aurantiogriseum foetidus-like virus | KT601100 | | *Alphayadokarivirus yonbani* | Penicillium digitatum yadokarivirus 1 | MK279488 | | *Alphayadokarivirus gobani* | Plasmopara viticola lesion associated yadokari virus 1 | MN551121 | | *Alphayadokarivirus rokubani* | Picoa juniperi yadokari virus 1 | MT876192 | | **genus *Betayadokarivirus*** | | | | *Betayadokarivirus ichibani* | Fusarium poae mycovirus 2 | LC150617 | | *Betayadokarivirus nibani* | yadokari virus 2 | MF375888 | | *Betayadokarivirus sanbani* | yadokari virus 3 | LC333757 | | *Betayadokarivirus yonbani* | yadokari virus 4 | LC333754 |   A phylogenetic tree generated based on an RdRP sequence alignment and presented in Fig. 2 shows clear separation between member viruses into two clades corresponding to two proposed genera, *Alphayadokarivirus* and *Betayadokarivirus*. These members can also be differentiated by RdRP sequence identity levels: within-genus identity (27.3 to 64.5% for alphayadokariviruses and 34.2 to 67.5% for betayadokariviruses) vs. inter-genus identity (19.1 to 25.4%) (Fig. 6A).  The interspecies RdRP sequence identity ranges from 19.1% to 67.5% among members, while the protein derived from the 3’-proximal portion of the polyprotein or encoded by ORF2 shows 7.6% to 41.9% interspecies sequence identity (Fig. 6B). The exemplar isolates of the 10 proposed species can readily be distinguishable by sequence divergence, genome organization, and/or original host.  **Origin of the new family name:** “Yadokari” - a Japanese word literally meaning borrowing (Kari) a house to stay (Yado), or biologically, hermit crab. Thus, “Yadokari” well describes the lifestyle (hetero-encapsidation) of yadokariviruses. | |

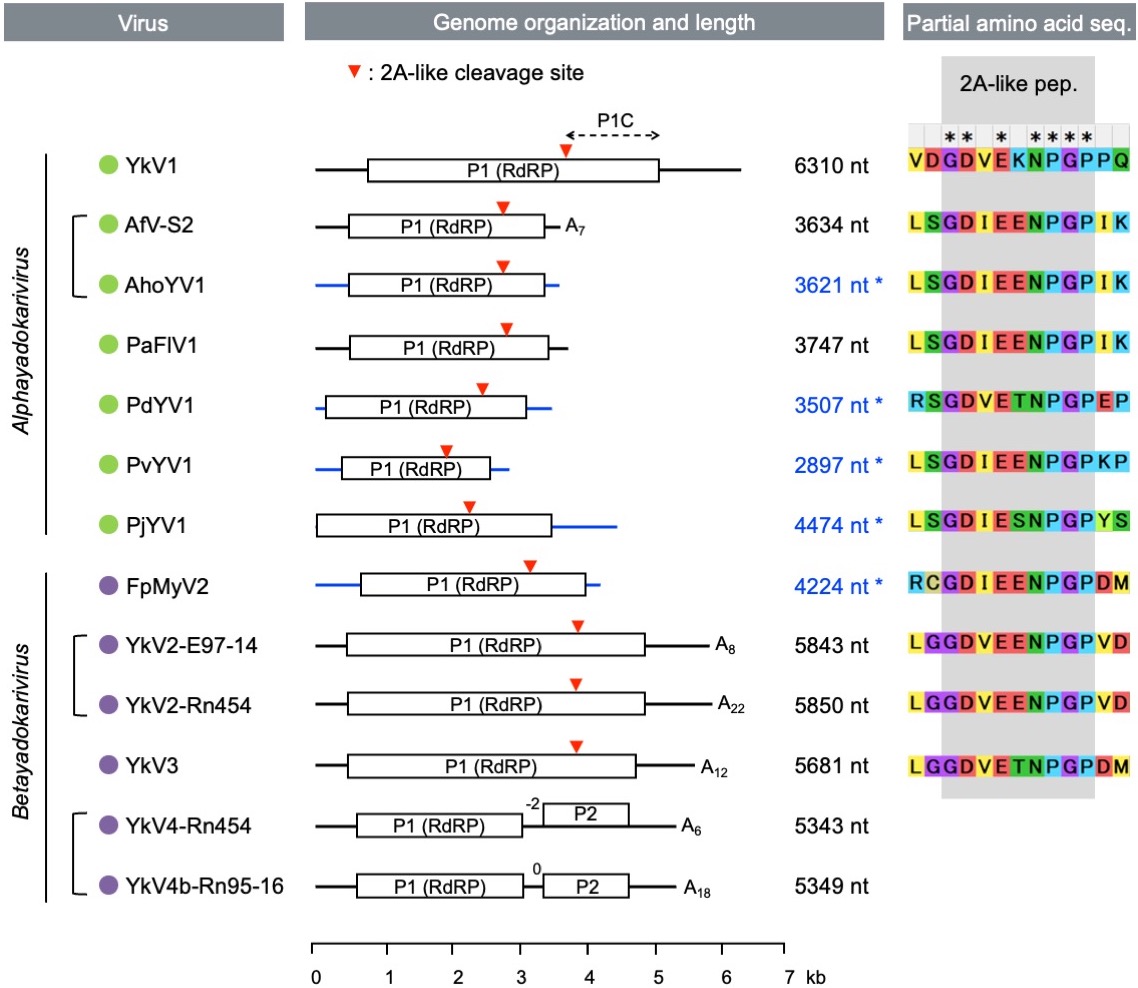
**Supporting evidence**

**Table 1.** Proposed family *Yadokariviridae*.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Genus** | **Species** |  | **Strain** | | | **Genome**  **info.** | **GenBank accession** | | Reference |
| ***a*** | **Virus name** | **Isolate** | **Abbre-**  **viation** | **Genome** | **P1 (RdRP)** |
| *Alphayadokarivirus* | *Alphayadokarivirus ichibani* | ★ | yadokari virus 1 | W1032 | YkV1 | Complete | [LC006253.1](https://www.ncbi.nlm.nih.gov/nuccore/LC006253.1) | [BAT50982.1](https://www.ncbi.nlm.nih.gov/protein/BAT50982.1) | [Zhang et al. *Nat Microbiol* 2016](https://www.nature.com/articles/nmicrobiol20151) |
| *Alphayadokarivirus nibani* | ★ | Aspergillus foetidus  slow virus 2 | IMI 41871 | AfV-S2 | Complete | [HE588148.1](https://www.ncbi.nlm.nih.gov/nuccore/HE588148.1) | [CCD33025.1](https://www.ncbi.nlm.nih.gov/protein/CCD33025.1?report=genbank&log$=prottop&blast_rank=5&RID=04FW5FA2013) | [Kozlakidis et al. *Arch Virol* 2013](https://link.springer.com/article/10.1007/s00705-013-1779-3) |
|  | Aspergillus homomorphus  yadokarivirus 1 | CBS  101889 | AhoYV1 | Complete?*b* | [MK279487.1](https://www.ncbi.nlm.nih.gov/nuccore/MK279487.1) | [AZT88626.1](https://www.ncbi.nlm.nih.gov/protein/AZT88626.1?report=genbank&log$=prottop&blast_rank=3&RID=04FW5FA2013) | [Gilbert et al. *PLoS ONE* 2019](https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0219207) |
| *Alphayadokarivirus sanbani* | ★ | Penicillium aurantiogriseum  foetidus-like virus | MUT4330 | PaFlV1 | Complete | [KT601100.1](https://www.ncbi.nlm.nih.gov/nuccore/KT601100.1) | [ALO50127.1](https://www.ncbi.nlm.nih.gov/protein/ALO50127.1) | [Nerva et al. *Virus Res* 2015](https://www.sciencedirect.com/science/article/abs/pii/S0168170215301076?via%3Dihub) |
| *Alphayadokarivirus yonbani* | ★ | Penicillium digitatum  yadokarivirus 1 | HS-F6 | PdYV1 | Complete?*b* | [MK279488.1](https://www.ncbi.nlm.nih.gov/nuccore/MK279488.1) | [AZT88627.1](https://www.ncbi.nlm.nih.gov/protein/AZT88627.1?report=genbank&log$=prottop&blast_rank=7&RID=04FW5FA2013) | [Gilbert et al. *PLoS ONE* 2019](https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0219207) |
| *Alphayadokarivirus gobani* | ★ | Plasmopara viticola  lesion associated  yadokari virus 1 | DMG-B-  DN53434 | PvYV1 | Complete?*b* | [MN551121.1](https://www.ncbi.nlm.nih.gov/nuccore/MN551121.1) | [QHD64758.1](https://www.ncbi.nlm.nih.gov/protein/QHD64758.1?report=genbank&log$=prottop&blast_rank=8&RID=04FW5FA2013) | [Chiapello et al. *Virus Evol* 2020](https://academic.oup.com/ve/article/6/2/veaa058/5892778) |
| *Alphayadokarivirus rokubani* | ★ | Picoa juniperi yadokari  virus 1 | ANK\_  VIR-91 | PjYV1 | Complete?*b* | [MT876192.1](https://www.ncbi.nlm.nih.gov/nuccore/MT876192.1) | [QOI17269.1](https://www.ncbi.nlm.nih.gov/protein/QOI17269.1?report=genbank&log$=prottop&blast_rank=11&RID=04FW5FA2013) | [Sahin et al. *Virology* 2021](https://www.sciencedirect.com/science/article/pii/S0042682220301938) |
| *Betayadokarivirus* | *Betayadokarivirus ichibani* | ★ | Fusarium poae mycovirus 2 | MAFF  240374 | FpMyV2 | Complete?*b* | [LC150617.1](https://www.ncbi.nlm.nih.gov/nuccore/LC150617.1) | [BAV56311.1](https://www.ncbi.nlm.nih.gov/protein/YP_009272910.1?report=genbank&log$=prottop&blast_rank=12&RID=04FW5FA2013) | [Osaki et al. *Virus Genes* 2016](https://link.springer.com/article/10.1007%2Fs11262-016-1379-x) |
| *Betayadokarivirus nibani* |  | yadokari virus 2 | E97-14 | YkV2-  E97-14 | Complete | [MF375888.2](https://www.ncbi.nlm.nih.gov/nuccore/MF375888.2) | [AVD68673.2](https://www.ncbi.nlm.nih.gov/protein/AVD68673.2?report=genbank&log$=prottop&blast_rank=14&RID=04FW5FA2013) | [Velasco et al. *Virology* 2019](https://www.sciencedirect.com/science/article/pii/S0042682219300923?via%3Dihub) |
| ★ | yadokari virus 2 | Rn454 | YkV2-  Rn454 | Complete | [LC333755.2](https://www.ncbi.nlm.nih.gov/nuccore/LC333755.2) | [BBB86807.2](https://www.ncbi.nlm.nih.gov/protein/BBB86807.2?report=genbank&log$=prottop&blast_rank=15&RID=04FW5FA2013) | [Arjona-Lopez et al. *Environ Microbiol* 2018](https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/1462-2920.14065) |
| *Betayadokarivirus sanbani* | ★ | yadokari virus 3 | Rn454 | YkV3 | Complete | [LC333757.2](https://www.ncbi.nlm.nih.gov/nuccore/LC333757.2) | [BBB86810.2](https://www.ncbi.nlm.nih.gov/protein/BBB86810.2?report=genbank&log$=prottop&blast_rank=13&RID=04FW5FA2013) | [Arjona-Lopez et al. *Environ Microbiol* 2018](https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/1462-2920.14065) |
| *Betayadokarivirus yonbani* | ★ | yadokari virus 4 | Rn454 | YkV4-  Rn454 | Complete | [LC333754.2](https://www.ncbi.nlm.nih.gov/nuccore/LC333754.2) | [BBB86805.1](https://www.ncbi.nlm.nih.gov/protein/BBB86805.1?report=genbank&log$=prottop&blast_rank=16&RID=04FW5FA2013) | [Arjona-Lopez et al. *Environ Microbiol* 2018](https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/1462-2920.14065) |
|  | yadokari virus 4 | Rn95-16 | YkV4-  Rn95-16 | Complete | [LC333741.2](https://www.ncbi.nlm.nih.gov/nuccore/LC333741.2) | [BBB86788.1](https://www.ncbi.nlm.nih.gov/protein/BBB86788.1?report=genbank&log$=prottop&blast_rank=17&RID=04FW5FA2013) | [Arjona-Lopez et al. *Environ Microbiol* 2018](https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/1462-2920.14065) |

*a* Star indicates the exemplar virus for each species.

*b* Completeness of terminal sequence of the genome remains ambiguous due to the lack of the description about experiments for terminal sequence determination in the references.

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**Figure 1:** Genome organization and partial amino acid sequence of putative 2A-like peptides of yadokariviruses. The seven isolates (green circles) of six species in the genus *Alphayadokarivirus* and the six representatives (purple circles) of four species in the genus *Betayadokarivirus* are shown. The viruses belonging to the same species are shown connected with a bracket. Complete names and GenBank accession numbers of all the viruses are listed in Table 1. Genome represented with the blue line and the asterisk means that their terminal sequences might have not be validated by RACE (rapid amplification of cDNA ends) since no mentions about that in original papers. Poly(A) nucleotide sequence at 3’-terminal of the genome is shown as A*n* where *n* indicates the number of the A residues in the reference sequence. The conserved RNA-dependent RNA polymerase (RdRP) domain predicted in proteins (P1) is encoded by the first open reading frame of all the yadokariviruses. With the exception of YkV4, P1 of all the yadokariviruses also contains a 2A-like self-cleaving peptide sequence at the position approximately indicated with the red triangle. The RdRP domain was located at the N-terminal part ahead of the 2A-like cleavage site. YkV4 lacks a 2A-like sequence and alternatively has the second open reading frame (P2). The alignment of amino acid sequences around 2A-like peptides was excerpted in the right panel.

**Table 2.** Proteins showing homology to YkV1-RdRP in BLASTp analyses*a*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Description** | **Virus Name** | **Max Score** | **Total Score** | **Query Cover** | **E value** | **Per. ident** | **Acc. Len** | **Accession** |
| RNA dependent RNA polymerase [yadokari virus 1] | yadokari virus 1*b* | 2973 | 2973 | 100% | 0 | 100 | 1430 | [YP\_009551451.1](https://www.ncbi.nlm.nih.gov/protein/YP_009551451.1?report=genbank&log$=prottop&blast_rank=1&RID=291W6YK7013" \t "_parent) |
| putative RNA-dependent RNA polymerase [yadokari virus 1] | yadokari virus 1*b* | 1308 | 1308 | 44% | 0 | 100 | 638 | [BAM36407.1](https://www.ncbi.nlm.nih.gov/protein/BAM36407.1?report=genbank&log$=prottop&blast_rank=2&RID=291W6YK7013) |
| RNA-dependent RNA polymerase [Aspergillus homomorphus yadokarivirus 1] | Aspergillus homomorphus yadokarivirus 1*b* | 630 | 630 | 45% | 0 | 51.9 | 963 | [AZT88626.1](https://www.ncbi.nlm.nih.gov/protein/AZT88626.1?report=genbank&log$=prottop&blast_rank=3&RID=291W6YK7013) |
| 115 kDa protein [Penicillium aurantiogriseum foetidus-like virus] | Penicillium aurantiogriseum foetidus-like virus*b* | 621 | 621 | 49% | 0 | 48.73 | 975 | [YP\_009182156.1](https://www.ncbi.nlm.nih.gov/protein/YP_009182156.1?report=genbank&log$=prottop&blast_rank=4&RID=291W6YK7013) |
| RNA dependent RNA polymerase [Aspergillus foetidus slow virus 2] | Aspergillus foetidus slow virus 2*b* | 616 | 616 | 45% | 0 | 51.14 | 962 | [CCD33025.1](https://www.ncbi.nlm.nih.gov/protein/CCD33025.1?report=genbank&log$=prottop&blast_rank=5&RID=291W6YK7013) |
| hypothetical protein [yadokari virus 1] | yadokari virus 1*b* | 594 | 594 | 19% | 0 | 99.29 | 283 | [BAM36406.1](https://www.ncbi.nlm.nih.gov/protein/BAM36406.1?report=genbank&log$=prottop&blast_rank=6&RID=291W6YK7013) |
| RNA-dependent RNA polymerase [Penicillium digitatum yadokarivirus 1] | Penicillium digitatum yadokarivirus 1*b* | 577 | 577 | 46% | 0 | 46.78 | 987 | [AZT88627.1](https://www.ncbi.nlm.nih.gov/protein/AZT88627.1?report=genbank&log$=prottop&blast_rank=7&RID=291W6YK7013) |
| RdRp [Plasmopara viticola lesion associated yadokari virus 1] | Plasmopara viticola lesion associated yadokari virus 1*b* | 451 | 451 | 30% | 1.00E-137 | 51.73 | 732 | [QHD64758.1](https://www.ncbi.nlm.nih.gov/protein/QHD64758.1?report=genbank&log$=prottop&blast_rank=8&RID=291W6YK7013) |
| RNA-dependent RNA polymerase [Rhizoctonia solani mycovirus 1] | Rhizoctonia solani mycovirus 1*c* | 309 | 309 | 22% | 3.00E-91 | 51.1 | 311 | [ANR02697.1](https://www.ncbi.nlm.nih.gov/protein/ANR02697.1?report=genbank&log$=prottop&blast_rank=9&RID=291W6YK7013) |
| hypothetical protein [yadokari virus 1] | yadokari virus 1*b* | 267 | 267 | 9% | 5.00E-79 | 98.48 | 135 | [BAM36405.1](https://www.ncbi.nlm.nih.gov/protein/BAM36405.1?report=genbank&log$=prottop&blast_rank=10&RID=291W6YK7013) |
| RNA-dependent RNA polymerase [Picoa juniperi yadokari virus 1] | Picoa juniperi yadokari virus 1*b* | 292 | 292 | 44% | 2.00E-77 | 33.08 | 1155 | [QOI17269.1](https://www.ncbi.nlm.nih.gov/protein/QOI17269.1?report=genbank&log$=prottop&blast_rank=11&RID=291W6YK7013) |
| RNA-dependent RNA polymerase [Fusarium poae mycovirus 2] | Fusarium poae mycovirus 2*d* | 140 | 140 | 25% | 3.00E-29 | 31.5 | 1107 | [YP\_009272910.1](https://www.ncbi.nlm.nih.gov/protein/YP_009272910.1?report=genbank&log$=prottop&blast_rank=12&RID=291W6YK7013) |
| RNA-dependent RNA polymerase [yadokari i virus 3] | yadokari virus 3*d* | 138 | 138 | 29% | 1.00E-28 | 28.07 | 1434 | [BBB86810.2](https://www.ncbi.nlm.nih.gov/protein/BBB86810.2?report=genbank&log$=prottop&blast_rank=13&RID=291W6YK7013) |
| replicase [yadokari i virus 2] | yadokari virus 2*d* | 133 | 133 | 27% | 5.00E-27 | 29.44 | 1469 | [AVD68673.2](https://www.ncbi.nlm.nih.gov/protein/AVD68673.2?report=genbank&log$=prottop&blast_rank=14&RID=291W6YK7013) |
| RNA-dependent RNA polymerase [yadokari virus 2] | yadokari virus 2*d* | 131 | 131 | 27% | 1.00E-26 | 28.61 | 1461 | [BBB86807.2](https://www.ncbi.nlm.nih.gov/protein/BBB86807.2?report=genbank&log$=prottop&blast_rank=15&RID=291W6YK7013) |
| RNA-dependent RNA polymerase [yadokari i virus 4] | yadokari virus 4*d* | 127 | 127 | 36% | 2.00E-25 | 26.84 | 835 | [BBB86805.1](https://www.ncbi.nlm.nih.gov/protein/BBB86805.1?report=genbank&log$=prottop&blast_rank=16&RID=291W6YK7013) |
| RNA-dependent RNA polymerase [yadokari virus 4] | yadokari virus 4*d* | 124 | 124 | 34% | 2.00E-24 | 26.8 | 835 | [BBB86788.1](https://www.ncbi.nlm.nih.gov/protein/BBB86788.1?report=genbank&log$=prottop&blast_rank=17&RID=291W6YK7013) |
| ORF1 polyprotein [Sapovirus GII.5] | Sapovirus GII.5*e* | 52.4 | 52.4 | 15% | 0.026 | 25.64 | 2279 | [AWK57648.1](https://www.ncbi.nlm.nih.gov/protein/AWK57648.1?report=genbank&log$=prottop&blast_rank=18&RID=291W6YK7013) |
| ORF1 polyprotein [Sapovirus GII.5] | Sapovirus GII.5*e* | 52.4 | 52.4 | 15% | 0.026 | 25.64 | 2279 | [AWK57654.1](https://www.ncbi.nlm.nih.gov/protein/AWK57654.1?report=genbank&log$=prottop&blast_rank=19&RID=291W6YK7013) |
| ORF1 polyprotein [Sapovirus GII.5] | Sapovirus GII.5*e* | 52.4 | 52.4 | 15% | 0.027 | 25.64 | 2279 | [AWK57650.1](https://www.ncbi.nlm.nih.gov/protein/AWK57650.1?report=genbank&log$=prottop&blast_rank=20&RID=291W6YK7013) |
| ORF1 polyprotein [Sapovirus GII.5] | Sapovirus GII.5*e* | 52.4 | 52.4 | 15% | 0.027 | 25.64 | 2279 | [AWK57652.1](https://www.ncbi.nlm.nih.gov/protein/AWK57652.1?report=genbank&log$=prottop&blast_rank=21&RID=291W6YK7013) |
| ORF1 polyprotein [Sapovirus GII.5] | Sapovirus GII.5*e* | 52.4 | 52.4 | 15% | 0.027 | 25.64 | 2279 | [AWK57656.1](https://www.ncbi.nlm.nih.gov/protein/AWK57656.1?report=genbank&log$=prottop&blast_rank=22&RID=291W6YK7013) |
| ORF1 polyprotein [Sapovirus Hu/GII/JP/2010/Kashiwa1] | Sapovirus Hu/GII/JP/2010/Kashiwa1*e* | 52.4 | 52.4 | 15% | 0.028 | 25.64 | 2279 | [BAX24515.1](https://www.ncbi.nlm.nih.gov/protein/BAX24515.1?report=genbank&log$=prottop&blast_rank=23&RID=291W6YK7013) |
| polyprotein [Sapovirus GII.3] | Sapovirus GII.3*e* | 51.2 | 51.2 | 16% | 0.045 | 25.62 | 902 | [QKO00687.1](https://www.ncbi.nlm.nih.gov/protein/QKO00687.1?report=genbank&log$=prottop&blast_rank=24&RID=291W6YK7013) |
| polyprotein [Sapovirus GV] | Sapovirus GV*e* | 51.2 | 51.2 | 19% | 0.048 | 27.7 | 1847 | [AWU66034.1](https://www.ncbi.nlm.nih.gov/protein/AWU66034.1?report=genbank&log$=prottop&blast_rank=25&RID=291W6YK7013) |

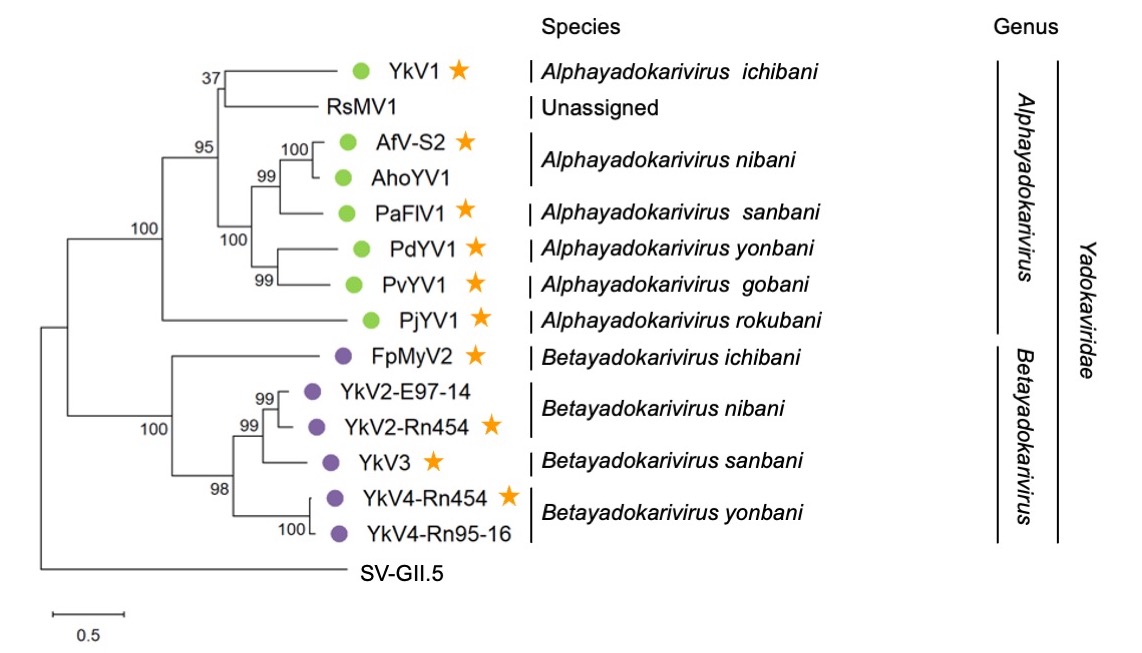
*a* Proteins that shows amino acid sequence homology to YkV1-RdRP (YP\_009551451.1) was screened by Protein BLAST search of “non-redundant protein sequences (nr)” database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>). The search was performed by blastp (protein-protein BLAST) algorithm with default scoring parameters. All the hits with an e-value lower than 0.05 were listed. The search was conducted on 11th Feb 2020.

*b* Viruses in the genus Alphayadokarivirus (see Table 1).

*c* An unassigned alphayadokari-like virus with partial genome sequence information.

*d* Viruses in the genus *Betayadokarivirus* (see Table 1).

*e* Viruses in the genus *Sapovirus* in the family *Caliciviridae*.

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**Figure 2:** Phylogenetic relationships among yadokarivirids. The phylogenetic tree was constructed by the maximum likelihood (ML) method based on the alignment of amino acid sequence of RdRP. Full names and accession numbers of yadokariviruses are listed in Table 1. The green and purple circles indicate viruses in the genus *Alphayadokarivirus* or *Betayadokarivirus*, respectively. The orange stars indicate the exemplar virus for each yadokarivirus species. RdRP of a sapovirus (sapovirus GII.5, AWK57648.1) was used as an outgroup (Table 2). The amino acid sequence alignment was performed online by MAFFT version 7.475 with the L-INS-i method and default parameters (scoring matrix: BLOSUM62, gap opening penalty: 1.53) [5]. The ML tree was generated in MEGA X version 10.2.3 by the best fit model “LG with Freps.” with “Gamma Distributed With Invariant Sites (G+I)” (No of Discrete Gamma Categories = 5) option [7]. All sites were used without trimming. The final dataset had a total of 3026 positions. Scale bar and branch length indicate the number of amino acid substitutions per site. The phylogeny was tested by the bootstrap method with 500 replications, with % support indicated at branching nodes.

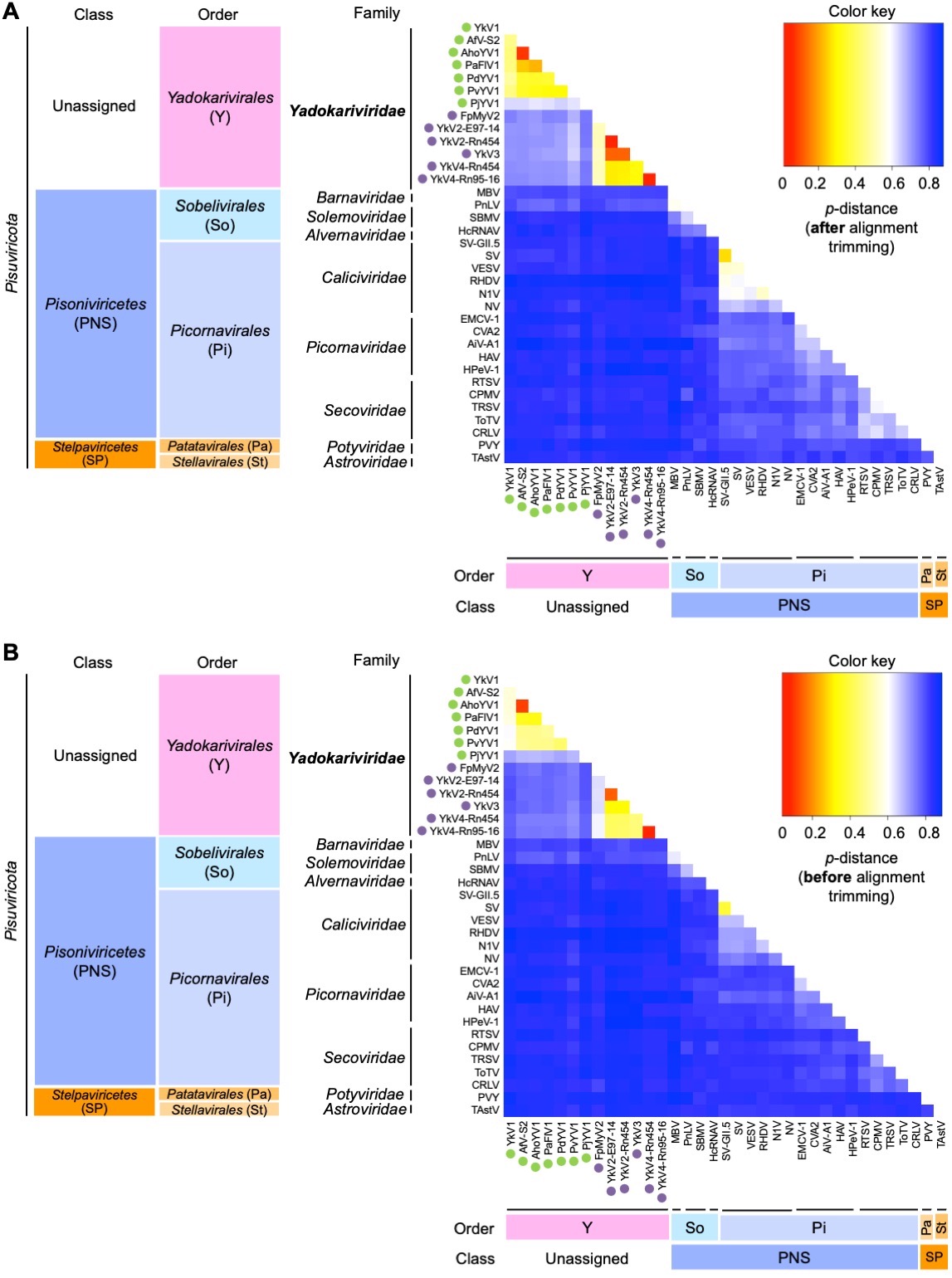
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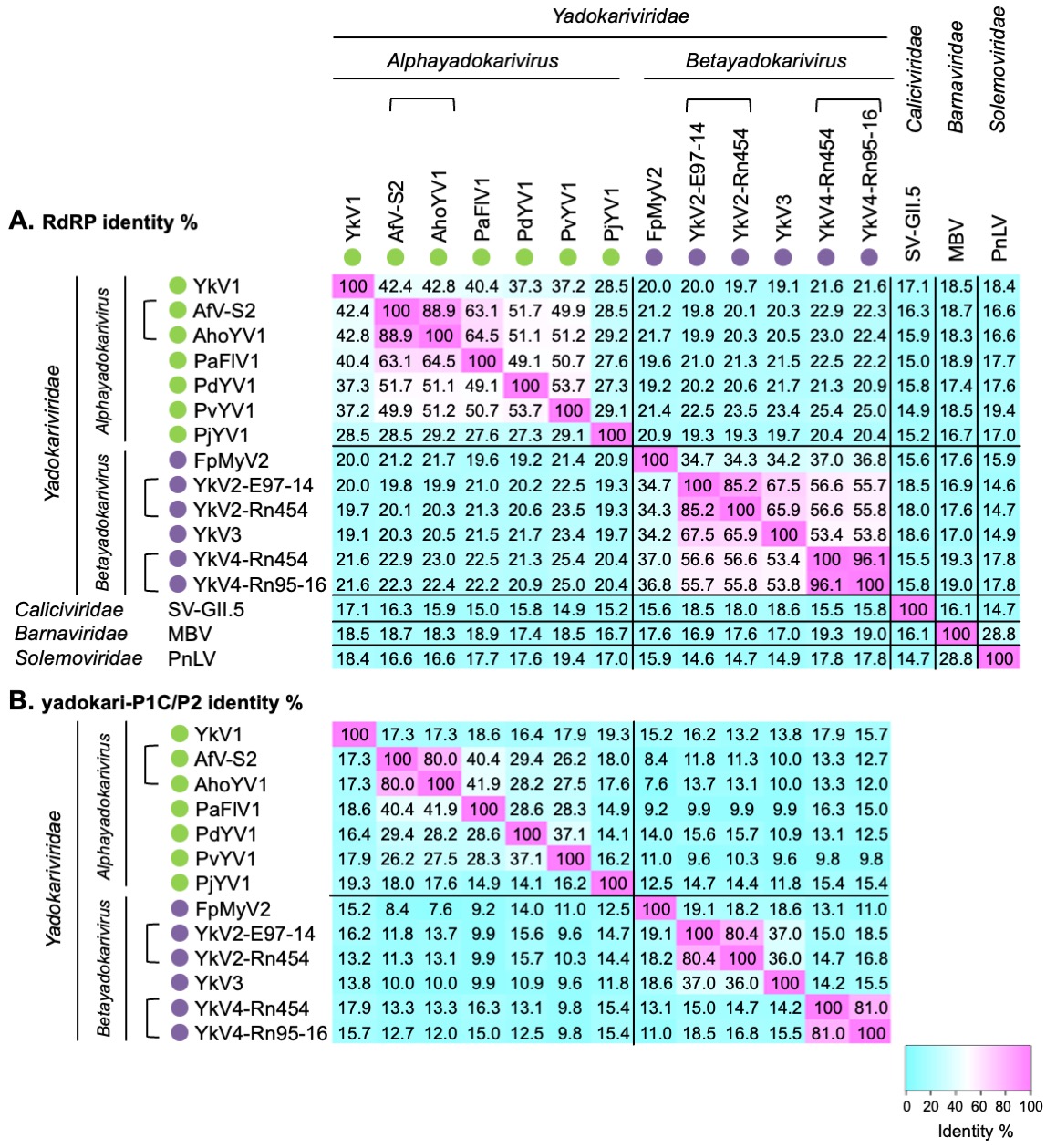
**Figure 3:** Phylogenetic relationships of yadokarivirids with the viruses in the phylum *Pisuviricota*. Amino acid sequences of RdRPs of the yadokarivirids (Table 1) and the viruses listed in Table S1 were aligned online by MAFFT version 7.475 with the FFT-NS-i method and default parameters [5]. Core RdRP motifs (A-C, E, F) were properly aligned (Fig. 4). The ambiguously aligned sites were removed by online trimAl version 1.3 with the Gappyout method [2]. The ML tree was generated in MEGA X version 10.2.3 by the best fit model “LG” with “G+I” (No of Discrete Gamma Categories = 5) option [7]. There was a total of 438 positions in the final dataset. The green and purple circles indicate viruses in the proposed genera *Alphayadokarivirus* or *Betayadokarivirus*, respectively. The phylogeny was tested by the bootstrap method with 1000 replications, with percentage (%) support indicated at the nodes.

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自動的に生成された説明Figure 4**: Conserved RdRP motifs in the yadokaviruses and the other viruses in the phylum *Pisuviricota*. Parts of multiple sequence alignment for the phylogenetic analysis (Fig. 3) were shown. The sequence alignment was visualized in MEGA X [7]. Motif D was not well aligned in the dataset.



**Figure 5**: Pairwise distance matrix based on the alignment of amino acid sequences of RdRP after (A) or before the trimming (B). The color key was based on *p*-distance, the number of amino acid differences per site between sequences. The multiple sequence alignment and the sequence trimming were performed as described in the Fig. 3 caption. The evolutionary distance was computed in MEGA X by the *p*-distance method with the “G+I” (Gamma parameter = 1) option [7]. All ambiguously aligned sites were removed for each sequence pair. There was a total of 438 or 6363 positions in the final dataset for (A) and (B), respectively. The heatmaps were generated by R package “gplots” version 3.1.1.

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**Figure 6:** Pairwise percent identity matrix based on the global alignment of the amino acid sequence of RdRP (A) or yadokari-P1C/P2 (B). For (A), the identity of whole polyproteins that contain the RdRP domain was analyzed. Full names and RdRP accession numbers of the yadokarivirids and viruses from the other three families (*Caliciviridae*, *Barnaviridae* and *Solemoviridae*) in the class *Pisoniviricetes* are listed in Table 1 or Table S1. For (B), the identity of yadokari-P1C (C-terminal part of yadokarivirus P1 polyprotein behind the 2A-like cleavage site) and -P2 (which was only found in YkV4s) was analyzed. The multiple sequence alignment was performed by Clustal Omega version 1.2.4 with default settings [13]. The heatmap was generated by R package “gplots” version 3.1.1.

**Table S1.** Taxonomy and accession numbers of the viruses used in the phylogenetic analyses.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Phylum** | **Class** | **Order** | | **Family** | **Genus** | **Species** | **Virus abbrev.** | **RdRP accession** |
| *Pisuviricota* | *Pisoniviricetes* | *Picornavirales* | | *Caliciviridae* | *Lagovirus* | *Rabbit hemorrhagic disease virus* | RHDV | NP\_062875.1 |
| *Nebovirus* | *Newbury 1 virus* | N1V | YP\_529550.1 |
| *Norovirus* | *Norwalk virus* | NV | NP\_056820.1 |
| *Sapovirus* | *Sapporo virus* | SV | YP\_077278.1 |
| SV-GII.5 | AWK57648.1 |
| *Vesivirus* | *Vesicular exanthema of swine virus* | VESV | NP\_066255.1 |
| *Picornaviridae* | *Cardiovirus* | *Cardiovirus A* | EMCV-1 | NP\_056777.1 |
| *Enterovirus* | *Enterovirus C* | CVA2 | YP\_009505603.1 |
| *Hepatovirus* | *Hepatovirus A* | HAV | NP\_041007.1 |
| *Kobuvirus* | *Aichivirus A* | AiV-A1 | NP\_047200.1 |
| *Parechovirus* | *Parechovirus A* | HPeV-1 | YP\_009505617.1 |
| *Secoviridae* | *Comovirus* | *Cowpea mosaic virus* | CPMV | NP\_613283.1 |
| *Nepovirus* | *Tobacco ringspot virus* | TRSV | NP\_919040.1 |
| *Cheravirus* | *Cherry rasp leaf virus* | CRLV | YP\_081444.1 |
| *Torradovirus* | *Tomato torrado virus* | ToTV | YP\_001039627.1 |
| *Waikavirus* | *Rice tungro spherical virus* | RTSV | NP\_042507.1 |
| *Sobelivirales* | | *Alvernaviridae* | *Dinornavirus* | *Heterocapsa circularisquama RNA virus 01* | HcRNAV | YP\_386495.1 |
| *Barnaviridae* | *Barnavirus* | *Mushroom bacilliform virus* | MBV | NP\_042510.2 |
| *Solemoviridae* | *Polemovirus* | *Poinsettia latent virus* | PnLV | YP\_002308462.1 |
| *Sobemovirus* | *Southern bean mosaic virus* | SBMV | AAC15983.1 |
| *Stelpaviricetes* | *Patatavirales* | *Potyviridae* | | *Potyvirus* | *Potato virus Y* | PVY | AAB50573.1 |
| *Stellavirales* | *Astroviridae* | | *Avastrovirus* | *Avastrovirus 1* | TAstV | NP\_853540.1 |

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