BEATRIZ DIAS JANUÁRIO

CARACTERIZAÇÃO BIOLÓGICA E MOLECULAR DE DOIS BACTERIÓFAGOS QUE INFECTAM *Ralstonia solanacearum E Ralstonia pseudosolanacearum*

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Magister Scientiae*.

Orientadora: Poliane Alfenas-Zerbini

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Poliane Alfenas-Zerbini
Orientadora

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RESUMO

JANUÁRIO, Beatriz Dias, M.Sc., Universidade Federal de Viçosa, julho de 2024. **CARACTERIZAÇÃO BIOLÓGICA E MOLECULAR DE DOIS BACTERIÓFAGOS QUE INFECTAM *Ralstonia solanacearum* E *Ralstonia pseudosolanacearum***. Orientadora: Poliane Alfenas-Zerbini.

A murcha bacteriana, causada por bactérias do complexo de espécies *Ralstonia solanacearum* (RSSC). O RSSC é um grupo de bactérias gram-negativas, classificadas nas espécies *Ralstonia solanacearum, Ralstonia pseudosolanacearum* e *Ralstonia syzygii.* Estas bactérias têm uma ampla gama de hospedeiros, incluindo muitas culturas de relevância econômica como tomate, berinjela e eucalipto. O controle desta doença é particularmente desafiador devido à persistência das bactérias no solo e na água, à sua grande diversidade genética e à ausência de tratamentos químicos eficazes. Diante desse cenário, a utilização de bacteriófagos, é uma alternativa para o biocontrole de *Ralstonia spp.*. O presente trabalho teve como objetivo realizar a caracterização biológica e molecular de dois bacteriófagos isolados do solo brasileiro, que infectam bactérias das espécies *Ralstonia solanacearum* e *Ralstonia pseudosolanacearum*. Os fagos, denominados RS-Phage-AB1 e RS-Phage-CA1, foram sequenciados e analisados, revelando diversidade genética e de mecanismos de infecção. O fago RS-Phage-AB1, possui um genoma de 41.668 pb com 46 ORFs e conteúdo GC de 64,3%. Foi classificado na família *Peduoviridae*, sugerindo a criação de um novo gênero "Acarajevirus" e a espécie "Acarajevirus *bahia*". Identificado como fago temperado com genes como integrase e metiltransferase de DNA, refletindo estratégias distintas de sobrevivência e infecção. O fago RS-Phage-CA1, possui um genoma de 46.254 pb com 59 ORFs e conteúdo GC de 62,3%, não foi classificado em nenhuma família descrita, propondo-se uma nova família viral "Anamaviridae*"* com as subfamílias *Kantovirinae* e *"*Mascarenevirinae*".* Contém genes como resolvase e endonuclease, indicando mecanismos diferentes de inserção/excisão e resistência a superinfecções. Ambos os fagos mostraram infecção específica para *R. solanacearum* e *R. pseudosolanacearum* e possuem genes relacionados à lise bacteriana, como endolisina e holina. Estes resultados contribuem paro o entendimento da diversidade de fagos e seus potenciais aplicações no controle biológico de fitopatógenos.

Palavras-chave: *Ralstonia solanacearum; Ralstonia pseudosolanacearum;* Murcha bacteriana; Classificação taxonômica; Bacteriófagos.

ABSTRACT

JANUÁRIO, Beatriz Dias, M.Sc., Universidade Federal de Viçosa, July 2024. **BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF TWO BACTERIOPHAGES THAT INFECT *Ralstonia solanacearum* AND *Ralstonia pseudosolanacearum***. Advisor: Poliane Alfenas-Zerbini.

Bacterial wilt is caused by bacteria of the *Ralstonia solanacearum* species complex (RSSC). RSSC is a group of gram-negative bacteria composed of *Ralstonia solanacearum, Ralstonia pseudosolanacearum,* and *Ralstonia syzygi*i. These bacteria have a broad host range, including many economically important crops such as tomato, eggplant, and eucalyptus. Control of this disease is particularly challenging due to the persistence of the bacteria in soil and water, their great genetic diversity, and the lack of effective chemical treatments. Given this scenario, using bacteriophages is an alternative for the biocontrol of *Ralstonia spp.*. The present study aimed to perform the biological and molecular characterization of two bacteriophages isolated from Brazilian soil, specifically targeted to infect *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum*. The phages, RS-Phage-AB1 and RS-Phage-CA1, were sequenced and analyzed, revealing genetic diversity and infection mechanisms. The phage RS-Phage-AB1 has a genome of 41,668 bp with 46 ORFs and a GC content of 64.3%. It was classified in the *Peduoviridae* family, suggesting the creation of a new genus, "Acarajevirus" and the species "Acarajevirus bahia." Identified as a temperate phage with genes such as integrase and DNA methyltransferase, reflecting distinct survival and infection strategies. The phage RS-Phage-CA1, has a genome of 46,254 bp with 59 ORFs and a GC content of 62.3%, was not classified in any described family, proposing a new viral family "Anamaviridae" with the subfamilie *Kantovirinae* and a new subfamily "Mascarenevirinae". It contains genes such as resolvase and endonuclease, indicating different insertion/excision mechanisms and resistance to superinfections.

Both phages are specific to *R. solanacearum* and *R. pseudosolanacearum* and possess genes related to bacterial lysis, such as endolysin and holin. These results expand the understanding of phage diversity and their potential applications in the biological control of plant pathogens.

Keywords: *Ralstonia solanacearum. Ralstonia pseudosolanacearum*. Bacterial wilt. Taxonomic classification. Bacteriophages.

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INTRODUÇÃO

A murcha bacteriana causada por bactérias do complexo de espécies *Ralstonia solanacearum* se destaca como uma das doenças em plantas mais devastadoras em âmbito global. O complexo de espécies *R. solanacearum*, do inglês *Ralstonia solanacearum* species complex (RSSC), é um grupo constituído por três espécies de bactérias: *Ralstonia solanacearum*, *Ralstonia pseudosolanacearum* e *Ralstonia syzygii*. São bactérias gram-negativas pertencentes ao filo Proteobacteria, classe β-Proteobacteria, ordem Burkholderiales e família *Burkholderiaceae* (GARĆIA; KERNS; THIESSEN, 2019). Estão relacionadas filogeneticamente, mas diferem em importantes características fenotípicas, como por exemplo gama de hospedeiros (SHARMA et al., 2022) e genotípicas, como variabilidade dos genes de efetores do tipo III (T3Es). Cada espécie no complexo RSSC tem seu próprio grupo de T3Es, essenciais para sua patogenicidade e interação com seus hospedeiros.Além disso formam clados bem distintos, com base na análise Average Nucleotide Identity (ANI) (GENG et al., 2022) O grupo RSSC é cosmopolita e afeta diferentes culturas em todo o mundo. É capaz de infectar diversas espécies de plantas classificadas em cerca de 50 famílias botânicas distintas (VAILLEAU; GENIN, 2023), incluindo *Eucalyptus spp.*(FERREIRA; MAFIA; ALFENAS, 2018).

No Brasil, o fitopatógeno *R. solanacearum* causa umas das mais importantes doenças do eucalipto, a murcha vascular (ALFENAS et al., 2006). O complexo de espécies de *Ralstonia solanacearum* (RSSC) é formado por grupos de bactérias fenotipicamente diferentes que podem ser subdivididas em quatro filotipos. Os isolados de *Ralstonia solanacearum* que afetam o eucalipto pertencem ao filotipo II (SAFNI et al., 2014). Os demais filotipos foram classificadas em espécies distintas: *R. pseudosolanacearum* (filotipo I e III) e *R. syzygii* (filotipo IV) (PRIOR et al., 2016). *R. pseudosolanacearum* também causa murcha de eucalipto no Brasil, essa espécie está associada a sintomas de murcha em plantas solanáceas, principalmente nas regiões Norte e Nordeste (FREITAS et al., 2020; LOPES; ROSSATO, 2018).

A murcha bacteriana causada por bactérias do RSSC é de difícil controle devido à capacidade de sobrevivência no solo e na água sem a presença de uma planta hospedeira. Além disso, a grande diversidade genética e fenotípica do complexo de espécies torna o controle pelo uso de variedades resistentes inviável e a falta de tratamentos químicos eficazes é um desafio no desenvolvimento de

tecnologias de controle bem-sucedidas para esta doença (ABDURAHMAN et al., 2019).

Em espécies de *Eucalyptus spp*. a murcha bacteriana causada por RSSC ocorre em regiões quentes e úmidas. Os principais sintomas da doença em eucaliptos no campo incluem a necrose das folhas, escurecimento e fratura da casca na base do tronco, alteração na coloração do xilema, bem como a murcha e morte das raízes (ALFENAS et al., 2006). Mudas clonais provenientes de estaqueamento podem abrigar infecções latentes, onde o xilema exibe quantidades limitadas de células bacterianas, e ao serem transplantadas para o campo, apresentam sinais de murcha cerca de 3 a 4 meses após o plantio (FERREIRA; MAFIA; ALFENAS, 2018).As espécies de *Eucalyptus* spp. fornecem matéria prima com propriedades físicas e químicas diversas com diferentes aplicações industriais, como siderurgia, moveleira, celulose e papel (GUERINO et al., 2022). No contexto brasileiro, as florestas plantadas de eucalipto são uma das mais produtivas do mundo. Com destaque para produção de celulose, sendo o Brasil um dos maiores produtores (IBÁ,2019).

O manejo de doenças causadas por fitopatógenos possui grande impacto no desenvolvimento da produção agrícola. Doenças bacterianas que afetam as culturas agrícolas, causam grandes perdas econômicas. Décadas de utilização de agroquímicos, como estreptomicina, oxitetraciclina e produtos à base de cobre visando prevenir e tratar tais doenças desempenharam um papel significativo com aumento da resistência entre patógenos de plantas a esses agentes (MILLER; FERREIRA; LEJEUNE, 2022). As doenças de plantas, propagadas via solo são consideradas de difícil manejo, devido a longa persistência no solo, vasta diversidade genética e gama de hospedeiros, impactando a produção de diversas culturas (KHAN, 2023; WANG et al., 2023).

Os fagos possuem grande relevância biotecnológica, para variados propósitos, como uma alternativa do uso de antimicrobianos contra bactérias resistentes a antibióticos, agentes de biocontrole na agricultura, como agentes de biocontrole de patógenos alimentares (SHARMA et al., 2017; MAHONY et al., 2011).

Os fagos no tratamento de infecções bacterianas em humanos oferecem uma alternativa viável aos tratamentos tradicionais, ajudando a mitigar a disseminação de doenças emergentes e a reduzir o uso de antibióticos tanto em humanos quanto em animais (MAIMAITI et al., 2023). Os fagos geralmente são específicos para um pequeno conjunto de cepas da mesma espécie (HSIEH et al., 2011), e podem ser

usados contra bactérias organizadas em biofilmes. Os fagos enfraquecem a estrutura do biofilme ao lisar células bacterianas (SZAFRAŃSKI; WINKEL; STIESCH, 2017). Aplicações terapêuticas de fagos líticos na medicina humana é autorizada para uso no tratamento de doenças infecciosas na Polônia, Rússia e Geórgia. Os fagos também podem ser usados para diagnosticar espécies microbianas ou guiar diferentes medicamentos para as células (GUO et al., 2020).

Na medicina veterinária a mastite em vacas leiteiras é uma das doenças que mais ameaça produtores de leite. O uso excessivo de antibióticos é um grande problema no tratamento da mastite bovina. Um estudo inicial usando camundongos como modelo foi desenvolvido, com resultados significativo usando coquetéis de fagos, apresentando uma diminuição no número de unidades formadoras de colônias e uma melhora na patologia da mastite (GENG et al, 2020).

Bacteriófagos oferecem grande potencial para uso como agentes de biocontrole e detecção de patógenos em alimentos, na indústria alimentícia pode prevenir a deterioração de produtos e a disseminação de doenças bacterianas e, promover ambientes seguros na produção, processamento e manuseio de alimentos de origem animal e vegetal (SILLANKORVA; OLIVEIRA; AZEREDO, 2012). Os efeitos de um coquetel de bacteriófagos específicos contra *Escherichia coli* em folhas verdes frescas armazenadas sob diferentes condições atmosféricas a 4 e 10°C, foi eficaz na redução significativa da bactéria em espinafre, alface verde, tendo em vista temperatura e composição atmosférica semelhantes com processamento, armazenamento e transporte de produtos industriais (BOYACIOGLU et al., 2013). Um produto já comercializado à base de fagos chamado PhageGuard Listex™, desenvolvido para combater *Listeria monocytogenes,* bactéria patogênica que pode causar listeriose, uma infecção que causa diarreia, vômitos, e pode ser transmitida por alimentos frescos como, verduras e legumes. Com a aplicação da concentração adequada, o produto é eficiente no tratamento pós-processamento não causou nenhum impacto prejudicial na qualidade sensorial (GÓMEZ-GALINDO et al., 2023).

Os vírus que infectam bactérias têm demonstrado ser nanomáquinas biológicas altamente eficazes, elaboradas para invadir seus hospedeiros com elevada especificidade e eficácia para o controle de doenças em plantas, animais e humanos (KILCHER; LOESSNER, 2019; ELHALAG et al., 2018). Os bacteriófagos têm a capacidade de regular processos ecológicos e evolutivos relacionados à bactéria patogênica. O mecanismo ecológico primordial envolve a modulação da densidade

dos patógenos. A ação dos fagos ao diminuir a quantidade de patógenos pode limitar as chances de infecção e influenciar a expressão de genes responsáveis pela virulência bacteriana (RUTHERFORD; BASSLER, 2012). É importante entender os mecanismos evolutivos que determinam o surgimento da resistência bacteriana contra a infecção por fagos. Um exemplo de resistência, é o impedimento da adsorção dos fagos na superfície bacteriana, devido a mudança das moléculas de superfície alvo que atuam como receptores do fago (ROHDE et al., 2018). Essas moléculas de superfície alvo possuem outras funções, como motilidade, integridade da membrana celular, transporte de nutrientes, e sua alteração ou perda está associada a custos adaptativos, o que pode limitar o sucesso de mutantes resistentes na ausência de fagos (SCANLAN; BUCKLING; HALL, 2015). Isto pode envolver mutações que afetam a regulação ou biossíntese da molécula de superfície, levando à perda ou alteração da estrutura do receptor (WRIGHT et al., 2019).

Assim como ocorre com outras abordagens de controle biológico, uma vantagem da fagoterapia é a diminuição da necessidade de utilizar produtos químicos para combater patógenos. Isso contribui para evitar questões relacionadas à contaminação do meio ambiente, à alteração dos ecossistemas e ao acúmulo de substâncias químicas nos cultivos (BALOGH et al., 2010). A aplicação da terapia fágica em contextos agrícolas tem sido extensivamente investigada durante as últimas cinco décadas como um método para controlar os fitopatógenos (HOLTAPPELS et al., 2021; MARONGIU et al., 2022).

Wang e colaboradores (2019), demonstraram que é possível utilizar os bacteriófagos como uma ferramenta altamente precisa para aprimorar a saúde das plantas, tanto em ambientes de estufa quanto em campo aberto. Foi observado que os efeitos do controle biológico realizado pelos bacteriófagos foram desencadeados por mecanismos ecológicos e por mecanismos evolutivos, já que a redução das doenças esteve ligada à seleção de agentes patogênicos altamente resistentes, porém de crescimento lento (WANG et al., 2019).

Elhalag e colaboradores (2018), isolaram o fago podovírus RsPod1EGY, com ação específica contra *R. solanacearum* e demonstraram que foi eficaz na eliminação da bactéria em condições de casa de vegetação. Todas as plantas de tomateiro tratadas não apresentaram sintomas de murcha ou qualquer infecção latente durante o período experimental, enquanto todas as plantas não tratadas murcharam 10 dias após a infecção.

Os fagos que infectam *R. solanacearum* provaram ser possíveis agentes de controle contra a murcha. Os bacteriófagos exibem uma ação específica, eliminando apenas as espécies bacterianas patogênicas desejadas, o que evita uma consequência indesejada de atingir microrganismos não desejados que podem ter uma relevância dentro do agroecossistema (ASKORA et al., 2017; FUJIWARA et al., 2011).

A fagoterapia envolve a utilização de bacteriófagos como agentes de controle de bactérias, e possui algumas vantagens. Os bacteriófagos têm a capacidade de coevoluir com as bactérias hospedeiras, além da crescente disponibilidade de fagos direcionados a doenças específicas devido à sua vasta variedade, e na sua segurança com o meio ambiente (BUTTIMER et al., 2017). O produto contendo fagos, “AgriPhage”(desenvolvido por Omnilytics Inc.) se mostrou eficiente para controlar doenças bacterianas em culturas como tomate e pimentão, combatendo patógenos como *Xanthomonas campestres* (D’ACCOLTI et al., 2021). O produto “EcoShield” (desenvolvido por Intralytix Inc.) usado como parte de um coquetel de bacteriófagos eficaz no controle de patógenos em frutas e vegetais frescos contaminados. EcoShield, também demonstrou reduzir significativamente os níveis de *Escherichia coli,* e *Salmonella spp*. em brócolis, melões e morangos (MAGNONE et al., 2013). O isolamento e a caracterização biológica e molecular de bacteriófagos são fundamentais para garantir a eficácia e segurança na fagoterapia. A caracterização que envolve a determinação da gama de hospedeiros dos fagos, e a ausência de genes de toxinas para que possam efetivamente combater bactérias patogênicas sem causar efeitos adversos (HYMAN, 2019). Portanto, uma etapa determinante para o sucesso da fagoterapia é o isolamento e a caracterização, levando em consideração o tipo de amostra e o hospedeiro (VILLALPANDO-AGUILAR et al., 2023).

Com a necessidade de supervisão da nomenclatura e classificação de vírus o “International Committee on Nomenclature of Virus” foi fundado em 1966 e, em 1977, foi renomeado para “International Committee on Taxonomy of Viruses” (ICTV). O desenvolvimento, e manutenção de uma taxonomia universal de vírus é responsabilidade do ICTV. Esta organização é responsável pela aprovação de nomes e classificações de novos vírus, para garantir que a nomenclatura seja padronizada (ADAMS et al., 2017; LEFKOWITZ et al., 2018).

Simmonds e colaboradores (2023), recomendam quatro princípios para orientar a construção de uma taxonomia de vírus coerente e atualizada. Primeiro, a

classificação dos vírus deve estar de acordo sua história evolutiva. A maioria dos vírus pode ser categorizada em realms virais independentes, cada um com uma origem evolutiva distinta inferida. Os membros desses realms compartilham conjuntos de genes ortólogos ancestrais, que geralmente correspondem a módulos de replicação ou formação de vírions em seus genomas. Em segundo lugar, destacam que as características dos vírus podem orientar a atribuição de suas classificações, que deve seguir padrões baseados em propriedades evolutivas, genômicas e fenotípicas. A taxonomia desenvolvida pelo ICTV fornece uma estrutura abrangente para a classificação dos vírus. No entanto, classificações alternativas, como aquelas baseadas em propriedades clínicas ou epidemiológicas ou em requisitos regulatórios, têm suas próprias utilidades em contextos específicos. E por fim, atribuições taxonômicas de vírus inferidas de sequências metagenômicas exigem um rigoroso controle de qualidade das sequências, pois devem ser precisas e completas (SIMMONDS et al., 2023).

Os bacteriófagos, ou fagos, são vírus que possuem como hospedeiras células bacterianas. Possuem material genético na forma de DNA ou RNA, encapsidado por uma capa de proteína, e desempenham um papel crucial na evolução de seu hospedeiro (HARADA et al., 2018). A taxonomia dos bacteriófagos tem sofrido mudanças há mais de quatro décadas. Antes dos métodos moleculares modernos, os fagos eram classificados com base em sua morfologia (TURNER; KROPINSKI; ADRIAENSSENS, 2021). Em 2021 foram aprovadas mudanças para classificação taxonômica de fagos. A classificação das famílias com base na morfologia foi abolida em favor de uma classificação baseada em informações genéticas e moleculares mais precisas (TURNER et al., 2023). O ICTV também aprovou tornar obrigatório o formato binomial para a nomeação de espécies de vírus. Dessa forma, o nome do gênero e um epíteto da espécie juntos formam um nome de espécie único, similares ao sistema usado para a classificação de outros organismos. Isso ajuda a padronizar a nomenclatura e facilita a identificação e estudo das espécies de fagos (SIDDELL et al., 2020). Assim, os membros da ordem (*Caudovirales*) foram atribuídos à classe *Caudoviricetes*. As antigas famílias Myoviridae, Podoviridae e Siphoviridae, baseadas em estruturas foram extintas e foram criadas uma série de novas famílias baseadas em relacionamento filogenético (ZHU et al., 2022). Além da mudança com as famílias, foram criadas ordens e classes para agrupar os bacteriófagos de maneira mais precisa. (TURNER et al., 2023).

Dessa forma, de acordo com Turner e colaboradores (2023), os critérios atuais para a classificação taxonômica de bacteriófagos priorizam a análise genômica. A classificação é baseada principalmente na sequência do genoma viral, refletindo as relações filogenéticas entre os fagos. A taxonomia baseada em morfologia foi abolida, e a nomenclatura binomial foi introduzida para as espécies de bacteriófagos, alinhando-se a um sistema mais sistemático. Embora características, como o ciclo de vida e o perfil de hospedeiro, ainda sejam relevantes, a ênfase principal está na genômica e na filogenia para determinar a classificação,

O objetivo principal deste trabalho foi sequenciar e caracterizar bacteriófagos que infectam o complexo de espécies de bactérias fitopatogênicas *Ralstonia solanacearum* (RSSC), a partir de amostras de solo e água associados à cultura de plantas de eucalipto. Em seguida extrair e sequenciar os genomas virais, montar e caracterizar os genomas virais, classificar taxonomicamente os genomas dos bacteriófagos e determinar a gama de hospedeiro. Foram obtidos dois fagos temperados descritos, isolados de amostras de solo, os dois são espécies novas, denominados RS-Phage-AB1 e RS-Phage-CA1. Ambos os fagos infectam *R. solanacearum* e *R. pseudosolanacearum*. O RS-Phage-AB1 tem um tamanho de genoma de 41.668 pb com 46 ORFs, e o RS-Phage-CA1 tem um tamanho de genoma de 46.254 pb com 59 ORFs. Foi proposta a criação de uma nova família viral, “Anamaviridae”, composta pela subfamília *Kantovirinae* e a criação de uma nova subfamília “Mascarenevirinae”. RS-Phage-AB1 foi sugerido ser nomeado como *“*Acarajevirus bahia” na família *Peduoviridae*, enquanto RS-Phage-CA1 ser nomeado como *“*Cocadavirus alagoinhas” pertencente a subfamília “Mascarenevirinae”, pertencente à família “Anamaviridae”.

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CAPÍTULO 1

Description of two novel *Caudoviricetes* isolated from Brazilian soil that infect *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum* and proposal of a novel viral family

Januário B. D., Rezende R. R., Morgan T., and Alfenas-Zerbini, P. Description of two novel *Caudoviricetes* isolated from Brazilian soil that infect *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum* and proposal of a novel viral family named “Anamaviridae”. To be submitted to the Journal of General Virology (Brief report).

Description of two novel *Caudoviricetes* isolated from Brazilian soil that infect *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum* and proposal of a novel viral family

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Abstract: This study reports the isolation and characterization of two new phages from Brazilian soil that infect *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum*. The phages, RS-Phage-AB1 and RS-Phage-CA1, were sequenced and analyzed, revealing genetic diversity and mechanisms of infection. RS-Phage-AB1 is a new virus species and was classified in the *Peduoviridae* family. The phage RS-Phage-CA1 was not classified in any described family. So, we propose a new viral family, *"*Anamaviridae" with the subfamily *Kantovirinae* and the new subfamily "Mascarenevirinae". RS-Phage-CA1 is also a new species classified in the subfamily "Mascarenevirinae". We propose the specie names "Cocadavirus alagoinhas" and "Acarajevirus bahia" for RS-Phage-CA1 and RS-Phage-AB1 respectively. These findings increase the understanding of phage diversity and potential applications in the biological control of plant pathogens**.**

Bacteriophages or phages are the most abundant microbes in the world, playing crucial ecological and evolutionary roles in nature (NAUREEN et al., 2020). They can use three replication strategies: lytic, temperate, and chronic (FERNÁNDEZ; RODRÍGUEZ; GARCÍA, 2018). After adsorbing to the bacteria, phages in a lytic multiplication use the cell machinery to produce new viral particles, which will be released by cell lysis, resulting in host death (ELOIS et al., 2023). Different from lytic phages, temperate phages may or may not cause immediate host death. Instead, these phages may integrate their DNA into the host's genome as prophages in a lysogenic cycle (SCHROVEN; AERTSEN; LAVIGNE, 2021). The prophage genome is replicated with the bacterial chromosome and transmitted vertically by cell division (BRADY et al., 2021). A temperate prophage can be excised from the host genome, starting a lytic cycle (MONTEIRO et al., 2019). Different situations, such as host starvation, phage population size, host damage, and others, rule the alternation between the lysogenic and lytic cycles (ZHANG et al., 2022).

Phage therapy is a promising approach to controlling bacterial infection using phages, and it was initially proposed in the 1920s. However, it was overshadowed by the discovery of antibiotics in the years following 1944 (SALMOND; FINERAN, 2015). Nevertheless, resistant bacteria have been a problem after using antibiotics, resulting in the recovery of attention to phage therapy as a potential alternative, especially to control multidrug-resistant bacteria (KORTRIGHT et al., 2019). Thus, studies about phage therapy are currently applied in diverse contexts, such as industry and health. However, phage therapy to control bacterial plant pathogens has not yet been widely explored (HOLTAPPELS et al., 2021).

The *Ralstonia solanacearum* species complex (RSSC) is a group of three bacteria species: *Ralstonia solanacearum*, *Ralstonia pseudosolanacearum*, and *Ralstonia syzygii*. Bacteria of the RSSC are cosmopolitan and affect a variety of plants. (VAILLEAU; GENIN, 2023). These bacteria infect the plant and colonize the xylem vessels, forming biofilms clogging water flow. Consequently, the plants are led to death due to dissection (LOWE-POWER; KHOKHANI; ALLEN, 2018). Taking preventive strategies to control these bacteria to protect crops is crucial. Unfortunately, bacteria of RSSC are resistant to usual strategies to control bacterial plant pathogens and are easily spread by water and farm equipment (CALDWELL; KIM; IYER-PASCUZZI, 2017; GARĆIA; KERNS; THIESSEN, 2019; KIM et al., 2015).

This work aimed to isolate phages that infect isolates of *R. solanacearum* and *R. pseudosolanacearum*. The bacteria strains *Ralstonia pseudosolanacearum* GMI1000, *Ralstonia solanacearum* 244/22, and *R. solanacearum* 1900/20 were used as prey in isolating phages. For this purpose, the bacteria were cultured in a CPG medium at 28 °C (KELMAN, 1954). The sample soils were obtained from two regions of eucalyptus cultivation with *R. solanacearum* and *R. pseudosolanacearum* incidence. A total of 1 mL of bacteria culture was grown overnight, and 5 mL of 1% glucose solution was added to 10 mL of the soil sample to increase phage concentration. The mixture was then incubated for 48 hours. After incubation, 20 mL of SM buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM $MgSO_4$, and 0.01% gelatin) was added to the mixture, homogenized, and let stand to decant the soil. Then, the supernatant was collected and filtered through a PES 0.22 µm membrane. Then, 100 µL of the increased phage from soil samples was incubated with 400 µL overnight-grown bacteria culture for 4 hours at 28 °C without shake to promote the adsorption. The mixture was then plated in a double layer of CPG and incubated for 24 hours. A single lysis plaque was picked and resuspended in the SM buffer. This process was repeated three times to ensure phage isolation.

To determine the nature of the phage genome, the total nucleic acids were treated with RNAse A, DNAse, and S1 nuclease (Promega) following the manufacturer's recommendations. Phage DNAs were prepared for whole-genome sequencing using an Illumina DNA Prep kit. Sequencing was performed on the Illumina NextSeq2000 platform using a 300-cycle flow cell kit to produce 2 x 150 bp paired reads. 1-2% PhiX control was spiked into the run to support optimal base calling. Raw data filtering was performed with Trimmomatic v0.39 (BOLGER; LOHSE; USADEL, 2014) using a 4-mer sliding window and mean Q equal to 15. Filtered reads were used for *de novo* genome assembly using SPAdes v.3.15.2 (BANKEVICH et al., 2012) using Kmer sizes ranging from 27 to 121. The genome quality/completeness was accessed with CheckV v.1.0.1 (NAYFACH et al., 2021) using default parameters. To perform the genome annotation, the phages' ORFs were predicted using Prokka with --Kingdom Viruses (SEEMANN, 2014). Following, the function of ORFs was predicted based on the report of BlastP and InterPro (Supplementary material -Table S1 and S2). Putative promoter regions were determined using Neural Network Promoter Prediction with a 0.9 minimum promoter score at https://www.fruitfly.org/seq_tools/promoter.html (REESE, 2001). Rho-independent terminators were determined using Arnold Finding

Terminators at http://rssf.i2bc.paris-saclay.fr/toolbox/arnold/ (NAVILLE et al., 2011).

We isolated two phages from eucalyptus cultivation soil from regions with eucalyptus plants. Their genomes were sequenced and annotated, and both showed similarities to other *Ralstonia* phages. The isolated phages were named RS-Phage-AB1 and RS-Phage-CA1. RS-Phage-AB1 was isolated using *Ralstonia solanacearum* 244/22 as prey, whereas RS-Phage-CA1 was isolated using *R. solanacearum* 1900/20 as prey. Thus, we sought to determine the host specificity of the RS phage by utilizing a collection of *Ralstonia* isolated from eucalyptus plants and other bacterial species. RS phages were shown to infect *R. solanacearum* and *R. pseudosolanacearum* (Table S7 and S8).

The RS phages were isolated in different regions from Bahia state in Brazil (Supplementary material – Figure S1). The RS-Phage-AB1 has a genome size of 41,668 bp with 46 ORFs, and the RS-Phage-CA1 has a genome size of 46,254 bp with 59 ORFs. Regarding CG composition, the RS-Phage-AB1 showed 64.3%, and the RS-Phage-CA1 showed 62.3%. The genomes of the RS phages were classified as complete and high-quality according to CheckV (NAYFACH et al., 2021). (Supplementary material – Table S3). RS-Phage-AB1 and RS-Phage-CA1 showed no significative identity, suggesting they are very different viruses.

The phage genomes usually are organized in modules of genes with related functionality. The RS phages have different genomic compositions, but it is possible to observe the presence of module genes; one comprises ORFs related to tail proteins, and the other comprises ORFs related to capsid and a few tail proteins (Figure 1). These two modules are separated by module genes related to acid nucleic metabolism, and both genomes showed direct terminal repeats (Figure 1). The identification of an integrase in the genome of RS-Phage-AB1 and a resolvase in the genome of RS-Phage-CA1 indicate a temperate lifestyle for both phages but suggest that they use different insertion/excision mechanisms. The integrase performs integration and excision of genome phage. The integrase recognizes the specific site in the bacterial genome denominated phage attachment site (attP) (FOGG et al., 2014). On the other hand, the resolvase is similar to transposon recombinases and has two functions. It catalyzes a site-specific recombination and negatively regulates the expression of two transposon genes (RICE, 2015).

The genomes of RS-Phage-AB1 and RS-Phage-CA1 show similar genes involved in lysis but differences in gene content associated with the processes of

lysogeny, reflecting their distinct survival and infection strategies. Both phages possess the lysis genes endolysin and holin. Endolysins catalyze the degradation of peptidoglycan in the bacterial cell wall, facilitating the rupture of the host cell. Holins control lysis timing by forming pores in the cell membrane, allowing the endolysins to access the peptidoglycan (LI et al., 2021). The genes related to lysogeny vary between the two phages. RS-Phage-AB1 has genes such as integrase, DNA-methyltransferase, and DNA primase. Integrase allows the viral genome to be integrated into the host cell's genome, establishing a lysogenic state where the phage can passively replicate together with the host's DNA. DNA methyltransferase protects phage DNA from degradation by bacterial restriction enzymes. DNA primase is crucial for initiating DNA synthesis and facilitating viral replication during the lytic or lysogenic phase (HUANG et al., 2021). RS-Phage-CA1 contains endonuclease, resolvase, DNA-methyltransferase, and exonuclease genes related to lysogeny. Endonuclease cleaves DNA at specific sites, potentially helping in phage genome integration or recombination. Resolvase is responsible for resolving recombination intermediates during viral DNA integration or excision. As in RS-Phage-AB1, DNA methyltransferase in RS-Phage-CA1 protects viral DNA. The exonuclease processively degrades the DNA from the ends, possibly aiding in the remodeling of the viral genome or in defending against bacterial restriction systems (AKRITIDOU; THURTLE-SCHMIDT, 2023; EDGELL; GIBB; BELFORT, 2010).

The bacteria employ different tools to survive the phage attack, such as the CRISPR-Cas systems. The CRISPR-Cas systems provide immunity against foreign nucleic acids, like phages (RAUCH et al., 2017). In RSSC, the CRISPR-Cas system has low frequency and generally does not confer protection against phages (DA SILVA XAVIER et al., 2019). Given the lack of effectiveness of RSSC CRISPR-Cas against phage, it was observed that RS-Phage-AB1 has a small ORF related to an anti-CRISPR-Cas protein (ACR). Thus, we deduced that the occurrence of ACR in RSSC phage may indicate that RSSC CRISPR-Cas affects phage infection at some level. In addition, we observed that both RS phages have ORFs related to methylase and methyltransferase. These proteins promote or remove DNA methylation, protecting the phage genome against restriction-modification (RM) systems (YUAN et al., 2023). The presence of methylase or methyltransferase may indicate that the restriction-modification systems are the primary phage defense system in RSSC (BIRKHOLZ et al., 2022).

In some cases, the prophages can increase bacterial fitness by new phenotypes generated by the phage presence, such as antibiotic resistance, effector proteins, toxin-antitoxin systems, and others (GONÇALVES et al., 2021;WENDLING; REFARDT; HALL, 2021). The RS phages have ORFs related to nucleic acid metabolism, transcriptional regulators, nutrient metabolism, and effectors. Related to nucleic acid metabolism, the RS-Phage-AB1 encodes a DNA primase. These proteins may act on phage genome replication, but these components also act on bacterial genome replication (KAZLAUSKAS; KRUPOVIC; VENCLOVAS, 2016; WEIGEL; SEITZ, 2006).

When a host is infected by two or more viruses simultaneously, the infection is denominated superinfection (CARVALHO et al., 2022). A host of temperate phages in a state prophage is susceptible to a second infection. The temperate phages have developed strategies to avoid a second infection, termed superinfection exclusion (CARVALHO et al., 2022; HUNTER; FUSCO, 2022; MAVRICH; HATFULL, 2019). The RS-Phage-CA1 genome contains a small protein related to superinfection immunity, indicating that during a prophage state, it confers resistance to RSSC against a second phage infection.

In the next step, we used VIRIDIC v.1.0r3.6 (MORARU; VARSANI; KROPINSKI, 2020) to obtain an overview of whole-sequence similarity between the sequenced phage genomes and similar sequences from RefSeq/GenBank. First, we queried the NCBI nt/nr database to search for the sequences most similar to RS phages. Next, we used VIRIDIC with default thresholds of intergenomic similarity to cluster sequences into species (95%) and genera (70%) (Figure 2A to C). The sequenced phages are considerably different from previously characterized ones and are classified into novel species and genera.

RS-Phage-AB1 is most similar to the Ralstonia phage RSY1 (*Arsyunavirus RSY1*) (~ 55% intergenomic similarity), which belongs to the genus *Arsyunavirus* (family: *Peduoviridae*, class: *Caudoviricetes*) (Figure 2A). The Ralstonia phage RSY1 is a temperate phage widespread in *R. solanacearum* (ASKORA et al., 2017). The RS-Phage-AB1 has the six orthologous core genes within monophyletic clades (Figure S2), which is the current *Peduoviridae* family demarcation criteria (https://ictv.global/taxonomy/taxondetails?taxnode_id=202200225&taxon_name=Peduoviridae). RS-Phage-AB1 was classified in the *Peduoviridae* family but in a new genus. So, we propose the genus “Acarajevirus” and the species name “Acarajevirus

bahia" (Figure 2A).

The RS-Phage-CA1 is most similar (~ 67% intergenomic similarity) to phages infecting *Ralstonia* sampled in Reunion and Mauritius islands, which belong to the genus *Bakolyvirus* (class: *Caudoviricetes*) (TROTEREAU et al., 2021). Until now, the genus *Bakolyvirus* has not been classified in any family. Given that the RS-Phage-CA1 showed < 70% with other related viruses, which are family orphans, we aimed to classify these groups. First, we downloaded viruses' genome sequences deposited in GenBank and performed tBLASTn (parameters: -evalue 1e-5) against RS-Phage-CA1 proteins predicted using Prokka v.1.14.5 (parameters: --kingdom Viruses --gcode 11) (SEEMANN, 2014). The tBLASTn results were filtered to keep only alignments showing query coverage >= 50% and identity >= 35%. As a result, a total of 310 viruses from the database were selected and subjected to redundancy removal using average nucleotide identity (https://bitbucket.org/berkeleylab/checkv/src/master/scripts/), resulting in 254 dereplicated viruses genomes that have some level of similarity to the RS-Phage-CA1. Next, based on principles of universal viral taxonomy, we decided to apply proteomic tree and protein orthology to classify the viruses at the family level and apply intergenomic distance to classify at genus and species levels(SIMMONDS et al., 2023). Thus, the intergenomic distance reveals six novel genera clustering and fifteen novel species (Figures 2B and 2C). The RS-Phage-CA1 was classified as a new species and genus. Next, we confirmed that RS-Phage-CA1 and its related phages did not belong to any known viral family according to the proteomic tree generated by VipTree (NISHIMURA et al., 2017) since they clustered into a clade of unclassified viruses (Figure S3). After that, we rebuilt the proteomic tree using only the 254 selected viruses' genomes to better visualize tree structure and clade formation (Figure 2D). A monophyletic clade (Clade I) was observed in the proteomic tree, harboring the RS-Phage-CA1 and phages that infect *Ralstonia*, *Burkholderia,* and *Xanthomonas*, which could be a novel viral family. In addition, two well-defined subclades (subclades I and II) were observed. Subclade I comprises phages that infect *Xanthomonas* and belong to the *Kantovirinae* subfamily, which is not associated with any family. Subclade II includes the RS-Phage-CA1 and viruses from the genus *Bakolyvirus* and the genus *Naesvirus*. In the next step, we sought homologous proteins inside the family and subfamilies to be used in demarcation criteria associated with the proteomic tree. For this, we ran ProinOrtho v.6.3.0 (parameters: -e=1e-05 -cov=50) (KLEMM; STADLER; LECHNER, 2023) using all proteomes of the 254 selected viruses to identify the protein

orthogroups (OGs). When not available in Genbank/RefSeq, genes were predicted using Prokka v.1.14.5. We identified five OGs exclusively found in all members of clade I, while two OGs were exclusively found in all members of each subclade in this sense, we proposed that clade I be classified as a new family and subclade II as a novel subfamily. (Supplementary Table S4 to S6). To facilitate the inclusion of new viruses into the family and subfamilies, we established the minimum sequence identity in pairwise alignments (query and subject coverage >= 50%) for a protein to be classified within the selected OGs. For this, we ran BLASTp between proteins within a given OG and between proteins of a given OG and proteins from viruses that do not belong to clade I enriched with proteins of all dsDNA viruses classified in ICTV. Using this approach, we obtained sequence identity thresholds for each selected OG. We confirmed these OGs were exclusively found in clade I, subclade I or subclade II viruses, except for Howlin OG from subclade II. However, by using the identity thresholds, the true orthologs belonging to Holin OG can be identified. The sequence identity thresholds for each OG can be checked in (Supplementary Figures S4 to S12).

The function of the proteins belonging to these OGs was predicted using BlastP and InterProScan. Some proteins give any significant hits and, thus, were kept as hypothetical proteins. The OGs harboring only hypothetical proteins were named using Greek letters (Supplementary Table S4 to S6). Under this result, we proposed a criation of the novel “Anamaviridae” family that is defined with the following demarcation criteria: all “Anamaviridae*”* members belong to monophyletic on the proteomic tree and have the five orthologous proteins above the identity thresholds (in parenthesis): Alpha OG (36.6%), Integrase OG (41.1%), Tail lysozyme OG (30.3%), Beta OG (41.1%) and Gamma OG (37.8%) (Supplementary Table S4). Proceeding, we propose that the *Kantovirinae* subfamily be classified inside the “Anamaviridae” family following demarcation criteria: the *Kantovirinae* members belong to the monophyletic subclade I inside the monophyletic clade of Anamaviridae on the proteomic tree and have the two orthologous proteins above the identity thresholds (in parenthesis): Delta OG (32.4%) and Epsilon OG (97.3%). Then, we propose a novel “Mascarenevirinae” subfamily being classified inside the “Anamaviridae*”* family following demarcation criteria: the “Mascarenevirinae*”* members belong to the monophyletic subclade II inside the monophyletic clade of “Anamaviridae*”* on the proteomic tree and have the two orthologous proteins above the identity thresholds (in parenthesis): Holin OG (59.5%) and Tail OG (31.7%). At last, we proposed that all

members of the “Anamaviridae" family be classified in genera and species using the intergenomic distances following demarcation criteria: members of the same genus clustering have intergenomic distance ≥ 70% and same species clustering have intergenomic distance ≥ 95%. The proposal to “Anamaviridae”, *Kantovirinae*, “Mascarenevirinae”, and the genera and species are summarized in Figure 3. Thus, RS-Phage-CA1, named “*C*ocada*virus* alagoinhas”, was classified as a novel species belonging to a novel genus named “*C*ocada*virus*”, which is included in the “Mascarenevirinae” subfamily from “Anamaviridae” family (Figure 3A). Then, the genome was deposited in Genbank as “Acarajevirus bahia” isolate RS-Phage-AB1 (PP316168) and “Cocadavirus alagoinhas” isolate RS-phage-CA1 (PP316169).

In summary, two RS phages were isolated and described. The genome composition showed that these phages are temperate with different strategies. Both RS phages are novel species sharing a low identity with other *Ralstonia* phages. The taxonomy analysis resulted in two novel taxonomy proposals. The species name proposed for RS-Phage-AB1 was “Acarajevirus bahia”*,* and its classification resulted in the proposal of a novel species set of novel genus belonging to the *Peduoviridae* family. The species name proposed for RS-Phage-CA1 was “Cocadavirus alagoinhas”, and its classification resulted in the proposal of a novel species set of a novel genus, in the proposed novel “Mascarenevirinae” subfamily and “Anamaviridae” family. This work expanded the knowledge about the phages that infected RSSC and improved the current viral taxonomy.

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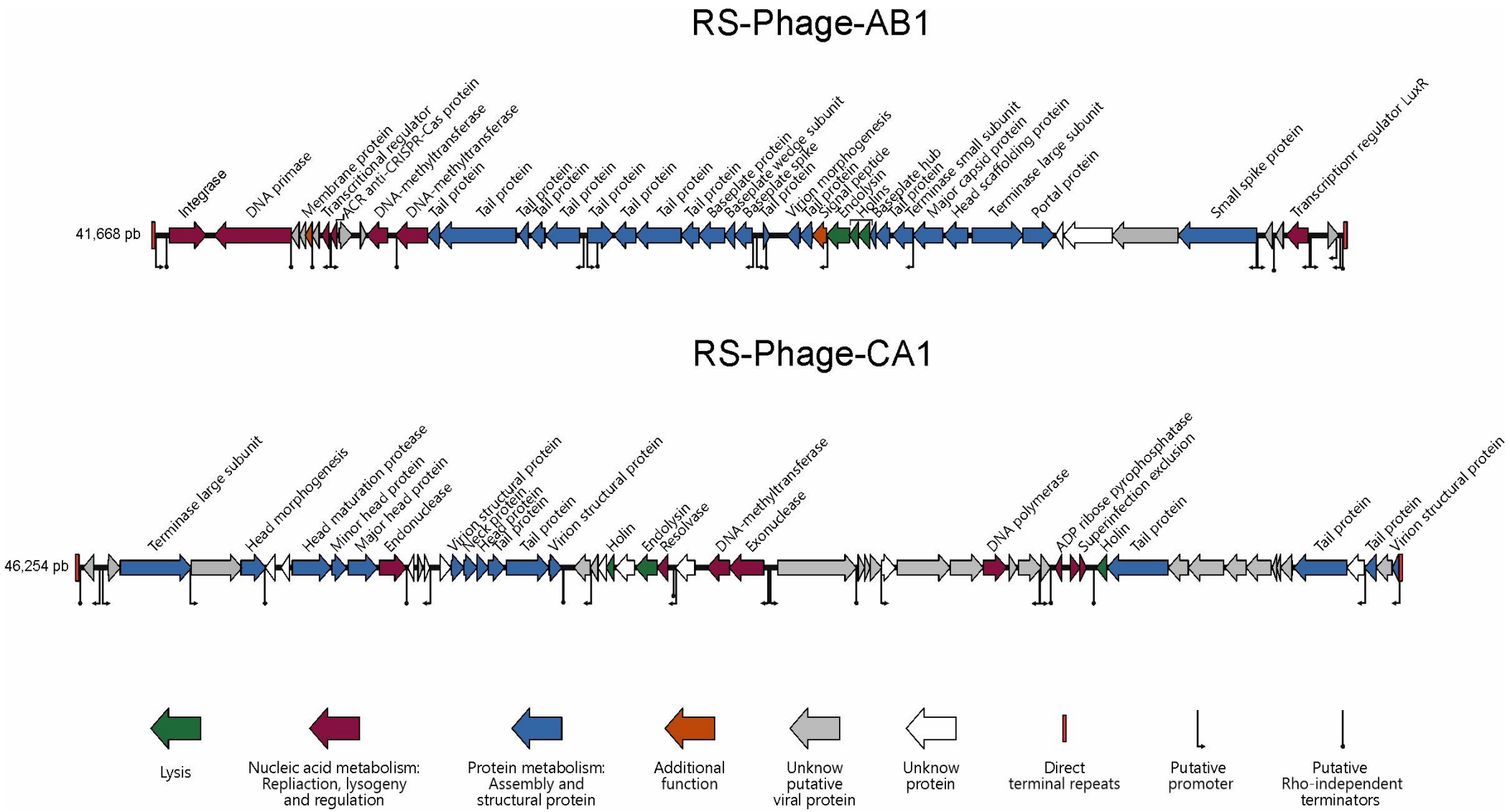

RS-Phage-AB1
41,668 pb
Integrase
DNA primase
Membrane protein
Transcritional regulator
ACR anti-CRISPR-Cas protein
DNA-methyltransferase
DNA-methyltransferase
Tail protein
Tail protein
Tail protein
Tail protein
Tail protein
Tail protein
Tail protein
Tail protein
Baseplate protein
Baseplate wedge subunit
Baseplate spike
Tail protein
Virion morphogenesis
Tail protein
Signal peptide
Endolysin
Holins
Baseplate hub
Tail protein
Terminase small subunit
Major capsid protein
Head scaffolding protein
Terminase large subunit
Portal protein
Small spike protein
Transcriptionr regulator LuxR
RS-Phage-CA1
46,254 pb
Terminase large subunit
Head morphogenesis
Head maturation protease
Minor head protein
Major head protein
Endonuclease
Virion structural protein
Neck protein
Head protein
Tail protein
Tail protein
Virion structural protein
Holin
Endolysin
Resolvase
DNA-methyltransferase
Exonuclease
DNA polymerase
ADP ribose pyrophosphatase
Superinfection exclusion
Holin
Tail protein
Tail protein
Tail protein
Virion structural protein
Lysis
Nucleic acid metabolism: Repliaction, lysogeny and regulation
Protein metabolism: Assembly and structural protein
Additional function
Unknow putative viral protein
Unknow protein
Direct terminal repeats
Putative promoter
Putative Rho-independent terminators

Figure 1

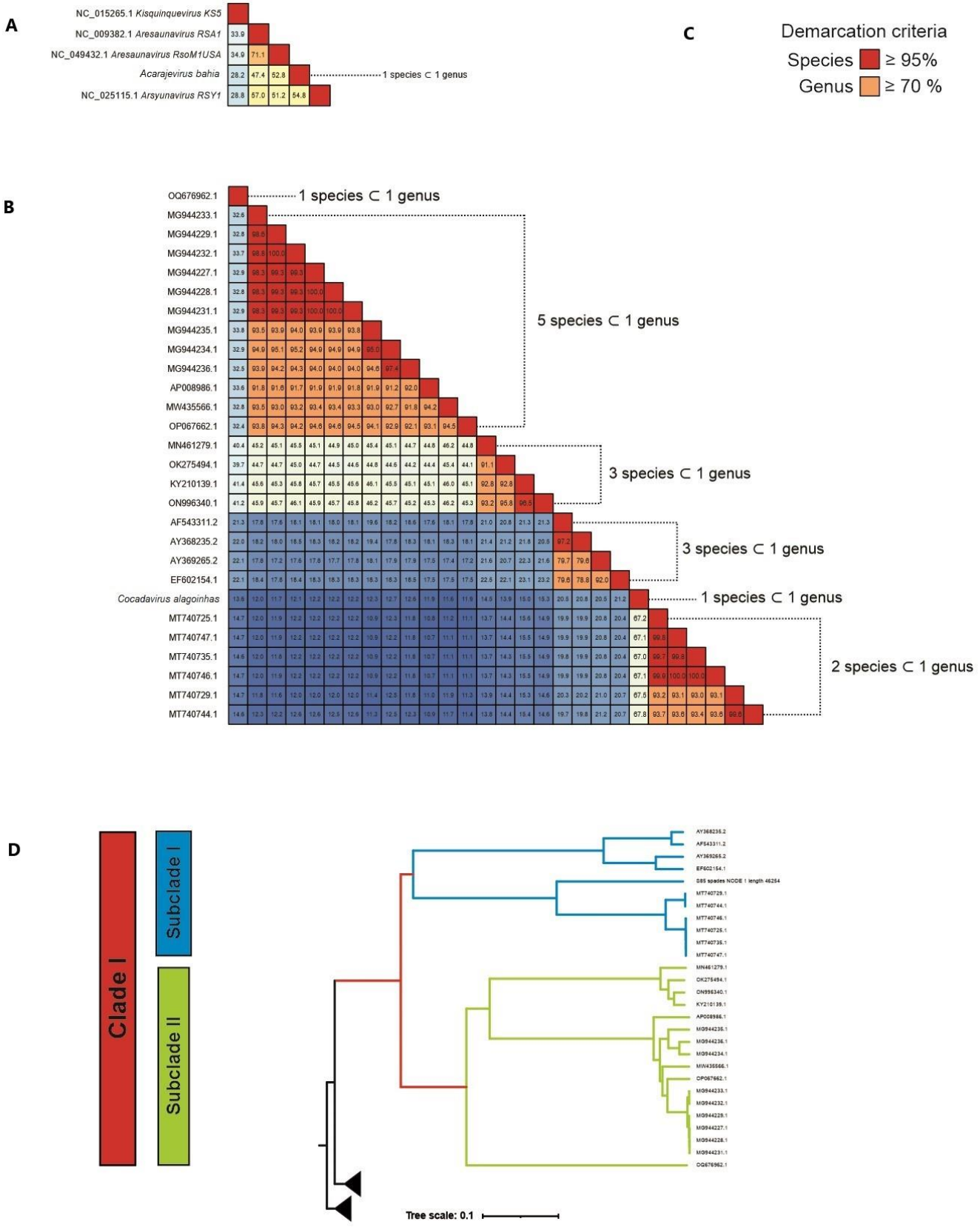

A
NC_015265.1 Kisquinquevirus KS5
NC_009382.1 Aresaunavirus RSA1
NC_049432.1 Aresaunavirus RsoM1USA
Acarajevirus bahia
NC_025115.1 Arsyunavirus RSY1
1 species ⊂ 1 genus
C
Demarcation criteria
Species ≥ 95%
Genus ≥ 70 %
B
1 species ⊂ 1 genus
5 species ⊂ 1 genus
3 species ⊂ 1 genus
3 species ⊂ 1 genus
1 species ⊂ 1 genus
2 species ⊂ 1 genus
Cocadavirus alagoinhas
D
Clade I
Subclade I
Subclade II
Tree scale: 0.1

Figure 2

A

Taxonomy proposal to *Anamaviridae* family					
Viruses information		**Demarcation criteria**			
Acession number	**Sequence name**	**Speceis** Members of the same species clustering have intergenomic distance ≥ 95%.	**Genus** Members of the same genus clustering have intergenomic distance ≥ 70%.	**Subfamily** The members of the *Kantovirinae* are part of the monophyletic subclade I within the monophyletic clade of *Anamaviridae* on the proteomic tree and have the two orthologous proteins Delta OG and Epsilon OG. The members of the *Mascarenevirinae* are part of the monophyletic subclade II within the monophyletic clade of *Anamaviridae* on the proteomic tree and have the two orthologous proteins hOLIN OG and Tail OG	**Family** Members of the *Anamaviridae* belong to a monophyletic group on the proteomic tree and have five orthologous proteins: Alpha OG, Integrase OG, Tail lysozyme OG, Beta OG, and Gamma OG.
		Classification			
	Cocadavirus alagoinhas	*Cocadavirus alagoinhas*	*Cocadavirus*		
MT740747.1	Ralstonia phage Simangalove	*Bakolyvirus bakoly*	*Bakolyvirus*	*Mascarenevirinae*	*Anamaviridae*
MT740735.1	Ralstonia phage Elie				
MT740725.1	Ralstonia phage Adzire				
MT740746.1	Ralstonia phage Sarlave				
MT740744.1	Ralstonia phage Jenny				
MT740729.1	Ralstonia phage Bakoly				
AF543311.2	Burkholderia phage Bcep781	*Naesvirus bcep*	*Naesvirus*		
AY368235.2	Burkholderia phage Bcep43				
EF602154.1	Burkholderia phage BcepNY3	*Naesvirus bcepNY*			
AY369265.2	Burkholderia phage Bcep1				
MG944231.1	Xanthomonas phage XPP6	*Tsukubavirus tsukuba*	*Tsukubavirus*	*Kantovirinae*	
MG944228.1	Xanthomonas phage XPP2				
MG944227.1	Xanthomonas phage XPP1				
MG944229.1	Xanthomonas phage XPP3				
MG944232.1	Xanthomonas phage XPP8				
MG944233.1	Xanthomonas phage XPP9				
OP067662.1	Xanthomonas phage pXoo2107				
MW435566.1	Xanthomonas phage X2				
MG944234.1	Xanthomonas phage XPV1				
MG944236.1	Xanthomonas phage XPV3				
MG944235.1	Xanthomonas phage XPV2				
AP008986.1	Xanthomonas oryzae phage OP2 DNA				
KY210139.1	Xanthomonas phage KPhi1	*Beograduvirus beogra*	*Beograduvirus*		
ON996340.1	Xanthomonas phage BsXeu269p/3				
OK275494.1	Xanthomonas phage MYK3				
MN461279.1	Xanthomonas virus phiXaf18				
OQ676962.1	Xanthomonas phage NEB7	*Xanthovirus NEB7*	Xanthovirus		

B

RS-Phage-AB1 classification				
Acession number	**Sequence name**	**Speceis**	**Genus**	**Family**
	RS-Phage-AB1	*Acarajevirus bahia*	*Acarajevirus*	*Peduoviridae*
NC_015265.1	Burkholderia phage KS5	*Kisquinquevirus KS5*	*Kisquinquevirus*	
NC_009382.1	Ralstonia phage phiRSA1	*Aresaunavirus RSA1*	*Aresaunavirus*	
NC_049432.1	Ralstonia phage RsoM1USA	*Aresaunavirus RsoM1USA*		
NC_025115.1	Ralstonia phage RSY1	*Arsyunavirus RSY1*	*Arsyunavirus*	

Figure 3

FIGURE LEGENDS

Figure 1. Representation of the genomic structure of the RS-Phage-AB1 and RS-Phage-CA1 phages identified in this study. The Open Reading Frames (ORFs) are represented by arrows. The ORFs have been colored according to the functional genomic blocks. The black line represents the genome, and the arrows represent the ORFs. The direction of the arrows indicates the direction of transcription, and they have been divided into categories according to their functions. Green arrows are ORFs related to lysis, purple arrows are related to nucleic acid metabolism, and blue arrows are related to the assembly of the virion structure. The orange arrows are ORFs with no defined function. The grey arrows represent the unknown putative viral proteins, which have no defined function, are similar to other virus proteins, are uncharacterized, and are found in other viruses' genomes. The white arrows represent the unknown proteins, which have no defined function and have not been found in other viruses. The smaller black arrows indicate the location of the putative promoter and putative Rho-independent terminators. In both genomes, Direct Terminal Repeats (DTRs), indicated by the red rectangle, are present at the ends of the genome, indicating regions essential for viral DNA replication and packaging.

Figure 2. Genomic similarity analysis and classification of RS phages, pairwise comparison to identify similarity with other previously described viruses.

(A) Intergenomic similarity of RS-Phage-AB1: Intergenomic similarity analysis using VIRIDIC shows the percentage of identity between the genome of RS-Phage-AB1 and other related phages from the RefSeq/GenBank database. The graph reveals that RS-Phage-AB1 is highly similar to other phages of the Arsyunavirus genus.

(B) Intergenomic similarity of RS-Phage-CA1: This analysis shows the genomic identity between RS-Phage-CA1 and related phages collected after Blastn, which had not been characterized and only had an assigned family then. The graph highlights the lack of significant similarity with previously described phages, suggesting the possibility of a new taxonomic grouping.

(C) Current criteria for demarcation: According to the current demarcation criteria, phages that share 95% or more genomic identities are of the same species, and those with 70% or more identities are considered to be of the same genus.

(D) Phyloproteomics performed to classify phages related to RS-Phage-CA1: The phylogeny, taking into account all proteins in the genome, revealed that the group forms a larger clade (Clade I), with the formation of two distinct subclades. This analysis reinforces that RS-Phage-CA1 and related phages represent a new taxonomic grouping.

Figure 3. Summary of phyloproteomic analyses.

A) Proposal for a new taxonomic classification for phages related to RS-Phage-CA1.

B) Suggested new taxonomic classification for RS-Phage-CA1-related phages, with a new genus and species.

Supplementary material

Supplementary Table S1. Annotation of the Functional Phage Genome (*Acarajevirus bahia*)

ORF	Predicted protein	Genomic coordinates	Strand	AA	BLASTP		INTERPRO	Prokka
					Best hit	E-value		
ORF 1	Integrase	261...1544	+	427	Integrase Arm-Type Dna-Binding Domain-Containing Protein [Acidovorax phage aval]	5,00e-173	Integrase, DNA-binding domain (IPR025166)	Integrase
ORF 2	DNA primase	1831...4515	-	894	DNA Primase [Ralstonia phage rsy1]	0.0	Archaeal primase DnaG/twinkle-like, TOPRIM domain (IPR034154)	DNA primase
ORF 3	Unknown viral protein	4517...4795	-	92	Hypothetical Protein Hyp59_Gp47 [Ralstonia phage rsom1usa]	1,00e-35	Unknown protein	Unknown viral protein
ORF 4	Unknown viral protein	4814...5017	-	67	Hypothetical Protein Rsc1899-Like [Ralstonia phage rsa1]	8,00e-19	Consensus disorder prediction	Unknown viral protein
ORF 5	Membrane protein viral	5010...5252	-	80	Membrane Protein [Ralstonia phage rsom1usa]	2,00e-40	Region of a membrane-bound protein predicted to be embedded in the membrane.	Membrane protein viral
ORF 6	Unknown viral protein	5252...5494	-	80	Hypothetical Protein Ma18_Gp42 [Ralstonia phage rsy1]	3,00e-12	Unknown protein	Unknown viral protein
ORF 7	Transcriptional regulator	5588...5836	-	82	Transcriptional Regulator [Ralstonia phage rsy1]	4,00e-42	Zinc finger, Ogr/Delta-type (IPR007684)	Transcriptional regulator
ORF 8	Arc anti CRISPR	5899...6108	-	69	Arc-Like Repressor [Ralstonia phage rsom1usa]	9,00e-06	Unknown protein	Arc-like DNA
ORF 9	Unknown viral protein	6191...6649	+	152	Hypothetical Protein Ma18_Gp38 [Ralstonia phage rsy1]	2,00e-42	Cro/C1-type helix-turn-helix domain (IPR001387)	Unknown viral protein
ORF 10	Unknown viral protein	6936...7142	+	68	Hypothetical Protein Ma18_Gp37 [Ralstonia phage rsy1]	2,00e-28	Region of a membrane-bound protein predicted to be outside the membrane, in the extracellular region.	Unknown viral protein
ORF 11	DNA-methyltransferase	7169...7891	-	240	Site-Specific Dna-Methyltransferase [Ralstonia phage rsom1usa]	1,00e-153	Restriction/modification DNA-methyltransferase (IPR001091)	DNA methyltransferase
ORF 12	DNA-methyltransferase	8161...9288	-	375	Tail Protein [Ralstonia Phage Rsom1usa]	0.0	Restriction/modification DNA-methyltransferase (IPR001091)	DNA methyltransferase

ORF 13	Tail Protein	9285...9707	-	140	Tail Protein [Ralstonia phage rsom1usa]	3,00e-98	Myoviridae, GpU (IPR009734) Bacteriophage P2, GpU (IPR016912)	Tail protein
ORF 14	Tail Protein	9710...12376	-	888	Tail Length Tape Measure Protein [Ralstonia phage rsom1usa]	0.0	Restriction/modification DNA-methyltransferase (IPR001091)	Tail protein
ORF 15	Tail Protein	12471...12797	-	108	Tail Protein [Ralstonia phage rsa1]	6,00e-65	Bacteriophage tail protein Gp41, putative (IPR019289)	Tail protein
ORF 16	Tail Protein	12874...13383	-	169	Head Closure [Ralstonia phage rsa1]	1,00e-102	Tail tube protein (IPR006498)	Tail protein
ORF 17	Tail Protein	13415...14590	-	391	Tail Sheath Protein [Ralstonia phage rsom1usa]	0.0	Bacteriophage tail protein Gp41, putative (IPR019289)	Tail protein
ORF 18	Tail Protein	14874...15773	+	299	ns	ns	Tail tube protein (IPR006498)	Hypothetical protein
ORF 19	Tail Protein	15802...16560	-	252	Tail Fiber Protein [Ralstonia phage rsom1usa]	2,00e-128	Myoviridae, GpU (IPR009734) Bacteriophage P2, GpU (IPR016912)	Tail protein
ORF 20	Tail Protein	16570...18144	-	524	Tail Protein [Ralstonia phage rsom1usa]	0.0	Phage tail fibre protein (IPR022225)	Tail protein
ORF 21	Tail Protein	18151...18768	-	205	Tail Protein [Ralstonia phage rsom1usa]	4,00e-145	Tail protein I (IPR006521)	Tail protein
ORF 22	Baseplate protein	18761...19669	-	302	Baseplate Protein [Ralstonia phage rsom1usa]	0.0	Baseplate protein J-like(IPR006949)	Baseplate protein
ORF 23	Baseplate Wedge Subunit	19672...20019	-	115	Baseplate Wedge Subunit [Ralstonia phage rsom1usa]	6,00e-74	Bacteriophage tail completion protein R (IPR009678) IraD/Gp25-like	Baseplate Wedge Subunit
ORF 24	Baseplate Spike	20016...20633	-	205	Baseplate Spike [Ralstonia phage rsom1usa]	5,00e-139	Gp5/Type VI secretion system Vgr protein, OB-fold domain(IPR006531)	Baseplate Spike
ORF 25	Tail Protein	20977...21201	+	74	ns	ns	Phage Tail Protein X-like (IPR008861)	Tail protein
ORF 26	Virion Morphogenesis Protein	21853...22299	-	148	Virion Morphogenesis Protein [Ralstonia phage rsom1usa]	1,00e-89	Phage virion morphogensis protein (IPR006522)	Virion Morphogenesis Protein
ORF 27	Tail Protein	22296...22730	-	144	Tail Terminator [Ralstonia phage rsom1usa]	1,00e-101	Bacteriophage tail completion protein R (IPR009678)(PF06891 P2 phage tail completion protein R (GpR)	Tail protein
ORF 28	Signal Peptide	22727...23227	-	166	Signal Peptide Motif [Ralstonia phage rsa1]	1,00e-96	Region of a membrane-bound protein predicted to be embedded in the membrane	Signal Peptide

ORF 29	Endolysin	23224...24030	-	268	Endolysin [Ralstonia phage rsom1usa]	0.0	N-acetylmuramidase (IPR002477) Peptidoglycan binding-like	Endolysin
ORF 30	Holin	24027...24341	-	104	Holin [Ralstonia phage rsom1usa]	9,00e-65	Putative 3TM holin, Phage holin 3 (IPR008473)	Holin
ORF 31	Holin	24338...24733	-	131	Holin [Ralstonia phage rsom1usa]	1,00e-79	Putative phage holin-like (IPR032637)	Holin
ORF 32	Baseplate hub	24749...24955	-	68	Baseplate Hub [Ralstonia phage rsom1usa]	1,00e-40	Phage Tail Protein X-like (IPR008861)	Baseplate hub
ORF 33	Tail Protein	24955...25434	-	159	Head-Tail Adaptor Ad1 [Ralstonia phage rsom1usa]	1,00e-108	Bacteriophage head completion protein GpL (IPR009225)	Tail protein
ORF 34	Terminase small subunit	25532...26254	-	240	Terminase Small Subunit [Ralstonia phage rsom1usa]	3,00e-155	Bacteriophage P2, GpM (IPR010270)	Terminase Small Subunit
ORF 35	Major capsid protein	26251...27288	-	345	Major Capsid Protein, P2 Family [Ralstonia phage rsom1usa]	0.0	Bacteriophage P2, capsid (IPR006441)	Major Capsid Protein
ORF 36	Head scaffolding protein	27341...28171	-	276	Head Scaffolding Protein [Ralstonia phage rsom1usa]	3,00e-178	Capsid scaffolding protein GpO (IPR009228)	Head Scaffolding Protein
ORF 37	Terminase large subunit	28315...30096	+	593	Terminase Large Subunit [Ralstonia phage rsom1usa]	0.0	ATPase terminase subunit, putative (IPR010332) Terminase, large subunit gp17-like, C-terminal	Terminase Large Subunit
ORF 38	Portal protein	30093...31181	+	362	Portal Protein [Ralstonia phage rsom1usa]	0.0	Bacteriophage/Gene transfer agent portal protein (IPR006944) Phage portal protein PBSX family (IPR006430) Phage portal protein PBSX family, Proteobacteria (IPR030935)	Portal Protein
ORF 39	PAAR domain	31232...31495	-	87	Paar Domain-Containing Protein [Rhizobium phage Rr1-B]	2,00e-12	PAAR motif (PF05488)	PAAR domain
ORF 40	Unknow protein	31513...33219	-	568	ns	ns	DUF2875, N-terminal domain (IPR021531)	N-terminal domain
ORF 41	Unknown protein	33213...35513	-	766	Duf3274 domain-containing protein [Ralstonia phage Rsy1]	2,00e-20	Protein of unknown function DUF3274 (IPR021692)	Unknown viral protein
ORF 42	Small spike protein	35525...38260	-	911	Vgr-Related Protein [Acinetobacter phage Md-2021a]	2,00e-112	Type VI secretion system, RhsGE-associated Vgr protein (IPR006533)	VGR-related protein

ORF 43	Unknown viral protein	38541...38807	-	88	ns	ns	Signal peptide region	Unknown protein
ORF 44	Unknown viral protein	38929...39192	-	87	ns	ns	Region of a membrane-bound protein predicted to be outside the membrane, in the extracellular region.	Unknown protein
ORF 45	Transcription regulator LuxR	39302...40060	-	252	Luxr Family Transcriptional Regulator Protein [Rhizobium phage rhph_Y25]	0.001	Transcription regulator LuxR, C-terminal (IPR000792)	Transcription regulator LuxR
ORF 46	Unknown viral protein	40749...41126	+	125	Tpa: Protein of Unknown Function (DUF2591) [Caudoviricetes Sp.]	6,00e-24	NinX, bacteriophage P22 (IPR019701)	Unknown viral protein

Supplementary Table S2. Annotation of the Functional Phage Genome (*Cocadavirus alagoinhas*)

ORF	Predicted protein	Genomic coordinates	Strand	AA	BLASTP		INTERPRO	Prokka
					Best hit	E-value		
ORF1	Unknown viral protein	203...580	-	125	Baseplate wedge subunit [Ralstonia phage Adzire]	9,00e-69	Unknown protein	Unknown viral protein
ORF2	Unknown viral protein	1074...1505	+	143	Hypothetical protein KE332_gp33 [Ralstonia phage Adzire]	2,00e-84	Unknown protein	Unknown viral protein
ORF3	Terminase large subunit	1502...3988	+	828	Terminase large subunit [Ralstonia phage Adzire]	0.0	Terminase, large subunit gp17-like, C-terminal (IPR035421)	Terminase large subunit
ORF4	Unknown viral protein	3985...5757	+	590	Portal protein [Ralstonia phage Adzire]	0.0	Anti-CBASS protein Acb1-like (IPR024459)	Unknown viral protein
ORF5	Head morphogenesis	5723...6592	+	289	Head morphogenesis [Ralstonia phage Adzire]	0.0	Phage Mu protein F like protein (PF04233)	Head morphogenesis
ORF6	Unknown protein	6570...6911	-	113	ns	ns	Coil	Unknown protein
ORF7	Unknown protein	7122...7415	-	97	ns	ns	Coil	Unknown_protein
ORF8	Head maturation protease	7514...8869	+	451	Head maturation protease [Ralstonia phage Adzire]	0.0	Uncharacterised conserved protein (IPR016913)	Head maturation protease
ORF9	Minor head protein	8905...9411	+	168	Minor head protein [Ralstonia phage Adzire]	2,00e-100	Unknown protein	Minor head protein
ORF10	Major head protein	9470...10483	+	337	Major head protein [Ralstonia phage Adzire]	0.0	Unknown protein	Major head protein
ORF11	Endonuclease	10558...11496	+	312	Grplintron_endo, group I intron endonuclease [uncultured Caudovirales phage]	5,00e-16	GIY-YIG endonuclease (IPR000305)	Endonuclease
ORF12	Unknown protein	11557...11766	-	69	ns	ns	Unknown protein	Unknown protein
ORF13	Unknown viral protein	11906...12073	+	55	Hypothetical protein KE332_gp23 [Ralstonia phage Adzire]	1,00e-17	Unknown protein	Unknown viral protein
ORF14	Unknown protein	12137...12319	+	60	ns	ns	Unknown protein	Unknown protein
ORF15	Unknown protein	12682...13056	+	124	Hypothetical protein KE332_gp21 [Ralstonia phage Adzire]	2,00e-78	Consensus disorder prediction	Unknown protein
ORF16	Virion structural protein	13084...13512	+	142	Virion structural protein [Ralstonia phage Adzire]	4,00e-90	Protein of unknown function DUF4054 (IPR025127)	Virion structural protein
ORF17	Neck protein	13515...13976	+	153	Neck protein 1 [Ralstonia phage Adzire]	3,00e-89	Unknown protein	Neck protein

ORF18	Head protein	13976...14371	+	131	Head protein [Ralstonia phage Adzire]	1,00e-80	Unknown protein	Head protein
ORF19	Tail protein	14368...14934	+	188	Tail completion protein 1 [Ralstonia phage Adzire]	9,00e-119	Unknown protein	Tail protein
ORF20	Tail protein	14992...16485	+	497	Tail sheath [Ralstonia phage Adzire]	0.0	Protein of unknown function DUF3383 (IPR021808)	Tail protein
ORF21	Virion structural protein	16498...16938	+	146	Virion structural protein [Ralstonia phage Adzire]	6,00e-99	Unknown protein	Virion structural protein
ORF22	Unknown viral protein	17359...17934	-	191	Hypothetical protein KE332_gp14 [Ralstonia phage Adzire]	5,00e-80	Spanin, inner membrane subunit (IPR004929)	Unknown viral protein
ORF23	Unknown viral protein	17931...18197	-	88	Hypothetical protein KE332_gp13 [Ralstonia phage Adzire]	1,00e-37	Unknown protein	Unknown viral protein
ORF24	Unknown protein	18194...18493	-	99	ns	ns	Unknown protein	Unknown protein
ORF25	Holin	18490...18756	-	88	Holin [Ralstonia phage Bakoly]	3,00e-43	Unknown protein	Holin
ORF26	Unknown protein	18815...19471	-	218	Hypothetical protein UFOVP679_19 [uncultured Caudovirales phage]	7,00e-17	Unknown protein	Unknown protein
ORF27	Endolysin	19522...20268	-	248	Endolysin [Ralstonia phage Bakoly]	2,00e-136	Peptidoglycan binding-like (IPR002477)	Endolysin
ORF28	Resolvase	20270...20641	-	123	RusA-like Holliday junction resolvase [Ralstonia phage Adzire]	3,00e-66	Holliday junction resolvase RusA-like (IPR008822) Crossover junction endodeoxyribonuclease, RusA (IPR016281)	RusA like Holliday junction resolvase
ORF29	Unknown protein	20908...21585	-	225	Hypothetical protein KE332_gp04 [Ralstonia phage Adzire]	3,00e-105	Nucleic acid-binding, OB-fold (IPR012340)	Unknown protein
ORF30	DNA-methyltransferase	22017...22793	-	258	Putative site-specific DNA-methyltransferase [Pseudomonas phage MR14]	2,00e-125	Restriction/modification DNA-methyltransferase (IPR001091)	Restriction/modification DNA-methyltransferase
ORF31	Exonuclease	22790...23989	-	399	Exonuclease [Ralstonia phage Adzire]	0.0	Protein of unknown function DUF2800 (IPR021229)	Exonuclease
ORF32	Unknown viral protein	24479...27232	+	917	Hypothetical protein KE332_gp02 [Ralstonia phage Adzire]	0.0	Consensus disorder prediction	Unknown viral protein
ORF33	Unknown viral protein	27291...27521	+	76	ns	ns	Unknown protein	Unknown viral protein
ORF34	Unknown viral protein	27521...27709	+	62	Hypothetical protein KE333_gp02 [Ralstonia phage Bakoly]	2,00e-15	Unknown protein	Unknown viral protein
ORF35	Unknown viral protein	27712...28116	+	134	Hypothetical protein KE332_gp56 [Ralstonia phage Adzire]	2,00e-55	Unknown protein	Unknown viral protein
ORF36	Unknown protein	28116...28601	+	161	TPA: zinc finger domain-containing protein [Caudoviricetes sp.]	9,00e-39	Protein of unkown function DUF3268 (IPR021686)	Zinc finger domain

ORF37	Unknown viral protein	28661...30511	+	616	Hypothetical protein KE332_gp55 [Ralstonia phage Adzire]	0.0	Helicase superfamily 1/2, ATP-binding domain(PR014001) Helicase/UvrB, N-terminal (IPR006935)	Unknown viral protein
ORF38	Unknown viral protein	30518...31666	+	382	Hypothetical protein KE332_gp54 [Ralstonia phage Adzire]	0.0	DNA/RNA polymerase superfamily (IPR043502)	Unknown viral protein
ORF39	DNA polymerase	31670...32485	+	271	DNA polymerase [Ralstonia phage Adzire]	4,00e-177	DNA-directed DNA polymerase, family A, palm domain (IPR001098)	DNA polymerase
ORF40	Unknown viral protein	32567...32848	+	93	Hypothetical protein KE333_gp07 [Ralstonia phage Bakoly]	2,00e-50	Consensus disorder prediction	Unknown viral protein
ORF41	Unknown viral protein	32901...33692	+	263	Hypothetical protein KE333_gp08 [Ralstonia phage Bakoly]	1,00e-89	Consensus disorder prediction	Unknown viral protein
ORF42	Unknown viral protein	33689...34015	+	108	Hypothetical protein [Burkholderia phage BCSR129]	0.012	Unknown protein	Unknown viral protein
ORF43	ADP-ribose pyrophosphatase	34182...34409	-	75	TPA: ADP-ribose pyrophosphatase [Caudoviricetes sp.]	4,00e-07	NUDIX hydrolase-like domain superfamily (IPR015797)	ADP-ribose pyrophosphatase
ORF44	Unknown viral protein	34711...35052	+	113	Hypothetical protein RsoM2USA_147 [Ralstonia phage RsoM2USA]	2,00e-04	Unknown protein	Unknown viral protein
ORF45	Superinfection exclusion	35049...35273	+	74	Immunity to superinfection [Ralstonia phage Adzire]	2,00e-30	Bacteriophage-associated immunity protein (IPR016410) Superinfection immunity protein (PF14373)	Immunity superinfection
ORF46	Holin	35623...35982	-	119	Holin [Ralstonia phage Adzire]	1,00e-61	LydA-like holin (IPR032126) LydA holin phage, holin superfamily III(PF16083)	Holin
ORF47	Tail protein	35999...38116	-	705	Tail fiber protein [Ralstonia phage Adzire]	0.0	Unknown protein	Tail protein
ORF48	Unknown viral protein	38136...38831	-	231	Hypothetical protein 30B_00050 [Ralstonia phage Jenny]	3,00e-144	Protein of unknown function DUF2612 (IPR021283)	Unknown viral protein
ORF49	Unknown viral protein	38831...40072	-	413	Hypothetical protein KE332_gp44 [Ralstonia phage Adzire]	0.0	Baseplate protein J-like (IPR006949)	Unknown viral protein
ORF50	Unknown viral protein	40120...40851	-	243	Hypothetical protein KE332_gp43 [Ralstonia phage Adzire]	3,00e-147	Phage protein Gp138 N-terminal domain (IPR041599)	Unknown viral protein
ORF51	Unknown viral protein	40853...41719	-	288	Hypothetical protein 30B_00047 [Ralstonia phage Jenny]	0.0	Unknown protein	Unknown viral protein

ORF52	Unknown viral protein	41767...41925	-	52	Hypothetical protein KE332_gp41 [Ralstonia phage Adzire]	1,00e-22	Region of a membrane-bound protein predicted to be embedded in the membrane.	Unknown viral protein
ORF53	Unknown viral protein	41927...42055	-	42	ns	ns	Region of a membrane-bound protein predicted to be embedded in the membrane.	Unknown viral protein
ORF54	Unknown viral protein	42057...42458	-	133	Hypothetical protein KE332_gp40 [Ralstonia phage Adzire]	1,00e-73	SignalP-TM	Unknown viral protein
ORF55	Tail protein	42527...44371	-	614	Tail length tape measure protein [Ralstonia phage Adzire]	0.0	Phage tail lysozyme (IPR041219)	Tail protein
ORF56	Unknown protein	44373...44990	-	205	Hypothetical protein KE332_gp38 [Ralstonia phage Adzire]	1,00e-102	Dit-like phage tail protein, N-terminal domain (IPR048494)	Unknown protein
ORF57	Tail protein	44994...45401	-	135	Tail assembly chaperone [Ralstonia phage Adzire]	1,00e-75	Unknown protein	Unknown viral protein
ORF58	Unknown viral protein	45398...45958	-	186	Hypothetical protein KE332_gp36 [Ralstonia phage Adzire]	2,00e-111	Unknown protein	Unknown viral protein
ORF59	Virion structural protein	46034...46246	-	70	Virion structural protein [Ralstonia phage Adzire]	8,00e-38	Unknown protein	Virion structural protein

Table S3. Summary information of RSSC phages

Isolate	Sample	City/State	Genome size (bp)	Number of ORFs	%GC	CheckV quality	CheckV completeness (%)
RS-Phage-AB1	Soil	Itanagra/Bahia	41,668	46	64.3	Complete	100
RS-Phage-CA1	Soil	Alagoinhas/Bahia	46,254	59	62.3	Complete	100

Supplementary Table S4. Annotation of proteins (*Anamaviridae* Family).

	Proteins	α (alpha) OG	Integrase OG	Tail lysozyme OG	β (beta) OG	γ (gamma) OG
Virus	**Xanthomonas phage NEB7**	WHB31186_21	WHB31188_23	WHB31172_7	WHB31169_4	WHB31221_56
	Xanthomonas phage pXoo2107	UUR56270_37	UUR56274_41	WHB31172_7	WHB31169_4	WHB31221_56
	Xanthomonas phage BsXeu269p/3	UUW40452_65	UUW40388_1	UUR56251_18	UUR56248_15	UUR56241_8
	Xanthomonas phage MYK3	UGL62943_64	UGL62947_68	UUW40437_50	UUW40433_46	UUW40426_39
	Xanthomonas phage X2	QRI46315_14	QRI46319_18	UGL62928_49	UGL62924_45	UGL62917_38
	Ralstonia phage Simangalove	QMV33706_4	QMV33735_33	QRI46370_70	QRI46367_67	QRI46358_58
	Ralstonia phage Sarlave	QMV33661_17	QMV33690_46	QMV33741_39	QMV33738_36	QMV33758_56
	Ralstonia phage Jenny	QMV33511_4	QMV33545_38	QMV33696_52	QMV33693_49	QMV33655_11
	Ralstonia phage Elie	QMV32949_4	QMV32978_33	QMV33551_44	QMV33548_41	QMV33567_60
	Ralstonia phage Adzire	QMV32632_59	QMV32598_25	QMV32984_39	QMV32981_36	QMV33001_56
	Ralstonia phage Adzire,	QMV32321_4	QMV32350_33	QMV32592_19	QMV32595_22	QMV32576_3
	Xanthomonas virus phiXaf18	QFR59556_63	QFR59561_1	QMV32356_39	QMV32353_36	QMV32373_56
	Xanthomonas phage XPV3	AVO24324_9	AVO24320_5	QFR59551_47	QFR59593_43	QFR59590_36
	Xanthomonas phage XPV2	AVO24264_23	AVO24260_19	AVO24343_28	AVO24346_31	AVO24353_38
	Xanthomonas phage XPV1	AVO24212_48	AVO24260_19	AVO24282_41	AVO24285_44	AVO24293_52
	Xanthomonas phage XPP9	AVO24097_13	AVO24216_52	AVO24193_29	AVO24190_26	AVO24182_18
	Xanthomonas phage XPP8	AVO24033_23	AVO24101_17	AVO24151_67	AVO24148_64	AVO24140_56
	Xanthomonas phage XPP6	AVO23988_50	AVO24029_19	AVO24053_43	AVO24056_46	AVO24064_54
	Xanthomonas phage XPP3	AVO23862_75	AVO23992_54	AVO23969_31	AVO23966_28	AVO23959_21
	Xanthomonas phage XPP3	AVO23789_2	AVO23793_6	AVO23843_56	AVO23840_53	AVO23832_45
	Xanthomonas phage XPP2	AVO23784_69	AVO23716_1	AVO23765_50	AVO23762_47	AVO23755_40
	Xanthomonas phage XPP1	AVO23657_15	AVO23661_19	AVO23710_68	AVO23707_65	AVO23700_58
	Xanthomonas phage KPhi1	APQ41913_34	APQ41907_28	APQ41930_51	APQ41934_55	APQ41940_61
	Burkholderia phage BcepNY3	ABR10555_21	ABR10553_19	ABR10579_45	ABR10582_48	ABR10590_56
	Burkholderia phage Bcep1	AAQ73368_22	AAQ73366_20	AAQ73392_45	AAQ73395_48	AAQ73403_56
	Burkholderia phage Bcep43	AAR89312_21	AAR89310_19	AAR89335_43	AAR89338_46	AAR89346_53
	Xanthomonas oryzae phage OP2 DNA	BAE72781_17	BAE72778_14	BAE72798_34	BAE72801_37	BAE72808_44
	Burkholderia phage Bcep781	AAN38022_21	AAN38020_19	AAN38043_44	AAN38046_47	AAT37988

Supplementary Table S5. Annotation of proteins (*Mascarenevirinae* subfamily).

Proteins	Vírus											
	Ralstonia phage Simangalove	Ralstonia phage Sarlave	Ralstonia phage Jenny	Ralstonia phage Elie	Ralstonia phage Bakoly	Ralstonia phage Adzire	Burkholderia phage BcepNY3	Burkholderia phage Bcep1	Burkholderia phage Bcep1	Burkholderia phage Bcep43	Burkholderia phage Bcep43	Burkholderia phage Bcep781	Burkholderia phage Bcep781
Holin OG	QMV33749_47	QMV33646_2	QMV33559_52	QMV32992_47	QMV32584_11	QMV32364_47	ABR10563_29	AAQ73378_31	AAQ73377_30	AAR89322_30	AAR89321_29	AAN38031_30	AAN38030_29
Tail OG	QMV33753_51	QMV33753_51	QMV33753_51	QMV33753_51	QMV33650_6	QMV33562_55	QMV32996_51	QMV32581_8	QMV32368_51	ABR10603_69	AAQ73415_69	AAR89355_64	AAN38063_65

Supplementary Table S6. Annotation of proteins (*Kantovirinae* subfamily).

Proteins	Vírus															
	Xanthomonas phage NEB7	Xanthomonas phage pXoo2107	Xanthomonas phage BsXeu269p/3	Xanthomonas phage MYK3	Xanthomonas phage X2	Xanthomonas virus phiXaf18	Xanthomonas phage XPV3	Xanthomonas phage XPV2	Xanthomonas phage XPV1	Xanthomonas phage XPP9	Xanthomonas phage XPP8	Xanthomonas phage XPP6	Xanthomonas phage XPP3	Xanthomonas phage XPP2	Xanthomonas phage XPP1	Xanthomonas phage KPhi1	Xanthomonas oryzae phage OP2 DNA
δ (delta) OG	WHB31207_42	UUR56292_59	UUW40409_22	UGL62899_20	QRI46338_37	QFR59582_20	AVO24376_61	AVO24314_73	AVO24236_72	AVO24121_37	AVO24083_73	AVO23940_2	AVO23813_26	AVO23736_21	AVO23681_39	APQ41888_9	BAE72822_58
ε (epsilon) OG	WHB31174_9	UUR56253_20	UUW40439_52	UGL62930_51	QRI46372_72	QFR59596_49	AVO24341_26	AVO24280_39	AVO24195_31	AVO24153_69	AVO24051_41	AVO23971_33	AVO23845_58	AVO23767_52	AVO23712_70	APQ41928_49	BAE72796_32

Table S7. Bacterial isolates used in this study and *Cocadavirus alagoinhas* host range.

Isolates	Source	Host	Phyl-seq	Plaque formation
***Ralstonia solanacearum* 1900/20**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 244/22**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 2199/21**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 924/19**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 1432/20**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 2113/20**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 701/21**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 203/18**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 2854/21**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 1395/20**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 518**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 519**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 523**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 783**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 2410**	Virus Laboratory/UFV	Eucalyptus	I	+
***Ralstonia pseudosolanacearum* GMI1000**	Virus Laboratory/UFV	Eucalyptus	I	+
Bacillus cereus	Industrial Microbiology Laboratory/UFV	-------	-------	-
Xanthomonas vesicatoria	Forest Pathology Laboratory/UFV	Tomato	-------	-
Clavibacter michiganensis subsp. Michiganensis	Forest Pathology Laboratory/UFV	Tomato	-------	-
Pseudomonas syringae pv. Garcae	Forest Pathology Laboratory/UFV	Coffee	-------	-
Pectobacterium brasilienses	Forest Pathology Laboratory/UFV	Tomato	-------	-
Erwinia psidii	Forest Pathology Laboratory/UFV	Eucalyptus	-------	-

Table S8. Bacterial isolates used in this study and *Acarajevirus bahia* host range.

Isolates	Source	Host	Phyl-seq	Plaque formation
***Ralstonia solanacearum* 1900/20**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 244/22**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 2199/21**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 924/19**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 1432/20**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 2113/20**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 701/21**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 203/18**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 2854/21**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 1395/20**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 518**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 519**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 523**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 783**	Virus Laboratory/UFV	Eucalyptus	II	+
***Ralstonia solanacearum* 2410**	Virus Laboratory/UFV	Eucalyptus	I	+
***Ralstonia pseudosolanacearum* GMI1000**	Virus Laboratory/UFV	Eucalyptus	I	+
Bacillus cereus	Industrial Microbiology Laboratory/UFV	-------	-------	-
Xanthomonas vesicatoria	Forest Pathology Laboratory/UFV	Tomato	-------	-
Clavibacter michiganensis subsp. Michiganensis	Forest Pathology Laboratory/UFV	Tomato	-------	-
Pseudomonas syringae pv. Garcae	Forest Pathology Laboratory/UFV	Coffee	-------	-
Pectobacterium brasilienses	Forest Pathology Laboratory/UFV	Tomato	-------	-
Erwinia psidii	Forest Pathology Laboratory/UFV	Eucalyptus	-------	-

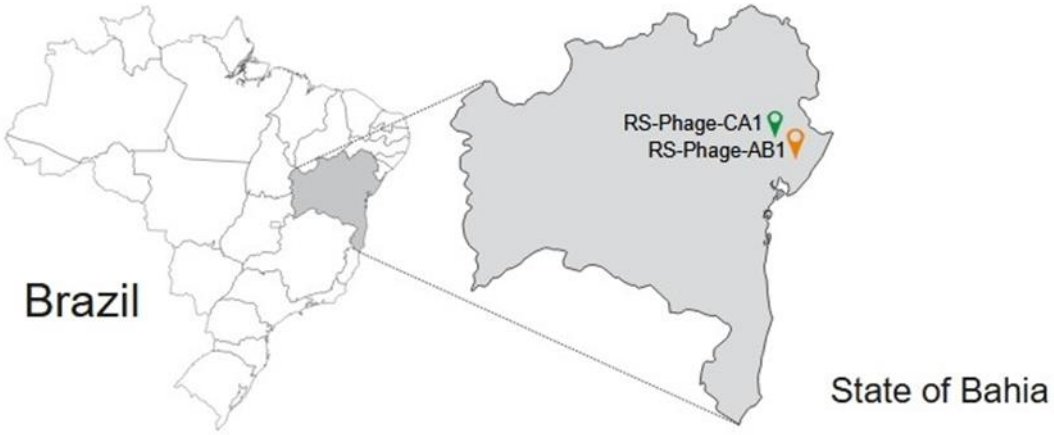
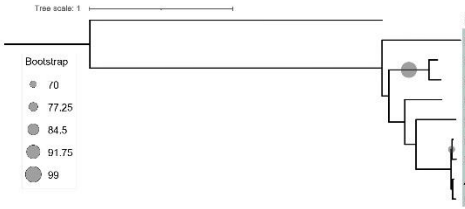


Figure S1. Brazil map with highlights on Bahia states. The pins indicate the sample collection site of each RS phages.

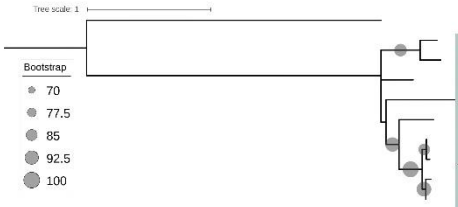
Orthogroup 1: Terminase, large subunit



Enterobacteria phage P21 Terminase large subunit [P36693.2]
NC_008201.1 Mannheimia phage PHL101 [YP_655470.1]
NC_070905.1 Escherichia virus P2 [YP_010663807.1]
NC_047833.1 Erwinia phage EtG [YP_009790832.1]
NC_019488.1 Salmonella phage RE-2010 [YP_007003509.1]
NC_015265.1 Burkholderia phage KS5 [YP_004306409.1]
NC_025115.1 Ralstonia phage RSY1 [YP_009067084.1]
NC_009382.1 Ralstonia phage phiRSA1 [YP_001165255.1]
***Acarajevirus bahia* [protein_00037]**
NC_049432.1 Ralstonia phage RsoM1USA [YP_009880091.1]

Peduoviridae

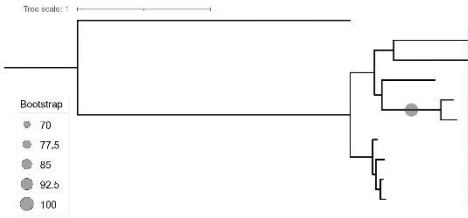
Orthogroup 2: Portal protein



Enterobacteria phage HK97 Portal protein [P49859.1]
NC_047833.1 Erwinia phage EtG [YP_009790830.1]
NC_070905.1 Escherichia virus P2 [YP_010663806.1]
NC_019488.1 Salmonella phage RE-2010 [YP_007003508.1]
NC_008201.1 Mannheimia phage PHL101 [YP_655469.1]
NC_015265.1 Burkholderia phage KS5 [YP_004306410.1]
NC_049432.1 Ralstonia phage RsoM1USA [YP_009880090.1]
***Acarajevirus bahia* [protein_00038]**
NC_025115.1 Ralstonia phage RSY1 [YP_009067083.1]
NC_009382.1 Ralstonia phage phiRSA1 [YP_001165254.1]

Peduoviridae

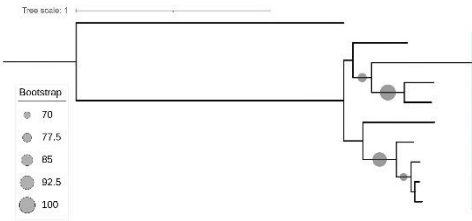
Orthogroup 3: Capsid scaffolding protein



Enterobacteria phage HK97 Head maturation protease [NP_037700.1]
NC_008201.1 Mannheimia phage [YP_655471.1]
NC_019488.1 Salmonella phage RE-2010 [YP_007003510.1]
NC_015265.1 Burkholderia phage KS5 [YP_004306408.1]
NC_047833.1 Erwinia phage EtG [YP_009790833.1]
NC_070905.1 Escherichia virus P2 [YP_010663808.1]
***Acarajevirus bahia* [protein_00036]**
NC_049432.1 Ralstonia phage RsoM1USA [YP_009880092.1]
NC_025115.1 Ralstonia phage RSY1 [YP_009067085.1]
NC_009382.1 Ralstonia phage phiRSA1 [YP_001165256.1]

Peduoviridae

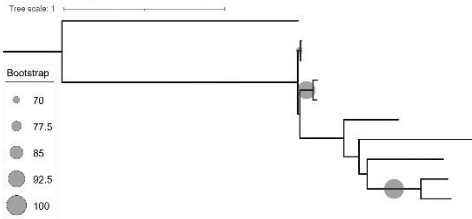
Orthogroup 4: Major capsid protein



Escherichia phage T5 Major capsid protein [Q6QGD8.1]
NC_019488.1 Salmonella phage RE-2010 [YP_007003511.1]
NC_008201.1 Mannheimia phage PHL101 [YP_655472.1]
NC_047833.1 Erwinia phage EtG [YP_009790834.1]
NC_070905.1 Escherichia virus P2 [YP_010663809.1]
NC_015265.1 Burkholderia phage KS5 [YP_004306407.1]
***Acarajevirus bahia* [protein_00035]**
NC_009382.1 Ralstonia phage phiRSA1 [YP_001165257.1]
NC_049432.1 Ralstonia phage RsoM1USA [YP_009880093.1]
NC_025115.1 Ralstonia phage RSY1 [YP_009067086.1]

Peduoviridae

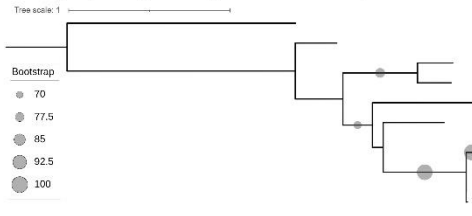
Orthogroup 5: Terminase, small subunit



Escherichia phage T4 Terminase small subunit [AAD42421.1]
NC_009382.1 Ralstonia phage phiRSA1 [YP_001165258.1]
NC_025115.1 Ralstonia phage RSY1 [YP_009067087.1]
***Acarejevirus bahia* [protein_00034]**
NC_049432.1 Ralstonia phage RsoM1USA [YP_009880094.1]
NC_015265.1 Burkholderia phage KS5 [YP_004306406.1]
NC_008201.1 Mannheimia phage PHL101 [YP_655473.1]
NC_019488.1 Salmonella phage RE-2010 [YP_007003512.1]
NC_070905.1 Escherichia virus P2 [YP_010663810.1]
NC_047833.1 Erwinia phage EtG [YP_009790835.1]

Peduoviridae

Orthogroup 6: Capsid completion/stabilization protein



Escherichia phage T4 Head completion protein [AAD42481.1]
NC_019488.1 Salmonella phage RE-2010 [YP 007003513.1]
NC_070905.1 Escherichia virus P2 [YP 010663811.1]
NC_047833.1 Erwinia phage EtG [YP 009790836.1]
NC_008201.1 Mannheimia phage PHL101 [YP 655474.1]
NC_015265.1 Burkholderia phage KS5 [YP 004306405.1]
NC_025115.1 Ralstonia phage RSY1 [YP 009067088.1]
NC_009382.1 Ralstonia phage phiRSA1 [YP 001165259.1]
NC_049432.1 Ralstonia phage RsoM1USA [YP 009880095.1]
***Acarajevirus bahia* [protein_00033]**

Peduoviridae

Figure S2. All members of *Peduoviridae* encode a set of 6 orthologous core genes: Terminase, large subunit; Portal protein; Capsid scaffolding protein; Major capsid protein; Terminase, small subunit; Capsid completion/stabilization protein (https://ictv.global/taxonomy/taxondetails?taxnode_id=202200225& taxon_ name=Peduoviridae). Multiple sequence alignments were done for each orthologous protein using Mafft v.7.471. The best evolutionary models were identified using ModelTest-NG v.0.1.7. Maximum likelihood trees were built using RaxML-NG v1.0.3 with 100 searches for the best-scoring tree and 1000 bootstrap replicates. The amino acid substitution model used was LG+G4, except for Capsid completion/stabilization protein, which was WAG+G4.

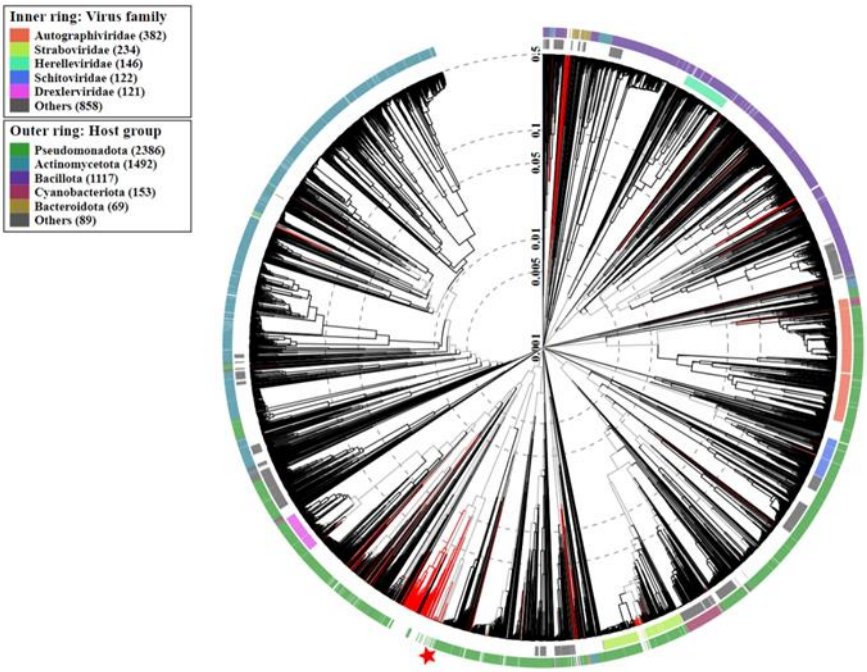


Figure S3. Running the VipTree with the reference sequences together with the 254 selected virus genomes and the RS-phage-CA1 genome confirmed that these do not belong to any know family (ICTV). The branch representing RS-phage-CA1 is marked with a star. The 254 sequences we provided to Viptree have red branches.

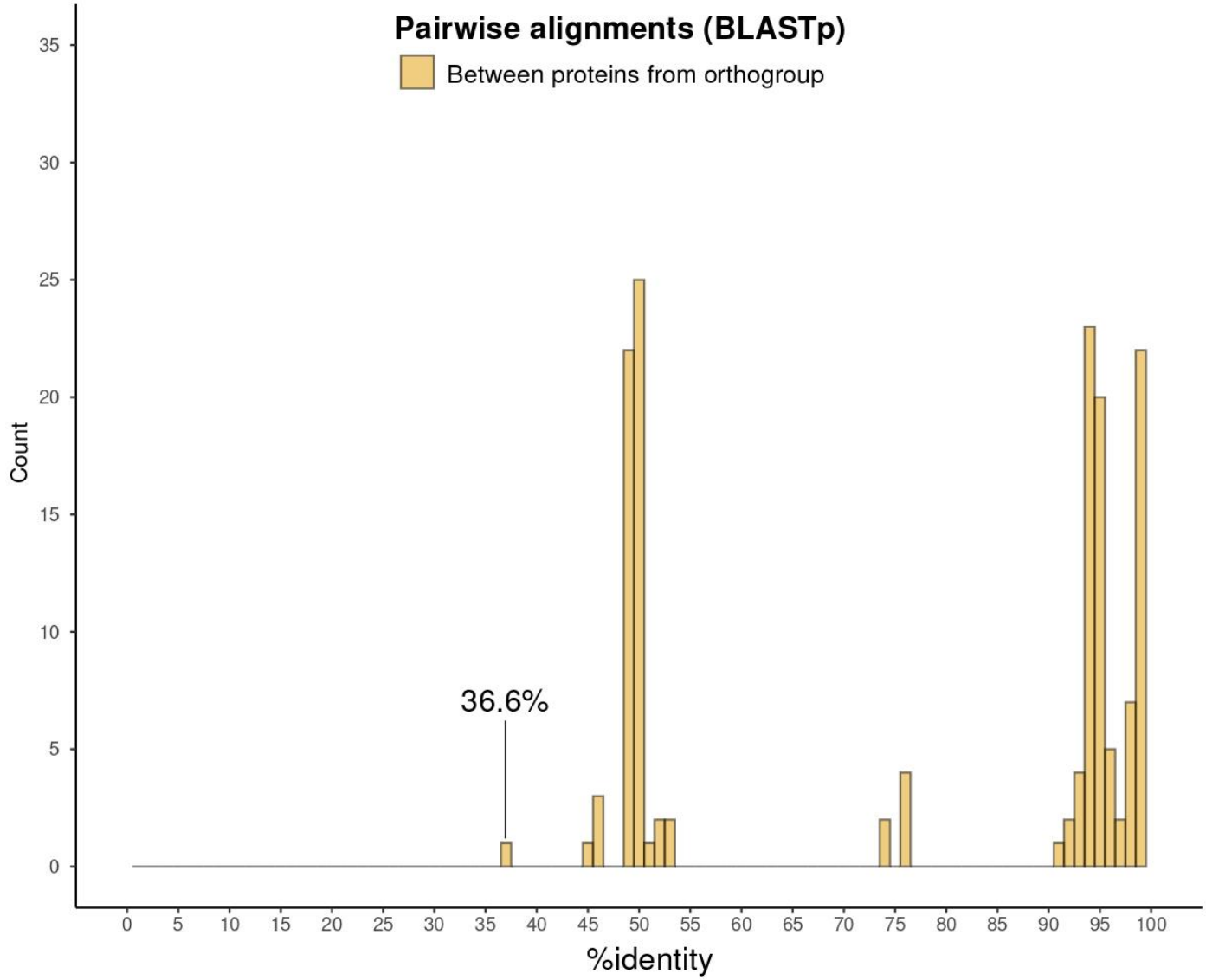


Figure S4. α (alpha) OG

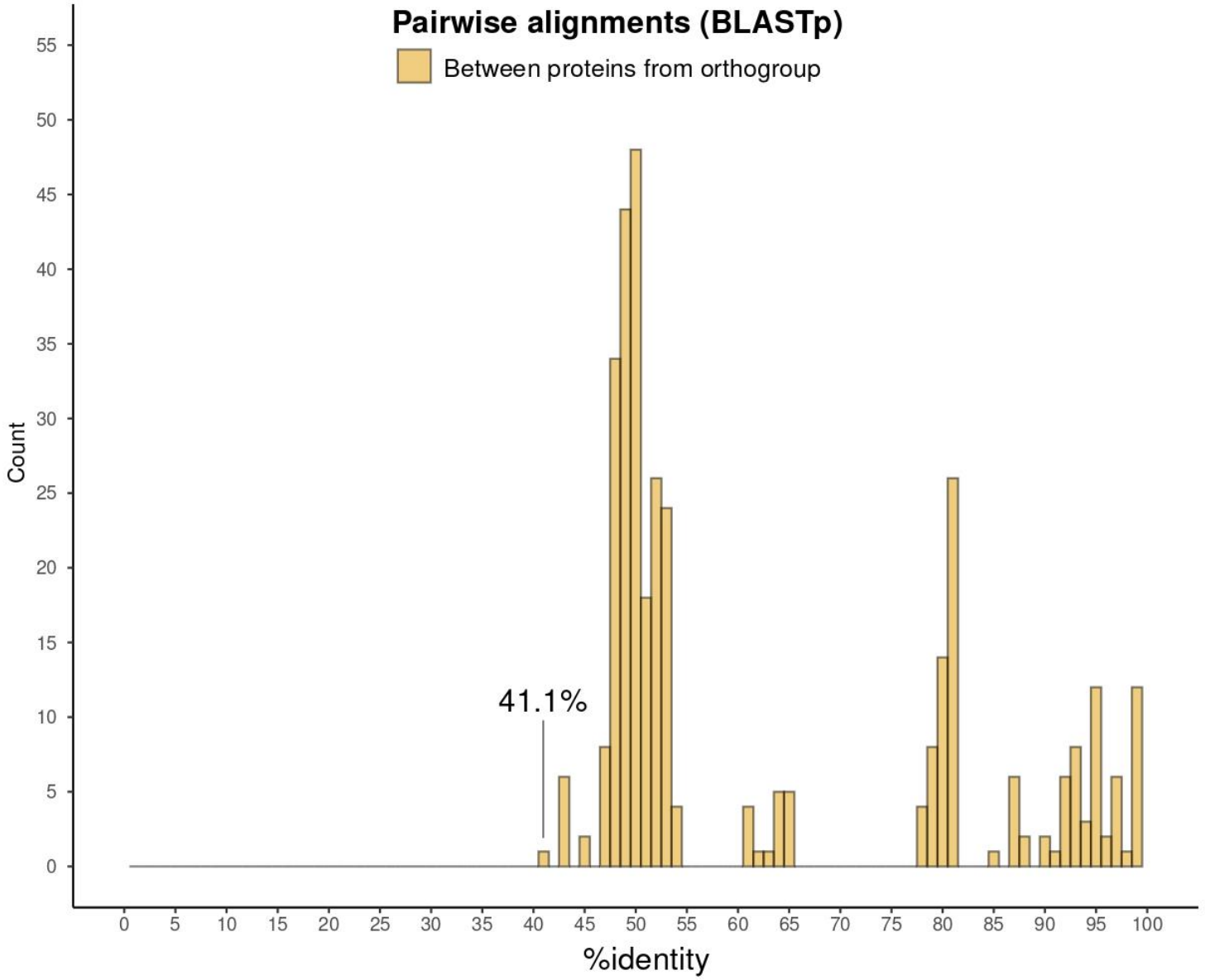


Figure S5. Integrase OG.

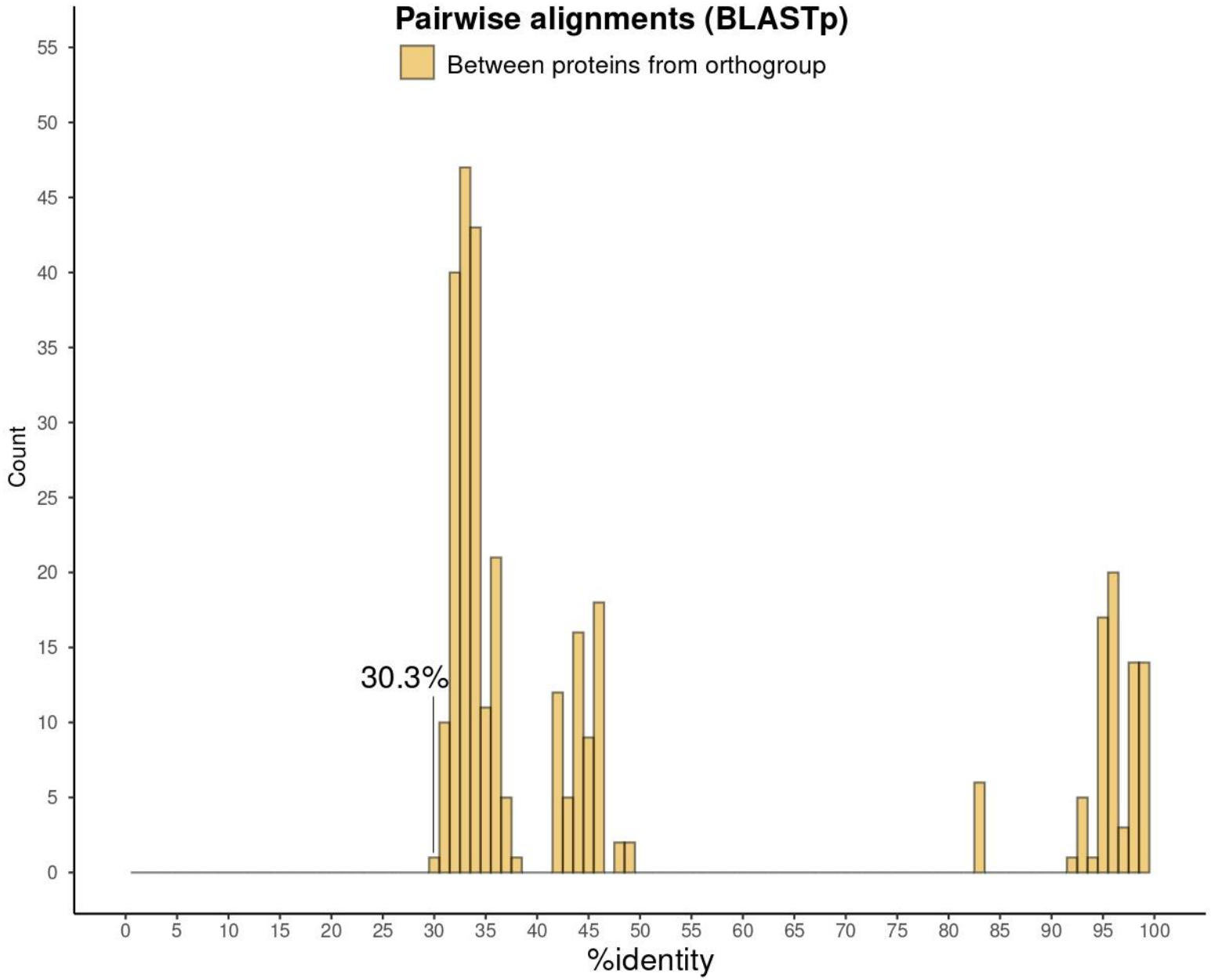


Figure S6. Tail lysozyme OG.

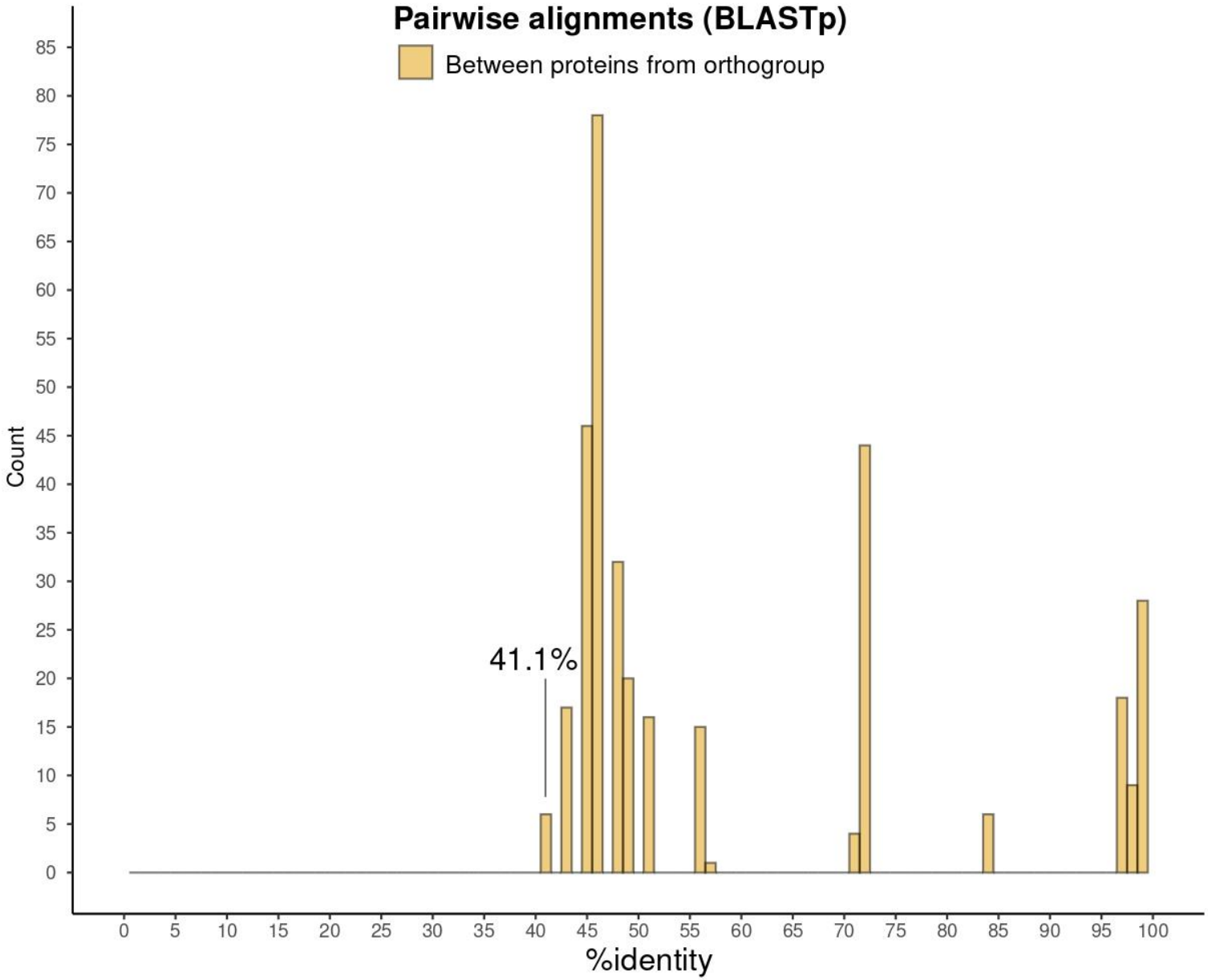


Figure S7. β (beta) OG.

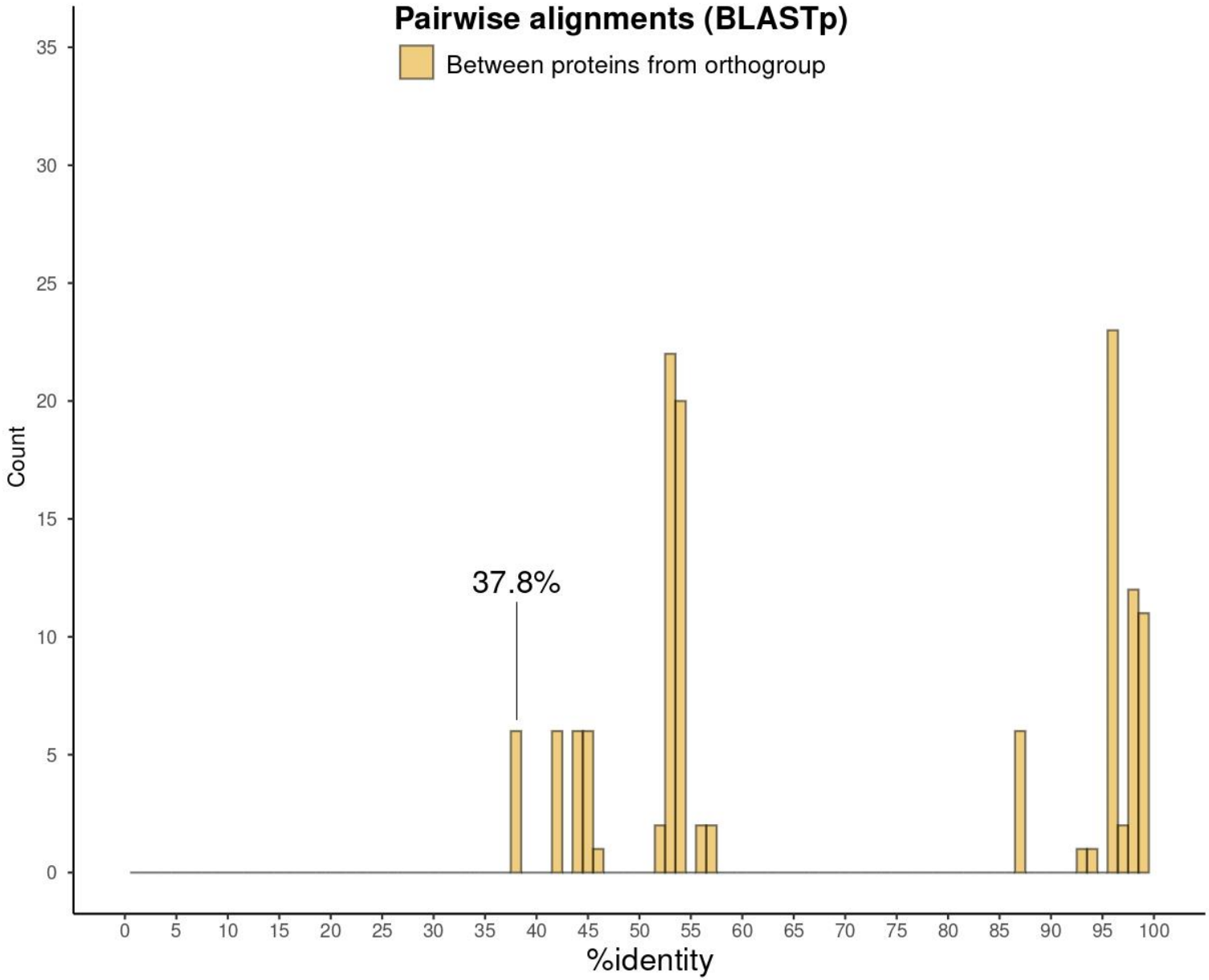


Figure S8. γ (gamma) OG.

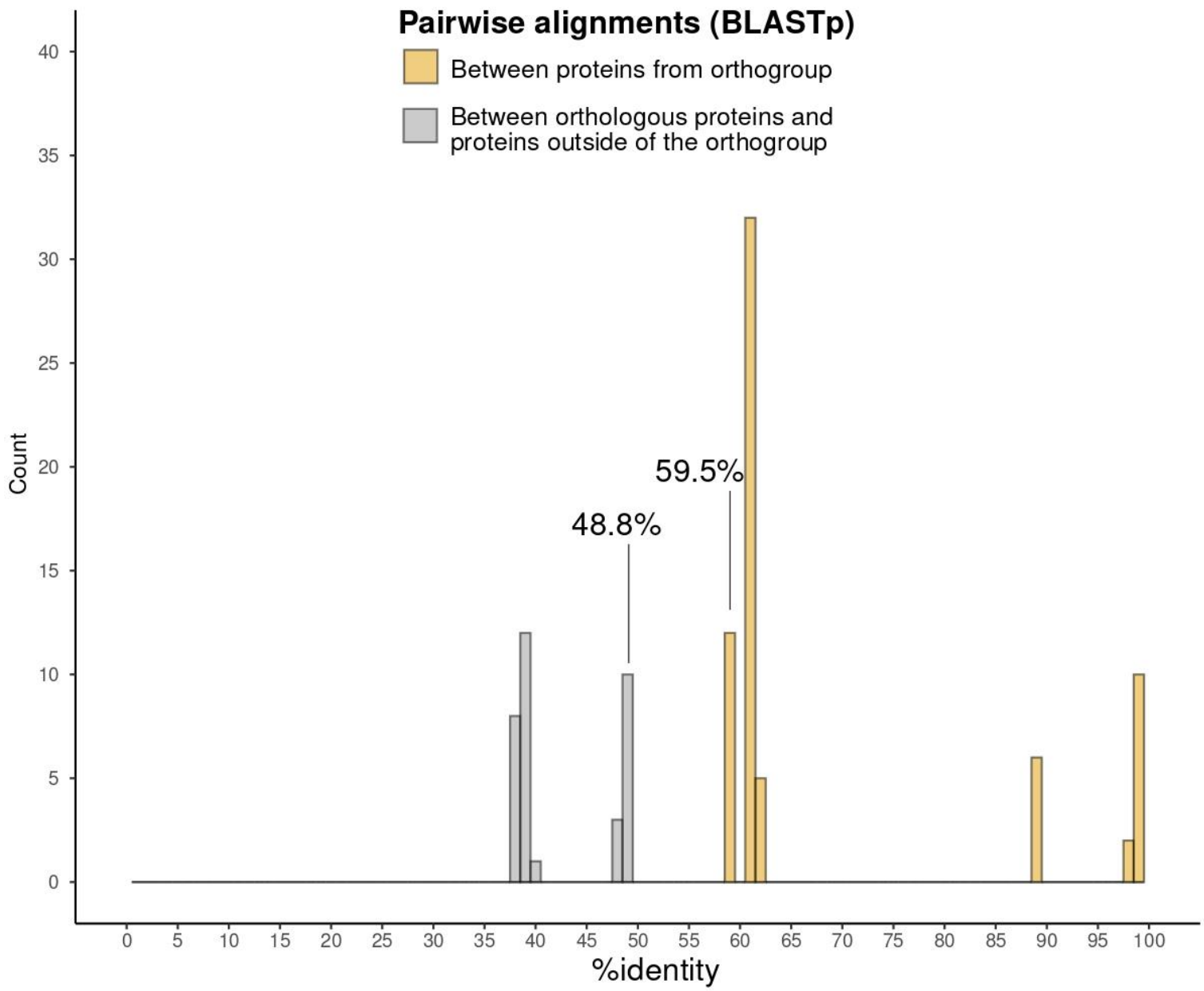


Figure S9. Holin OG.

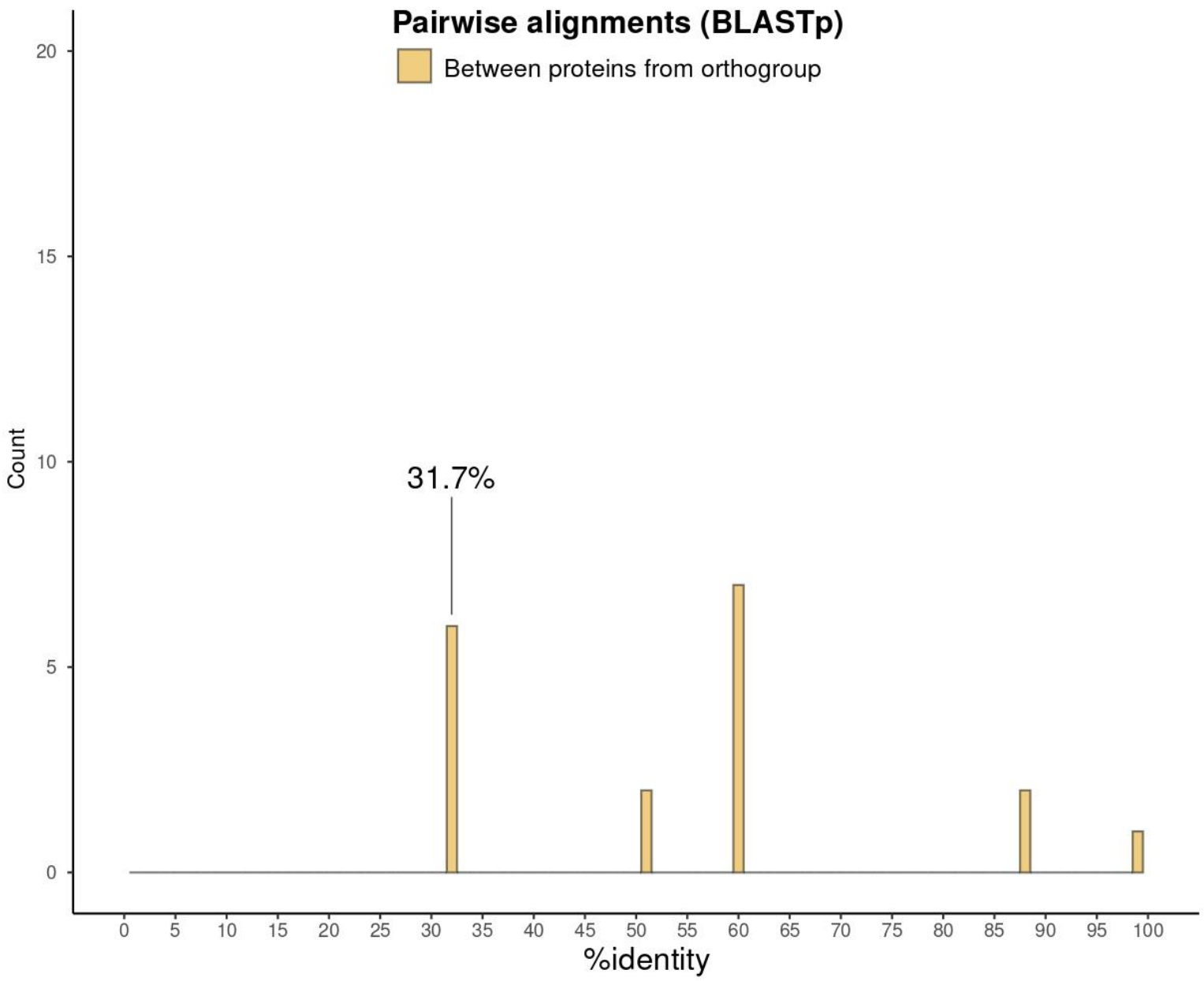


Figure S10. Tail Protein OG.

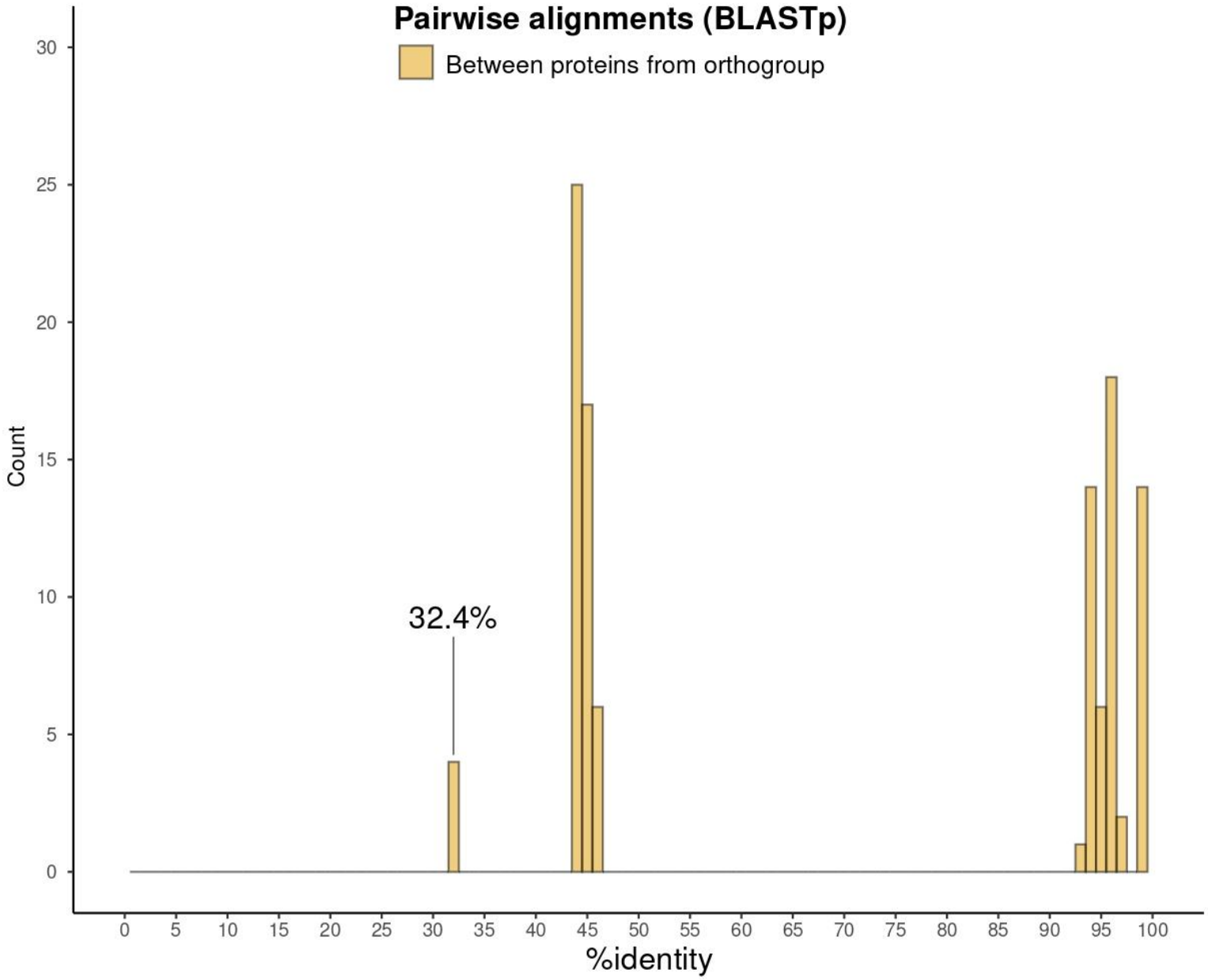


Figure S11. δ (delta) OG.

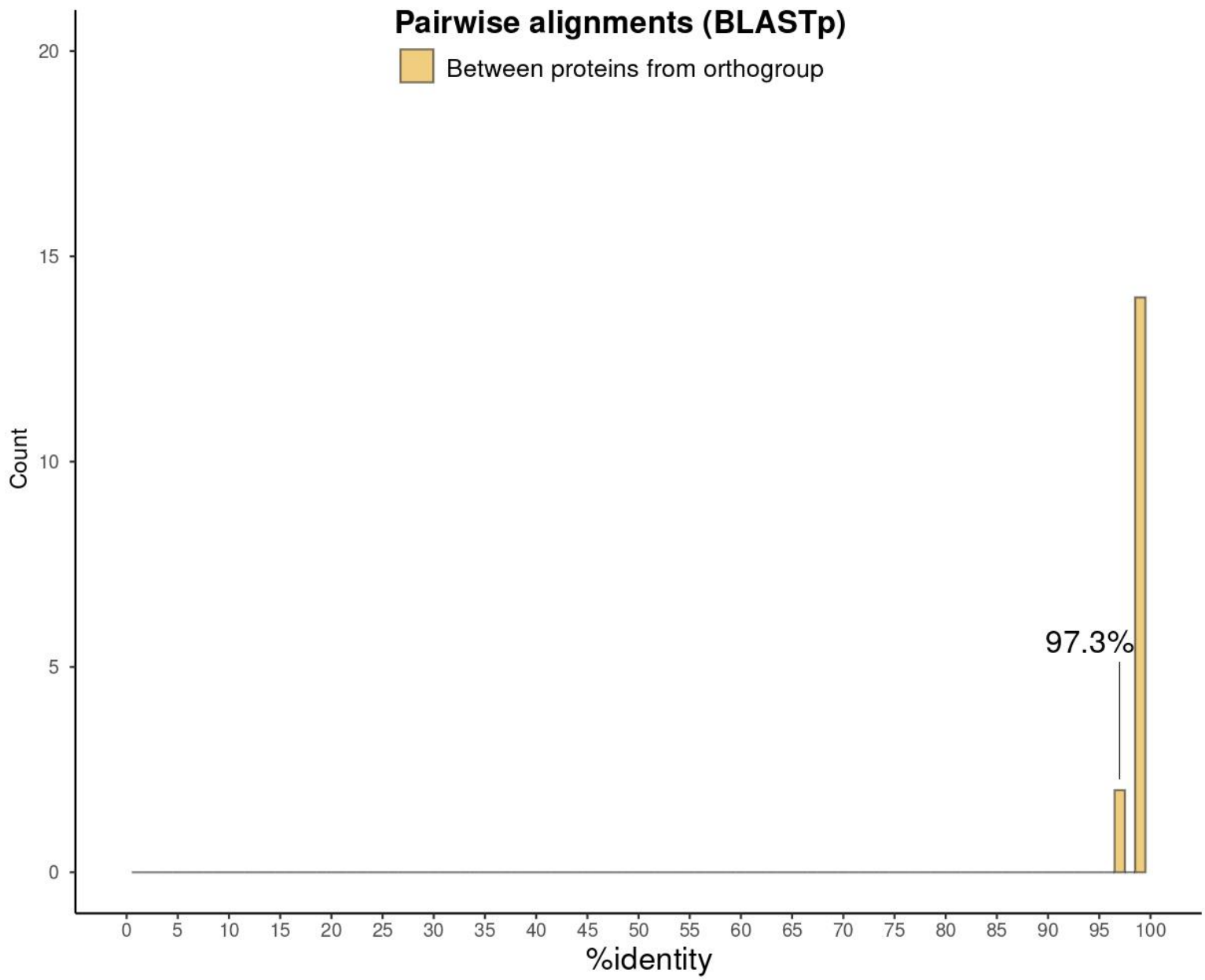


Figure S12. ε (epsilon) OG.

APÊNDICE A - Notas

The International Committee on Taxonomy of Viruses
Taxonomy Proposal Form, 2024

Taxonomy Proposal Submission:

This Word module should be used for all formal proposals to ICTV.

- **Part 1** should be completed for ALL proposals.
- **Part 2** should be completed ONLY for proposals involving changes to ICTV procedures, rules, statutes, policy or structure.
- **Part 3** should be completed for proposals to establish new taxa or modify assignments or nomenclature of existing taxa.

Do not edit the grey filled cells in the tables

Delete the unused **Part 2** or **Part 3** sections before submission.

The blue help text can be left in the document as it will be automatically removed after submission

Except where indicated, all fields should be completed

Please submit the completed Word module, together with the accompanying Excel spreadsheet module for taxonomy proposals (named in **Part 3**) to the appropriate ICTV Subcommittee Chair.

Taxonomy proposals must be accompanied by the Excel module. This is a critical document for taxonomy proposals that will be used to implement the proposed taxonomic changes once they are approved and ratified. If proposals presented in **Part 3** of the Word module are not presented accurately in the Excel module, the taxonomic changes cannot proceed and the proposal will be returned for revision.

For further guidance, see the notes in blue below and the Help Notes (Taxonomic_Proposals_Help_2024) available at https://ictv.global/taxonomy/templates.

Part 1a: Details of taxonomy proposals

Title:	Create a novel species (*Acarajevirus bahia*) and new genus in the Peduoviridae family
Code assigned:	<to be assigned by ICTV officers>

Author(s), affiliation and email address(es): One author per row - add additional rows as necessary			
Name Format name as Smith DB	**Affiliation** Include and limit to Department, Institution, City, Country	**Email address** One only	**Corresponding author(s)** Mark with X
Alfenas-Zerbini P.	Departamento de Microbiologia, Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa, Viçosa, MG, Brazil.	palfenas@ufv.br	X
Rezende R.R.	Departamento de Microbiologia, Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO),	rafael.r.rezende@ufv.br	

	Universidade Federal de Viçosa, Viçosa, MG, Brazil.		
Morgan T.	Departamento de Microbiologia, Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa, Viçosa, MG, Brazil.	tulio.morgan@ufv.br	
Januário D.B.	Departamento de Microbiologia, Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa, Viçosa, MG, Brazil.	beatriz.d.januario@ufv.br	

Part 1b: Taxonomy Proposal Submission <To be completed on initial submission>

ICTV Subcommittee: Enter an "X" against the subcommittee(s) most appropriate to deal with the taxonomy proposal. If in doubt, contact the appropriate subcommittee chair for clarification (see https://ictv.global/sc).			
Animal DNA Viruses and Retroviruses		Bacterial viruses	X
Animal minus-strand and dsRNA viruses		Fungal and protist viruses	
Animal positive-strand RNA viruses		Plant viruses	
Archaeal viruses		General -Submit to Proposals Secretary	

List the ICTV Study Group(s) that have seen or have been involved in creating this proposal: A list of study groups and contacts is provided at https://ictv.global/sc.

Optional – complete only if formally voted on by an ICTV Study Group: <To be completed by Study Group>			
Study Group	**Number of members**		
	Votes in support	**Votes against**	**No vote**

Submission date:	DD/MM/YYYY	Enter date of the initial submission

Part 1c: Feedback from ICTV Executive Committee (EC) meeting <To be completed by the subcommittee chair after EC evaluation>

Executive Committee Meeting Decision code:	X
A – Accept	
Ac – Accept subject to revision by relevant subcommittee chair. No further vote required	
U – Accept without revision but with re-evaluation and email vote by the EC	
Uc – Accept subject to revision and re-evaluation and email vote by the EC	
Ud – Deferred to the next EC meeting, with an invitation to revise based on EC comments	
J - Reject	
W - Withdrawn	

Comments from the Executive Committee:

Part 1d: Revised Taxonomy Proposal Submission <To be completed for the revised version>

Response of proposer: Please describe in detail how you have responded to the EC meeting feedback

Revision date:	DD/MM/YYYY	Enter date of the revised version.

Part 2: GENERAL PROPOSAL

Please use this section only for proposals regarding ICTV procedures, rules, statutes, policy or structure, that do not involve the establishment of new taxonomy.

Abstract for General Proposal: Please provide a structured summary of your proposal using the headings provided (maximum 250 words). Ensure that the abstract briefly describes the changes to ICTV procedures and the reasons and justification for proposed changes. The abstract should provide sufficient context to be understandable without reference to the full text of the proposal as it will be used as written for the automatic population of public documents such as taxonomy summary publications.
Brief description of current situation: *Proposed changes:* *Justification:*

Text of General Proposal: Please include the reasons for the changes you are proposing.
Background: *Proposed changes:* *Justification:*

References:

Tables, Figures: The use of Figures and Tables as supporting evidence is strongly recommended (note that using material from publications will require permission from the copyright holder if the publication is not open access).

<Start here>

Part 3: TAXONOMIC PROPOSAL

Please use this section for proposing taxonomic changes. The changes MUST also be presented in an accompanying Excel module, TP_Template_Excel_module_2024, available at https://ictv.global/taxonomy/templates.

Name of accompanying Excel module: Use the same stem filename as the Word document
PROPOSED TAXONOMY *(Acarajevirus bahia)*.xlsx

Taxonomic changes proposed: Enter an "X" against one or more actions			
Establish new taxon	**x**	Split taxon	
Abolish taxon		Merge taxon	
Move taxon		Promote taxon	
Rename taxon		Demote taxon	
Move and rename			

Is any taxon name used here derived from that of a living person: If you propose taxon name(s) which are derived from the name of a living person or persons, you must attach documents to verify that permission has been obtained from those persons and that they agree to the form in which their name or an element of it is to be used. These documents will not be posted online but will be retained by ICTV as evidence of authorization.		Y/N
Taxon name	**Person from whom the name is derived**	**Attached X**

Abstract of Taxonomy Proposal: Please provide a structured summary of your taxonomic proposal using the headings provided (maximum 250 words). The abstract should provide sufficient context to be understandable without reference to the full text of the proposal as it will be used as written for the automatic population of public documents such as taxonomy summary publications.
Taxonomic rank(s) affected: *Description of current taxonomy*: *Proposed taxonomic change(s):* *Justification*:

Text of Taxonomy proposal: Please explain the reasons for the taxonomic changes you are proposing.

Species:

- Explain how to distinguish the newly proposed species from established ones in a genus and use defined criteria, such as nucleotide sequence identity, for species demarcation.
- If criteria have not been previously established, and if more than one species in the genus is envisioned, please state the **species demarcation criteria** you are proposing.
- If demarcation criteria have been previously established, provide a reference for those criteria (e.g. previously accepted proposal, ICTV Report Chapter, or publication).
- Names for new or renamed species must follow a genus + species epithet binomial format (see https://pubmed.ncbi.nlm.nih.gov/35043230/).

Higher taxa:
- If criteria have not been previously established, and if more than one taxon in the rank is envisioned, please state the **demarcation criteria** you are proposing.
- If demarcation criteria have been previously established, provide a reference for those criteria (e.g. previously accepted proposal, ICTV Report Chapter, or publication).
- Defining the unique characteristics of the taxon is desirable and can assist evaluation of the proposal.

Origin of names:
- Please indicate the origin of names (etymology) of new taxa.

Availability of genome sequences:
- Proposals for the establishment of new taxa require that annotated and coding complete genome sequences of the species exemplars are available from an INSDC database (*eg.* GenBank).
- Short read archive (SRA) records (unassembled sequences) are not acceptable.

Taxonomic rank(s) affected: New species and genus.

Description of current taxonomy:

Proposed taxonomic change(s): New species (*Acarajevirus bahia*) and new genus (*Acarajevirus*) in the *Peduoviridae* family.

Demarcation criteria:

Justification: Phage (*Acarajevirus bahia*) is most similar to the Ralstonia phage RSY1 (Arsyunavirus RSY1) (~55% intergenomic similarity), which belongs to the genus Arsyunavirus (family: Peduoviridae, class: Caudoviricetes) (Figure 1). Ralstonia phage RSY1 is a widespread temperate phage in R. solanacearum. *Acarajevirus bahia* has the six orthologous core genes within the monophyletic clades (Figure 2), which is the current demarcation criterion of the Peduoviridae family (http://ictv.global/taxonomy/taxondetails?taxnode_id=202200225&taxon_name=Peduoviridae).
Thus, it was named *Acarajevirus bahia* and classified as a new species and genus in the Peduoviridae family.

References:

ASKORA, Ahmed et al. Lysogenic Conversion of the Phytopathogen *Ralstonia solanacearum* by the P2virus ϕRSY1. Frontiers in Microbiology, v. 8, p. 308454, 2017.

Tables, Figures:

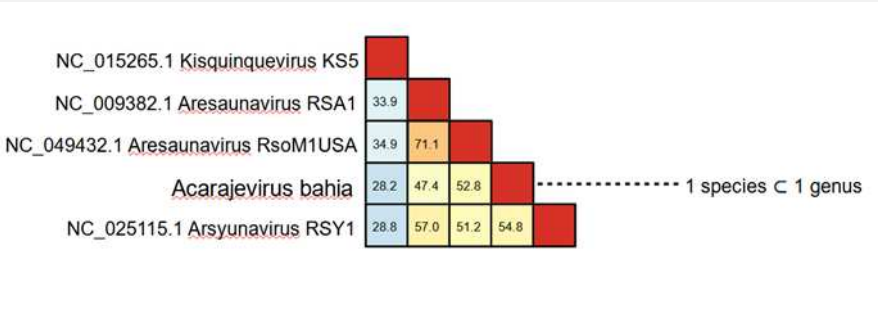


Figure 1.

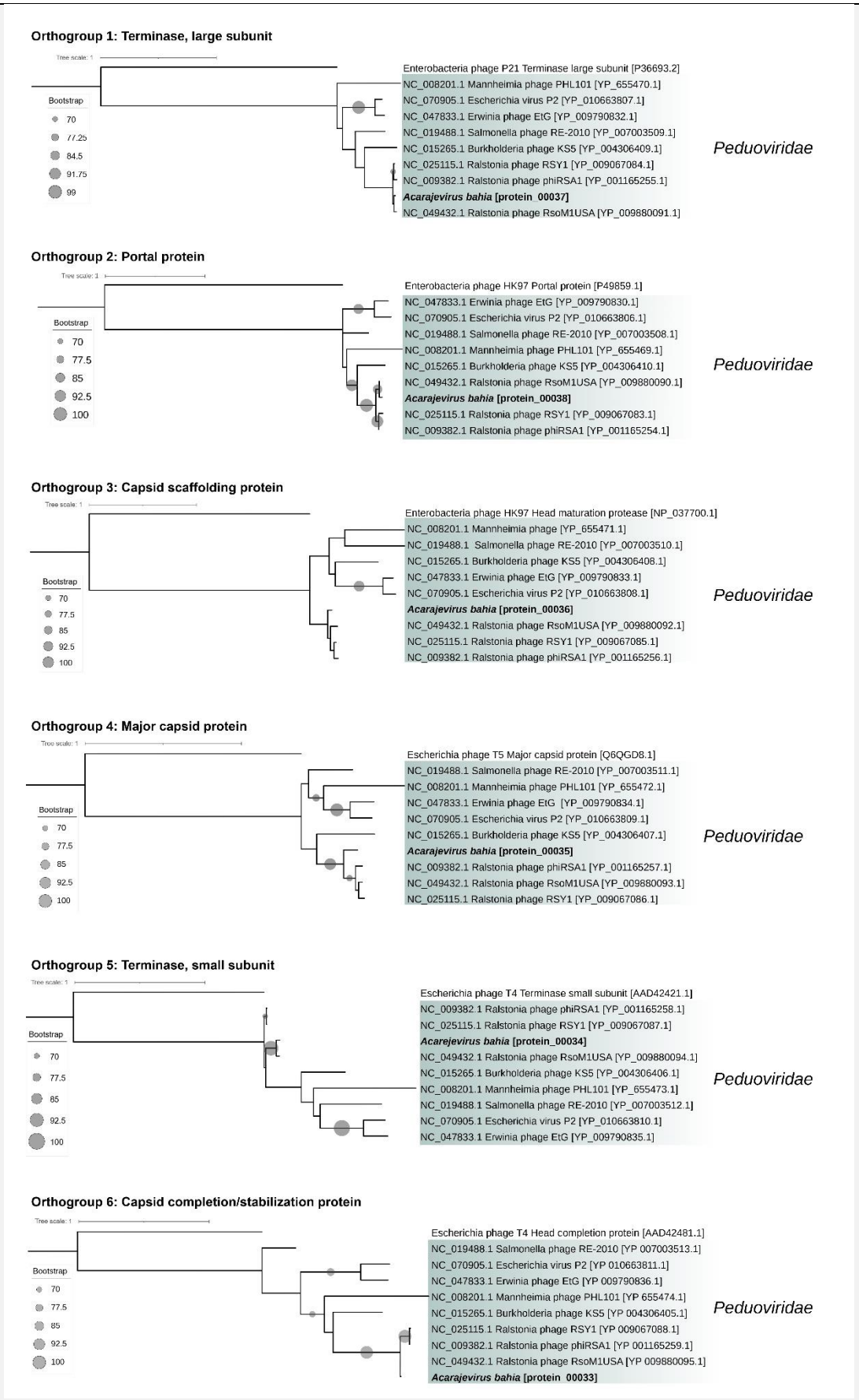

Orthogroup 1: Terminase, large subunit
Tree scale: 1
Bootstrap
70
77.25
84.5
91.75
99
Enterobacteria phage P21 Terminase large subunit [P36693.2]
NC_008201.1 Mannheimia phage PHL101 [YP_655470.1]
NC_070905.1 Escherichia virus P2 [YP_010663807.1]
NC_047833.1 Erwinia phage EtG [YP_009790832.1]
NC_019488.1 Salmonella phage RE-2010 [YP_007003509.1]
NC_015265.1 Burkholderia phage KS5 [YP_004306409.1]
NC_025115.1 Ralstonia phage RSY1 [YP_009067084.1]
NC_009382.1 Ralstonia phage phiRSA1 [YP_001165255.1]
Acarajevirus bahia [protein_00037]
NC_049432.1 Ralstonia phage RsoM1USA [YP_009880091.1]
Peduoviridae
Orthogroup 2: Portal protein
Tree scale: 1
Bootstrap
70
77.5
85
92.5
100
Enterobacteria phage HK97 Portal protein [P49859.1]
NC_047833.1 Erwinia phage EtG [YP_009790830.1]
NC_070905.1 Escherichia virus P2 [YP_010663806.1]
NC_019488.1 Salmonella phage RE-2010 [YP_007003508.1]
NC_008201.1 Mannheimia phage PHL101 [YP_655469.1]
NC_015265.1 Burkholderia phage KS5 [YP_004306410.1]
NC_049432.1 Ralstonia phage RsoM1USA [YP_009880090.1]
Acarajevirus bahia [protein_00038]
NC_025115.1 Ralstonia phage RSY1 [YP_009067083.1]
NC_009382.1 Ralstonia phage phiRSA1 [YP_001165254.1]
Peduoviridae
Orthogroup 3: Capsid scaffolding protein
Tree scale: 1
Bootstrap
70
77.5
85
92.5
100
Enterobacteria phage HK97 Head maturation protease [NP_037700.1]
NC_008201.1 Mannheimia phage [YP_655471.1]
NC_019488.1 Salmonella phage RE-2010 [YP_007003510.1]
NC_015265.1 Burkholderia phage KS5 [YP_004306408.1]
NC_047833.1 Erwinia phage EtG [YP_009790833.1]
NC_070905.1 Escherichia virus P2 [YP_010663808.1]
Acarajevirus bahia [protein_00036]
NC_049432.1 Ralstonia phage RsoM1USA [YP_009880092.1]
NC_025115.1 Ralstonia phage RSY1 [YP_009067085.1]
NC_009382.1 Ralstonia phage phiRSA1 [YP_001165256.1]
Peduoviridae
Orthogroup 4: Major capsid protein
Tree scale: 1
Bootstrap
70
77.5
85
92.5
100
Escherichia phage T5 Major capsid protein [Q6QGD8.1]
NC_019488.1 Salmonella phage RE-2010 [YP_007003511.1]
NC_008201.1 Mannheimia phage PHL101 [YP_655472.1]
NC_047833.1 Erwinia phage EtG [YP_009790834.1]
NC_070905.1 Escherichia virus P2 [YP_010663809.1]
NC_015265.1 Burkholderia phage KS5 [YP_004306407.1]
Acarajevirus bahia [protein_00035]
NC_009382.1 Ralstonia phage phiRSA1 [YP_001165257.1]
NC_049432.1 Ralstonia phage RsoM1USA [YP_009880093.1]
NC_025115.1 Ralstonia phage RSY1 [YP_009067086.1]
Peduoviridae
Orthogroup 5: Terminase, small subunit
Tree scale: 1
Bootstrap
70
77.5
85
92.5
100
Escherichia phage T4 Terminase small subunit [AAD42421.1]
NC_009382.1 Ralstonia phage phiRSA1 [YP_001165258.1]
NC_025115.1 Ralstonia phage RSY1 [YP_009067087.1]
Acarajevirus bahia [protein_00034]
NC_049432.1 Ralstonia phage RsoM1USA [YP_009880094.1]
NC_015265.1 Burkholderia phage KS5 [YP_004306406.1]
NC_008201.1 Mannheimia phage PHL101 [YP_655473.1]
NC_019488.1 Salmonella phage RE-2010 [YP_007003512.1]
NC_070905.1 Escherichia virus P2 [YP_010663810.1]
NC_047833.1 Erwinia phage EtG [YP_009790835.1]
Peduoviridae
Orthogroup 6: Capsid completion/stabilization protein
Tree scale: 1
Bootstrap
70
77.5
85
92.5
100
Escherichia phage T4 Head completion protein [AAD42481.1]
NC_019488.1 Salmonella phage RE-2010 [YP 007003513.1]
NC_070905.1 Escherichia virus P2 [YP 010663811.1]
NC_047833.1 Erwinia phage EtG [YP 009790836.1]
NC_008201.1 Mannheimia phage PHL101 [YP 655474.1]
NC_015265.1 Burkholderia phage KS5 [YP 004306405.1]
NC_025115.1 Ralstonia phage RSY1 [YP 009067088.1]
NC_009382.1 Ralstonia phage phiRSA1 [YP 001165259.1]
NC_049432.1 Ralstonia phage RsoM1USA [YP 009880095.1]
Acarajevirus bahia [protein_00033]
Peduoviridae

Figure 2.

The International Committee on Taxonomy of Viruses
Taxonomy Proposal Form, 2024

Taxonomy Proposal Submission:

This Word module should be used for all formal proposals to ICTV.

- **Part 1** should be completed for ALL proposals.
- **Part 2** should be completed ONLY for proposals involving changes to ICTV procedures, rules, statutes, policy or structure.
- **Part 3** should be completed for proposals to establish new taxa or modify assignments or nomenclature of existing taxa.

Do not edit the grey filled cells in the tables

Delete the unused **Part 2** or **Part 3** sections before submission.

The blue help text can be left in the document as it will be automatically removed after submission

Except where indicated, all fields should be completed

Please submit the completed Word module, together with the accompanying Excel spreadsheet module for taxonomy proposals (named in **Part 3**) to the appropriate ICTV Subcommittee Chair.

Taxonomy proposals must be accompanied by the Excel module. This is a critical document for taxonomy proposals that will be used to implement the proposed taxonomic changes once they are approved and ratified. If proposals presented in **Part 3** of the Word module are not presented accurately in the Excel module, the taxonomic changes cannot proceed and the proposal will be returned for revision.

For further guidance, see the notes in blue below and the Help Notes (Taxonomic_Proposals_Help_2024) available at https://ictv.global/taxonomy/templates.

Part 1a: Details of taxonomy proposals

Title:	Create new species in the genus *Cocadavirus* (Mascarenevirinae: Anamaviridae)
Code assigned:	<to be assigned by ICTV officers>

Author(s), affiliation and email address(es): One author per row - add additional rows as necessary			
Name Format name as Smith DB	**Affiliation** Include and limit to Department, Institution, City, Country	**Email address** One only	**Corresponding author(s)** Mark with X
Alfenas-Zerbini P.	Departamento de Microbiologia, Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa, Viçosa, MG, Brazil.	palfenas@ufv.br	X
Rezende R.R.	Departamento de Microbiologia, Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa, Viçosa, MG, Brazil.	rafael.r.rezende@ufv.br	X
Morgan T.	Departamento de Microbiologia, Instituto de Biotecnologia Aplicada à	tulio.morgan@ufv.br	X

	Agropecuária (BIOAGRO), Universidade Federal de Viçosa, Viçosa, MG, Brazil.		
Januário D.B.	Departamento de Microbiologia, Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa, Viçosa, MG, Brazil.	beatriz.d.januario@ufv.br	X

Part 1b: Taxonomy Proposal Submission <To be completed on initial submission>

ICTV Subcommittee: Enter an "X" against the subcommittee(s) most appropriate to deal with the taxonomy proposal. If in doubt, contact the appropriate subcommittee chair for clarification (see https://ictv.global/sc).			
Animal DNA Viruses and Retroviruses		Bacterial viruses	X
Animal minus-strand and dsRNA viruses		Fungal and protist viruses	
Animal positive-strand RNA viruses		Plant viruses	
Archaeal viruses		General -Submit to Proposals Secretary	

List the ICTV Study Group(s) that have seen or have been involved in creating this proposal: A list of study groups and contacts is provided at https://ictv.global/sc.

Optional – complete only if formally voted on by an ICTV Study Group: <To be completed by Study Group>			
Study Group	**Number of members**		
	Votes in support	**Votes against**	**No vote**

Submission date:	DD/MM/YYYY	Enter date of the initial submission

Part 1c: Feedback from ICTV Executive Committee (EC) meeting <To be completed by the subcommittee chair after EC evaluation>

Executive Committee Meeting Decision code:	X
A – Accept	
Ac – Accept subject to revision by relevant subcommittee chair. No further vote required	
U – Accept without revision but with re-evaluation and email vote by the EC	
Uc – Accept subject to revision and re-evaluation and email vote by the EC	
Ud – Deferred to the next EC meeting, with an invitation to revise based on EC comments	
J - Reject	
W - Withdrawn	

Comments from the Executive Committee:

Part 1d: Revised Taxonomy Proposal Submission <To be completed for the revised version>

Response of proposer: Please describe in detail how you have responded to the EC meeting feedback

Revision date:	DD/MM/YYYY	Enter date of the revised version.

Part 2: GENERAL PROPOSAL

Please use this section only for proposals regarding ICTV procedures, rules, statutes, policy or structure, that do not involve the establishment of new taxonomy.

Abstract for General Proposal: Please provide a structured summary of your proposal using the headings provided (maximum 250 words). Ensure that the abstract briefly describes the changes to ICTV procedures and the reasons and justification for proposed changes. The abstract should provide sufficient context to be understandable without reference to the full text of the proposal as it will be used as written for the automatic population of public documents such as taxonomy summary publications.
Brief description of current situation: *Proposed changes:* *Justification:*

Text of General Proposal: Please include the reasons for the changes you are proposing.
Background: *Proposed changes:* *Justification:*

References:

Tables, Figures: The use of Figures and Tables as supporting evidence is strongly recommended (note that using material from publications will require permission from the copyright holder if the publication is not open access).

<Start here>

Part 3: TAXONOMIC PROPOSAL

Please use this section for proposing taxonomic changes. The changes MUST also be presented in an accompanying Excel module, TP_Template_Excel_module_2024, available at https://ictv.global/taxonomy/templates.

Name of accompanying Excel module: Use the same stem filename as the Word document
PROPOSED TAXONOMY*(Cocadavirus alagoinhas)*.xlsx

Taxonomic changes proposed: Enter an "X" against one or more actions			
Establish new taxon		Split taxon	
Abolish taxon		Merge taxon	
Move taxon		Promote taxon	
Rename taxon		Demote taxon	
Move and rename			

Is any taxon name used here derived from that of a living person: If you propose taxon name(s) which are derived from the name of a living person or persons, you must attach documents to verify that permission has been obtained from those persons and that they agree to the form in which their name or an element of it is to be used. These documents will not be posted online but will be retained by ICTV as evidence of authorization.		Y/N
Taxon name	**Person from whom the name is derived**	**Attached X**

Abstract of Taxonomy Proposal: Please provide a structured summary of your taxonomic proposal using the headings provided (maximum 250 words). The abstract should provide sufficient context to be understandable without reference to the full text of the proposal as it will be used as written for the automatic population of public documents such as taxonomy summary publications.

Taxonomic rank(s) affected:

Description of current taxonomy:

Proposed taxonomic change(s):

Justification:

Text of Taxonomy proposal: Please explain the reasons for the taxonomic changes you are proposing.

Species:

- Explain how to distinguish the newly proposed species from established ones in a genus and use defined criteria, such as nucleotide sequence identity, for species demarcation.
- If criteria have not been previously established, and if more than one species in the genus is envisioned, please state the **species demarcation criteria** you are proposing.
- If demarcation criteria have been previously established, provide a reference for those criteria (e.g. previously accepted proposal, ICTV Report Chapter, or publication).

- Names for new or renamed species must follow a genus + species epithet binomial format (see https://pubmed.ncbi.nlm.nih.gov/35043230/).

Higher taxa:

- If criteria have not been previously established, and if more than one taxon in the rank is envisioned, please state the **demarcation criteria** you are proposing.
- If demarcation criteria have been previously established, provide a reference for those criteria (e.g. previously accepted proposal, ICTV Report Chapter, or publication).
- Defining the unique characteristics of the taxon is desirable and can assist evaluation of the proposal.

Origin of names:

- Please indicate the origin of names (etymology) of new taxa.

Availability of genome sequences:

- Proposals for the establishment of new taxa require that annotated and coding complete genome sequences of the species exemplars are available from an INSDC database (*eg.* GenBank).
- Short read archive (SRA) records (unassembled sequences) are not acceptable.

Taxonomic rank(s) affected: Novel subfamily and novel family, and novel species set of a novel genus.

Description of current taxonomy:

Proposed taxonomic change(s): Proposal of a novel species (Cocadavirus *alagoinhas*) set of a novel genus (*Cocadavirus*), the novel *Mascarenevirinae* subfamily and novel *Anamaviridae* family.

Demarcation criteria: We propose that the *Kantovirinae* subfamily be classified inside the *Anamaviridae* family following demarcation criteria: the *Kantovirinae* members belong to the monophyletic subclade I inside the monophyletic clade of *Anamaviridae* on the proteomic tree and have the two orthologous proteins above the identity thresholds (in parenthesis): Delta OG (32.4%) and Epsilon OG (97.3%)

Justification: we propose a novel *Mascarenevirinae* subfamily being classified inside the *Anamaviridae* family following demarcation criteria: the *Mascarenevirinae* members belong to the monophyletic subclade II inside the monophyletic clade of *Anamaviridae* on the proteomic tree and have the two orthologous proteins above the identity thresholds (in parenthesis): Holin OG (59.5%) and Tail OG (31.7%). At last, we proposed that all members of the *Anamaviridae* family be classified in genera and species using the intergenomic distances following demarcation criteria: members of the same genus clustering have intergenomic distance ≥ 70% and same species clustering have intergenomic distance ≥ 95%. The proposal to *Amanaviridae*, *Kantovirinae*, *Mascarenevirinae*, and the genera and species are summarized in (Figure 1). Thus, phage, named *Cocadavirus alagoinhas*, was classified as a novel species belonging to a novel genus named *Cocadavirus*, which is included in the *Mascarenevirinae* subfamily from *Anamaviridae* family.

References:

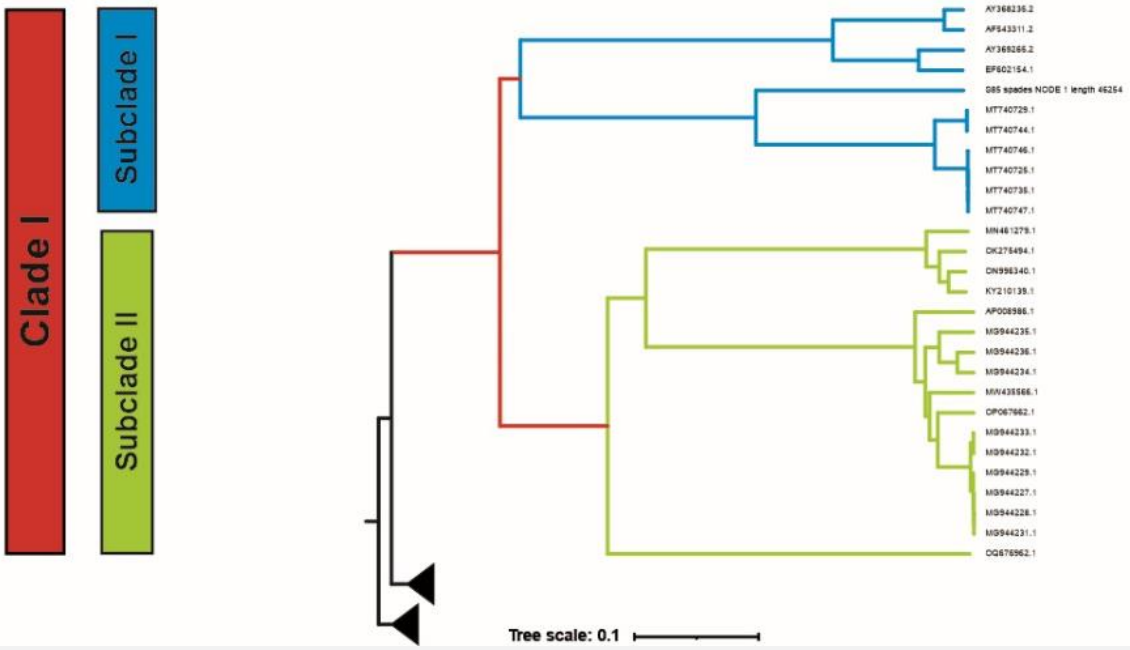
Trotereau, A., Boyer, C., Bornard, I. et al. High genomic diversity of novel phages infecting the plant pathogen Ralstonia solanacearum, isolated in Mauritius and Reunion islands. Scientific Reports, 2021, 11, 5382.

Tables, Figures:

Figure 1.

Taxonomy proposal to *Anamaviridae* family

Viruses information		Demarcation criteria			
		Species	Genus	Subfamily	Family
Acession number	Sequence name	Members of the same species clustering have intergenomic distance ≥ 95%.	Members of the same genus clustering have intergenomic distance ≥ 70%.	The members of the *Kantovirinae* are part of the monophyletic subclade I within the monophyletic clade of *Anamaviridae* on the proteomic tree and have the two orthologous proteins Delta OG and Epsilon OG. The members of the *Mascarenevirinae* are part of the monophyletic subclade II within the monophyletic clade of *Anamaviridae* on the proteomic tree and have the two orthologous proteins hOLIN OG and Tail OG	Members of the *Anamaviridae* belong to a monophyletic group on the proteomic tree and have five orthologous proteins: Alpha OG, Integrase OG, Tail lysozyme OG, Beta OG, and Gamma OG.
		Classification			
	Cocadavirus alagoinhas	*Cocadavirus alagoinhas*	*Cocadavirus*	*Mascarenevirinae*	*Anamaviridae*
MT740747.1	Ralstonia phage Simangalove	*Bakolyvirus bakoly*	*Bakolyvirus*		
MT740735.1	Ralstonia phage Elie				
MT740725.1	Ralstonia phage Adzire				
MT740746.1	Ralstonia phage Sarlave				
MT740744.1	Ralstonia phage Jenny				
MT740729.1	Ralstonia phage Bakoly				
AF543311.2	Burkholderia phage Bcep781	*Naesvirus bcep*	*Naesvirus*		
AY368235.2	Burkholderia phage Bcep43				
EF602154.1	Burkholderia phage BcepNY3	*Naesvirus bcepNY*			
AY369265.2	Burkholderia phage Bcep1				
MG944231.1	Xanthomonas phage XPP6	*Tsukubavirus tsukuba*	*Tsukubavirus*	*Kantovirinae*	
MG944228.1	Xanthomonas phage XPP2				
MG944227.1	Xanthomonas phage XPP1				
MG944229.1	Xanthomonas phage XPP3				
MG944232.1	Xanthomonas phage XPP8				
MG944233.1	Xanthomonas phage XPP9				
OP067662.1	Xanthomonas phage pXoo2107				
MW435566.1	Xanthomonas phage X2				
MG944234.1	Xanthomonas phage XPV1				
MG944236.1	Xanthomonas phage XPV3				
MG944235.1	Xanthomonas phage XPV2				
AP008986.1	Xanthomonas oryzae phage OP2 DNA				
KY210139.1	Xanthomonas phage KPhi1	*Beograduvirus beogra*	*Beograduvirus*		
ON996340.1	Xanthomonas phage BsXeu269p/3				
OK275494.1	Xanthomonas phage MYK3				
MN461279.1	Xanthomonas virus phiXaf18				
OQ676962.1	Xanthomonas phage NEB7	*Xanthovirus NEB7*	Xanthovirus		



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CONCLUSÕES

Neste estudo, isolamos e caracterizamos dois novos fagos de solos brasileiros capazes de infectar *Ralstonia solanacearum* e *Ralstonia pseudosolanacearum*. Os genomas dos fagos isolados, nomeados RS-Phage-AB1 e RS-Phage-CA1, foram sequenciados e anotados, com diferenças em seus tamanhos, composição de genes e estratégias de infecção. Ambos os fagos podem ser considerados temperados.

Propomos que RS-Phage-AB1 seja classificado como uma nova espécie ("*Acarajevirus bahia*") e gênero na família *Peduoviridae*, enquanto a RS-Phage-CA1 seja classificado como uma nova espécie ("*Cocadavirus alagoinhas*") e gênero, e ainda sugerimos a criação da nova família "Anamaviridae", incluindo as novas subfamílias "Kantovirinae" e "Mascarenevirinae", sendo o fago RS-Phage-CA1 pertencente a subfamília "Mascarenevirinae". A família "Anamaviridae" e suas subfamílias foram propostas e definidas com base em critérios de demarcação que incluem identidades de proteínas ortólogas. Os membros das subfamílias possuem proteínas ortólogas com identidades acima dos limiares estabelecidos, como Holin OG (59.5%) e Tail OG (31.7%) para "Mascarenevirinae", e Delta OG (32.4%) e Epsilon OG (97.3%) para "Kantovirinae".

A caracterização desses novos fagos fornece um pouco mais de entendimento sobre a diversidade e a evolução dos bacteriófagos que infectam RSSC.