UNIVERSIDADE ESTADUAL DE SANTA CRUZ

PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

DESCOBERTA DE UM COMPLEXO VIROMA ASSOCIADO AOS PATÓGENOS DO CACAU *Ceratocystis cacaofunesta* E *Ceratocystis fimbriata*

ROY BOGARDID ARDON ESPINAL

ILHÉUS – BAHIA – BRASIL
2023

ROY BOGARDID ARDON ESPINAL

DESCOBERTA DE UM COMPLEXO VIROMA ASSOCIADO AOS PATÓGENOS DO CACAU *Ceratocystis cacaofunesta* E *Ceratocystis fimbriata*

Dissertação apresentada à Universidade Estadual de Santa Cruz, como parte das exigências para obtenção do título de Mestre em Genética e Biologia Molecular.

Área de Concentração: Genética e Biologia Molecular

Orientador: Eric Roberto Guimarães Rocha Aguiar

ILHÉUS – BAHIA – BRASIL
2023

ROY BOGARDID ARDON ESPINAL

DESCOBERTA DE UM COMPLEXO VIROMA ASSOCIADO AOS PATÓGENOS DO CACAU *Ceratocystis cacaofunesta* E *Ceratocystis fimbriata*

Dissertação apresentada à Universidade Estadual de Santa Cruz, como parte das exigências para obtenção do título de Mestre em Genética e Biologia Molecular.

Área de Concentração: Genética e Biologia Molecular

APROVADO: 25 de agosto de 2023.

Dr. Thiago Mafra Batista (UFSB)	Dr. Gonçalo Santos Da Silva (UESC)
Dr. Ramon Costa Dominato (UESC)	Dr. Eric Roberto Guimarães Rocha Aguiar (UESC - Orientador)

"À imensa e misteriosa força celestial que orquestra os destinos do universo, à minha família querida, fonte inesgotável de afeto, encorajamento e estímulo. Aos estimados professores e colegas, dedico com humildade esta notável conquista, uma vez que cada passo traçado nesta jornada foi conduzido pela chama ardente do amor e pela inquebrantável fé depositada em meu potencial. Expresso minha gratidão por permanecerem inabaláveis ao meu lado, compartilhando sorrisos nos momentos de júbilo e oferecendo amparo nos desafios inerentes. Esta tese, profundamente entrelaçada com minhas horas de dedicação, é um espelho não somente do meu esforço incansável, mas também do caloroso carinho que emanou de todos vocês. Com a mais sincera afeição e reconhecimento, Roy Bogardid Ardón Espinal"

AGRADECIMENTOS

Gostaria de aproveitar este momento para expressar minha profunda gratidão a todas as pessoas e instituições que desempenharam um papel crucial no desenvolvimento e conclusão desta tese. Cada um de vocês contribuiu de maneira significativa para tornar possível esta conquista acadêmica.

Meu reconhecimento especial vai para meu orientador, Eric Roberto Guimarães Rocha Aguiar, cuja orientação especializada, apoio constante e contribuições enriquecedoras foram fundamentais para o sucesso deste trabalho. Sua orientação e mentoria foram inestimáveis em todo o processo.

Da mesma forma, gostaria de expressar minha sincera gratidão ao meu coorientador, Dr. Leandro Lopes Loguercio, por seu comprometimento, direcionamento e valiosos conhecimentos que complementaram de forma excepcional a direção da minha pesquisa.

Meus sinceros agradecimentos também se estendem a co-autora Sabrina Ferreira de Santana e aos colaboradores Vinícius Castro Santos, Gabriela Nicolle Ramos Lizardo, Dr. Raner José Santana Silva, Dr. Ronan Xavier Corrêa, Dr. Aristóteles Góes-Neto, Dr. Carlos Priminho Pirovani e Dr. Paula Luize Camargos Fonseca. Suas contribuições enriqueceram significativamente minha abordagem e perspectiva neste estudo.

Não posso deixar de reconhecer a Universidade Estadual de Santa Cruz por fornecer a infraestrutura e os recursos necessários para a realização desta pesquisa. Seu apoio foi essencial para alcançar os objetivos deste trabalho.

Também gostaria de expressar minha gratidão ao Laboratório de BioVirLab por proporcionar um ambiente propício para pesquisa e experimentação científica.

Minha gratidão se estende ao Programa de Pós-Graduação em Genética e Biologia Molecular pela oportunidade inestimável de cursar o programa de mestrado, assim como meu reconhecimento à CAPES pela generosa concessão das bolsas de mestrado que permitiram minha dedicação total a este projeto. Igualmente, agradeço ao CNPq pelo apoio financeiro crucial que respaldou esta pesquisa e seu desenvolvimento.

Por fim, quero expressar minha profunda gratidão a todos aqueles que, de maneira direta ou indireta, contribuíram para esta dissertação. Cada gesto de apoio, orientação e colaboração foi essencial e muito apreciado.

Não posso deixar de mencionar o OEA-Grupo Coimbra de Universidades Brasileiras (GCUB) por oferecer a oportunidade de estudos a jovens com aspirações de superação, como eu. Seu apoio teve um impacto positivo em minha trajetória acadêmica.

Mais uma vez, agradeço a todos por fazerem parte desta conquista e por sua valiosa contribuição para o meu crescimento e desenvolvimento acadêmico.

“Se cheguei até aqui foi porque me apoiei no ombro dos gigantes”

Isaac Newton

NDICE

RESUMO

ESPINAL, Roy Bogardid Ardón, M.S., Universidade Estadual de Santa Cruz, Ilhéus, agosto de 2023. **Descoberta de um Complexo Viroma Associado aos Patógenos do Cacau *Ceratocystis cacaofunesta* e *Ceratocystis fimbriata*.** Orientador: Eric Roberto Guimarães Rocha Aguiar. Co-orientador: Leandro Lopes Loguercio. Colaboradores: Sabrina Ferreira de Santana, Vinícius Castro Santos, Gabriela Nicolle Ramos Lizardo, Raner José Santana Silva, Ronan Xavier Corrêa, Aristóteles Góes-Neto, Carlos Priminho Pirovani, Paula Luize Camargos Fonseca.

A cultura do cacau tem uma história milenar e é considerada uma das principais culturas comerciais em várias regiões tropicais. Ela representa a principal fonte de renda para milhares de pequenos agricultores, que produzem mais de 80% do suprimento mundial de cacau. No entanto, as mudanças climáticas e o surgimento de patógenos representam desafios significativos para a produção sustentável dessa cultura. O gênero *Ceratocystis* é conhecido por causar a murcha de Ceratocystis no cacau, resultando em perdas expressivas para os agricultores. O controle desses fungos é trabalhoso e muitas vezes depende de fungicidas caros e/ou tóxicos para os seres humanos, o que tem motivado pesquisadores a buscar novas soluções para combater a proliferação desses patógenos, incluindo o uso de agentes biológicos como os micovírus. Os micovírus têm sido objeto de estudo como uma alternativa promissora no controle biológico de diversas doenças de plantas, incluindo aquelas causadas por fungos fitopatogênicos. Neste estudo, focamos na investigação da diversidade de microorganismos associados aos patógenos do *Theobroma cacao*, *Ceratocystis cacaofunesta* e *Ceratocystis fimbriata,* com um enfoque específico no viroma. nossa metodologia combinou o uso de dados de sequenciamento de RNA provenientes de fontes públicas com uma abordagem bioinformática detalhada. Isso incluiu aprimoramentos na sequência viral por meio de um processo de montagem integrado e a busca por correspondências em bases de dados externas. Essa abordagem multifacetada permitiu uma análise abrangente e precisa das características genéticas do vírus em questão. Por meio dessa estratégia, fomos capazes de identificar 4 vírus putativos dividido em 4 gêneros de *C. cacaofunesta* (*Hypovirus, Sclerotimonavirus, Alphapartitivirus e Narnavirus*) e 6 vírus putativos dividido em 4 gêneros de *C. fimbriata* (três *Alfaendornavirus*, um *Victorivirus*, um *Mitovirus* e um *Unuamitovirus*). Todas as sequências virais identificadas mostraram semelhança com genomas virais presentes em bases de dados públicas apenas a nível de aminoácidos, o que provavelmente indica que se trata de novas espécies virais. É relevante destacar que apresentamos o primeiro relato de vírus associados aos patógenos do cacau, *C. cacaofunesta* e *C. fimbriata*, e o segundo relato de espécies virais infectando membros da família *Ceratocystidaceae*. Nossas descobertas enfatizam a necessidade de condução de mais estudos prospectivos para descobrir a real diversidade de vírus que infectam fungos, o que pode contribuir significativamente para o desenvolvimento de novas estratégias de manejo. A identificação e compreensão desses micovírus podem abrir caminho para a criação de biofungicidas eficazes e ambientalmente amigáveis, que podem auxiliar no controle das doenças causadas pelos patógenos do cacau e, consequentemente, contribuir para a sustentabilidade dessa importante cultura comercial. Além disso, nossos achados ressaltam a importância da continuidade das pesquisas nessa área, visando o aprimoramento das técnicas de bioinformática e a identificação de novas espécies virais, promovendo avanços na agricultura e garantindo o futuro da produção de cacau de forma mais sustentável.

Palavras-chave: *Theobroma cacao*. Bioinformática. Micovírus. RNA de interferência.

ABSTRACT

ESPINAL, Roy Bogardid Ardón, M.S., Universidade Estadual de Santa Cruz, Ilhéus, August 2023. **Uncovering a Complex Virome Associated with the Cacao Pathogens *Ceratocystis cacaofunesta* and *Ceratocystis fimbriata*.** Advisor: Eric Roberto Guimarães Rocha Aguiar. Co-advisor: Leandro Lopes Loguercio. Collaborators: Sabrina Ferreira de Santana, Vinícius Castro Santos, Gabriela Nicolle Ramos Lizardo, Raner José Santana Silva, Ronan Xavier Corrêa, Aristóteles Góes-Neto, Carlos Priminho Pirovani, Paula Luize Camargos Fonseca.

The cocoa culture has a millennia-old history and is considered one of the main cash crops in various tropical regions. It represents the primary source of income for thousands of small-scale farmers who produce over 80% of the world's cocoa supply. However, climate change and the emergence of pathogens pose significant challenges to the sustainable production of this crop. The genus *Ceratocystis* is known to cause Ceratocystis wilt in cocoa, resulting in significant losses for farmers. Controlling these fungi is labor-intensive and often relies on expensive and/or toxic fungicides, motivating researchers to seek new solutions to combat the proliferation of these pathogens, including the use of biological agents like mycoviruses.
Mycoviruses have been studied as a promising alternative in the biological control of various plant diseases, including those caused by phytopathogenic fungi. In this study, we focused on investigating the diversity of microorganisms associated with *Theobroma cacao* pathogens, *Ceratocystis cacaofunesta* and *Ceratocystis fimbriata*, with a specific focus on the virome. Our methodology combined the use of RNA sequencing data from public sources with a detailed bioinformatic approach. This included viral sequence enhancement through an integrated assembly process and the search for matches in external databases. This multifaceted approach allowed for a comprehensive and accurate analysis of the genetic characteristics of the virus in question. Through this strategy, we were able to identify 4 putative viruses divided into 4 genera of C. *cacaofunesta* (*Hypovirus, Sclerotimonavirus, Alphapartitivirus*, and *Narnavirus*) and 6 putative viruses divided into 4 genera of *C. fimbriata* (three *Alfaendornaviruses*, one *Victorivirus*, one *Mitovirus*, and one *Unuamitovirus*). All identified viral sequences showed

similarity to viral genomes present in public databases only at the amino acid level, indicating they are likely new viral species. It is relevant to highlight that we present the first report of viruses associated with cocoa pathogens, *C. cacaofunesta* and *C. fimbriata*, and the second report of viral species infecting members of the *Ceratocystidaceae* family. Our findings emphasize the need for further prospective studies to discover the true diversity of viruses infecting fungi, which can significantly contribute to the development of new management strategies. The identification and understanding of these mycoviruses may pave the way for the creation of effective and environmentally friendly biofungicides that can assist in controlling diseases caused by cocoa pathogens and, consequently, contribute to the sustainability of this important cash crop. Furthermore, our findings underscore the importance of continued research in this field, aiming to improve bioinformatic techniques and identify new viral species, promoting advances in agriculture and ensuring the future of cocoa production in a more sustainable manner.

Keywords: *Theobroma cacao*. Bioinformatics. Mycovirus. RNA interference

1 INTRODUCÃO

O cultivo do *Theobroma cacao* L. possui uma relevância social significativa devido ao seu uso na alimentação e medicina ao longo de séculos, remontando a tempos anteriores à invasão espanhola entre os anos 1504 e 1525 (CILAS; BASTIDE, 2020). Nas antigas civilizações da América Central e México, essa cultura era considerada sagrada, sendo conhecida como a "bebida dos deuses" (CILAS; BASTIDE, 2020). Estudos recentes com análises moleculares revelaram que a origem e domesticação do cacau na região do alto amazonas , onde hoje se localizam o Equador e o Peru, datando de cerca de 5000 a.C. (ZARRILLO, et al, 2018).

Atualmente, o *T. cacao* L. é uma das principais culturas comerciais em diversas regiões tropicais do mundo, abrangendo uma área plantada de aproximadamente 8.2 milhões de hectares (BEKELE; PHILLIPS-MORA, 2019), e a produção de cacau atingiu 5,242 toneladas no período produtivo de 2020/21 (ICCO, 2023). A maior parte da produção de cacau concentra-se na África, responsável por cerca de 77.3% da produção global, seguida pela América com 17.8%, e Ásia juntamente com a Oceania com 4.8% (ICCO, 2023). As amêndoas de cacau são essenciais como matéria-prima na fabricação de chocolate, também são utilizadas pelas indústrias para a produção de produtos de beleza (FERNANDES et al., 2018).

Apesar da sua inegável importância econômica, a indústria do cultivo de cacau está atualmente enfrentando uma série de desafios substanciais, muitos dos quais estão intrinsecamente ligados às mudanças climáticas e ao aquecimento global. A interseção desses fatores tem criado um ambiente propício para o surgimento e ressurgimento de patógenos que exercem um impacto prejudicial na produtividade das plantações de cacau (ANDERSON; BAYER; EDWARDS, 2020).

De fato, as pressões ambientais e genéticas em curso estão atuando como impulsionadores para o aparecimento de novos patógenos e pragas de insetos. Essa convergência de estresses tornou-se uma ameaça substancial e iminente para a produção agrícola em escala global, representando um dilema crítico que requer atenção urgente

(CHALONER; GURR; BEBBER, 2021).

Entre os patógenos emergentes, o gênero Ceratocystis tem se destacado como um grupo de fungos ascomicetos que afetam diversas plantas economicamente importantes em todo o mundo (SCHOCH et al., 2020). Duas espécies de Ceratocystis merecem destaque: Ceratocystis fimbriata, que infecta quase 100 espécies hospedeiras, incluindo muitas plantas lenhosas levando à murcha e morte das árvores infectadas, e Ceratocystis cacaofunesta, um fungo especializado responsável pela murcha ou "Mal do facão" exclusivamente em plantas de Theobroma cacao e no gênero Herrania. (DE BEER et al., 2014; ENGELBRECHT; HARRINGTON; ALFENAS, 2007; NASUTION et al., 2019). A murcha de Ceratocystis ou Mal do facão no cacau é uma doença importante, levando à murcha e morte das árvores infectadas (ENGELBRECHT et al., 2007; NASUTION et al., 2019).

Nesse contexto, os micovírus têm sido estudados como possíveis agentes de controle biológico contra várias doenças de plantas causadas por fungos fitopatogênicos, sendo uma alternativa promissora para o manejo dessas doenças (MUNERET et al., 2019). Eles têm a capacidade de afetar fungos fitopatogênicos, resultando na indução de hipovirulência. Alguns exemplos bem-sucedidos de agentes de biocontrole (ABCs) viral incluem o *Cryphonectria hypovirus* 1 (CHV1) contra *Cryphonectria parasitica* e o *Fusarium oxysporum alternavírus* 1 (FoAV1) contra *Fusarium* (WAGEMANS et al., 2022).

Os avanços tecnológicos no sequenciamento de alto rendimento na área da bioinformática, juntamente com o sequenciamento de nova geração (NGS), têm possibilitado uma melhor caracterização dos micovírus em diversas espécies fúngicas (MU et al., 2017; NIBERT et al., 2014), desempenhando um papel significativo na descoberta de novos vírus em diversos organismos, incluindo fungos (MARZANO; DOMIER, 2016). As diferentes técnicas de NGS empregadas nessas análises são capazes de detectar a presença de várias sequências virais independentemente da amostra ou identificação prévia dos vírus presentes, eliminando a necessidade de estudos ou conhecimentos prévios sobre as sequências genômicas dos vírus (MOKILI; ROHWER; DUTILH, 2012; ROOSSINCK, 2015).

Nesta perspectiva, nossa abordagem de pesquisa concentrou-se na investigação do viroma associado a dois importantes patógenos do *Theobroma cacao*, o *C. cacaofunesta* e o *C. fimbriata*, utilizando dados públicos de sequenciamento de RNA. Através de uma estratégia de bioinformática meticulosa, conseguimos identificar diversos vírus em ambas as espécies do gênero *Ceratocystis*, incluindo membros das famílias *Hypoviridae*, *Alphaendornaviridae* e *Alphapartitiviridae*, os quais têm sido descritos como contendo vírus com potencial biotecnológico. É importante ressaltar que este é o primeiro registro de vírus *C. cacaofunesta* e *C. fimbriata*, além de ser o segundo relato de vírus infectando membros da família *Ceratocystidaceae*. Nossas descobertas enfatizam a necessidade de novas pesquisas para desvendar a real diversidade de micovírus que podem contribuir para o desenvolvimento de novos biofungicidas.

2 REVISÃO BIBLIOGRÁFICA

2.1 *Theobroma cacao através da história*

O cultivo do *Theobroma cacao,* juntamente com sua produção, domesticação, consumo e comercialização, tem uma história que remonta a vários séculos antes da invasão espanhola na América Central e no México, ocorrida entre 1504 e 1525 (CILAS; BASTIDE, 2020). O cacau era considerado sagrado pelas diferentes civilizações que habitavam essa região, sendo conhecido como a "bebida dos deuses". Acredita-se que a domesticação do cacaueiro tenha sido realizada pela civilização Olmeca a partir de 1000 a.C., sendo considerada a civilização mãe de toda a Mesoamérica (CILAS; BASTIDE, 2020). No entanto, estudos recentes indicam que o uso do cacau já era praticado na região do alto amazonas superior, onde atualmente estão localizados o Equador e o Peru, datando de cerca de 5000 a.C. (ZARRILLO et al, 2018).

Após a apropriação da planta do cacau e suas diversas aplicações pelos europeus, a cultura do cacau expandiu-se rapidamente. A primeira expansão ocorreu na América Central a partir do século XVI. Posteriormente, no final do século XVI e ao longo do século XVII, as plantações desenvolveram-se na América do Sul, especialmente na Colômbia, Equador e Venezuela. Nesse período, também foram estabelecidas algumas plantações no sudeste da Ásia (CILAS; BASTIDE, 2020).

A exportação de cacau, a partir do século XIX, teve como destinos principais a Ásia e a África. Essa expansão global da cultura do cacau teve um impacto significativo na economia e na cultura das regiões envolvidas. O cacau tornou-se uma mercadoria altamente valorizada e desejada, sendo amplamente utilizado na produção de chocolate e outras iguarias. As técnicas de cultivo e produção foram aprimoradas ao longo dos séculos, contribuindo para o aumento da produtividade e qualidade do cacau (CILAS; BASTIDE, 2020).

Atualmente, o *Theobroma cacao* L. é considerado uma das culturas comerciais mais importantes em diversas regiões tropicais do mundo, abrangendo uma área plantada de aproximadamente 8.2 milhões de hectares (BEKELE; PHILLIPS-MORA, 2019). Suas amêndoas são a principal matéria-prima utilizada na fabricação de chocolate pelas indústrias, bem como na produção de produtos de beleza (FERNANDES et al., 2018). A produção de cacau desempenha um papel vital como uma das principais fontes de renda para milhões de pequenos agricultores em diversos países. Esta cultura é responsável por mais de 80% da oferta global de cacau, conforme destacado por Bekele e Phillips-Mora (2019).

Em termos de estatísticas referentes ao período produtivo de 2020/21 divulgadas pela *International Cocoa Organization* (ICCO), um total de 5242 toneladas de amêndoas de cacau foram registradas. Quando analisamos a distribuição da produção entre os três principais continentes produtores, a África assume uma posição proeminente, representando 77.3% da produção total, seguida pela América com 17.8% e a Ásia e Oceania com 4.8%.

Ao explorar os principais países contribuintes para a produção global de cacau, a Costa do Marfim lidera o grupo, respondendo por 42.9% da produção, seguida por Gana com 20.0%, Equador com 7.0%, Camarões com 5.6%, Nigéria com 5.5%, Brasil com 3.8%, Indonésia com 3.2%, e Papua Nova Guiné com 0.8%. Além desses, outros países também contribuem com uma parcela total de 11.2% (ICCO, 2023). A distribuição entre esses países evidencia a complexa rede global de produção de cacau (Figura 1).

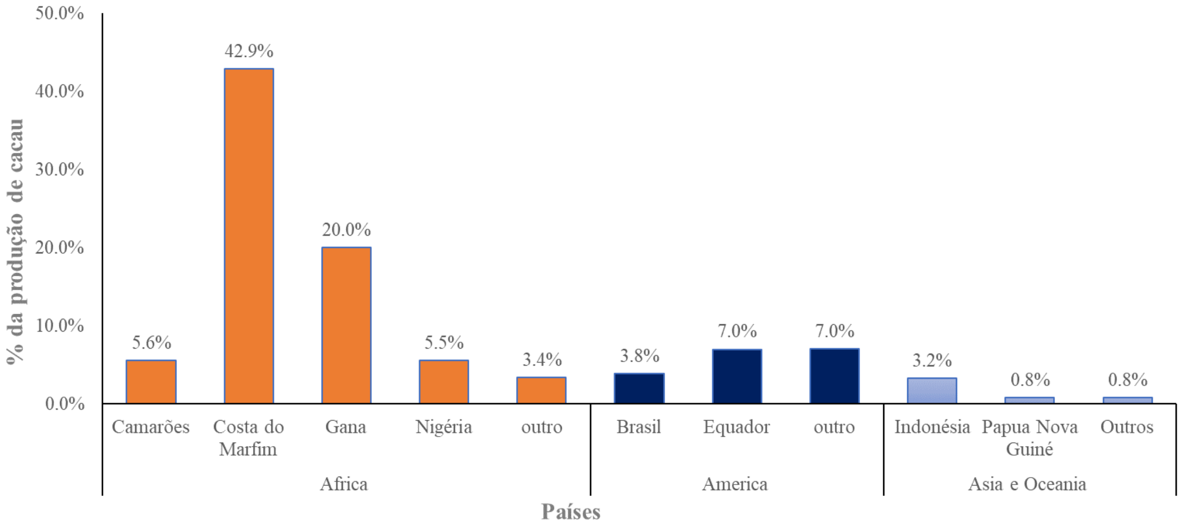


Figura 1. Porcentagem da produção de cacau dos principais países produtores no mundo, no período 2020/2021. Adaptado de ICCO (2023).

2.2 *Efeitos das mudanças climáticas na agricultura*

As atividades humanas, como industrialização, queimam de combustíveis fósseis, pecuária intensiva e desmatamento em larga escala de áreas inadequadas para cultivo, têm contribuído significativamente para o aumento na concentração de Gases de Efeito Estufa (GEE) na atmosfera (ANDERSON, BAYER, EDWARDS, 2020). Essas ações têm sido responsáveis pelo fenômeno do aquecimento global e pelas consequentes mudanças climáticas em escala global.

Além dos efeitos diretos no clima, essas atividades humanas também têm um impacto indireto na saúde dos ecossistemas e na agricultura. Patógenos que afetam as plantas, como *Puccinia graminis* (causador da ferrugem do trigo) (DEAN et al., 2012), *Xylella fastidiosa* (responsável pela doença de Pierce na videira) (ALMEIDA, NUNNEY, 2015), *Erwinia amylovora* (causador da doença do fogo bacteriano em árvores frutíferas) (NORELLI, JONES, ALDWINCKLE, 2003) e *Botrytis cinerea* (causador do mofo cinzento) (WILLIAMSON et al., 2007), são favorecidos pelas mudanças climáticas e podem ameaçar a segurança alimentar e a produção agrícola.

Esses patógenos podem se espalhar mais facilmente em condições climáticas em constante mudança e podem causar epidemias devastadoras em cultivos, afetando assim a disponibilidade de alimentos e a economia agrícola. Portanto, é essencial abordar tanto as emissões de GEE quanto a gestão de doenças de plantas para garantir a resiliência de nossos sistemas agrícolas e a saúde do planeta.(ANDERSON; BAYER; EDWARDS, 2020).

As mudanças climáticas a gerado consequências preocupantes para a segurança alimentar, uma vez que promovem o surgimento e ressurgimento de patógenos, exercendo grande pressão sobre a produção agrícola global (CHALONER; GURR; BEBBER, 2021). Os estresses bióticos e abióticos nas culturas, influenciados principalmente por fatores ambientais, como condições climáticas, e fatores genéticos, têm sido os principais impulsionadores do surgimento de novos patógenos e insetos pragas. Tais fatores criam um ambiente propício para o desenvolvimento e disseminação de doenças, ameaçando a produtividade das plantas cultivadas (HUSSAIN, 2015).

Esta influência fica clara na análise de diversos estudos científicos. Chakraborty e Newton (2011) oferecem uma visão abrangente de como as mudanças climáticas podem influenciar a epidemiologia de doenças vegetais, gerando preocupações diretas para a segurança alimentar. Bebber e colaboradores (2014) investigam como as alterações climáticas podem contribuir para a disseminação global de patógenos de plantas, o que representa uma ameaça à produção de cultivos em escala mundial. Anderson e colaboradores (2004) exploram a relação entre as mudanças climáticas, a contaminação por patógenos e o surgimento de doenças emergentes em plantas, ressaltando a complexidade dessa interação. Além disso, Pautasso e colaboradores (2010) discutem as implicações das mudanças climáticas na saúde das plantas e sua gestão em paisagens agrícolas e naturais, enfatizando a necessidade de estratégias de adaptação. Por fim, Garrett e sua equipe (2006) ampliam a perspectiva ao considerar como as mudanças climáticas podem afetar vários níveis, desde genomas até ecossistemas, em relação às doenças das plantas.

Os patógenos fitopatogênicos emergentes (PFE) são caracterizados por causarem novas doenças, apresentarem uma incidência anormalmente alta e infectarem novos hospedeiros, além de exibirem alterações ou variabilidade patogênica. Essas mudanças têm implicado em desafios adicionais para a agricultura, uma vez que novas doenças podem afetar culturas que antes eram consideradas resistentes ou imunes (CORREDOR-MORENO; SAUNDERS, 2020).

As infecções por patógenos em plantas podem ocorrer em diferentes velocidades, dependendo do ambiente em que a planta está localizada e das características específicas do próprio patógeno. Em condições ideais, a infecção do hospedeiro pode ocorrer rapidamente (CHALONER; GURR; BEBBER, 2020). A velocidade da infecção depende de uma série de fatores, incluindo a capacidade do patógeno de se estabelecer e se multiplicar no tecido da planta, a suscetibilidade da planta ao patógeno, a presença de condições ambientais favoráveis para a infecção e a resposta de defesa da planta (CHALONER; GURR; BEBBER, 2020).

Por exemplo, alguns patógenos são conhecidos por ter um ciclo de vida rápido e uma capacidade de reprodução eficiente, o que lhes permite infectar e se espalhar rapidamente dentro de uma população de plantas. Outros patógenos podem ter um ciclo de vida mais lento e depender de condições ambientais específicas, como umidade ou temperatura, para se desenvolver e se espalhar (CHALONER; GURR; BEBBER, 2020). Além disso, a suscetibilidade da planta desempenha um papel crucial na velocidade da infecção. Plantas geneticamente mais resistentes a um determinado patógeno podem resistir à infecção por mais tempo, enquanto plantas suscetíveis podem ser rapidamente sobrecarregadas pela infecção (CHALONER; GURR; BEBBER, 2020).

A resposta de defesa da planta também é um fator importante. As plantas têm mecanismos de defesa naturais para combater patógenos, como a ativação de genes de resistência ou a produção de substâncias antimicrobianas. A rapidez com que a planta pode montar uma resposta eficaz pode afetar significativamente a progressão da infecção. Nesse contexto, surge um desafio significativo para os melhoristas de plantas, que é gerar e aprimorar culturas mais resistentes (HUSSAIN, 2015), já que os percentuais de perdas produtivas por esses patógenos podem chegar a até 20% ou mais (PLOETZ, 2016).

2.3 *Gênero Ceratocystis*

Ceratocystis é um dos numerosos gêneros de fungos ascomicetos pertencentes à família *Ceratocystidaceae*, à ordem Microascales e à classe Sordariomycetes (SCHOCH et al., 2020). Esse gênero foi descrito em 1890 com base na espécie *C. fimbriata*, inicialmente descrita como agente causal da podridão negra da batata-doce (HALSTED, 1890; HOLLAND et al., 2019) encontrada em (*Ipomoea batatas*) nos Estados Unidos da América (EUA).

Até o momento, 66 espécies foram descritas nesse gênero (SCHOCH et al., 2020), incluindo várias espécies patogênicas agressivas que afetam uma ampla variedade de plantas economicamente importantes ao redor do mundo. A doença Ceratocystis Murcha do Cacau (CMC), popularmente conhecida como "doença mal do facão", tem origem nos patógenos como *C. fimbriata* que afeta a batata-doce, *C. platani* que prejudica as bananeiras, e *C. manginecans* que compromete as mangueiras. Esses agentes patogênicos, ao atacarem suas hospedeiras, frequentemente desencadeiam sintomas devastadores, incluindo podridão de raízes e tubérculos, aftas e murcha vascular. Essas manifestações de doenças, por sua vez, conduzem a perdas econômicas substanciais para os agricultores e, mais amplamente, colocam em risco tanto a viabilidade da produção agrícola quanto a saúde dos ecossistemas naturais circundantes (CABRERA et al., 2016; MOLANO et al., 2018).

A murcha de Ceratocystis do cacau é a principal doença causada por os patógeno *C. cacaofunesta* e *C. fimbriata*, resultando em murcha e morte das árvores infectadas (ENGELBRECHT et al., 2007; NASUTION et al., 2019). Os primeiros registros desse patógeno São reportados a 1918, no Equador, seguido pela Colômbia na década de 1940. Na década de 1950, foi observado em Trinidad, Venezuela e Costa Rica, embora possa estar presente desde 1926 (DELGADO-OSPINA et al., 2021). Na década de 1970, a doença começou a se espalhar da América Central para o Brasil, sendo registrado em 1978 no sudoeste da Amazônia, mais especificamente em Rondônia. Na década de 1990, ocorreu o primeiro registro de *Ceratocystis* na Bahia (DELGADO-OSPINA et al., 2021).

Em geral, entre as espécies de *Ceratocystis* relatadas até o momento, duas se destacam: *C. fimbriata*, que infecta quase 100 espécies hospedeiras, incluindo muitas plantas lenhosas e *C. cacaofunesta*, considerado um fungo especializado que causa murcha ou "Mal do facão" exclusivamente em plantas de *T.cacao* (Figura 2) e também em espécies do gênero *Herrania* (DE BEER et al., 2014; ENGELBRECHT; HARRINGTON; ALFENAS, 2007; NASUTION et al., 2019).

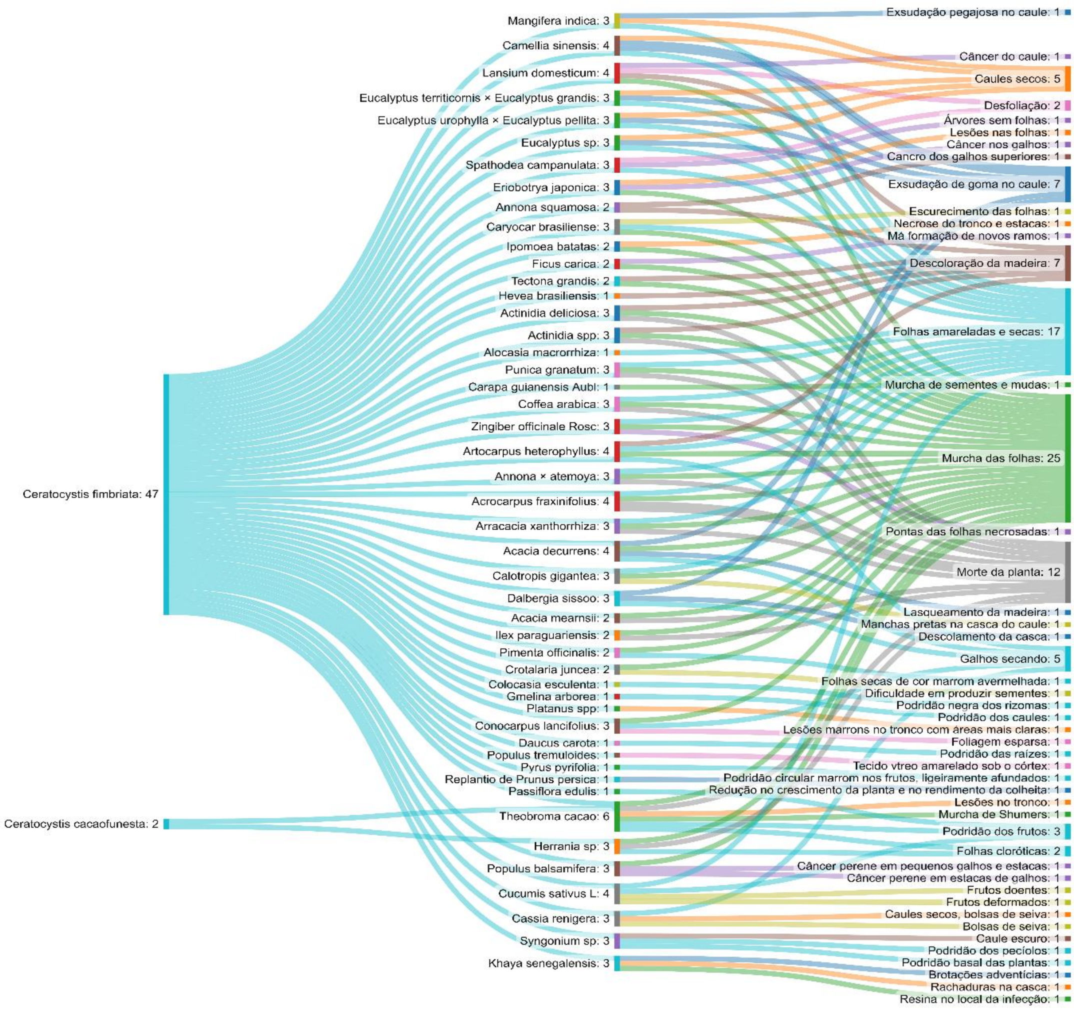


Figura 2. Impacto de *Ceratocystis Cacaofunesta* e *Ceratocystis fimbrita* identificada em diferentes espécies de plantas lenhosas (Fonte: autor).

2.4 *Morfologia e reprodução*

A literatura científica é uma fonte valiosa de informações sobre a relação entre características morfológicas de plantas e patógenos, bem como o impacto dos ciclos reprodutivos na disseminação de doenças vegetais. Agrios (2005) destaca a relevância das características morfológicas das plantas na interação com patógenos.

Além disso, os ciclos reprodutivos dos patógenos, especialmente dos fungos, desempenham um papel crucial na disseminação de ameaças para a saúde das plantas e ecossistemas (Fisher et al., 2012). Stukenbrock e colaboradores (2007) mostram como o ciclo reprodutivo influencia a especialização do patógeno em variedades de trigo. Essas referências enriquecem nossa compreensão das complexas interações entre características morfológicas e ciclos reprodutivos na propagação de patógenos e doenças vegetais, com implicações importantes para a pesquisa e prática agrícola.

Ceratocystis spp. possui dois estágios reprodutivos, sendo um deles a reprodução teleomórfica (sexual) e o outro a reprodução anamórfica (assexuada). O estágio teleomórfico é caracterizado pela presença de pequenas estruturas frutíferas, chamadas ascocarpos (ou ascomata), que variam em cor de escura a clara e têm forma subglobosa, globosa ou esférica. (Figura 3) (NASUTION et al., 2019).

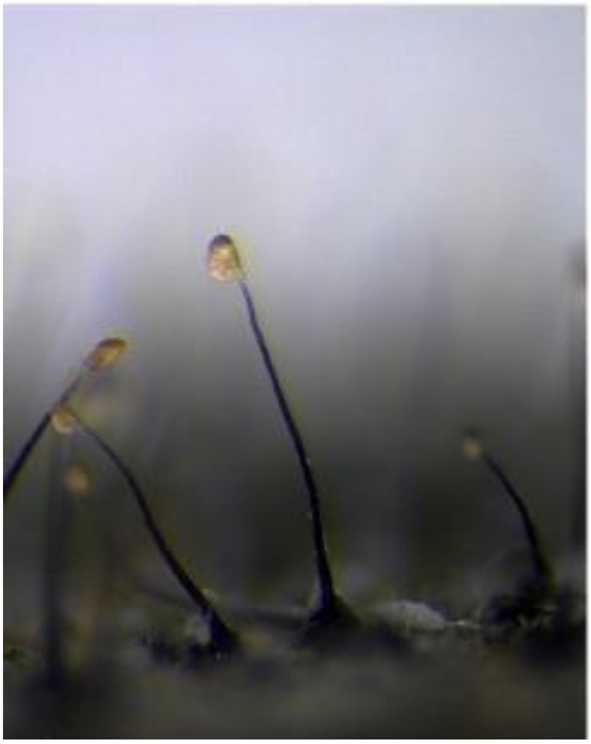


Figura 3. Morfologia dos ascomas da espécie *Ceratocystis* sl. Ascomata de *C. fimbriata* em substratos lenhosos com massas de ascósporos germinando de seus pescoços (DE BEER et al., 2014).

Os ascósporos são liberados dos ascocarpos por meio das hifas ostiolares localizadas nas pontas dos pescoços ascomáticos. Esses esporos são frequentemente envoltos por uma gota viscosa excretada pelo ascocarpo, o que facilita sua adesão a insetos (DE BEER et al., 2014).

O segundo estágio reprodutivo de *Ceratocystis*, conhecido como estágio anamórfico, é caracterizado pela presença de células conidiogênicas tubulares simples chamadas fiálidas. Essas fiálidas apresentam um estreitamento em direção ao ápice, resultando na produção de conídios retangulares ou conídios secundários escuros. Esses conídios têm uma forma de barril e são esporos assexuados (DE BEER et al., 2014; NASUTION et al., 2019). Algumas espécies de *Ceratocystis* são capazes de produzir clamidósporos ou aleuroconídios, que contribuem para a sobrevivência do fungo no solo. Esses esporos pigmentados possuem paredes espessas e se assemelham a clamidósporos, originando-se de conidióforos especializados (DE BEER et al., 2014; NASUTION et al., 2019).

2.5 *Progresso da CMC em cacaueiros*

2.5.1 Sobrevivência (fonte de inóculo)

As fontes de inóculo se referem às diferentes unidades infectantes do patógeno que podem ser encontradas em determinado local e têm potencial para causar danos patogênicos (HAYASAKA et al., 2015; PALADINES-REZABALA et al., 2022). No caso específico do patógeno *C. cacaofunesta*, as fontes de inóculo seriam as plantas doentes ou mortas, incluindo suas raízes, troncos e galhos (Figura 4). Essas partes da planta afetadas podem abrigar o fungo e ser uma fonte de propagação da doença, contribuindo para a disseminação da infecção para outras plantas saudáveis. É importante identificar e controlar essas fontes de inóculo para minimizar a propagação e os danos causados pelo patógeno *C. cacaofunesta* (HAYASAKA et al., 2015; PALADINES-REZABALA et al., 2022).

Figura 4. Ciclo CMC por *Ceratocystis cacaofunesta* em cacaueiros. Adaptação de Paladines-Rezabala et al. (2022).

2.5.2 Disseminação

Os patógeno *Ceratocystis* pode ser disperso por meio de ferramentas contaminadas, como facões, durante a poda de espigas e colheita (Figura 4) (SANTOS et al., 2013). Essa contaminação pode ocorrer quando as ferramentas entram em contato com plantas infectadas e carregam esporos ou outras estruturas do patógeno, que podem ser transferidos para árvores saudáveis, facilitando a disseminação da doença (SANTOS et al., 2013).

Além disso, insetos do gênero *Xyleborus* (Coleoptera: *Curculionidae*: *Scolytinae*) podem desempenhar um papel na disseminação do patógeno. Esses insetos podem se tornar vetores do *Ceratocysti*s, carregando esporos ou estruturas do fungo em seus corpos, contribuindo para a disseminação da doença (PALADINES-REZABALA et al., 2022).

2.5.3 Infecção.

As espécies do gênero *Ceratocystis* não possui estruturas especializadas penetrantes, como apressórios ou haustórios (CABRERA et al., 2016). A infecção por esse patógeno pode ser identificada por locais de necrose, como raízes com necrose, que indicam a presença da infecção (Figura 4) (CABRERA et al., 2016). Os aleuroconídios, que são esporos assexuados, podem ser transmitidos pelo solo a partir de raízes de árvores vizinhas infectadas, representando uma fonte potencial de transmissão subterrânea para árvores saudáveis (HARRINGTON, 2009).

Além disso, o *C. cacaofunesta* e *C. fimbriata* pode penetrar nas plantas de cacau por meio de ferramentas infectadas durante a colheita, bem como através de feridas causadas por insetos. Isso destaca a importância de tomar medidas de higiene durante a colheita e evitar ferimentos nas plantas, a fim de reduzir a disseminação do patógeno (SANTOS et al., 2013).

2.5.4 Colonização

Os patógeno *Ceratocystis* spp. apresenta dois estágios de colonização nos tecidos vegetais: um estágio biotrófico e um estágio necrotrófico (SUN et al., 2020). Na fase biotrófica, que ocorre em inoculações sem feridas nas folhas e caules, as hifas invadem os tecidos vegetais, tanto entre as células como dentro das células epidérmicas saudáveis. Na fase necrotrófica, ocorre a morte celular nas folhas e veias infectadas (SANTOS et al., 2013).

O *Ceratocystis* é caracterizado por ser um colonizador do xilema, o tecido vascular das plantas (PALADINES-REZABALA et al., 2022). Ao infectar os tecidos, o patógeno se espalha transversalmente pelos vasos do xilema, resultando em sintomas como murcha leve e amarelecimento das folhas. No entanto, a colonização e os sintomas variam dependendo do genótipo do cacau (SANTOS et al., 2013).

O período de colonização pelo patógeno pode levar entre 15 e 18 meses. No entanto, uma vez que os primeiros sintomas se manifestam, que geralmente ocorrem entre 10 e 30 dias após a infecção, a planta pode morrer repentinamente. As folhas afetadas, que estão completamente mortas, podem permanecer aderidas à planta por um longo período de tempo (Figura 5) (CABRERA et al., 2016).

2.5.5 Reprodução

C. cacaofunesta e *C. fimbriata* possui um sistema de acasalamento bipolar e é considerado um fungo homotálico devido aos mecanismos de mudança de acasalamento que ocorrem apenas em isolados Mat-2. Estudos genômicos recentes no loci Mat-1 de *C. fimbriata* revelaram a presença de três genes (Mat-1-1, Mat1-2 e Mat2-1) com domínios semelhantes aos genes MAT encontrados em outros fungos do grupo Ascomycota. No entanto, a distribuição desses genes em diferentes posições genéticas posições que determinam onde um gene está localizado em relação a outros genes e elementos genéticos no genoma e eles podem ser incomum e peculiares (CABRERA et al., 2016; WILKEN et al., 2014).

A temperatura desempenha um papel crucial no crescimento ótimo do patógeno. A faixa de temperatura ideal para a produção de ascósporos e crescimento micelial no hospedeiro, ou para a formação de aleuroconídios em diferentes substratos ou ambientes, situa-se entre 18°C e 28°C. Essa faixa de temperatura permite o desenvolvimento e a reprodução eficientes do *C. cacaofunesta* (SYAZWAN et al., 2021).

2.6 *Micovirus*

Há cerca de 60 anos, foi relatada a primeira ocorrência de micovírus no cogumelo comestível *Agaricus bisporus* (ANTON DE BARY, 1866).. Inicialmente, a identificação desses vírus era limitada à observação fenotípica das partículas virais por meio de microscopia eletrônica (GHABRIAL et al., 2015). No entanto, os avanços tecnológicos no sequenciamento de alto rendimento (SAR) permitiram uma melhor caracterização dos micovírus em diversas espécies fúngicas (MU et al., 2017; NIBERT et al., 2014).

Um estudo recente realizado por Villan Larios et al., (2023) identificou a existência de 267 espécies de micovírus, sendo que 189 dessas espécies são reconhecidas pelo Comitê Internacional de Taxonomia de Vírus (CITV). Dentre os micovírus descritos, a maioria possui genoma de RNA dupla fita (dsRNA), correspondendo a 38,6% da diversidade micoviral identificada. No entanto, os *Botourmiaviridae* são exemplos de micovírus que possuem genoma de RNA fita simples positiva (ssRNA+), representando aproximadamente 14% da diversidade micoviral conhecida.

A maioria das espécies de micovírus possui genomas de RNA, que podem ser RNA de fita simples (ssRNA) com sentido positivo (+), negativo (-) ou RNA de fita dupla (dsRNA) (DOMINGO; HOLLAND, 1997; VILLAN LARIOS et al. ., 2023). Os genomas de DNA de espécies de micovírus são encontrados em menor abundância (GHABRIAL et al., 2015). Um micovírus é considerado verdadeiro quando é capaz de infectar fungos saudáveis, e sua transmissão pode ocorrer de diferentes formas (VILLAN LARIOS et al., 2023). A transferência horizontal é um dos mecanismos mais comuns, ocorrendo quando há fusão citoplasmática entre hifas de fungos da mesma espécie. Já a transmissão vertical ocorre por meio de esporos assexuados, onde o micovírus é transmitido de geração em geração (VILLAN LARIOS et al., 2023).

A variabilidade genética das espécies fúngicas patogênicas resulta no surgimento dos micovírus como uma alternativa promissora no controle biológico de várias doenças de plantas (MUNERET et al., 2019). Devido à sua capacidade de influenciar a aptidão do hospedeiro de diferentes maneiras, os micovírus estão se tornando cada vez mais populares como agentes de biocontrole (ABCs), podendo ter efeitos benéficos, prejudiciais ou neutros. Além disso, eles têm a capacidade de afetar fungos fitopatogênicos, resultando na indução de hipovirulência (MUNERET et al., 2019).

A hipovirulência ocorre quando os patógenos são infectados pelos micovírus, resultando na perda de sua agressividade e redução da severidade da doença. Esse efeito é especialmente relevante, pois os micovírus têm o potencial de diminuir a capacidade dos patógenos de causarem doenças, proporcionando uma proteção eficaz para as plantas. Essas descobertas destacam a importância dos micovírus como agentes de biocontrole no manejo sustentável de doenças de plantas (SHARMA et al., 2018).

Um exemplo bem-sucedido de ABCs viral contra fungos fitopatogênicos é o *Cryphonectria hypovirus* 1 (CHV1), um micovírus que causa hipovirulência em *Cryphonectria parasitica.* Esse micovírus tem sido amplamente utilizado para reduzir a infecção de figo (WAGEMANS et al., 2022). Outro exemplo importante é o *Fusarium oxysporum alternavirus* 1 (FoAV1), que contribui para a redução do crescimento de fungos e dos sintomas da murcha de Fusarium, uma doença vascular em *Lilium brownie* (WEN et al., 2021). Além disso, o *Esclerotinia sclerotiorum partitivirus* 1 tem sido estudado em espécies fúngicas como *Sclerotinia sclerotiorum, S. nivalis, S. minor* e *Botrytis cinerea*, e foi observado que ele pode afetar a virulência do fungo hospedeiro nas plantas (XIAO et al., 2014). Esses exemplos destacam a eficácia dos micovírus como agentes de controle biológico no manejo de doenças causadas por fungos fitopatogênicos.

2.7 *Mecanismo de silenciamento de RNA como defesa antiviral em fungos.*

Devido à coexistência de diferentes espécies, os fungos desenvolveram mecanismos de proteção contra os patógenos que os atacam, incluindo os vírus. Esses mecanismos estão associados ao silenciamento de RNA de interferência (RNAi), que tem sido amplamente estudado em diversas espécies de fungos, como *Criponectria parasitica, Aspergillus nidulans* e *Roselinia necatrix* (SEGERS et al., 2007; YU et al., 2018). Nas espécies fúngicas, as vias de RNAi desempenham três funções principais: defesa genômica, formação de heterocromatina e regulação gênica (DANG et al., 2011), com a produção de pequenos RNA de interferência (vsiRNA) derivados do vírus (SEGERS et al., 2007). Esses estudos evidenciam a importância do RNAi como um mecanismo de defesa utilizado pelos fungos contra a infecção viral.

Vários estudos têm descrito a capacidade de diferentes espécies fúngicas em neutralizar múltiplas infecções virais através de mecanismos de RNAi com atividade antiviral, como observado na espécie *Cryphonectria parasitica* (NUSS, 2011). Esses mecanismos de silenciamento têm o poder de inibir a expressão de ácidos nucleicos invasores, incluindo os produzidos por vírus, transposons ou transgenes, em um amplo espectro de espécies de fungos (NICOLÁS; RUIZ-VÁZQUEZ, 2013; NUSS, 2011). Para que esses mecanismos funcionem, são necessários componentes celulares essenciais, como as proteínas dicer, Argonauta (AGO) e RNA polimerase dependente de RNA (RdRp), que estão presentes no maquinário de RNAi dos fungos (ZHANG et al., 2015).

No entanto, embora a maioria das espécies de fungos possua o mecanismo de RNAi, existem várias espécies de fungos filamentosos que aparentemente não possuem os componentes necessários para a produção de silenciamento de RNA, como observado em *Candida albicans* e *Ustilago maydis* (DANG et al., 2011; SEGERS et al., 2007), em como em leveduras *Saccharomyces cerevisiae* (DANG et al., 2011). Isso indica que alguns organismos unicelulares não requerem a via do RNAi (DANG et al., 2011). A ausência desse mecanismo de defesa contra micovírus nessas espécies permite que esses vírus causem fatores de hipovirulência em seus hospedeiros. A hipovirulência é um exemplo notável de interações multitróficas e, portanto, esses vírus têm um grande potencial como agentes de controle biológico contra várias doenças fúngicas (LIU et al., 2022; ZHU et al., 2018).

2.8 *Aplicação de ferramentas de sequenciamento de nova geração (NGS) na identificação de genomas virais.*

Os avanços tecnológicos na área da bioinformática, juntamente com o sequenciamento de nova geração (NGS), têm desempenhado um papel significativo na descoberta de novos vírus em diversos organismos, incluindo fungos (MARZANO; DOMIER, 2016). As diferentes técnicas de NGS empregadas nessas análises são capazes de detectar a presença de várias sequências virais independentemente da amostra ou identificação prévia dos vírus presentes, eliminando a necessidade de estudos ou conhecimentos prévios sobre as sequências genômicas dos vírus (MOKILI; ROHWER; DUTILH, ROOSSINCK, 2015).

Os dados de transcriptoma gerados a partir de diferentes fontes são extremamente valiosos e fornecem uma rica fonte de informações nas áreas evolutiva, genômica e funcional (ZHANG, 2018).. Eles têm desempenhado um papel importante na revelação de níveis significativos de diversidade genética em espécies virais anteriormente não exploradas *Ibid*.. Ao utilizar materiais essenciais para a construção de bibliotecas de sequenciamento de alto rendimento, é possível obter dados transcriptômicos que podem incluir sequências de vírus presentes nas amostras. Esses dados podem ser usados para caracterizar os vírus e realizar a montagem genômica *in silico* (ZHU et al., 2018).

Capítulo I

pathogens

MDPI

Article

Uncovering a Complex Virome Associated with the Cacao Pathogens *Ceratocystis cacaofunesta* and *Ceratocystis fimbriata*

Roy Bogardid Ardón Espinal [1,†], Sabrina Ferreira de Santana [1,2,†], Vinícius Castro Santos [3],Gabriela Nicolle Ramos Lizardo [1], Raner José Santana Silva [1], Ronan Xavier Corrêa [1], Leandro Lopes Loguercio [1], Aristóteles Góes-Neto [3], Carlos Priminho Pirovani [1],Paula Luize Camargos Fonseca [1] and Eric Roberto Guimarães Rocha Aguiar [1,*]

1 Center for Biotechnology and Genetics, Universidade Estadual de Santa Cruz, Ilhéus 45662-900, Brazil
2 Department of Microbiology, Universidade Federal da Bahia, Salvador 40170-115, Brazil
3 Department of Biochemistry and Immunology, Universidade Federal de Minas Gerais, Belo Horizonte 31270-901, Brazil
* Correspondence: ericgdp@gmail.com
† These authors contributed equally to this work.

Abstract: *Theobroma cacao* is one of the main crops of economic importance in the world as the source of raw material for producing chocolate and derivatives. The crop is the main source of income for thousands of small farmers, who produce more than 80% of the world's cocoa supply. However, the emergence, re-emergence and proliferation of pathogens, such as *Ceratocystis* spp., the causative agent of *Ceratocystis* wilt disease and canker disease, have been affecting the sustainability of many crops. Fungal control is laborious, often depending on fungicides that are expensive and/or toxic to humans, prompting researchers to look for new solutions to counteract the proliferation of these pathogens, including the use of biological agents such as mycoviruses. In this study, we investigated the diversity of microorganisms associated with the *T. cacao* pathogens *Ceratocystis cacaofunesta* and *Ceratocystis fimbriata* with a focus on the virome using RNA sequencing data available in public databases. We used a comprehensive bioinformatics pipeline containing several steps for viral sequence enrichment and took advantage of an integrated assembly step composed of different assemblers followed by sequence similarity searches using NCBI nonredundant databases. Our strategy was able to identify four putative *C. cacaofunesta* viruses (hypovirus, sclerotimonavirus, alphapartitivirus and narnavirus) and six *C. fimbriata* viruses (three alphaendornaviruses, one victorivirus and two mitoviruses). All the viral sequences identified showed similarity to viral genomes in public databases only at the amino acid level, likely representing new viral species. Of note, we present the first report of viruses associated with the cacao pathogens *C. cacaofunesta* and *C. fimbriata* and the second report of viral species infecting members of the *Ceratocystidaceae* family. Our findings highlight the need for further prospective studies to uncover the real diversity of fungus-infecting viruses that can contribute to the development of new management strategies.

Keywords: mycovirus; phytopathogen; virus; fungi; RNA interference

check for updates

Citation: Espinal, R.B.A.; de Santana, S.F.; Santos, V.C.; Lizardo, G.N.R.; Silva, R.J.S.; Corrêa, R.X.; Loguercio, L.L.; Góes-Neto, A.; Pirovani, C.P.; Fonseca, P.L.C.; et al. Uncovering a Complex Virome Associated with the Cacao Pathogens *Ceratocystis cacaofunesta* and *Ceratocystis fimbriata*. *Pathogens* **2023**, *12*, 287. https://doi.org/10.3390/pathogens12020287

Academic Editor: Rodrigo Rodrigues

Received: 27 December 2022
Revised: 10 January 2023

Accepted: 16 January 2023
Published: 9 February 2023

1. Introduction

Theobroma cacao L. is one of the most important cash crops in many tropical regions of the world, with planted areas comprising ~8.2 million ha [1]. The nuts are used to make chocolate and cosmetics [2]. The cacao crop is the main source of income for millions of small farmers in many countries (e.g., Ivory Coast, Ghana, Ecuador and Brazil), who account for more than 80% of the global supply of cocoa [3,4]. Overall, worldwide food security is threatened by the effects of biotic and abiotic stresses on crops, with environmental (mostly climatic) and genetic factors being the main causes of new pathogens and insect pests. Under these circumstances, a major challenge for plant breeders is the generation and improvement of resistant crops [5], since phytopathogens can reduce yields by up to 20% or more [6].

Ceratocystis (*Ascomycota* phylum, *Sordariomycetes* class) include aggressive pathogenic fungal species, which attack an array of economically important plant species worldwide. These pathogens cause the diseases Ceratocystis wilt and canker, which often kill the host plants, causing significant losses to farmers and jeopardizing the sustainability of agricultural production [7]. In the last two decades, emerging *Ceratocystis* diseases have increased considerably in Central and South America [7,8]; *Theobroma cacao* and *Eucalyptus* sp. can be infected by *Ceratocystis cacaofunesta* and *C. fimbriata*, respectively, which reduces the productive capacity of these plants [9]. The causal agent of *Ceratocystis* wilt of cacao (CWC, or 'machete disease') [10] is one of the three well-established host-specific pathogens in the genus [7], causing disease only to cacao plants [11]. Besides pruning, another transmission mechanism of this pathogen is through contact of the plant with different insect vectors, such as those of the *Xyleborus* genus (*Coleoptera Scolytidae*) [10,12,13]. This fungus penetrates the plant through holes and wounds. The CWC pathogenesis is characterized by irreversible damage to the plant's vascular system, leading to death on the order of 10^4 to 10^5 individuals per plantation, hence significantly reducing cocoa production per area [7,14]. This disease poses a serious threat to global cocoa production due to the high risk of its spread [7,15] by human activities, the main propagation mode described [3]. The control of this fungal pathogen is difficult, so the search for alternative economically viable and environmentally friendly solutions is necessary [14]. Previous research has described the potential of controlling oak wilt (caused by *C. fimbriata*) by using the bacterium *Pseudomonas chlororaphis* subsp. *aureofaciens*, based on the synthesis of volatile compounds by SPS-41, which showed significant inhibitory effects on mycelial growth and spore germination of *C. fimbriata* [16]. In terms of cacao (a perennial plant), one of the most efficient strategies to minimize losses is the selection of resistant varieties. However, the substitution of existing genotypes with resistant genotypes on plantations is a slow process. In addition, the various cacao pathogens and their specific modes of pathogenicity hamper the selection of plants simultaneously resistant to all of them [2]. Each pathogen affects different organs of the plant, e.g., *Moniliophthora perniciosa* in vegetative shoots, pods, and floral cushions [17]; *Phytophthora* spp. in pods and leaves; [18] and *Ceratocystis cacaofunesta* in the vascular system [11]. Finally, the use of biocontrol agents (BCAs) such as *Trichoderma* spp. has proven to be effective in inhibiting the *in vitro* growth and reducing spore germination of *C. cacaofunesta* [19]. The antifungal tebuconazole present in the medicinal plant *Adiantum latifolium* also had an important inhibition effect on mycelial growth and propagation of *C. cacaofunesta*, mainly in places where genetic selection and proper handling of instruments at the time of pruning fail altogether [14].

Among the BCAs, mycoviruses are good candidates, since they have been found to infect all major taxonomic groups of fungi, including phytopathogenic species [20]. Several mycoviruses cause abnormal symptoms in the host, such as hypovirulence and wasting [21]. A successful example of a viral BCA against phytopathogenic fungi is *Cryphonectria hypovirus* 1 (CHV1), which causes hypovirulence in *Cryphonectria parasitica* and has been used to reduce infection of figs [22]. Similarly, *Fusarium oxysporum alternavirus* 1 (FoAV1) was recently found to reduce the fungal growth and symptoms of *Fusarium* wilt (a vascular disease) in *Lilium brownii* [23]. For phytopathogenic fungi such as *Sclerotinia sclerotiorum*, *S. nivalis*, *S. minor* and *Botrytis cinerea*, *Sclerotinia sclerotiorum partitivirus* 1 can affect the virulence of the host fungus on the plant [24]. Thus, these and other mycovirus species are very promising as biological control agents against fungal diseases [21,25,26].

Fungi have developed different defense mechanisms against their own pathogens, including viruses. The conserved defense mechanisms based on RNA interference (RNAi) viral silencing have been studied in many fungal species, including *Cryponectria parasitica*, *Aspergillus nidulans* and *Rosellinia necatrix* [27,28]. These mechanisms act against invading nucleic acids, such as those produced by viruses, transposons or transgenes, in a wide spectrum of fungal species [26,29–31]. In fungi, the different RNAi pathways are related to three main functions: genomic defense, heterochromatin formation and gene regulation [32–35]. Although most fungal species possess the RNAi mechanism, there are several filamentous fungi (e.g., *Candida albicans* and *Ustilago maydis* [27,36]) and yeasts (e.g., *Saccharomyces cerevisiae* [32]) that seem to lack all or most of the components necessary for RNA silencing to occur [27,36]. This means that the RNAi pathway is not required by some unicellular organisms [32]. Fungal viruses are less explored than plant viruses, therefore their real diversity still unknown. Similarly, regarding plant viruses, most mycoviruses have been studied in the context of controlling fungal phytopathogens [37]. The application of bioinformatics and next-generation sequencing technologies (NGS) has contributed greatly to the discovery of new viruses in many organisms, including fungi [26]. NGS techniques have been used to detect the presence of viral sequences, regardless of the viral titer of the sample, since this method does not require prior knowledge of the genomic sequences of the candidate viruses [26,37,38].

We decided to investigate the virome associated with the important *T. cacao* pathogens *C. cacaofunesta* and *C. fimbriata* using public RNA sequencing data. Our bioinformatics strategy led to the identification of many viruses in both Ceratocysitis species, including members of *Hypoviridae*, *Alphaendornaviridae* and *Alphapartitiviridae* families that have been described as containing viruses with biotechnological potential. Of note, this is the first report of viruses associated with the cacao pathogens *C. cacaofunesta* and *C. fimbriata* and the second report of viruses infecting members of the *Ceratocystidaceae* family. Our findings highlight the need for new studies to uncover the real diversity of mycoviruses that can contribute to the development of new biofungicides.

2. Materials and Methods

2.1 Acquisition of RNA Libraries

Ceratocystis cacaofunesta and *Ceratocystis fimbriata* public RNA-seq libraries were downloaded from the Sequence Read Archive (SRA) database from NCBI. We used two libraries: one from *C. cacaofunesta* (SRR6217952) constructed from a medium enriched with cacaoxylem and deeply sequenced using the Illumina HiSeq 2500 Platform with a single layout [10] and one from *C. fimbriata* isolate CBS 114,723 (SRR8599076) constructed from a culture maintained at the Forestry and Agricultural Biotechnology Institute (FABI) of the University of Pretoria, which was deeply sequenced using the Ion Torrent Proton instrument, also with a single layout [39,40].

2.2 Bioinformatic Analysis

2.2.1 Transcriptome Assembly

Public RNA-seq libraries were preprocessed using the tools FastQC (version 0.11.9) [41]and Trim Galore (version 0.6.7 + galaxy0) [42]. Removal of host-derived sequences was performed by aligning preprocessed reads against each specific reference genome *C. cacaofunesta* (PEJQ01000008.1) and *C. fimbriata* (VNIJ01000001.1) using Bowtie2 (version 2.4.5 + galaxy1) [43] with default settings. The unmapped reads were used in the assembly step, taking advantage of different assemblers: SPAdes (version 3.15.4 + galaxy1) [44], rnaviralSPAdes (version 3.15.4 + galaxy1) [45] and Trinity (version 2.9.1 + galaxy2) [46],with default settings. The assembled transcripts derived from SPAdes, rnaviralSPAdes and Trinity were used as input for CAP3 (version 10.2011) [47] in order to consolidate the results and extend possible viral sequences.

2.2.2 Identification of Viral Sequences

The viral contigs were identified by sequence similarity search through the Diamond (version 2.0.15 + galaxy0) [48] with BlastX mode using the viral Refseq from NCBI as thedatabase. Statistical analysis of assembled contigs was performed with Fasta Statistics (version 2.0) [49] (Supplementary Table S1). All tools and programs used can be found online on the Galaxy Australia platform [50].

2.2.3 Manual Curation of Viral Genomes

Non-retroviral sequences were filtered by length >500 nt and checked by manual inspection including online Blast searches, structural annotation, and analysis of conserved domains. Nonredundant contigs were subjected to sequence similarity searches using the online Basic Local Alignment Search Tool [51] at the nucleotide and amino acid levels. Sequences identified with coverage greater than 30% with known species and E-values < $1x10^{-3}$ for protein and <$1x10^{-5}$ for nucleotide were further analyzed (Supplementary Table S2). Representative viral sequences from each group were selected, classified according to the highest bit score and used in subsequent analyses. Structural annotation regarding predicted open reading frames (ORFs) was carried out using the online tool ORFfinder [52] except for viruses related to the *Mitoviridae* and *Narnaviridae*families, for which genetic code 4 was used (Mold, Mitochondrial Protozoa and Coelenterates and Mycoplasma/Spiroplasma), with acceptance of "ATG" and alternative initiation codons. The ORFs were used for the identification of conserved protein domains as well as homologous superfamilies of the different amino acids of replication-associated proteins in the viruses *C. cacaofunesta* and *C. fimbriata* using the online tools InterProScan [53] and HMMER [54–56].

2.2.4 Integrative Genome Assembly

In order to improve possible viral genomes, an integrative assembly strategy was also performed running the different tools rnaviralSPAdes [45], metaSPAdes [57], Oases [58], Metavelvet [59] and IDBA_UB [60]. The results were consolidated using CD-HIT [61] to join sequences with more than 90% similarity. The assembled sequences were manually investigated and used along with raw read alignment to correct misassembled regions. Manually curated viral sequences identified in our work were deposited in the NCBI GenBank Third Party Annotation database under accession numbers BK062942-BK062946, BK063053-BK063059, and can also be found in the Supplementary Data.

2.3 Phylogenetic Analysis

Viral sequences that presented similarity to polymerase or polyprotein-coding genes were used in phylogenetic analysis. Datasets composed of public protein sequences (Supplementary Table S3) related to *Hypoviridae*, *Fusariviridae*, *Nyamiviridae*, *Mymonaviridae*, *Potyviridae*, *Partitiviridae*, *Fiersviridae*, *Botourmiaviridae*, *Narnaviridae*, *Mitoviridae*, *Tymoviridae*, *Endornaviridae*, *Cystoviridae* and *Totiviridae* were created considering close amino acid viral sequences and ICTV reference sequences. For each dataset, a global alignment was cre- ated using the online tool MAFFT [62]. The alignment was visualized and trimmed with AliView [63]. The edited alignment was used as input in the program ModelTest to select the best evolutionary amino acid model according to the Akaike information criterion (AIC), and then a maximum likelihood phylogeny tree was inferred with 1000 bootstrap replicates.This last step was performed with the CIPRES Science Gateway [64]. The trees were visualized in the online tool Interactive Tree of Life (ITOL v6) [65]. Lastly, to search for conserved RNA-dependent RNA polymerase (RdRp) motifs within the putative viral proteins, we used the NCBI CDD, v3.20-59693 PSSM search tool with standard parameters [66].

2.4 Genetic Diversity of Species and Abundance of Viral Sequences

The assembled consolidated transcripts derived from SPAdes, rnaviralSPAdes and Trinity assemblies were analyzed using the Kaiju platform [67] with standard parameters (Supplementary Table S4). In addition, to produce the reference transcriptome, we performed a second contig assembly using the programs SPAdes (version 3.15.4 + galaxy1) [44],rnaviralSPAdes (version 3.15.4 + galaxy1) [45] and Trinity (version 2.9.1 + galaxy2) [46],with raw reads followed by TransDecoder (version 5.5.0 + galaxy2) [68] to identify high-confidence transcripts. Finally, the Salmon quant software (version 1.5.1 + galaxy0) [69]was executed using the identified transcripts and viral sequences to assess viral abundance.

The host mitochondrial ribosomal protein S3 and nuclear beta-tubulin genes were selected endogenonus and standard genes for comparison with viral quantification.

2.5 Characterization of RNAi-Related Proteins in C. fimbriata

Identification: The proteins involved in the RNAi pathway, Dicer proteins 1 and 2 (DCL1 and DCL1), RNase III domain-containing protein (MRPL3), quelling deficient element genes (QDE), QDE-2-interacting protein (QIP), suppressor of ascus dominance (SAD) and suppressor of meiotic silencing (SMS) were identified based on annotation derivedfrom *Neurospora crassa* [32]. The *N. crassa* proteins were aligned against the *Ceratocystis fimbriata* annotation (Cfim3.0 NCBI) using sequence similarity searches with the Blast software. The homologous proteins identified were evaluated for the presence of conserved domain characteristics of each class of proteins using the InterProScan 5.59–91.0 tool. *Dendrogram*: Homologous proteins from *N. crassa* and *C. fimbriata* were aligned by the ClustalW tool [70], and analyzed with the parameters of distance calculation, fractional dissimilarity and bootstrap value of 100. The location and name of the identified domains of each sequence were used to construct the domain plot of the dendrogram with the Archeopteryx 0.9914 tool [71].*Abundance*: Transcripts corresponding to RNAi-related proteins in *C. fimbriata* were identified in their corresponding genome (Cfim3.0 NCBI) and used as a reference for quantification. The *C. fimbriata* transcriptome library (SRR16204154) was used for quantification, using the Salmon quant tool of the Galaxy platform with standard parameters.

3. Results

3.1 Transcriptome Assembly

The *C. cacaofunesta* (Cc) library presented 55,951,393 raw reads. After the quality filtering, 55,529,085 reads with Phred quality >20 were retained for further analysis. A total of 91.15% of the reads were aligned with the fungal reference genome. The reads that were not aligned with the reference genome were used in the transcriptome assembly. The assembly produced 1750 SPAdes transcripts, 965 rnaviralSPAdes transcripts and 480 Trinity transcripts. The consolidation and transcript extension step with CAP3 resulted in 1002 unambiguous sequences with N50 of 1932 and an average length of 892.9 nt. The *C. fimbriata* (Cf) library consisted of 42,480,313 raw reads. A total of 68.55% of the reads were aligned with the reference genome. The remaining reads were used in the assembly step, producing 7398 SPAdes transcripts, 10,709 rnaviralSPAdes transcripts and 64,961 Trinity transcripts. Transcript consolidation resulted in 52,493 nonredundant sequences with N50 of 729 and an average length of 593 nt (Supplementary Table S1).

3.2 Metagenomic Analysis

Transcriptomic data resulting from the consolidation of the data from the three different assemblers were used for initial screening using a metagenomics-based strategy with Kaiju. We observed transcripts derived mainly from fungi (Cc: 98.4%, Cf: 99.8%) and viruses (Cc: 1.6%, Cf: 0.1%) in *Ceratocystis* samples, with the exception of *C. fimbriata*, inwhich we also observed sequences derived from bacteria (0.1%) (Figure 1A,B). Regarding fungal diversity, in the sample derived from *C. cacaofunesta*, we observed the presence of transcripts assigned to species from different genera, such as *Marasmius*, *Gelatoporia* and *Armilaria*, while in *C. fimbriata*, we detected sequences related to *Thielaviopsis*, *Fusarium*,*Trichoderma* and *Colletotrichum*, among others (Figure 1C,D). The viral sequences detected included members of alphapartitivirus, sclerotimonavirus, an unclassified genus in *C. cacafunesta* and mitovirus, victorivirus and an unclassified genus in *C. fimbriata* (Figure 1C,D). Sequences derived from bacteria were only identified in *C. fimbriata* and were restricted to species related to *Escherichia* and *Clostridioides* (Figure 1D).

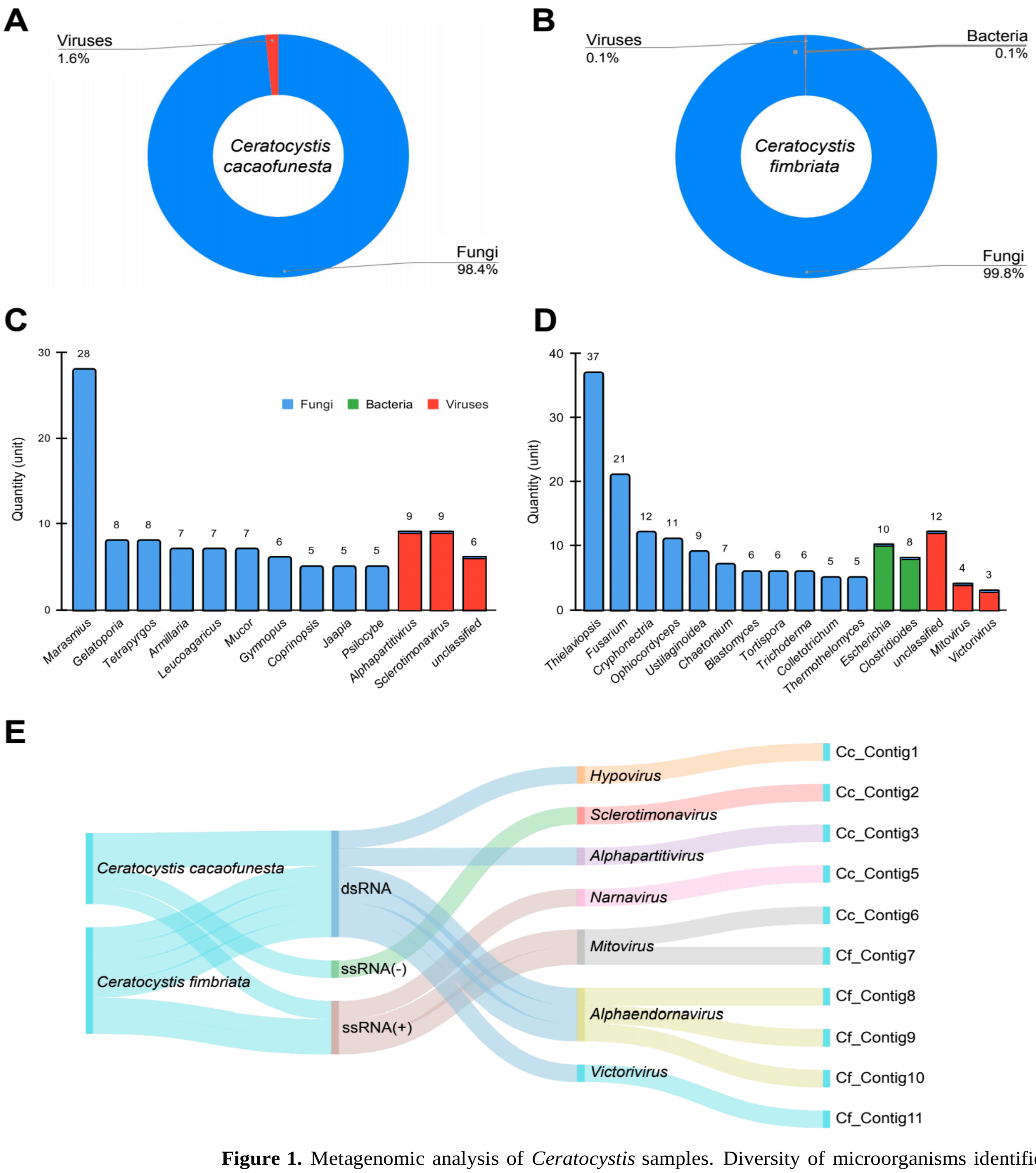


Figure 1. Metagenomic analysis of *Ceratocystis* samples. Diversity of microorganisms identified in *C. cacaofunesta* and *C. fimbriata* at the kingdom (**A**,**B**) and genus (**C**,**D**) level, respectively. (**E**) Sankey plot referring to the general diversity of transcripts derived from viruses identified in *Ceratocystis*samples classified by genome composition and viral family.

3.3 Virome Characterization

We identified a considerable number of viral sequences in the metagenomic analysis,so we further explored the presence of viruses in *Ceratocystis* samples. Therefore, we performed regular sequence similarity searches against NCBI nonredundant databases. For *C. cacaofunesta* and *C. fimbriata*, viral analysis identified the presence of double-stranded RNA viruses (dsRNA) and positive single-stranded RNA viruses (ssRNA+), while only in the case of *C. cacaofunesta*, we also identified elements relative to negative single-stranded RNA viruses (ssRNA−) (Figure 1E). At the genus level, members of the dsRNA genome included hypovirus, alphapartitivirus, alphaendornavirus and victorivirus, while ssRNA(+) was represented by narnavirus and mitovirus and, finally, ssRNA(−) by sclerotimonavirus (Figure 1E).

- *Hypoviridae*

The transcript assembled from the library of *C. cacaofunesta*, Cc_Contig1, presented 9920 nt and had similarity to viruses of the family *Hypoviridae* at the amino acid level. Structural annotation using NCBI ORFfinder indicated a large open reading frame (ORF) of 9450 nt surrounded by UTRs of 5′ and 3′ with 347 nt and 124 nt, respectively (Figure 2A). In Cc_Contig1, we detected the domains Helicase_ATP-bd (IPR014001) and Helicase_C (IPR001650) superimposed on the homologous superfamily P-loop_NTPase (IPR027417) and the DNA/ARN_pol_sf superfamily (IPR043502) (Figure 2A). According to the phylogenetic analysis, the putative virus was grouped with sequences from the genus *Hypovirus*(*Hypoviridae*), closely related to *Mycosphaerella hypovirus A* (MHV-A), with 100% bootstrap (Figure 2B). Indeed, analysis of conserved domains of the closely related MHV-A revealed the same domains identified in the Cc_Contig1, with the exception of the RNA-dir_pol_PSvirus domain (IPR007094), only identified in the MHV-A (Figure 2A and Supplementary Figure S1).This new virus was named Hypovirus cacaofunestae (HVc) to reflect the host origin.

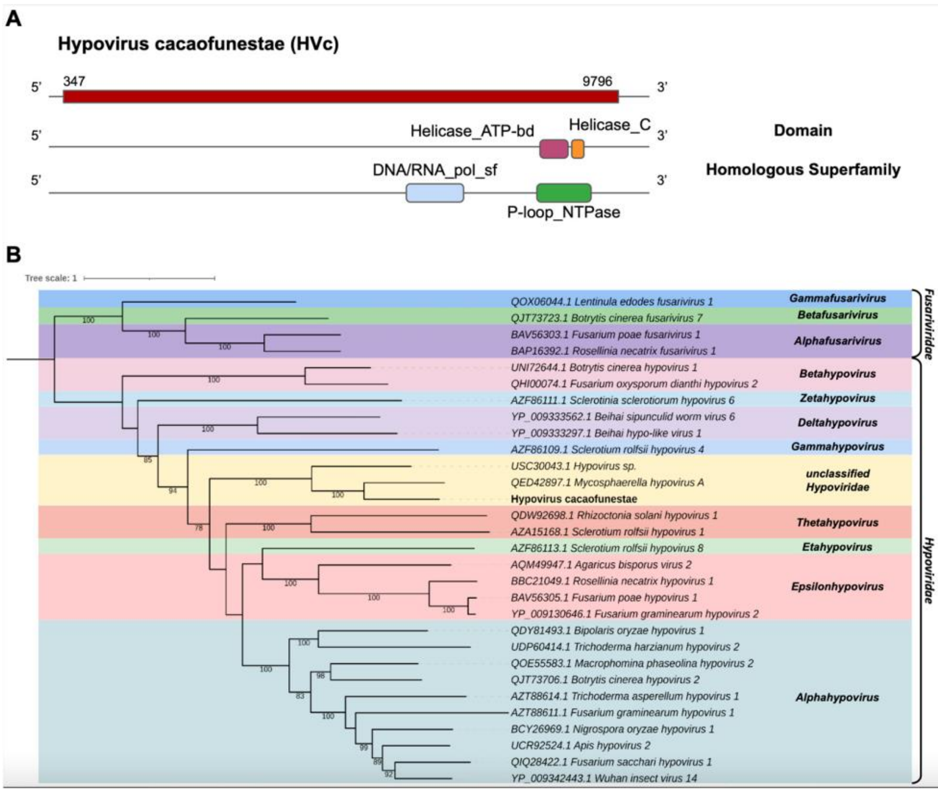


Figure 2. Characterization of the new *Hypovirus*. (**A**) Schematic representation of the novel hypovirus HVc with the ORF and domains represented. (**B**) Phylogenetic analysis using the ModelTest-NG according to the Akaike information criterion (AIC) indicated that the best evolutionary model was BLOSUM62 + F. Bootstrap values were established from 1000 replicates. Bootstrap values (%) lower than 70 are not shown.

Of note, in the HVc viral contig, we also identified conserved domains (CDD) that presented a central catalytic domain of RdRp of RNA viruses belonging to the Hypoviridae family, ps-ssRNAv_Hypoviridae_RdRp (cd23170), which is a member of the ps-ssRNAv_RdRp- like superfamily (cl40470) located in the interval 2079–23038 and with an E-value of 1.53 $x10^{-20}$. This region represents conserved polymerase motifs called conserved polymerase motif A, located at position 2149–2163 TRFSADITAYDANTP; polymerase B motif, located at positions 2217–2233 and 2235–2240 GGTGQSSTSWDNHWGMR..AMIMIW; and conserved polymerase C motif, located at positions 2255–2258 and 2259–2269 NSVH..TGDDNIWGTD, sites that are found in seven (there are only six identified) viral species, namely, *Cryphonectria hypovirus* 3 (AAF13604), *Phomopsis longicolla hypovirus* (YP_009051683), *Valsa ceratosperma hypovirus* 1 (YP_005476604), *Sclerotinia sclerotiorum hypovirus* 1 (YP_004782527), *Cryphonectria hypovirus* 4 (YP_138519) and *Setosphaeria turcica hypovirus* 1 (AZT8861) (Supplementary Figure S2). In addition to the previously mentioned conserved motifs, this viral sequence presented a DEAD-like_helicase_N superfamily (smart00487) located in the interval 2722–2880 with an E-value of 3.82×10^{-9}, which contains a DEAD box helicase motif DEFH, located at position 2818–2821.

- *Mymonaviridae*

One transcript assembled from the *C. cacaofunesta* library, Cc_Contig2, with a size of 5.652 nt, showed similarity to viruses from the *Mymonaviridae* family at the amino acid level. Structural annotation indicated a large ORF of 5496 nt surrounded by 5′ and 3′UTRs of 109 nt and 48 nt, respectively (Figure 3A). Conserved domains were identified in Cc_Contig2, such as Mononeg_RNA_pol_cat (IPR014023) and Mononeg_mRNAcap (IPR026890) (Figure 3A). Analysis of conserved domains of the closely related *Alternaria tenuissima negative-stranded RNA virus* 1 (AtNSRV1) revealed a very similar ORF length and the presence of the same domains identified in Cc_Contig2 (Figure 3A and Supplementary Figure S1). Phylogenetic analysis of the putative virus indicated a relationship with members of the genus *Sclerotimonavirus* (*Mymonaviridae*), closely related to *Alternaria tenuissima negative-stranded RNA virus* 1, with 99% bootstrap (Figure 3B). This new virus was named Sclerotimonavirus cacaofunestae (SVc).

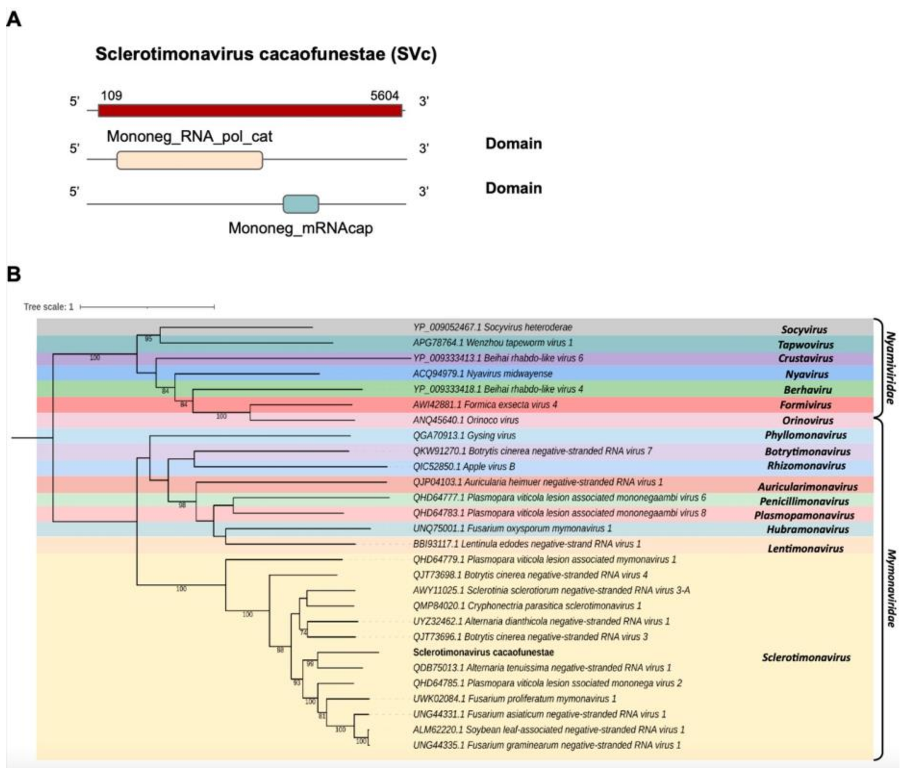


Figure 3. Conserved domains and phylogenetic analysis of sequences showing similarity to *Sclerotimonavirus*. (**A**) Schematic representation of the novel sclerotimonavirus SVc with the ORF and domains represented. (**B**) Phylogenetic analysis using the ModelTest-NG according to the Akaike information criterion (AIC) indicated that the best evolutionary model was BLOSUM62 + F. Bootstrap values were established with 1000 replicates. Bootstrap values (%) lower than 70 are not shown.

- *Partitiviridae*

The transcript Cc_Contig3 assembled in the *C. cacaofunesta* library with a size of 1998 nt was closely related to viruses from the *Partitiviridae* family at the amino acid level. Structuralannotation indicated a large ORF of 1866 nt surrounded by 5′ and 3′ UTRs of 85 nt and 48 nt, respectively. Another transcript from the *C. cacaofunesta* library, Cc_Contig4-Cp, with a size of 1818 nt, also showed similarity to viruses of the *Partitiviridae* family. This transcript was related to a coat protein whose structural annotation indicated an ORF of 1521 nt surrounded by 5′ and 3′ UTRs of 172 nt and 126 nt, respectively. Analysis of conserved domains in Cc_Contig3 revealed the presence of RNA-dir_pol_C (IPR001205) superimposed on the homologous superfamily DNA/RNA_pol_sf (IPR043502). On the other hand, Cc_Contig4-Cp did not have conserved domains (Figure 4A). Search for domains in the closest virus *Amasya cherry disease-associated mycovirus* (AcDAV) RdRp (currently *Cherry chlorotic rusty spot associated partitivirus*) revealed the presence of the same domains identified in Cc_Contig3 (Figure 4A and Supplementary Figure S1). The coat segment of AcDAV did not present domains, which was also true for Cc_contig4-Cp. According to the phylogenetic analysis, the putative virus is related to sequences from the genus *Alphapartitivirus* (*Partitiviridae*), closely related to the *Amasya cherry disease-associated mycovirus* (AcDAV) grouping with 98% bootstrap (Figure 4B). Therefore, the virus was named Alphapartitivirus cacaofunestae (APVc-RdRp).

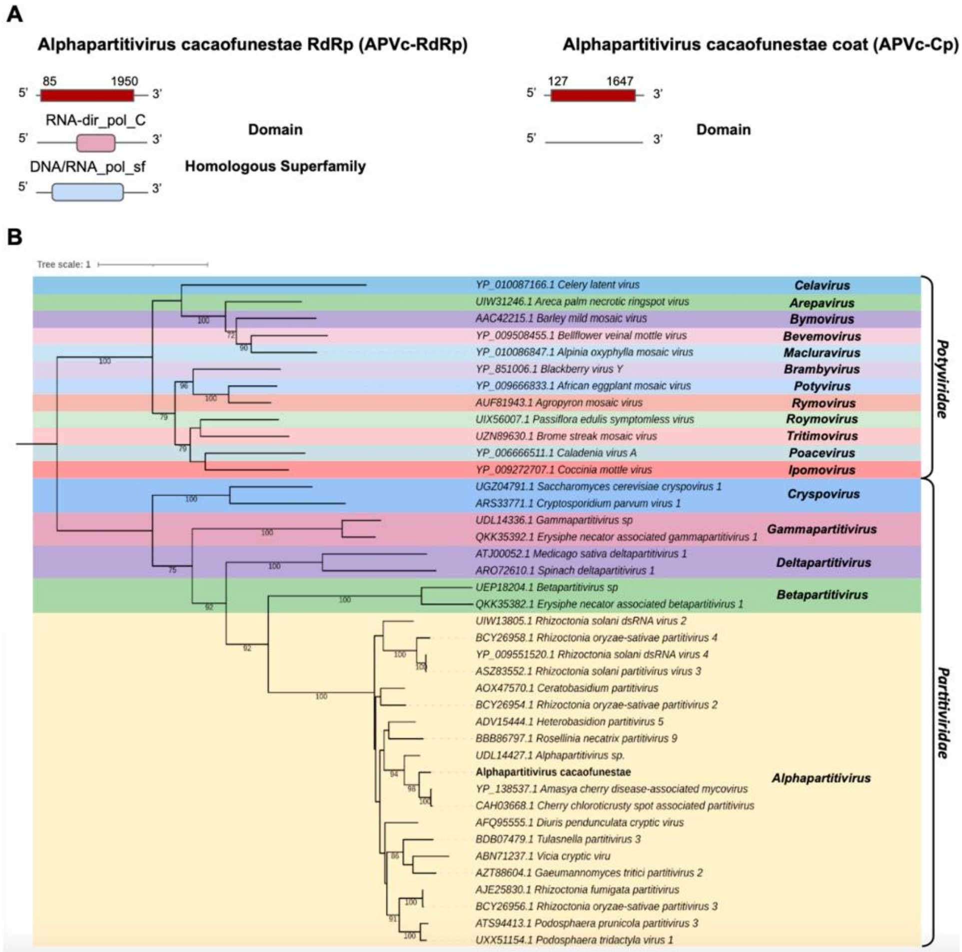


Figure 4. Characterization of the virus related to members of the *Partitiviridae* family. (**A**) Schematic representation of the novel partitivirus segments APVc-RdRp and APVc-Cp with the ORF and domains represented. (**B**) Phylogenetic analysis using the ModelTest-NG according to the Akaike information criterion (AIC) indicated that the best evolutionary model was VT + F. Bootstrap values were established with 1000 replicates. Bootstrap values (%) lower than 70 are not shown.

- *Narnaviridae and Mitoviridae*

Two transcripts assembled from the *C. fimbriata* library and the *C. cacaofunesta* library showed sequence similarity to members of the families *Narnaviridae* and *Mitoviridae*. The contig belonging to the *C. cacaofunesta* library, Cc_Contig5, with a size of 2409 nt, showed similarity to viruses of the *Narnaviridae* at the amino acid level. Structural annotation of Cc_Contig5 indicated an ORF of 2381 nt surrounded by UTRs of 3′ and 5′ with 28 nt and 102 nt, respectively. The second contig assembled from the *C. fimbriata* library, Cf_Contig6, presented a length of 3268 nt and showed a large ORF of 2322 nt surrounded by UTRs of 3′ and 5′ with 275 nt and 672 nt, respectively. The other contig assembled from the *C.fimbriata* library, Cf_Contig7, presented 2395 nt, and the structural annotation indicated an ORF of 1740 nt surrounded by UTRs of 5′ and 3′ of 324 nt and 332 nt, respectively. These two transcripts showed similarity to viruses of the *Mitoviridae* at the amino acid level. Of note, all three transcripts only presented large ORFs in genetic code 4 (Mold, Protozoan Mitochondrial and Coelenterate Mitochondrial and the Mycoplasma/Spiroplasma) consistent with the replication strategy of narnaviruses and mitoviruses. Cc_Contig5 did not present conserved domains. However, for Cf_Contig6 and Cf_Contig7 sequences, we observed the presence of the RNA_pol_mitovir family (IPR008686), which overlapped with the homologous DNA/RNA_pol_sf superfamily (IPR043502) (Figure 5A).

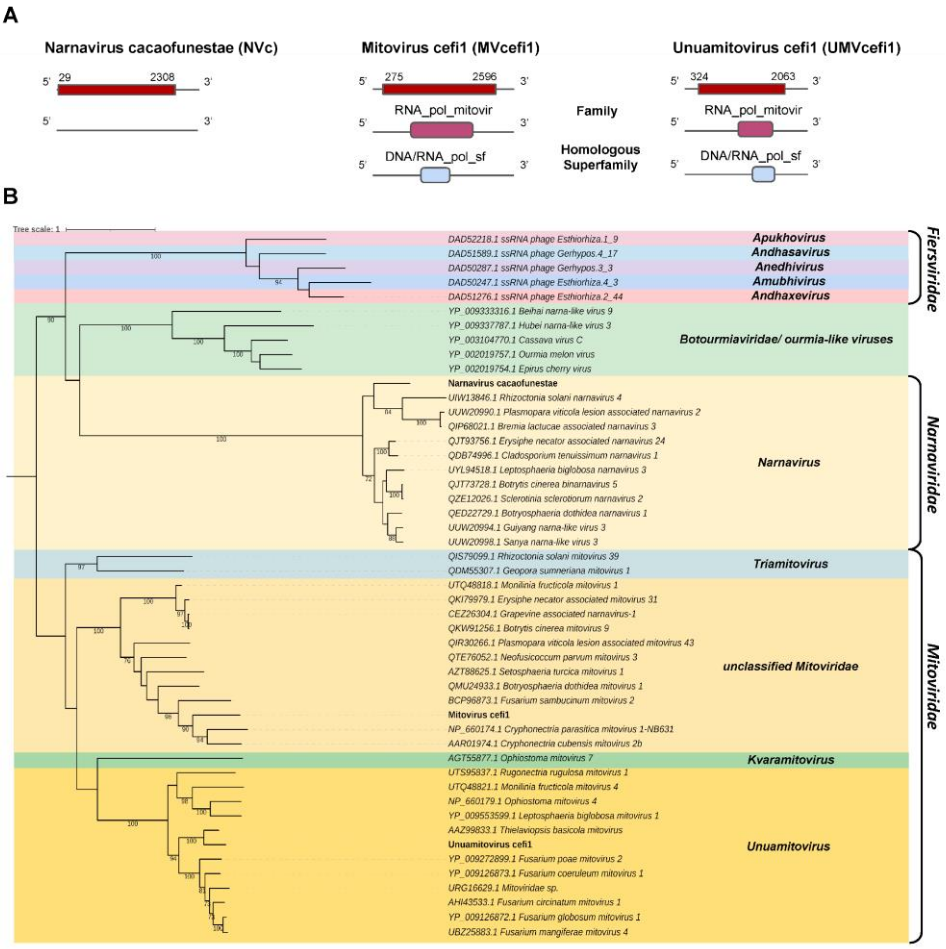


Figure 5. Phylogenetic analysis of sequences showing similarity to *Narnavirus*, *Mitovirus* and *Unuamitovirus*. (**A**) Schematic representation of the novel narnavirus NVc and mitoviruses MVcefi1 and UMVcefi1 with the ORF and domains represented. (**B**) Phylogenetic analysis using the ModelTest-NG according to the Akaike information criterion (AIC) indicated that the best evolutionary model was VT + F. Bootstrap values were established with 1000 replicates. Bootstrap values (%) lower than 70 are not shown.

Analysis of conserved domains of the closely related *Botrytis cinerea binarnavirus* 5 (BcBNV5), *Cryphonectria parasitica mitovirus* 1-NB63 1(CpMV1) and *Thielaviopsis basicola mitovirus* (TbMV) revealed the same domain profile observed in the transcripts Cc_Contig5, Cc_Contig6 and Cc_Contig7, respectively. The exception was the RT_dom domain (IPR000477), only identified in *Thielaviopsis basicola mitovirus* (TbMV) (Figure 5A and Supplementary Figure S1).

According to the phylogenetic analysis, the putative virus represented by the transcript Cc_Contig5 was grouped with members of the genus *Narnavirus* (*Narnaviridae*) within the clade containing BcBNV5 (Figure 5B). Cf_Contig6 formed a clade containing members of *Mitoviridae* but without genus designation. Finally, Cf_Contig7 was grouped with sequences from the genus *Unuamitovirus* (*Mitoviridae*), with 100% bootstrap. (Figure 5B). These new viruses were named Narnavirus cacaofunestae (NVc), Mitovirus cefi1 (MVcefi1) and Unuamitovirus cefi1 (UMVcefi1), respectively.

Further analysis regarding conserved motifs revealed that the NVc viral transcript presented a central conserved RNA-dependent RNA polymerase (RdRp) catalytic domain ps- ssRNAv_RdRp-like superfamily (cl40470), located in the interval 527–736 with an E-value of 2.35×10^{-7}. This region contained two conserved polymerase motifs called conserved polymerase A motifs, located at positions 612 and 631–644 D..FSSTDYQEATDAMQ, and conserved polymerase B motifs, located at positions 698–724 and 726–729 VLMGDPLTKPVLH- LVNI..VRRI. Both motifs were found in the ps-ssRNAv_Botourmiaviridae_RdRp domain (cd23183) belonging to the *Botourmiaviridae* family, order *Ourlivirales* (Supplementary Figure S2).

- *Endornaviridae*

We identified three transcripts in the *C. fimbriata* library showing sequence similarity to members of the *Endornaviridae* family. Cf_Contig8, with a length of 10,432 nt, presented an ORF of 10,431 nt surrounded by UTRs of 5′ and 3′ with 1 nt and 834 nt, respectively. For Cf_Contig9, we only reconstituted a fragment with an expected length of 5328 nt, likely representing a fragment of the large polyprotein gene. Similarly, Cf_Contig10 of 5790 nt denoted a partial ORF. All of them showed similarity to *Endornaviruses* at the amino acid level. Regarding the presence of conserved domains, Cf_Contig8 and Cf_Contig9 contained the RNA_virus_helicase_core_dom domain (IPR027351) superimposed on the homologous superfamily P-loop_NTPase (IPR027417), while Cf_Contig10 showed the RNA-dep_RNA_pol_C_virus (IPR001788) domain with integration of the conserved RNA-dir_pol_PSvirus (IPR007094) domains found to overlap with the homologous superfamily Rev_trsase/Diguanyl_cyclase (IPR043128) integrated with DNA/RNA_pol_sf (IPR043502) (Figure 6A).

Analysis of conserved domains of the *Tvarminne alphaendornavirus*, the closest viruses to Cf_Contig8, revealed the presence of shared domains, with the exception of the RNA-dep_RNA_pol_C_virus (IPR001788), RNA-dir_pol_PSvirus (IPR007094) and DNA/RNA_pol_sf (IPR043502) domains. The analysis of conserved domains of *Alphaendornavirus* sp., the closest virus to Cf_Contig9, revealed that they share two domains, with the exception of Mycovirus_RNAse (IPR046747), RNA-dep_RNA_pol_C_virus (IPR001788), RNA-dir_pol_PSvirus (IPR007094) and DNA/RNA_pol_sf (IPR043502). The analysis of conserved domains of *Morchella importuna endornavirus* 2 (MiEV2), the closest virus to Cf_Contig10, revealed that they also share two domains, with the exception of (+)_RNA_virus_helicase_core_dom (IPR027351) and P-loop_NTPase (IPR027417) only identified in MiEV2 and RNA-dir_pol_PSvirus (IPR007094) and Rev_trsase/Diguanyl_cyclase (IPR043128) only identified in Cf_Contig10 (Figure 6A and Supplementary Figure S1).

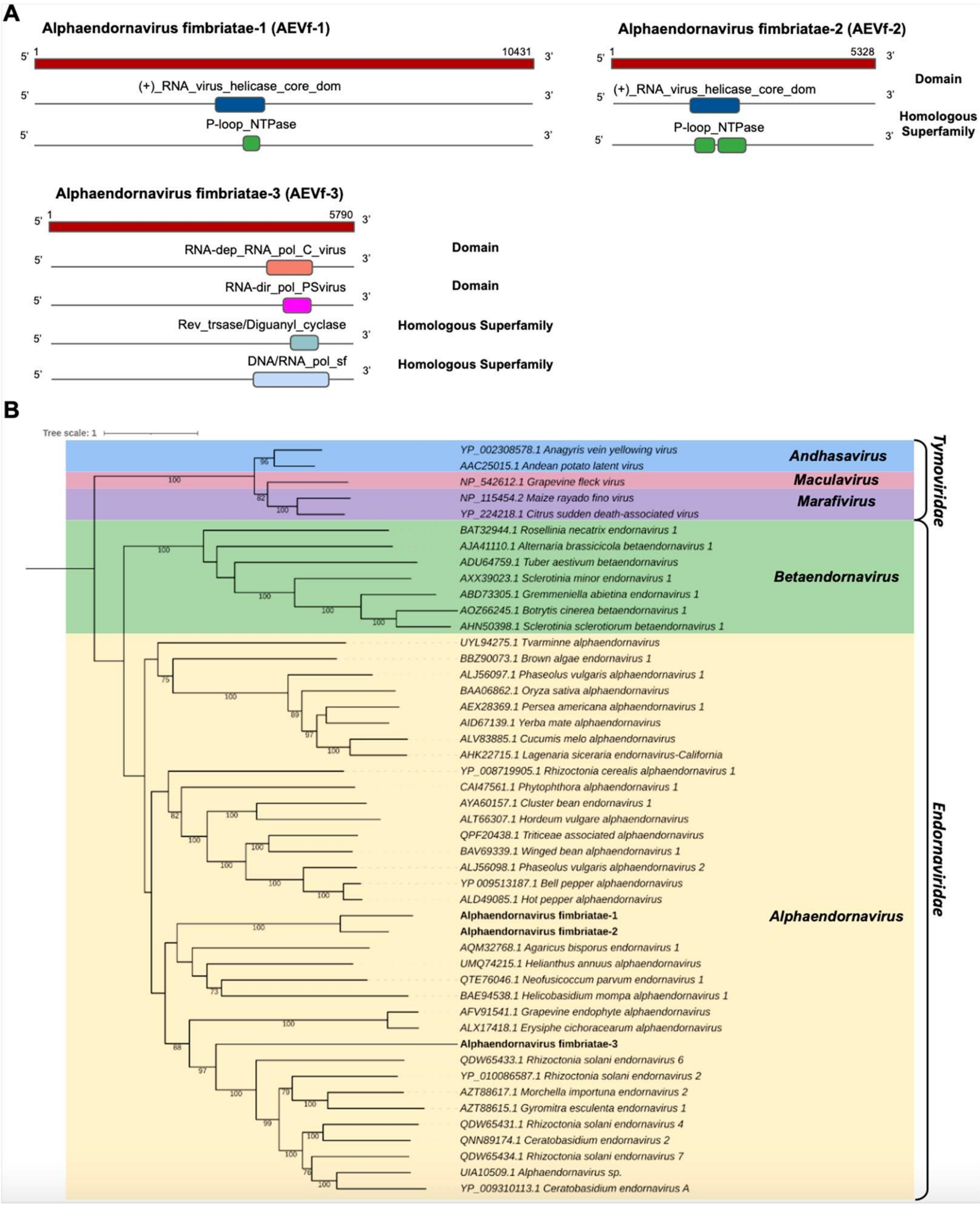


Figure 6. Conserved domains and phylogenetic analysis of sequences showing similarity to *Alphaendornavirus*. (**A**) Schematic representation of the novel alphaendornaviruses AEVf-1, AEVf-2 and AEVf-3 with the ORF and domains represented. (**B**) Phylogenetic analysis using the ModelTest-NGaccording to the Akaike information criterion (AIC) indicated that the best evolutionary model wasBLOSUM62 + F. Bootstrap values were established with 1000 replicates. Bootstrap values (%) lower than 70 are not shown.

The phylogeny of the putative viruses revealed that Cf_Contig8 and Cf_Contig9 were grouped together and, like Cf_Contig10, were closely related to members of the *Alphaendornavirus* genus (*Endornaviridae*) (Figure 6B). These new viruses were named Alphaendornavirus fimbriatae-1 (AEVf-1), Alphaendornavirus fimbriatae-2 (AEVf-2) and Alphaendornavirus fimbriatae-3 (AEVf-3), respectively.

AEVf-3 also presented a conserved central catalytic domain of the RNA-dependent RNA polymerase (RdRp) ps-ssRNAv_RdRp-like super family (cl40470), located in the interval 1491–1736 with an E-value of 1.52 x10^{-118}. This region represents conserved polymerase motifs called conserved polymerase A motif, located at position 2613–2627 LFVED- DLEKQDRQTD; conserved polymerase B motif, located at position 1673–1696 RLTGQAT-TALGNVITNLLVHSRLV; and conserved polymerase C motif, located at position 1704–1718 VIMMCLGDDNLMVCR. All motifs are found in the Endornaviridae_RdRp (cd23255) domain, belonging to the *Endornaviridae* family (Supplementary Figure S4).

- *Totiviridae*

One of the transcripts assembled in the *C. fimbriata* library, Cf_Contig11, with a length of 3995 nt was related to viruses from the *Totiviridae* family at the amino acid level. Cf_Contig11 contained an ORF of 3285 nt surrounded by 5′ and 3′ UTRs with 393 nt and 318 nt, respectively. We observed that the presence of the conserved domain RNA-dir_pol_luteovirus family (IPR001795) overlapped with the homologue superfamily DNA/RNA_pol_sf (IPR043502). Another transcript in the *C. fimbriata* library, Cf_Contig12, with a size of 2493 nt also showed similarity to viruses of the *Totiviridae* at the amino acid level, with an ORF of 2034 nt surrounded by 5′ and 3′ UTRs with 459 nt and 1 nt, respectively, and presenting the conserved domain for the Totivirus_coat family (IPR008871) (Figure 7A). The analysis of conserved domains of the closely related *Totiviridae* sp. sequence revealed the presence of the same domains observed for Cf_Contig11 (Figure 7A and Supplementary Figure S1).

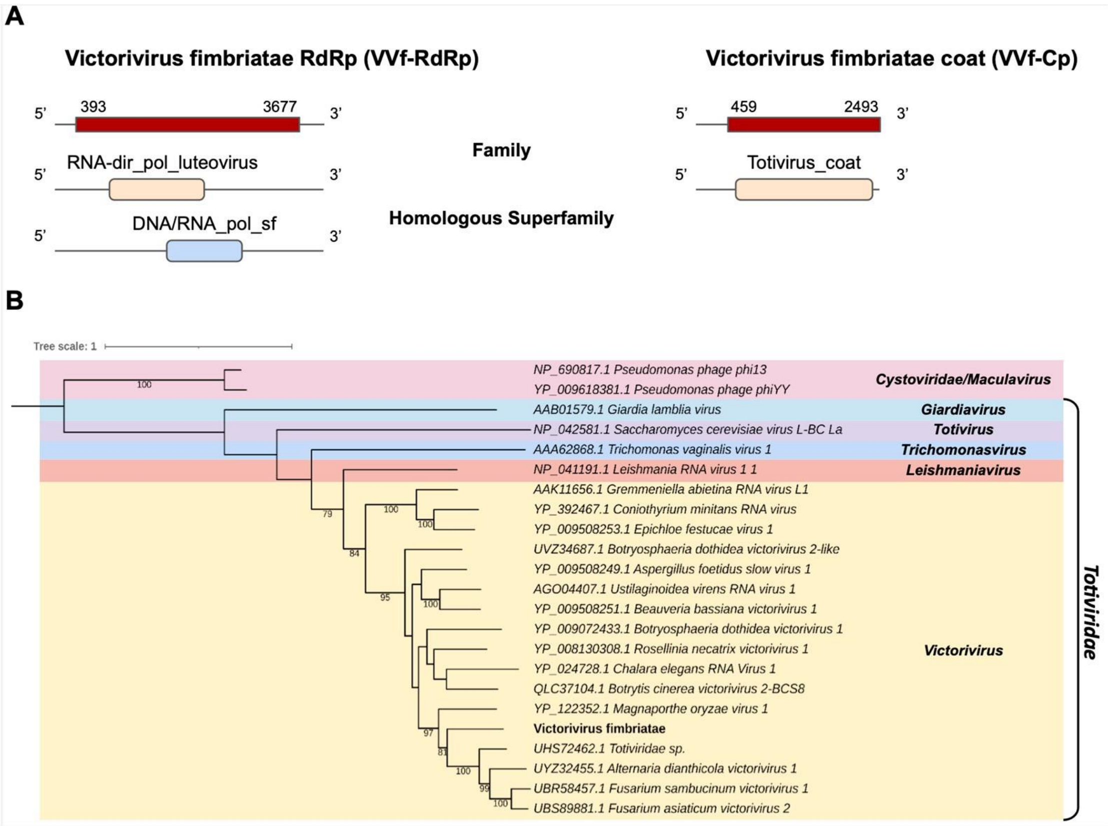


Figure 7. Conserved domains and phylogenetic analysis of sequence showing similarity to *Victorivirus*. (**A**) Schematic representation of the novel victorivirus segments VVf-RdRp and VVf-Cp with the ORF and domains represented. (**B**) Phylogenetic analysis using the ModelTest-NG according to the Akaike information criterion (AIC) indicated that the best evolutionary model was BLOSUM62 + F. Bootstrap values were established with 1000 replicates. *Cystovirus* was used as an outgroup to root the tree. Bootstrap values (%) lower than 70 are not shown.

According to the phylogenetic analysis, the putative virus was grouped with sequences from the genus *Victorivirus* (*Totiviridae*), closely related to *Totiviridae* sp. (Figure 7B). This new virus was named Victorivirus fimbriatae (VVf).

3.4 Abundance of Viral Sequences

Unexpectedly, we observed a complex diversity of viruses infecting the fungal species. Therefore, we decided to investigate the abundance of these viral species in comparison with endogenous nuclear and mitochondrial genes. Regarding the putative viruses identified in *C. cacaofunesta*, HVc showed the highest abundance with 404.689 TPM, while SVc presented the lowest with 15.258 TPM (Figure 8A). Of note, the constitutive genes beta-tubulin and mitochondrial RPS3 accounted for 0.081 and 0.253 TPM, respectively. Our results suggest that the transcriptional activity of the *C. cacaofunesta*-infecting viruses is ~180 to ~5000 times higher than the constitutive nuclear gene (Figure 8A). In the case of viruses identified in *C. fimbriata*, VVf had the greatest abundance with 11.289 TPM, while AEVf-2 showed the lowest with 0.846 TPM (Figure 8B). Unlike what was observed for *C. cacaofunesta*, *C. fimbriata* infecting viruses showed a transcriptional activity closer to that detected for the marker genes beta-tubulin and mitochondrial RPS3, with less than a10-fold difference (Figure 8B).

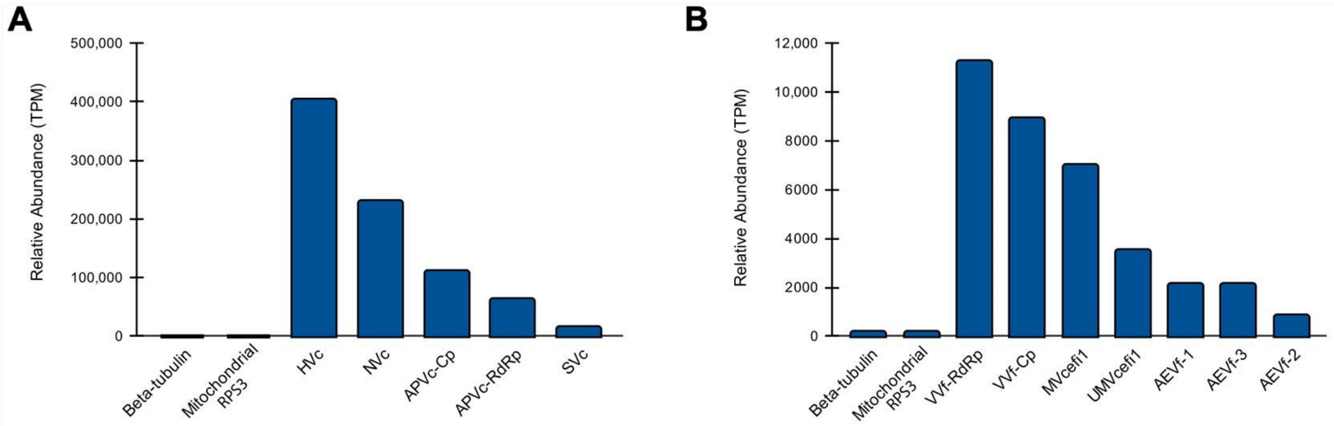


Figure 8. Abundance of viral sequences in Ceratocystis samples. Relative abundance of new viruses identified in *C. cacaofunesta* (**A**) and *C. fimbriata* (**B**). For both organisms, we included the quantification of the constitutive genes beta-tubulin and mitochondrial RPS3.

3.5 Characterization of RNA-Interference-Related Genes in Ceratocystis Fimbriata

Other studies have shown that fungi can contain RNA interference (RNAi) machinery with important roles in genome stability and antiviral response [32]. Therefore, we hypothesized that RNAi could be involved in the management of multiple viral infections by fungi. Thus, we investigated whether their genomes contained the RNAi core genes and whether they were transcriptionally active. Since the genome of *C. cacaofunesta* is not annotated, we performed characterization of RNAi genes in *C. fimbriata*. Using proteins identified and validated experimentally in the model fungus *Neurospora crassa*, we searched for RNAi-related genes including the RNA-dependent RNA polymerases QDE-1 and SAD-1, Argonaute-2 orthologs, QDE-2 and SMS2 and Dicer, among others (Supplementary Table S5 and Figure S5A). The RNAi-related proteins identified had similar lengths and presence of conserved domains, including all canonical elements expected for Argonaute and Dicer genes (Supplementary Table S5 and Supplementary Figure S5A). Quantification of RNAi-related genes detected transcriptional activity for all of the genes assessed, with Argonaute orthologues showing higher abundance and SAD and SMS2 presenting the lowest number of transcripts detected.

4. Discussion

Ceratocystis in general accommodates many important pathogens of agricultural crops and woody plants. *C. fimbriata* was the first to be described and can be found in plantations mainly of *Ipomoea batatas* [11,72–74], *Mangifera indica* [75,76], *Eucalyptus* sp. [75,77,78], *Ficus carica* [75,79], *Punica granatum* [80,81], *Colocasia esculenta* [80,82,83], *Theobroma cacao* [11], *Gmelina arborea* [84,85], *Syngonium* sp. [86,87] and *Ilex paraguariensis* [88,89]. In contrast, *C. cacaofunesta* has only been described in *Theobroma cacao* [90] and in species from the genus *Herrania* sp. [91].

Metatranscriptomic analyses indicated the presence of several microorganisms in the libraries, including elements derived from bacteria and viruses. This diversity of microorganisms may be related to the culture medium used for fungal growth, which was enriched with cocoa xylem of *C. cacaofunesta* [10] and was extracted from an isolate preserved in a culture collection and subjected to genome sequencing of *C. fimbriata* [39,40].Nevertheless, the possible presence of these species does not interfere with the result, since the viral sequences assembled in our study are from families described as infecting fungi, not bacteria. Furthermore, their abundance was hundreds of times lower than that of *C. cacaofunesta* and *C. fimbriata*, inconsistent with the abundance of viruses in the sample.

Hypoviridae is a family of capsidless viruses that present a positive-sense ssRNA genome of 9.1 to 12.7 kb encoding one or two ORFs [92,93]. These characteristics are similar to those found in our research. In addition, when comparing the sequence called HVc with *Mycosphaerella hypovirus* A, we observed similarities in terms of the conserved domains found, demonstrating the probable membership of the new virus in the *Hypoviridae* family. It is worth mentioning that other studies have identified *Hypovirus* in other fungal species, such as the brown rot fungus *Monilinia fructicola* reported by De Miccolis Angelini and collaborators (2022) [94], who named them *Monilinia fructicola hypovirus* 1 and *Moniliniafructicola hypovirus* 2, or MfrcHV1 and MfrcHV2, respectively. In MfrcHV1, the polyprotein had three conserved domains (an RdRp, a putative peptidase PPPDE and a C-terminal helicase), while MfrcHV2 had two conserved domains in the polyproteins (the superfamilies 1 and 2 oftype 1 ATP binding and C-terminal helicases), presenting similarity with the conserved domains present in the viral contigs described in our study.

Other researchers who have reported the identification and characterization of hypoviruses are Velasco and collaborators (2018) [95], studying *Entoleuca* sp.; Li and collaborators (2020) [96], studying *Bipolaris oryzae;* Wang and collaborators (2022) [97], who studied *Fusarium graminearum hypovirus* 1 (FgHV1) and demonstrated that it has proteins with characteristics such as suppression of host RNA silencing; and Aulia and collaborators (2021) [98], who studied the fungus *Cryphonectria parasitica* and showed that it facilitates infection by inducing hypovirulence. The viral contig HVc was the first hypovirus detected in *Ceratocystis cacaofunesta.*

The viruses from the *Mymonaviridae* produce filamentous and enveloped virions containing a single negative-sense linear RNA molecule of about 10 kb. Mimonaviruses usually infect filamentous fungi, and one of the negative-strand RNAs of one virus, *Sclerotinia sclerotiorum* virus 1, induces hypovirulence in the fungal host [99]. Our phylogenetic analysis suggests a new virus genome belonging to this family, SVc. Furthermore, a previous study by Li and collaborators (2022) [100] characterized a new ssRNA( ) mycovirus isolated from the fungus *Auricularia heimuer* CCMJ1222, while another study, by Hao, Wu & Li (2018) [101], described an isolate of the phytopathogenic fungus *Botrytis cinerea.* The viral contig SVc was the first sclerotimonavirus detected in *Ceratocystis cacaofunesta.*

According to Vainio and collaborators (2018) [102], *Partitiviridae* is a family of small, isometric, nonenveloped viruses with bisegmented double-stranded (ds) RNA genomes of 3–4.8 kbp. The two genome segments are individually encapsulated and have a single large ORF or two ORFs, as in the current study where an ORF was presented, identifying and characterizing a new virus belonging to the *Partitiviridae* family, which presented a fragment of RdRp and a coat protein segment, which were named APVc-RdRp and APVc- CP, respectively, due to their phylogenetic proximity to alphapartitiviruses. Furthermore, Jiang and collaborators (2021) [103] studied the fungus *Aspergillus nidulans*, and Moriyama and collaborators (2021) [104] analyzed the phytopathogen *Rosellinia necatrix*, the causative agent of root rot, also identifying viral sequences related to this family. Finally, Deng and Boland (2007) [105] reported viruses belonging to the genus *Partiviridae* isolated from *Ceratocystis resinifera*, called named *Ceratocystis partitivirus* 1 CPV1. This virus has the same characteristics as the virus present in *Ceratocystis polonica.* The viral contigs APVc-RdRp and APVc-CP represent the first alphapartitivirus detected in *Ceratocystis cacaofunesta.*

In addition, a new viral contig called NVc belonging to *Narnaviridae* was identified. The contig showed similarity to individual RNA virus genomes ranging from 2.3 to 3.6 kb encoding only a single polypeptide having an RdRp domain. This feature was found in the study by Botella and collaborators (2022) [106], who described narnaviruses in the fungus *Phytophthora palustris*, viruses that are related to the traditional positive-coding monopartite, included in the genus *Narnavirus*. Also of note, Kinsella and collaborators (2022) [107] identified narnaviruses in the hosts *R. oryzae* and *A. lentulus*, which are pathogenic fungi, and Zou et al. (2021) [108] analyzed the fungus *Botryosphaeria dothidea*, which is the causal agent of the disease pear ring rot. The viral contig NVc is the first narnavirus detected in *Ceratocystis cacaofunesta.*

Two new viral contigs, named UMVcefi1 and MVcefi1, belonging to the *Mitoviridae* were characterized in our study. Members of this family are positive-stranded ssRNA viruses of 2.5 to 2.9 kb. Generally, the genome has a single ORF encoding an RdRp. Previous studies have also identified viral contigs in different fungal phytopathogens, such as Wang and collaborators (2021 A-B) [109,110] and de Rezende and collaborators (2021) [111]. Shafik and collaborators (2021) [112] identified a new mycovirus, named *Melanconiella theae mitovirus* 1 (MtMV1), a virus that infects the phytopathogenic MtMV1 and shares most of the characteristics of the members of the *Mitoviridae* family. The UMVcefi1 and MVcefi1 sequences represents the first mitoviruses detected in *Ceratocystis fimbriata*.

AEVf-1, AEVf-2 and AEVf-3 are similar to *Endornaviridae*, which has a linear dsRNA genome of about 14 kb to 17.6 kb. This genome encodes an ORF potentially divided into several polypeptides. Here, two major ORF-associated domains were found: *Tymovirus*, RdRp, and (+) RNA virus helicase core dom, which have been identified in several virus types [113] including a variety of plant virus families [114]. In addition, Luo and collaborators (2022) [115], analyzing *S. sclerotiorum*, identified a new *endornavirus* called *Scelrotinia sclerotiorum endornavirus* 11 (SsEV11), suggesting that there is remarkable phylogenetic diversity in the *Endornaviridae* family and corroborating previous findings that viruses of this family can be found in fungi. In this respect, Cao and collaborators (2022) [116] studied *Ceratobasidium* infected with three endornaviruses with significantly upregulated micro-RNA-like RNAs (Cer-milRNAs) and identified transcription factors, suggesting an effect of viral infection on the control of regulatory networks and growth and development of the fungus. Moreover, Marais (2021) [117] investigated *Neofusicoccum parvum* and found the presence of co-infecting viruses from *Totiviridae*, *Victorivirus*, *Endornaviridae*, *Mitoviridae* and *Narnaviridae*. The viral contigs AEVf-1, AEVf-2 and AEVf-3 are the first alphaendornaviruses belonging to the *Endornaviridae* family detected in *Ceratocystis fimbriata.*

Finally, the *Totiviridae* has the main feature of being a nonenveloped icosahedral virion composed of a single coat protein (Cp), with a diameter of about 40 nm and a linear dsRNA genome of 4.6 to 6.7 kb. In addition, it has two overlapping ORFs, gag and pol, respectively encoding Cp and RdRp. In the viral contig assembled in our study (VVf), we identified a conserved domain of RdRp *Luteovirus*, which is an essential protein encoded in the genomes of all RNA containing viruses with no DNA stage [118,119]. The viral contigs VVf-RdRp and VVf-Cp were the first representants of a *Victorivirus* detected in *Ceratocystis fimbriata*. Many fungal species have been found able to counteract multiple viral infections relying on the RNA interference (RNAi) mechanisms with antiviral activity, such as de- scribed for *Cryphonectria parasitica* [29]. When the host's RNAi mechanism targets the viral genome, an overlapping population of virus-derived vsiRNA can be produced. In *C. parasitica*, the dicer-like (dcl-2) and argonaute-like (agl-2) genes are necessary for antiviral silencing [27,120]. Shinji Honda (2020) [121] demonstrated that in the fungus *Neurospora crassa*, viral infection positively regulates the transcription of the various components of RNAi, where the Dicer (DCL-1 and DCL-2) and Argonaute (QDE-2) proteins participate in the suppression of viral replication. Mekhala Maiti (2007) [122] reported that the Argonaute QDE-2 homologue was necessary for the generation of single-stranded siRNA and gene silencing *in vivo*.

Although various RNAi pathways have been lost in several fungal species and lineages, most species possess this ancient mechanism [27,36]. However, there are several filamentous fungi that seem to lack the necessary components for RNA silencing to occur [27], which means that by lacking this machinery to defend against mycoviruses, these viruses produce different hypovirulence factors in their hosts. Mycovirus-mediated hypovirulence is an excellent example of multitrophic interactions, making these viruses promising for the biological control of fungal diseases [26,123].

In our study, the highest abundance of RNAi occurred in two Argonaute genes, QDE2 and SMS2, with a value of around 300 TPM, as previously indicated by Lee and collaborators (2009) [124] in their study of QDE genes induced by DNA damage. On the other hand, Li and collaborators. (2022) [100], studying *Fusarium oxysporum* f. sp. *cubense*, noted that by knocking out QDE2, the fungus showed reduced virulence, suggesting its involvement in pathogenesis. Another noteworthy fact is that the biogenesis of milR4 requires MRPL3 [32], which may explain its presence in the data analyzed. We can conclude that the genes have all the components of the RNAi pathway and are transcriptionally active, suggesting that the pathway may help the fungus combat viral infection.

Overall, of the *Ceratocystis* species reported to date, two stand out: *Ceratocystis fimbriata*, which has been reported to infect nearly 100 host species, including many woody plants [125,126], and *Ceratocystis cacaofunesta*, which has been reported in *Theobroma cacao* and in the genus *Herrania* [90,125,126]. Therefore, our study reveals new insights into the mycovirome diversity of *Ceratocystis*, an important pathogen that causes irreversible damage to the vascular system of cocoa plants, reducing yield and, in severe cases, killing the plant.

Supplementary Materials: The following supporting information can be downloaded at: https://www. mdpi.com/article/10.3390/pathogens12020287/s1, Table S1: Overview of RNA deep sequencing li- braries used in the work; Table S2: Best hits at nucleotide and amino acid levels for viral transcripts assembled in our work; Table S3: Viral genomes used in phylogenetic analyses; Table S4: Metage- nomic analysis of RNA sequencing libraries using Kaiju software; Table S5: RNAi-related genes; Figure S1: Genomic characteristics of viral genomes closely related to the viruses identified in our work; Figure S2: Conserved motifs and catalytic site in the Hypovirus cacaofunestae viral sequence; Figure S3: Conserved motifs and catalytic site in the Narnavirus cacaofunestae viral sequence; Figure S4: Conserved motifs and catalytic site in the Alphaendornavirus fimbriatae-3 viral sequence; Figure S5: Characterization of RNAi-related genes in *Ceratocystis fimbriata*.

Author Contributions: Conceptualization, E.R.G.R.A.; methodology, R.B.A.E., S.F.d.S., P.L.C.F., R.J.S.S. and V.C.S.; software, R.B.A.E., S.F.d.S. and V.C.S.; validation, R.B.A.E., S.F.d.S., P.L.C.F. and E.R.G.R.A.; formal analysis, R.B.A.E., S.F.d.S., P.L.C.F. and E.R.G.R.A.; investigation, R.B.A.E., S.F.d.S. and P.L.C.F.; resources, C.P.P. and E.R.G.R.A.; data curation, R.B.A.E. and S.F.d.S.; writing—original draft preparation, R.B.A.E., S.F.d.S., G.N.R.L., R.X.C., L.L.L., C.P.P., A.G.-N. and E.R.G.R.A.; writing—review and editing, R.B.A.E., S.F.d.S. and E.R.G.R.A.; visualization, R.B.A.E. and S.F.d.S.; supervision, E.R.G.R.A.; project administration, E.R.G.R.A.; funding acquisition, E.R.G.R.A. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated and analyzed during the current study are available in the NCBI GenBank Third Party Annotation database under accession numbers BK062942- BK062946 and BK063053-BK063059.

Acknowledgments: We thank the all the members of Virus Bioinformatics Laboratory–UESC for the fruitful discussions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bekele, F.; Phillips-Mora, W. Cacao (*Theobroma Cacao* L.) Breeding. In *Advances in Plant Breeding Strategies: Industrial and Food Crops: Volume 6*; Al-Khayri, J.M., Jain, S.M., Johnson, D.V., Eds.; Springer International Publishing: Cham, Switzerland, 2019; pp. 409–487. [CrossRef]
2. Fernandes, L.D.S.; Royaert, S.; Corrêa, F.M.; Mustiga, G.M.; Marelli, J.-P.; Corrêa, R.X.; Motamayor, J.C. Mapping of a Major QTL for *Ceratocystis* Wilt Disease in an F1 Population of *Theobroma Cacao*. *Front. Plant Sci.* **2018**, *9*, 155. [CrossRef] [PubMed]
3. Marelli, J.-P.; Guest, D.I.; Bailey, B.A.; Evans, H.C.; Brown, J.K.; Junaid, M.; Barreto, R.W.; Lisboa, D.O.; Puig, A.S. Chocolate Under Threat from Old and New Cacao Diseases. *Phytopathology* **2019**, *109*, 1331–1343. [CrossRef] [PubMed]
4. International Cocoa Organization—Statistics 2021. Available online: https://www.icco.org/statistics/ (accessed on 17 October 2021).
5. Hussain, B. Modernization in Plant Breeding Approaches for Improving Biotic Stress Resistance in Crop Plants. *Turk. J. Agric. For.* **2015**, *39*, 515–530. [CrossRef]
6. Ploetz, R. The Impact of Diseases on Cacao Production: A Global Overview. In *Cacao Diseases: A History of Old Enemies and New Encounters*; Bailey, B.A., Meinhardt, L.W., Eds.; Springer International Publishing: Cham, Switzerland, 2016; pp. 33–59. [CrossRef]
7. Cabrera, O.G.; Molano, E.P.L.; José, J.; Álvarez, J.C.; Pereira, G.A.G. *Ceratocystis* Wilt Pathogens: History and Biology—Highlighting *C. Cacaofunesta*, the Causal Agent of Wilt Disease of Cacao. In *Cacao Diseases: A History of Old Enemies and New Encounters*; Bailey, B.A., Meinhardt, L.W., Eds.; Springer International Publishing: Cham, Switzerland, 2016; pp. 383–428. [CrossRef]
8. Delgado-Ospina, J.; Molina-Hernández, J.B.; Chaves-López, C.; Romanazzi, G.; Paparella, A. The Role of Fungi in the Cocoa Production Chain and the Challenge of Climate Change. *J. Fungi* **2021**, *7*, 202. [CrossRef]
9. Santos, S.A.; Vidigal, P.M.P.; Thrimawithana, A.; Betancourth, B.M.L.; Guimarães, L.M.S.; Templeton, M.D.; Alfenas, A.C. Comparative Genomic and Transcriptomic Analyses Reveal Different Pathogenicity-Related Genes among Three *Eucalyptus* Fungal Pathogens. *Fungal Genet. Biol.* **2020**, *137*, 103332. [CrossRef]
10. Molano, E.P.L.; Cabrera, O.G.; Jose, J.; do Nascimento, L.C.; Carazzolle, M.F.; Teixeira, P.J.P.L.; Alvarez, J.C.; Tiburcio, R.A.; Tokimatu Filho, P.M.; de Lima, G.M.A.; et al. *Ceratocystis Cacaofunesta* Genome Analysis Reveals a Large Expansion of Extracellular Phosphatidylinositol-Specific Phospholipase-C Genes (PI-PLC). *BMC Genom.* **2018**, *19*, 58. [CrossRef]
11. Engelbrecht, C.J.B.; Harrington, T.C. Intersterility, Morphology and Taxonomy of *Ceratocystis Fimbriata* on Sweet Potato, Cacao and Sycamore. *Mycologia* **2005**, *97*, 57–69. [CrossRef]
12. Raffa, K.F.; Hobson, K.R.; Lafontaine, S.; Aukema, B.H. Can Chemical Communication Be Cryptic? Adaptations by Herbivores to Natural Enemies Exploiting Prey Semiochemistry. *Oecologia* **2007**, *153*, 1009–1019. [CrossRef]
13. Smith, S.M.; Hulcr, J. Chapter 12—Scolytus and Other Economically Important Bark and Ambrosia Beetles. In *Bark Beetles*; Vega, F.E., Hofstetter, R.W., Eds.; Academic Press: San Diego, CA, USA, 2015; pp. 495–531. [CrossRef]
14. da Cruz, M.B.; de Sousa, D.F.; de Almeida Oliveira, L.; de França, J.P.; de França, L.P.; Conceição, A.O.; Fonseca Chaves, A.L. Interference of Aqueous and Ethanolic Solutions of *Adiantum Latifolium* Lam. (*Pteridaceae*) Leaves on in Vitro *Ceratocystis Cacaofunesta* Mycelial Growth. *Arq. Inst. Biol.* **2019**, *86*. [CrossRef]
15. Mora-Ocampo, I.Y.; Pirovani, C.P.; Luz, E.D.M.N.; Rêgo, A.P.B.; Silva, E.M.A.; Rhodes-Valbuena, M.; Corrêa, R.X. *Ceratocystis Cacaofunesta* Differentially Modulates the Proteome in Xylem-Enriched Tissue of Cocoa Genotypes with Contrasting Resistance to *Ceratocystis* Wilt. *Planta* **2021**, *254*, 94. [CrossRef]
16. Zhang, Y.; Li, T.; Xu, M.; Guo, J.; Zhang, C.; Feng, Z.; Peng, X.; Li, Z.; Xing, K.; Qin, S. Antifungal Effect of Volatile Organic Compounds Produced by *Pseudomonas Chlororaphis* Subsp. *Aureofaciens SPS-41 on Oxidative Stress and Mitochondrial Dysfunction ofCeratocystis Fimbriata*. *Pestic. Biochem. Physiol.* **2021**, *173*, 104777. [CrossRef] [PubMed]
17. Aime, M.C.; Phillips-Mora, W. The Causal Agents of Witches' Broom and Frosty Pod Rot of Cacao (Chocolate, *Theobroma Cacao*) Form a New Lineage of *Marasmiaceae*. *Mycol.* **2005**, *97*, 1012–1022. [CrossRef]
18. Surujdeo-Maharaj, S.; Sreenivasan, T.N.; Motilal, L.A.; Umaharan, P. Black Pod and Other Phytophthora Induced Diseases of Cacao: History, Biology, and Control. In *Cacao Diseases: A History of Old Enemies and New Encounters*; Bailey, B.A., Meinhardt, L.W., Eds.; Springer International Publishing: Cham, Switzerland, 2016; pp. 213–266. [CrossRef]
19. Rodrigues, G.d.S.; Magalhães, D.M.A.; da Costa, A.M.; Luz, E.D.M.N. Antagonism of *Trichoderma* Spp. To the Etiological Agent of *Ceratocystis* Wilt in Cacao. *Summa Phytopathol.* **2018**, *44*, 72–78. [CrossRef]
20. Torres-Trenas, A.; Prieto, P.; Cañizares, M.C.; García-Pedrajas, M.D.; Pérez-Artés, E. Mycovirus *Fusarium Oxysporum* f. Sp. Dianthi Virus 1 Decreases the Colonizing Efficiency of Its Fungal Host. *Front Cell Infect. Microbiol.* **2019**, *9*, 51. [CrossRef]
21. Sharma, M.; Guleria, S.; Singh, K.; Chauhan, A.; Kulshrestha, S. Mycovirus Associated Hypovirulence, a Potential Method for Biological Control of *Fusarium* Species. *Virus Dis.* **2018**, *29*, 134–140. [CrossRef] [PubMed]
22. Wagemans, J.; Holtappels, D.; Vainio, E.; Rabiey, M.; Marzachì, C.; Herrero, S.; Ravanbakhsh, M.; Tebbe, C.C.; Ogliastro, M.; Ayllón, M.A.; et al. Going Viral: Virus-Based Biological Control Agents for Plant Protection. *Annu. Rev. Phytopathol.* **2022**, *60*, 21–42. [CrossRef]
23. Wen, C.; Wan, X.; Zhang, Y.; Du, H.; Wei, C.; Zhong, R.; Zhang, H.; Shi, Y.; Xie, J.; Fu, Y.; et al. Molecular

Characterization of the First *Alternavirus* Identified in *Fusarium Oxysporum*. *Viruses* **2021**, *13*, 2026. [CrossRef]
24. Xiao, X.; Cheng, J.; Tang, J.; Fu, Y.; Jiang, D.; Baker, T.S.; Ghabrial, S.A.; Xie, J. A Novel *Partitivirus* That Confers Hypovirulence on Plant Pathogenic Fungi. *J. Virol.* **2014**, *88*, 10120–10133. [CrossRef]
25. Zhu, J.Z.; Guo, J.; Hu, Z.; Zhang, X.T.; Li, X.G.; Zhong, J. A Novel *Partitivirus* That Confer Hypovirulence to the Plant Pathogenic Fungus *Colletotrichum Liriopes*. *Front Microbiol* **2021**, *12*, 653809. [CrossRef] [PubMed]
26. Zhu, J.Z.; Zhu, H.J.; Gao, B.D.; Zhou, Q.; Zhong, J. Diverse, Novel Mycoviruses From the Virome of a Hypovirulent *Sclerotium Rolfsii* Strain. *Front Plant Sci.* **2018**, *9*, 1738. [CrossRef]
27. Segers, G.C.; Zhang, X.; Deng, F.; Sun, Q.; Nuss, D.L. Evidence That RNA Silencing Functions as an Antiviral Defense Mechanism in Fungi. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 12902–12906. [CrossRef] [PubMed]
28. Yu, J.; Lee, K.-M.; Cho, W.K.; Park, J.Y.; Kim, K.-H. Differential Contribution of RNA Interference Components in Response to Distinct *Fusarium Graminearum* Virus Infections. *J. Virol.* **2018**, *92*, e01756-e17. [CrossRef] [PubMed]
29. Nuss, D.L. Mycoviruses, RNA Silencing, and Viral RNA Recombination. *Adv. Virus Res.* **2011**, *80*, 25–48. [CrossRef] [PubMed]
30. Nicolás, F.E.; Ruiz-Vázquez, R.M. Functional Diversity of RNAi-Associated SRNAs in Fungi. *Int. J. Mol. Sci.* **2013**, *14*, 15348–15360. [CrossRef]
31. Nicolás, F.E.; Vila, A.; Moxon, S.; Cascales, M.D.; Torres-Martínez, S.; Ruiz-Vázquez, R.M.; Garre, V. The RNAi Machinery Controls Distinct Responses to Environmental Signals in the Basal Fungus Mucor Circinelloides. *BMC Genom.* **2015**, *16*, 237. [CrossRef]
32. Dang, Y.; Yang, Q.; Xue, Z.; Liu, Y. RNA Interference in Fungi: Pathways, Functions, and Applications. *Eukaryot. Cell* **2011**, *10*, 1148–1155. [CrossRef]
33. Liu, Y. RNA Interference Pathways in Filamentous Fungi. *Cell Mol. Life Sci.* **2010**, *67*, 3849–3863. [CrossRef]
34. Khatri, M.; Rajam, M.V. Targeting Polyamines of *Aspergillus Nidulans* by SiRNA Specific to Fungal Ornithine Decarboxylase Gene. *Med. Mycol.* **2007**, *45*, 211–220. [CrossRef]
35. Mukherjee, K.; Campos, H.; Kolaczkowski, B. Evolution of Animal and Plant Dicers: Early Parallel Duplications and Recurrent Adaptation of Antiviral RNA Binding in Plants. *Mol. Biol. Evol.* **2013**, *30*, 627–641. [CrossRef]
36. Nakayashiki, H.; Kadotani, N.; Mayama, S. Evolution and Diversification of RNA Silencing Proteins in Fungi. *J. Mol. Evol.* **2006**, *63*, 127–135. [CrossRef]
37. Roossinck, M.J. Metagenomics of Plant and Fungal Viruses Reveals an Abundance of Persistent Lifestyles. *Front Microbiol.* **2015**, *5*, 767. [CrossRef] [PubMed]
38. Mokili, J.L.; Rohwer, F.; Dutilh, B.E. Metagenomics and Future Perspectives in Virus Discovery. *Curr. Opin. Virol.* **2012**, *2*, 63–77. [CrossRef] [PubMed]
39. Simpson, M.C.; Wilken, P.M.; Coetzee, M.P.A.; Wingfield, M.J.; Wingfield, B.D. Analysis of Microsatellite Markers in the Genome of the Plant Pathogen *Ceratocystis Fimbriata*. *Fungal. Biol.* **2013**, *117*, 545–555. [CrossRef] [PubMed]
40. Wilken, P.M.; Steenkamp, E.T.; Wingfield, M.J.; de Beer, Z.W.; Wingfield, B.D. IMA Genome-F 1: *Ceratocystis Fimbriata:* Draft Nuclear Genome Sequence for the Plant Pathogen, *Ceratocystis Fimbriata*. *IMA Fungus* **2013**, *4*, 357–358. [CrossRef]
41. Wingett, S.W.; Andrews, S. FastQ Screen: A Tool for Multi-Genome Mapping and Quality Control. *F1000Res* **2018**, *7*, 1338. [CrossRef] [PubMed]
42. BI Ageing Clock Team; Stubbs, T.M.; Bonder, M.J.; Stark, A.-K.; Krueger, F.; von Meyenn, F.; Stegle, O.; Reik, W. Multi-Tissue DNA Methylation Age Predictor in Mouse. *Genome Biol.* **2017**, *18*, 68. [CrossRef]
43. Langmead, B.; Salzberg, S.L. Fast Gapped-Read Alignment with Bowtie 2. *Nat. Methods* **2012**, *9*, 357–359. [CrossRef]
44. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Prjibelski, A.D.; et al. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477. [CrossRef]
45. Prjibelski, A.D.; Vasilinetc, I.; Bankevich, A.; Gurevich, A.; Krivosheeva, T.; Nurk, S.; Pham, S.; Korobeynikov, A.; Lapidus, A.; Pevzner, P.A. ExSPAnder: A Universal Repeat Resolver for DNA Fragment Assembly. *Bioinformatics* **2014**, *30*, i293–i301. [CrossRef]
46. Haas, B.J.; Papanicolaou, A.; Yassour, M.; Grabherr, M.; Blood, P.D.; Bowden, J.; Couger, M.B.; Eccles, D.; Li, B.; Lieber, M.; et al. De Novo Transcript Sequence Reconstruction from RNA-Seq Using the Trinity Platform for Reference Generation and Analysis. *Nat. Protoc.* **2013**, *8*, 1494–1512. [CrossRef]
47. Huang, X.; Madan, A. CAP3: A DNA Sequence Assembly Program. *Genome Res.* **1999**, *9*, 868–877. [CrossRef] [PubMed]
48. Buchfink, B.; Xie, C.; Huson, D.H. Fast and Sensitive Protein Alignment Using DIAMOND. *Nat. Methods* **2015**, *12*, 59–60. [CrossRef] [PubMed]
49. Kyran, A. Fasta Statistics: Display Summary Statistics for a Fasta File 2021. Available online: https://github.com/galaxyproject/tools-iuc (accessed on 5 November 2022).
50. Afgan, E.; Baker, D.; Batut, B.; van den Beek, M.; Bouvier, D.; Čech, M.; Chilton, J.; Clements, D.; Coraor, N.; Grüning, B.A.; et al. The Galaxy Platform for Accessible, Reproducible and Collaborative Biomedical Analyses: 2018 Update. *Nucleic Acids Res.* **2018**, *46*, W537–W544. [CrossRef] [PubMed]
51. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic Local Alignment Search Tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef]
52. Rombel, I.T.; Sykes, K.F.; Rayner, S.; Johnston, S.A. ORF-FINDER: A Vector for High-Throughput Gene Identification. *Gene* **2002**, *282*, 33–41. [CrossRef]
53. Blum, M.; Chang, H.-Y.; Chuguransky, S.; Grego, T.; Kandasaamy, S.; Mitchell, A.; Nuka, G.; Paysan-Lafosse, T.;

Qureshi, M.; Raj, S.; et al. The InterPro Protein Families and Domains Database: 20 Years On. *Nucleic Acids Res.* **2021**, *49*, D344–D354. [CrossRef]
54. Finn, R.D.; Clements, J.; Eddy, S.R. HMMER Web Server: Interactive Sequence Similarity Searching. *Nucleic Acids Res.* **2011**, *39* (Suppl. S2), W29–W37. [CrossRef]
55. Finn, R.D.; Clements, J.; Arndt, W.; Miller, B.L.; Wheeler, T.J.; Schreiber, F.; Bateman, A.; Eddy, S.R. HMMER Web Server: 2015 Update. *Nucleic Acids Res.* **2015**, *43*, W30–W38. [CrossRef]
56. Potter, S.C.; Luciani, A.; Eddy, S.R.; Park, Y.; Lopez, R.; Finn, R.D. HMMER Web Server: 2018 Update. *Nucleic Acids Res.* **2018**, *46*, W200–W204. [CrossRef]
57. Mitchell, A.L.; Almeida, A.; Beracochea, M.; Boland, M.; Burgin, J.; Cochrane, G.; Crusoe, M.R.; Kale, V.; Potter, S.C.; Richardson, L.J.; et al. MGnify: The Microbiome Analysis Resource in 2020. *Nucleic Acids Res.* **2020**, *48*, D570–D578. [CrossRef]
58. Schulz, M.H.; Zerbino, D.R.; Vingron, M.; Birney, E. Oases: Robust de Novo RNA-Seq Assembly across the Dynamic Range of Expression Levels. *Bioinformatics* **2012**, *28*, 1086–1092. [CrossRef] [PubMed]
59. Afiahayati; Sato, K.; Sakakibara, Y. MetaVelvet-SL: An Extension of the Velvet Assembler to a de Novo Metagenomic Assembler Utilizing Supervised Learning. *DNA Res.* **2015**, *22*, 69–77. [CrossRef]
60. Peng, Y.; Leung, H.C.M.; Yiu, S.M.; Chin, F.Y.L. IDBA-UD: A de Novo Assembler for Single-Cell and Metagenomic Sequencing Data with Highly Uneven Depth. *Bioinformatics* **2012**, *28*, 1420–1428. [CrossRef] [PubMed]
61. Li, W.; Godzik, A. Cd-Hit: A Fast Program for Clustering and Comparing Large Sets of Protein or Nucleotide Sequences. *Bioinformatics* **2006**, *22*, 1658–1659. [CrossRef] [PubMed]
62. Katoh, K.; Standley, D.M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef]
63. Larsson, A. AliView: A Fast and Lightweight Alignment Viewer and Editor for Large Datasets. *Bioinformatics* **2014**, *30*, 3276–3278. [CrossRef]
64. Miller, M.A.; Pfeiffer, W.; Schwartz, T. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. In Proceedings of the 2010 Gateway Computing Environments Workshop (GCE), New Orleans, LA, USA, 14 November 2010; pp. 1–8. [CrossRef]
65. Letunic, I.; Bork, P. Interactive Tree Of Life (ITOL) v5: An Online Tool for Phylogenetic Tree Display and Annotation. *Nucleic Acids Res.* **2021**, *49*, W293–W296. [CrossRef]
66. Lu, S.; Wang, J.; Chitsaz, F.; Derbyshire, M.K.; Geer, R.C.; Gonzales, N.R.; Gwadz, M.; Hurwitz, D.I.; Marchler, G.H.; Song, J.S.; et al. CDD/SPARCLE: The Conserved Domain Database in **2020**. *Nucleic Acids Res.* **2020**, *48*, D265–D268. [CrossRef]
67. Menzel, P.; Ng, K.L.; Krogh, A. Fast and Sensitive Taxonomic Classification for Metagenomics with Kaiju. *Nat. Commun* **2016**, *7*, 11257. [CrossRef]
68. Grabherr, M.G.; Haas, B.J.; Yassour, M.; Levin, J.Z.; Thompson, D.A.; Amit, I.; Adiconis, X.; Fan, L.; Raychowdhury, R.; Zeng, Q.; et al. Full-Length Transcriptome Assembly from RNA-Seq Data without a Reference Genome. *Nat. Biotechnol.* **2011**, *29*, 644–652. [CrossRef]
69. Patro, R.; Duggal, G.; Love, M.I.; Irizarry, R.A.; Kingsford, C. Salmon Provides Fast and Bias-Aware Quantification of Transcript Expression. *Nat. Methods* **2017**, *14*, 417–419. [CrossRef] [PubMed]
70. Thompson, J.D.; Gibson, T.J.; Higgins, D.G. Multiple Sequence Alignment Using ClustalW and ClustalX. *Curr. Protoc. Bioinform.* **2003**, Unit 2.3. [CrossRef]
71. Han, M.V.; Zmasek, C.M. PhyloXML: XML for Evolutionary Biology and Comparative Genomics. *BMC Bioinform.* **2009**, *10*, 356. [CrossRef] [PubMed]
72. Marincowitz, S.; Barnes, I.; de Beer, Z.W.; Wingfield, M.J. Epitypification of *Ceratocystis Fimbriata*. *Fungal Syst. Evol.* **2020**, *6*, 289–298. [CrossRef] [PubMed]
73. Sun, Y.; Li, M.; Wang, Y.; Li, L.; Wang, M.; Li, X.; Xu, M.; Loake, G.J.; Guo, M.; Jiang, J. *Ceratocystis Fimbriata* Employs a Unique Infection Strategy Targeting Peltate Glandular Trichomes of Sweetpotato (*Ipomoea Batatas*) Plants. *Phytopathology* **2020**, *110*, 1923–1933. [CrossRef]
74. Stahr, M.; Quesada-Ocampo, L.M. Assessing the Role of Temperature, Inoculum Density, and Wounding on Disease Progression of the Fungal Pathogen *Ceratocystis Fimbriata* Causing Black Rot in Sweetpotato. *Plant Dis.* **2020**, *104*, 930–937. [CrossRef] [PubMed]
75. Harrington, T.C.; Thorpe, D.J.; Alfenas, A.C. Genetic Variation and Variation in Aggressiveness to Native and Exotic Hosts among Brazilian Populations of *Ceratocystis Fimbriata*. *Phytopathology* **2011**, *101*, 555–566. [CrossRef]
76. Kumari, N.; Shukla, P.K.; Singh, H.; Fatima, T.; Bajpai, A. Development of Species-Specific PCR Based Detection Assay for *Ceratocystis Fimbriata*, Mango Wilt Pathogen. *Indian Phytopathol.* **2022**, *75*, 215–220. [CrossRef]
77. Fourie, A.; Wingfield, M.J.; Wingfield, B.D.; Barnes, I. Molecular Markers Delimit Cryptic Species in *Ceratocystis Sensu Stricto*. *Mycol. Prog.* **2014**, *14*, 1020. [CrossRef]
78. Harrington, T.C.; Kazmi, M.R.; Al-Sadi, A.M.; Ismail, S.I. Intraspecific and Intragenomic Variability of ITS RDNA Sequences Reveals Taxonomic Problems in *Ceratocystis Fimbriata Sensu Stricto*. *Mycologia* **2014**, *106*, 224–242. [CrossRef] [PubMed]
79. Yakushiji, H.; Morita, T.; Jikumaru, S.; Ikegami, H.; Azuma, A.; Koshita, Y. Interspecific Hybridization of Fig (*Ficus Carica* L.) and *Ficus Erecta* Thunb., a Source of *Ceratocystis* Canker Resistance. *Euphytica* **2012**, *183*, 39–47. [CrossRef]
80. Li, Q.; Harrington, T.C.; McNew, D.; Li, J.; Huang, Q.; Somasekhara, Y.M.; Alfenas, A.C. Genetic Bottlenecks for Two Populations of *Ceratocystis Fimbriata* on Sweet Potato and Pomegranate in China. *Plant Dis.* **2016**, *100*, 2266–2274.

[CrossRef]
81. Huang, Q.; Zhu, Y.Y.; Chen, H.R.; Wang, Y.Y.; Liu, Y.L.; Lu, W.J.; Ruan, X.Y. First Report of Pomegranate Wilt Caused by *Ceratocystis Fimbriata* in Yunnan, China. *Plant Dis.* **2003**, *87*, 1150. [CrossRef] [PubMed]
82. Huang, Q.; Wang, Y.Y.; Zhao, Y.Y.; Jiao, Y.X.; Li, X.F.; Chen, H.R.; Zhu, Y.Y. First Report of Taro Black Rot Caused by *CeratocystisFimbriata* in China. *Plant Pathol.* **2008**, *57*, 780. [CrossRef]
83. Harrington, T.C.; Thorpe, D.J.; Marinho, V.L.A.; Furtado, E.L. First Report of Black Rot of *Colocasia Esculenta* Caused by *CeratocystisFimbriata* in Brazil. *Fitopatol. Bras.* **2005**, *30*, 88–89. [CrossRef]
84. Baker, C.J.; Harrington, T.C.; Krauss, U.; Alfenas, A.C. Genetic Variability and Host Specialization in the Latin American Clade of *Ceratocystis Fimbriata*. *Phytopathology* **2003**, *93*, 1274–1284. [CrossRef]
85. Méndez-Álvarez, D.; de Souza Cândido, T.; Couto Alfenas, A.; Murillo, O.; Badilla, Y.; Ferreira Alfenas, R. First Report of *Ceratocystis Fimbriata* Causing Wilt on *Gmelina Arborea* in Costa Rica. *For. Pathol.* **2020**, *50*, e12628. [CrossRef]
86. Thorpe, D.J.; Harrington, T.C.; Uchida, J.Y. Pathogenicity, Internal Transcribed Spacer-RDNA Variation, and Human Dispersal of *Ceratocystis Fimbriata* on the Family *Araceae*. *Phytopathology* **2005**, *95*, 316–323. [CrossRef]
87. Walker, J.; Tesoriero, L.; Pascoe, I.; Forsberg, L.I. Basal Rot of *Syngonium* Cultivars and the First Record of *Ceratocystis Fimbriata* from Australia. *Australas. Plant Pathol.* **1988**, *17*, 22–23. [CrossRef]
88. De Brito, N.M.; Duarte, H.S.S.; Bühner, C. de B.; Auer, C.G.; Santos, Á.F. dos. Morphophysiological Characterization of *Ceratocystis Fimbriata* Isolates from Yerba Mate. Cienc. *Rural* **2021**, *51*. [CrossRef]
89. De Brito, N.M.; Duarte, H.d.S.S.; Auer, C.G.; Wendling, I.; Alfenas, R.F.; Dos Santos, Á.F. Aggressiveness and Screening for Host Responses in Yerba Mate (*Ilex Paraguariensis*) Clones to a Wilt Disease Caused by *Ceratocystis Fimbriata*. *For. Pathol.* **2021**, *51*, e12682. [CrossRef]
90. Engelbrecht, C.J.; Harrington, T.C.; Alfenas, A. Ceratocystis Wilt of Cacao—A Disease of Increasing Importance. *Phytopathology* **2007**, *97*, 1648–1649. [CrossRef] [PubMed]
91. Engelbrecht, C.J.B.; Harrington, T.C.; Alfenas, A.C.; Suarez, C. Genetic Variation in Populations of the Cacao Wilt Pathogen, *Ceratocystis Cacaofunesta*. *Plant Pathol.* **2007**, *56*, 923–933. [CrossRef]
92. Suzuki, N.; Ghabrial, S.A.; Kim, K.-H.; Pearson, M.; Marzano, S.-Y.L.; Yaegashi, H.; Xie, J.; Guo, L.; Kondo, H.; Koloniuk, I.; et al.ICTV Virus Taxonomy Profile: *Hypoviridae*. *J. Gen. Virol.* **2018**, *99*, 615–616. [CrossRef] [PubMed]
93. Li, H.; Bian, R.; Liu, Q.; Yang, L.; Pang, T.; Salaipeth, L.; Andika, I.B.; Kondo, H.; Sun, L. Identification of a Novel Hypovirulence-Inducing *Hypovirus* From *Alternaria Alternata*. *Front. Microbiol.* **2019**, *10*, 1076. [CrossRef] [PubMed]
94. De Miccolis Angelini, R.M.; Raguseo, C.; Rotolo, C.; Gerin, D.; Faretra, F.; Pollastro, S. The Mycovirome in a Worldwide Collectionof the Brown Rot Fungus *Monilinia Fructicola*. *J. Fungi* **2022**, *8*, 481. [CrossRef]
95. Velasco, L.; Arjona-Girona, I.; Ariza-Fernández, M.T.; Cretazzo, E.; López-Herrera, C. A Novel *Hypovirus* Species From *Xylariaceae* Fungi Infecting Avocado. *Front. Microbiol.* **2018**, *9*, 778. [CrossRef]
96. Li, Q.; Huang, W.; Hai, D.; Wang, Y.; Xie, J.; Wang, M. The Complete Genome Sequence of a Novel *Hypovirus* Infecting *BipolarisOryzae*. *Arch. Virol.* **2020**, *165*, 1027–1031. [CrossRef]
97. Wang, S.; Zhang, J.; Nzabanita, C.; Zhang, M.; Nie, J.; Guo, L. Fungal Virus, FgHV1-Encoded P20 Suppresses RNA Silencingthrough Single-Strand Small RNA Binding. *J. Fungi* **2022**, *8*, 1171. [CrossRef]
98. Aulia, A.; Hyodo, K.; Hisano, S.; Kondo, H.; Hillman, B.I.; Suzuki, N. Identification of an RNA Silencing Suppressor Encoded bya Symptomless Fungal *Hypovirus*, *Cryphonectria Hypovirus* 4. *Biology* **2021**, *10*, 100. [CrossRef]
99. Cotmore, S.F.; Agbandje-McKenna, M.; Canuti, M.; Chiorini, J.A.; Eis-Hubinger, A.-M.; Hughes, J.; Mietzsch, M.; Modha, S.;Ogliastro, M.; Pénzes, J.J.; et al. ICTV Virus Taxonomy Profile: *Parvoviridae*. *J. Gen. Virol.* **2019**, *100*, 367–368. [CrossRef] [PubMed]
100. Li, X.; Liu, Q.; Li, S.; Sossah, F.L.; Han, X.; Zhu, G.; Li, Y.; Li, C.; Fu, Y. A Novel Fungal Negative-Stranded RNA Virus Related toMymonaviruses in Auricularia Heimuer. *Arch. Virol.* **2022**, *167*, 2223–2227. [CrossRef]
101. Hao, F.; Wu, M.; Li, G. Molecular Characterization and Geographic Distribution of a *Mymonavirus* in the Population of *BotrytisCinerea*. *Viruses* **2018**, *10*, 432. [CrossRef] [PubMed]
102. Vainio, E.J.; Chiba, S.; Ghabrial, S.A.; Maiss, E.; Roossinck, M.; Sabanadzovic, S.; Suzuki, N.; Xie, J.; Nibert, M. ICTV VirusTaxonomy Profile: Partitiviridae. *J. Gen. Virol.* **2018**, *99*, 17–18. [CrossRef] [PubMed]
103. Jiang, Y.; Yang, B.; Liu, J.; Wang, Q.; Zhang, Q.; Yu, W.; Hsiang, T. A Novel Mycovirus Infecting *Aspergillus Nidulans* That IsClosely Related to Viruses in a New Genus of the Family *Partitiviridae*. *Arch. Virol.* **2021**, *166*, 659–664. [CrossRef] [PubMed]
104. Moriyama, H.; Kotta-Loizou, I.; Kim, K.-H.; Xie, J. Editorial: Mycoviruses and Related Viruses Infecting Fungi, Lower Eukaryotes,Plants and Insects. *Front. Microbiol.* **2021**, *12*, 798598. [CrossRef]
105. Deng, F.; Boland, G.J. Natural Occurrence of a *Partitivirus* in the Sapstaining Fungus *Ceratocystis Resinifera*. *Can. J. Plant Pathol.* **2007**, *29*, 182–189. [CrossRef]
106. Botella, L.; Jung, M.H.; Rost, M.; Jung, T. Natural Populations from the Phytophthora Palustris Complex Show a High Diversityand Abundance of SsRNA and DsRNA Viruses. *J. Fungi* **2022**, *8*, 1118. [CrossRef]
107. Kinsella, C.M.; Deijs, M.; Gittelbauer, H.; van der Hoek, L.; van Dijk, K. Human Clinical Isolates of Pathogenic Fungi Are Host to Diverse Mycoviruses. *Microbiol. Spectr.* **2022**, *10*, e01610–e01622. [CrossRef]
108. Zou, Q.; Gao, Y.; Wang, Q.; Yang, Y.; Wang, F.; Hong, N.; Wang, G.; Wang, L. The Full-Length Genome Sequence of a Novel *Mitovirus* from *Botryosphaeria Dothidea*, the Causal Agent of Pear Ring Rot Disease. *Arch. Virol.* **2021**, *166*, 2881–2885. [CrossRef]
109. Wang, H.; Liu, H.; Zhou, Q. The Complete Genome Sequence of a New *Mitovirus* from the Phytopathogenic Fungus *Colletotrichum Higginsianum*. *Arch. Virol.* **2021**, *166*, 1481–1484. [CrossRef] [PubMed]
110. Wang, J.; Ni, Y.; Liu, X.; Zhao, H.; Xiao, Y.; Xiao, X.; Li, S.; Liu, H. Divergent RNA Viruses in *Macrophomina*

Phaseolina Exhibit Potential as Virocontrol Agents. *Virus Evol.* **2021**, *7*, veaa095. [CrossRef] [PubMed]
111. de Rezende, R.R.; de Oliveira Souza, F.; Leal, L.L.; Morgan, T.; Bermudez, J.M.M.; Cascardo, R.d.S.; de Barros, D.R.; Alfenas- Zerbini, P. Characterization of a New *Mitovirus* Infecting the Phytopathogenic Fungus *Microdochium Albescens*. *Arch. Virol.* **2021**, *166*, 3455–3459. [CrossRef] [PubMed]
112. Shafik, K.; Umer, M.; You, H.; Aboushedida, H.; Wang, Z.; Ni, D.; Xu, W. Characterization of a Novel *Mitovirus* Infecting *Melanconiella Theae* Isolated From Tea Plants. *Front Microbiol.* **2021**, *12*, 757556. [CrossRef]
113. Koonin, E.V.; Dolja, V.V.; Morris, T.J. Evolution and Taxonomy of Positive-Strand RNA Viruses: Implications of Comparative Analysis of Amino Acid Sequences. *Crit. Rev. Biochem. Mol. Biol.* **1993**, *28*, 375–430. [CrossRef]
114. Nishikiori, M.; Sugiyama, S.; Xiang, H.; Niiyama, M.; Ishibashi, K.; Inoue, T.; Ishikawa, M.; Matsumura, H.; Katoh, E. Crystal Structure of the Superfamily 1 Helicase from Tomato Mosaic Virus. *J. Virol.* **2012**, *86*, 7565–7576. [CrossRef]
115. Luo, X.; Jiang, D.; Xie, J.; Jia, J.; Duan, J.; Cheng, J.; Fu, Y.; Chen, T.; Yu, X.; Li, B.; et al. Genome Characterization and Phylogenetic Analysis of a Novel *Endornavirus* That Infects Fungal Pathogen *Sclerotinia Sclerotiorum*. *Viruses* **2022**, *14*, 456. [CrossRef]
116. Cao, C.T.H.; Derbyshire, M.C.; Regmi, R.; Li, H.; Jones, M.G.K.; Wylie, S.J. Small RNA Analyses of *a Ceratobasidium* Isolate Infected with Three Endornaviruses. *Viruses* **2022**, *14*, 2276. [CrossRef]
117. Marais, A.; Faure, C.; Comont, G.; Candresse, T.; Stempien, E.; Corio-Costet, M.-F. Characterization of the Mycovirome of the Phytopathogenic Fungus, *Neofusicoccum Parvum*. *Viruses* **2021**, *13*, 375. [CrossRef]
118. Koonin, E.V.; Gorbalenya, A.E.; Chumakov, K.M. Tentative Identification of RNA-Dependent RNA Polymerases of DsRNA Viruses and Their Relationship to Positive Strand RNA Viral Polymerases. *FEBS Lett.* **1989**, *252*, 42–46. [CrossRef]
119. Zanotto, P.M.; Gibbs, M.J.; Gould, E.A.; Holmes, E.C. A Reevaluation of the Higher Taxonomy of Viruses Based on RNA Polymerases. *J. Virol.* **1996**, *70*, 6083–6096. [CrossRef] [PubMed]
120. Zhang, X.; Nuss, D.L. A Host Dicer Is Required for Defective Viral RNA Production and Recombinant Virus Vector RNA Instability for a Positive Sense RNA Virus. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 16749–16754. [CrossRef] [PubMed]
121. Honda, S.; Eusebio-Cope, A.; Miyashita, S.; Yokoyama, A.; Aulia, A.; Shahi, S.; Kondo, H.; Suzuki, N. Establishment of *Neurospora Crassa* as a Model Organism for Fungal Virology. *Nat. Commun.* **2020**, *11*, 5627. [CrossRef] [PubMed]
122. Maiti, M.; Lee, H.-C.; Liu, Y. QIP, a Putative Exonuclease, Interacts with the *Neurospora* Argonaute Protein and Facilitates Conversion of Duplex SiRNA into Single Strands. *Genes Dev.* **2007**, *21*, 590–600. [CrossRef]
123. Liu, H.; Wang, H.; Liao, X.L.; Gao, B.; Lu, X.; Sun, D.; Gong, W.; Zhong, J.; Zhu, H.; Pan, X.; et al. Mycoviral Gene Integration Converts a Plant Pathogenic Fungus into a Biocontrol Agent. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2214096119. [CrossRef]
124. Lee, H.-C.; Chang, S.-S.; Choudhary, S.; Aalto, A.P.; Maiti, M.; Bamford, D.H.; Liu, Y. QiRNA Is a New Type of Small Interfering RNA Induced by DNA Damage. *Nature* **2009**, *459*, 274–277. [CrossRef]
125. de Beer, Z.W.; Duong, T.A.; Barnes, I.; Wingfield, B.D.; Wingfield, M.J. Redefining *Ceratocystis* and Allied Genera. *Stud. Mycol* **2014**, *79*, 187–219. [CrossRef]
126. Nasution, A.; Glen, M.; Beadle, C.; Mohammed, C. Ceratocystis Wilt and Canker—A Disease That Compromises the Growing of Commercial Acacia-Based Plantations in the Tropics. *Aust. For.* **2019**, *82* (Suppl. S1), 80–93. [CrossRef]

5. CONCLUSÕES

A adequada aplicação das ferramentas de bioinformática, assim como as diferentes tecnologias de sequenciamento de próxima geração, tem contribuído significativamente para a descoberta de novos vírus nas bibliotecas públicas de RNA-seq dos patógenos de cacau *C. cacaofunesta* e *C. fimbriata*, os quais são patógenos de grande importância econômica em nível mundial.

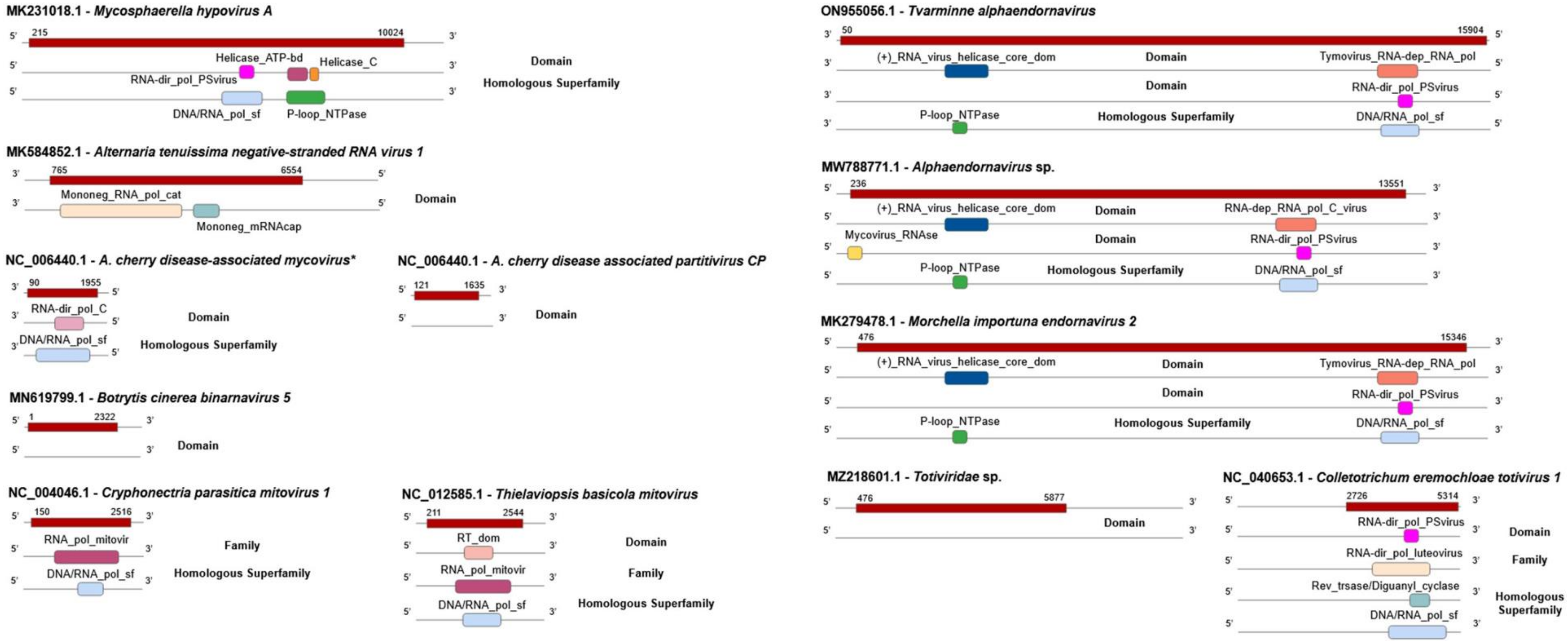
Nossa estratégia de bioinformática resultou na identificação de quatro vírus putativos em *C. cacaofunesta* (*Hypovirus, Sclerotimonavirus, Alphapartitivirus* e *Narnavirus*) e seis vírus em *C. fimbriata* (três *Alfaendornavirus*, um *Victorivirus* e dois *Mitovirus*), resultados muito relevantes, já que dentro das famílias micovirais encontradas neste estudo, já foram descritos vírus com potencial biotecnológico, fornecendo uma visão mais ampla para futuras investigações científicas em áreas de genética e biologia molecular.

Com base nos resultados obtidos, é evidente que a aplicação da bioinformática na análise de sequências de RNA-seq foi fundamental para o avanço do conhecimento sobre a presença de vírus em *C. cacaofunesta* e *C. fimbriata*, fornecendo informações valiosas para o controle e manejo desses patógenos no cultivo do cacau. A identificação desses vírus é um marco importante para a ciência e para a indústria do cacau, abrindo portas para futuras pesquisas que possam aproveitar o potencial biotecnológico desses microrganismos para o desenvolvimento de estratégias mais eficazes de controle de doenças em plantações de cacau.

Isso torna este estudo o primeiro relato de vírus associados aos patógenos de cacau *C. cacaofunesta* e *C. fimbriata*, e o segundo relato de vírus que infectam membros da família *Ceratocistidaceae.* No entanto, essas descobertas destacam a necessidade de novos estudos para descobrir a diversidade real de micovírus que podem contribuir para o desenvolvimento de novos biofungicidas.

Portanto, este estudo representa um avanço significativo no campo da virologia de fungos, contribuindo para a compreensão da diversidade viral em importantes patógenos do cacau. Espera-se que os resultados aqui apresentados impulsionem novas investigações no campo da biologia molecular e da genética, visando a descoberta de novos micovírus com potencial aplicação na agricultura e na produção sustentável de cacau, beneficiando a indústria cacaueira e a economia global.

6. MATERIAL SUPLEMENTAR



* *Amasya cherry disease-associated mycovirus* is a below-species classification of *Cherry chlorotic rusty spot associated partitivirus*

Figure S1. Conserved structures of the hit organisms for e-value of the studied sequences based on BlastX similarity analysis. TheORFfinder program was used to identify the largest ORF and InterProScan program was used to identify conserved structures.

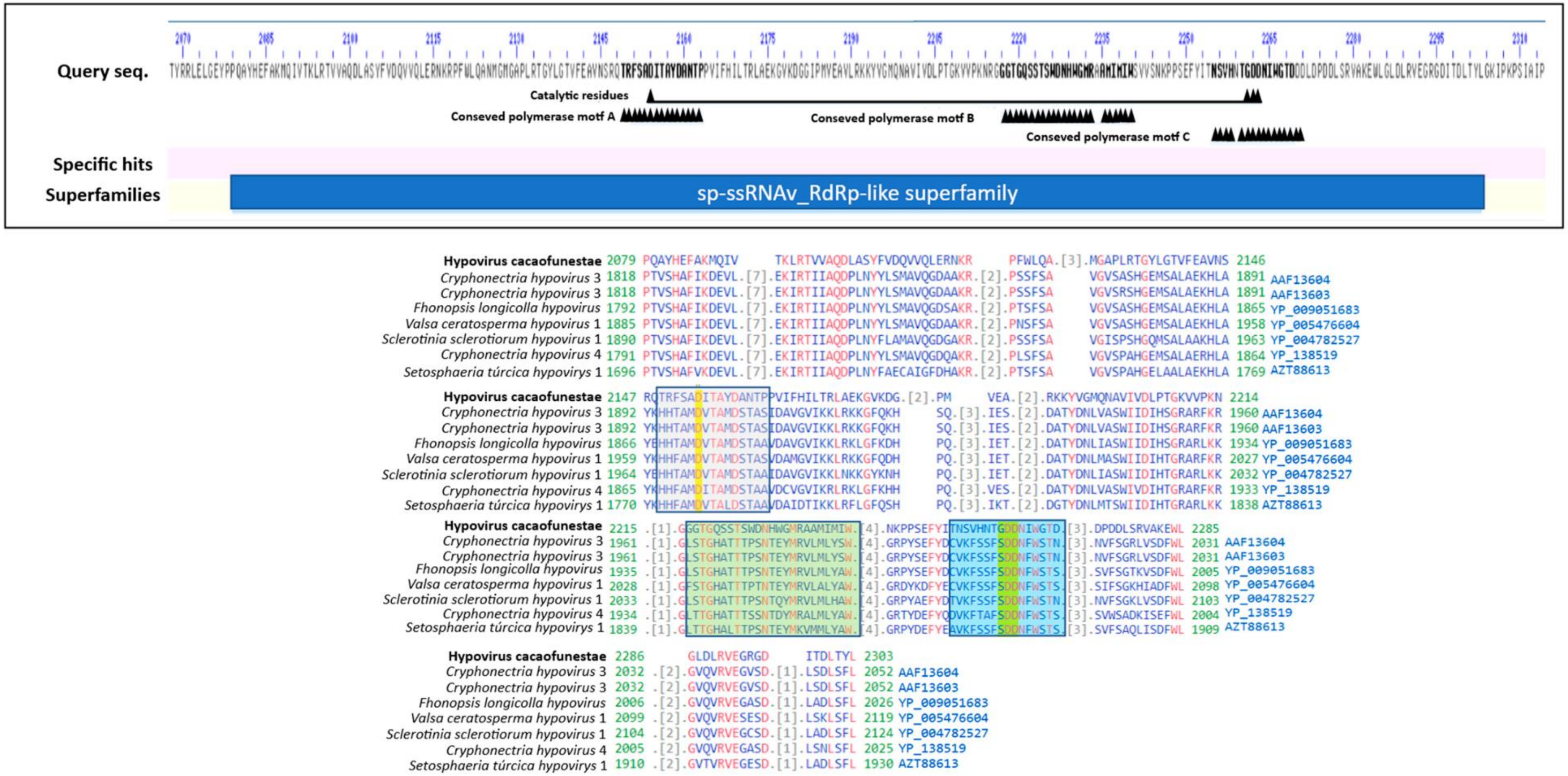


Figure S2. Conserved motifs and catalytic site in the Hypovirus cacaofunestae viral contig. A. Superfamily cl40470 similar to ps- ssRNAv_RdRp located in the interval 2079-2303, conserved polymerase motif A, located at position 2149-2163, conserved polymerase motif B,located at position 2217-2233 and 2235-2240, conserved polymerase motif C, located at position 2255-2258 and 2259-2269 B. Species of the family *Hypoviridae* with similarity of motifs conserved with HVc, Catalytic site (Yellow), Motif A (Light Grey), Motif B (Light Green), Motif C (LemonGreen).

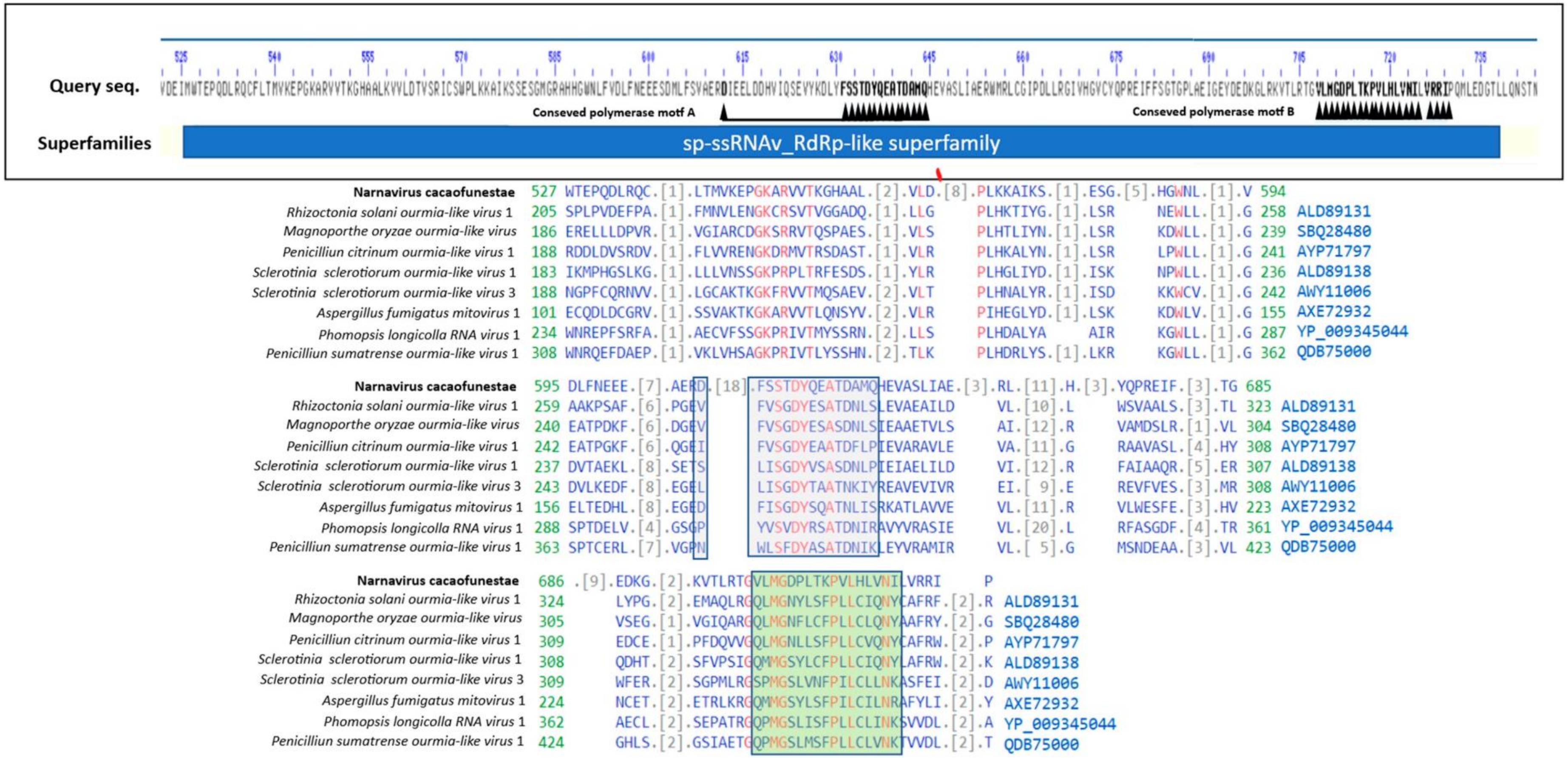


Figure S3. Conserved motifs and catalytic site in the Narnavirus cacaofunestae viral contig. A. Superfamily cl40470 similar to ps- ssRNAv_RdRp located in the interval 527-736, conserved polymerase motif A, located at positions 612 and 631-644 , conserved polymerase motif B, located at positions 698-724 and 726-729. B. Species of the family Botourmiaviridae and Mitoviridae with similarity of motifs conserved with NVf, Motif A (light grey), Motif B (light green).

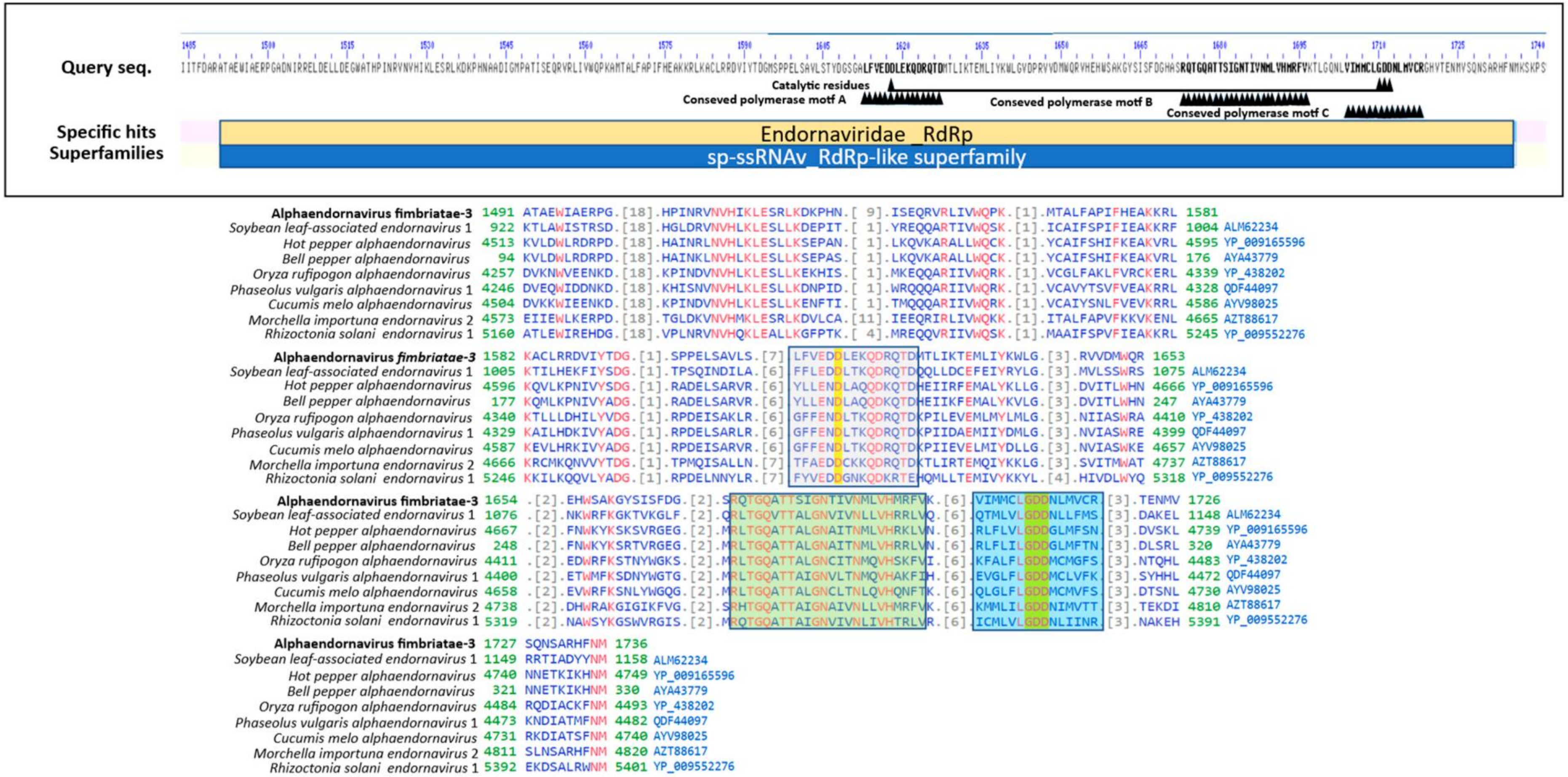


Figure S4. Conserved motifs and catalytic site in the Alphaendornavirus fimbriatae-3 viral contig. A. Superfamily cl40470 similar to ps- ssRNAv_RdRp located in the interval 1491-1736, conserved polymerase motif A, located at position 1613-1627, conserved polymerase motif B,located at position 1673-1696, conserved polymerase motif C, located at position 1704-1718 B. Species of the family *Endornaviridae* with similarity of motifs conserved with AEVf-3, Catalytic site (Yellow), Motif A (Light Grey), Motif B (Light Green), Motif C (Blue).

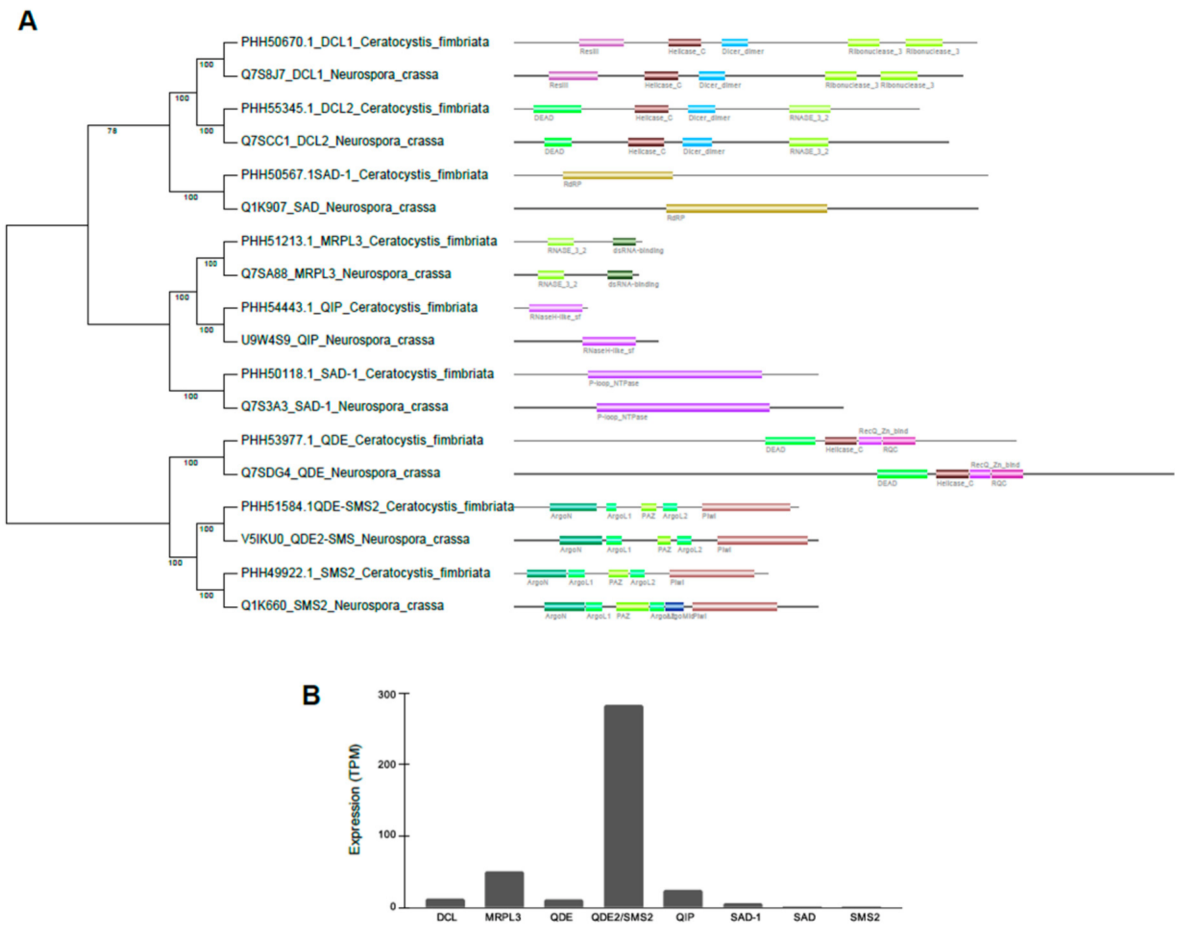


Figure S5. Characterization of RNAi-related genes in *Ceratocystis fimbriata*. (A) Dendrogram based on sequence similarity. (B) Evaluationof RNAi-related genes transcriptional activity. Quantification was normalized by Transcripts Per Million (TPM).

Table S1: Overview of RNA deep sequencing li- braries used in the work.

Bioproject	Organism	Run	Total Sequences	Bowtie2 - Aligned 0 times	Bowtie2 - Aligned exactly 1 time	Bowtie2 - Aligned >1 times	SPAdes	rnaviralSPAdes	Trinity	CAP3
PRJNA415812	*Ceratocystis cacaofunesta*	SRR6217952	55,951,393	3.549.075 (6.39%)	50.613.134 (91.15%)	1.366.876 (2.46%)	1,750	965	480	1,002
PRJNA67151	*Ceratocystis fimbriata CBS 114723*	SRR8599076	42,480,313	9.524.414 (23.58%)	27.690.238 (68.55%)	3.178.582 (7.87%)	7.398	10.709	64,961	52,493

Table S2: Best hits at nucleotide and amino acid levels for viral transcripts assembled in our work.

Contig	Length	BlastN					BlastX				
		Query Cover	E value	Per. Ident	Hit	Accession	Query Cover	E value	Per. Ident	Hit	Accession
Cc_Contig1	9920	-	-	-	No significant similarity found	-	94%	0.0	42.58%	unknown [*Mycosphaerella hypovirus* A]	QED42897.1
Cc_Contig2	5652	30%	3E-148	67.83%	*Fusarium graminearum negative-stranded RNA virus* 1 strain HN1, complete genome	MF276904.1	98%	0.0	55.14%	RNA-dependent RNA polymerase [*Alternaria tenuissima negative-stranded RNA virus* 1]	QDB75013.1
Cc_Contig3	1998	98%	0.0	78.19%	*Cherry chlorotic rusty spot associated partitivirus* RdRp gene for RNA-dependent RNA polymerase, segment 1, genomic RNA	AJ781401.1	93%	0.0	83.55%	RNA-dependent RNA polymerase [*Amasya cherry disease-associated mycovirus*]	YP_138537.1
Cc_Contig4	1818	46%	3E-79	71.69%	*Amasya cherry disease associated partitivirus* CP gene for putative coat protein, genomic RNA	AJ781167.1	80%	0.0	58.61%	putative coat protein [*Amasya cherry disease-associated mycovirus*]	YP_138536.1
Cc_Contig5	2409	-	-	-	No significant similarity found	MN605460.1	91%	0.0	50.00%	RNA-dependent RNA polymerase [*Botrytis cinerea binarnavirus* 5]	QJT73728.1
Cf_Contig6	2395	-	-	-	No significant similarity found	LN827943.1	53%	4E-113	48.97%	putative RNA-dependent RNA polymerase [*Cryphonectria parasitica mitovirus* 1-NB631]	NP_660174.1
Cf_Contig7	3268	77%	0.0	69.50%	*Thielaviopsis basicola mitovirus* RNA-dependent RNA polymerase gene, complete cds	AY563138.1	64%	0.0	66.00%	RNA-dependent RNA polymerase [*Thielaviopsis basicola mitovirus*]	AAZ99833.1
Cf_Contig8	10431	-	-	-	No significant similarity found	-	38%	2E-55	23.85%	putative polyprotein [*Tvarminne*	UYL94275.1

										alphaendornavirus]	
Cf_Contig10	5328	-	-	-	No significant similarity found	-	51%	4E-40	23.25%	polyprotein [*Alphaendornavirus* sp.]	UIA10510.1
Cf_Contig8	5790	-	-	-	No significant similarity found	-	53%	1E-125	50.71%	polyprotein [*Morchella importuna endornavirus* 2]	AZT88617.1
Cf_Contig11	3995	19%	3E-38	65.59%	*Totiviridae* sp. isolate 237-k141_22307 genomic sequence	MZ218601.1	66%	0.0	48.79%	putative RNA-dependent RNA polymerase [*Totiviridae* sp.]	UHS72462.1
Cf_Contig12	2493	41%	2E-69	66.45%	*Fusarium asiaticum victorivirus* 1 isolate F16176, complete genome	NC_040653.1	77%	0.0	54.05%	coat protein [*Colletotrichum eremochloae totivirus* 1]	AZT88632.1

Table S3: Viral genomes used in phylogenetic analyses.

Hypoviridae	
Organism	**Accession number**
Wuhan insect virus 14	YP_009342443.1
Fusarium sacchari hypovirus 1	QIQ28422.1
Apis hypovirus 2	UCR92524.1
Nigrospora oryzae hypovirus 1	BCY26969.1
Fusarium graminearum hypovirus 1	AZT88611.1
Trichoderma asperellum hypovirus 1	AZT88614.1
Botrytis cinerea hypovirus 2	QJT73706.1
Macrophomina phaseolina hypovirus 2	QOE55583.1
Trichoderma harzianum hypovirus 2	UDP60414.1
Bipolaris oryzae hypovirus 1	QDY81493.1
Fusarium graminearum hypovirus 2	YP_009130646.1
Fusarium poae hypovirus 1	BAV56305.1
Rosellinia necatrix hypovirus 1	BBC21049.1
Agaricus bisporus virus 2	AQM49947.1
Sclerotium rolfsii hypovirus 8	AZF86113.1
Sclerotium rolfsii hypovirus 1	AZA15168.1
Rhizoctonia solani hypovirus 1	QDW92698.1
Mycosphaerella hypovirus A	QED42897.1
Hypovirus sp.	USC30043.1
Sclerotium rolfsii hypovirus 4	AZF86109.1
Beihai hypo-like virus 1	YP_009333297.1
Beihai sipunculid worm virus 6	YP_009333562.1
Sclerotinia sclerotiorum hypovirus 6	AZF86111.1
Fusarium oxysporum dianthi hypovirus 2	QHI00074.1
Botrytis cinerea hypovirus 1	UNI72644.1
Rosellinia necatrix fusarivirus 1	BAP16392.1
Fusarium poae fusarivirus 1	BAV56303.1
Botrytis cinerea fusarivirus 7	QJT73723.1
Lentinula edodes fusarivirus 1	QOX06044.1

Mymonaviridae	
Organism	**Accession number**
Soybean leaf-associated negative-stranded RNA virus 1	ALM62220.1
Fusarium graminearum negative-stranded RNA virus 1	UNG44335.1
Fusarium asiaticum negative-stranded RNA virus 1	UNG44331.1
Fusarium proliferatum mymonavirus 1	UWK02084.1
Plasmopara viticola lesion associated mononega virus 2	QHD64785.1
Alternaria tenuissima negative-stranded RNA virus 1	QDB75013.1
Alternaria dianthicola negative-stranded RNA virus 1	UYZ32462.1
Botrytis cinerea negative-stranded RNA virus 3	QJT73696.1
Cryphonectria parasitica sclerotimonavirus 1	QMP84020.1
Sclerotinia sclerotiorum negative-stranded RNA virus 3-A	AWY11025.1
Botrytis cinerea negative-stranded RNA virus 4	QJT73698.1
Plasmopara viticola lesion associated mymonavirus 1	QHD64779.1
Plasmopara viticola lesion associated mononegaambi virus 6	QHD64777.1
Plasmopara viticola lesion associated mononegaambi virus 8	QHD64783.1
Fusarium oxysporum mymonavirus 1	UNQ75001.1
Lentinula edodes negative-strand RNA virus 1	BBI93117.1
Auricularia heimuer negative-stranded RNA virus 1	QJP04103.1
Botrytis cinerea negative-stranded RNA virus 7	QKW91270.1
Apple virus B	QIC52850.1
Gysing virus	QGA70913.1
Orinoco virus	ANQ45640.1
Formica exsecta virus 4	AWI42881.1
Beihai rhabdo-like virus 4	YP_009333418.1
Nyavirus midwayense	ACQ94979.1
Beihai rhabdo-like virus 6	YP_009333413.1
Wenzhou tapeworm virus 1	APG78764.1
Socyvirus heteroderae	YP_009052467.1

Partitiviridae	
Organism	**Accession number**
Podosphaera tridactyla virus 1	UXX51154.1
Podosphaera prunicola partitivirus 3	ATS94413.1
Rhizoctonia oryzae-sativae partitivirus 3	BCY26956.1
Rhizoctonia fumigata partitivirus	AJE25830.1
Gaeumannomyces tritici partitivirus 2	AZT88604.1
Vicia cryptic viru	ABN71237.1
Tulasnella partitivirus 3	BDB07479.1
Diuris pendunculata cryptic virus	AFQ95555.1
Cherry chloroticrusty spot associated partitivirus	CAH03668.1
Amasya cherry disease-associated mycovirus	YP_138537.1
Alphapartitivirus sp.	UDL14427.1
Rosellinia necatrix partitivirus 9	BBB86797.1
Heterobasidion partitivirus 5	ADV15444.1
Rhizoctonia oryzae-sativae partitivirus 2	BCY26954.1
Ceratobasidium partitivirus	AOX47570.1
Rhizoctonia solani partitivirus virus 3	ASZ83552.1
Rhizoctonia solani dsRNA virus 4	YP_009551520.1
Rhizoctonia oryzae-sativae partitivirus 4	BCY26958.1
Rhizoctonia solani dsRNA virus 2	UIW13805.1
Erysiphe necator associated betapartitivirus 1	QKK35382.1
Betapartitivirus sp	UEP18204.1
Spinach deltapartitivirus 1	ARO72610.1
Medicago sativa deltapartitivirus 1	ATJ00052.1
Erysiphe necator associated gammapartitivirus 1	QKK35392.1
Gammapartitivirus sp	UDL14336.1
Cryptosporidium parvum virus 1	ARS33771.1
Saccharomyces cerevisiae cryspovirus 1	UGZ04791.1
Coccinia mottle virus	YP_009272707.1
Caladenia virus A	YP_006666511.1
Brome streak mosaic virus	UZN89630.1
Passiflora edulis symptomless virus	UIX56007.1
Agropyron mosaic virus	AUF81943.1
African eggplant mosaic virus	YP_009666833.1
Blackberry virus Y	YP_851006.1
Alpinia oxyphylla mosaic virus	YP_010086847.1
Bellflower veinal mottle virus	YP_009508455.1
Barley mild mosaic virus	AAC42215.1
Areca palm necrotic ringspot virus	UIW31246.1
Celery latent virus	YP_010087166.1

Narnaviridae and Mitoviridae	
Organism	**Accession number**
Fusarium mangiferae mitovirus 4	UBZ25883.1
Fusarium globosum mitovirus 1	YP_009126872.1
Fusarium circinatum mitovirus 1	AHI43533.1
Mitoviridae sp.	URG16629.1
Fusarium coeruleum mitovirus 1	YP_009126873.1
Fusarium poae mitovirus 2	YP_009272899.1
Thielaviopsis basicola mitovirus	AAZ99833.1
Leptosphaeria biglobosa mitovirus 1	YP_009553599.1
Ophiostoma mitovirus 4	NP_660179.1
Monilinia fructicola mitovirus 4	UTQ48821.1
Rugonectria rugulosa mitovirus 1	UTS95837.1
Ophiostoma mitovirus 7	AGT55877.1
Cryphonectria cubensis mitovirus 2b	AAR01974.1
Cryphonectria parasitica mitovirus 1-NB631	NP_660174.1
Fusarium sambucinum mitovirus 2	BCP96873.1
Botryosphaeria dothidea mitovirus 1	QMU24933.1
Setosphaeria turcica mitovirus 1	AZT88625.1
Neofusicoccum parvum mitovirus 3	QTE76052.1
Plasmopara viticola lesion associated mitovirus 43	QIR30266.1
Botrytis cinerea mitovirus 9	QKW91256.1
Grapevine associated narnavirus-1	CEZ26304.1
Erysiphe necator associated mitovirus 31	QKI79979.1
Monilinia fructicola mitovirus 1	UTQ48818.1
Geopora sumneriana mitovirus 1	QDM55307.1
Rhizoctonia solani mitovirus 39	QIS79099.1
Sanya narna-like virus 3	UUW20998.1
Guiyang narna-like virus 3	UUW20994.1
Botryosphaeria dothidea narnavirus 1	QED22729.1
Sclerotinia sclerotiorum narnavirus 2	QZE12026.1
Botrytis cinerea binarnavirus 5	QJT73728.1
Leptosphaeria biglobosa narnavirus 3	UYL94518.1
Cladosporium tenuissimum narnavirus 1	QDB74996.1
Erysiphe necator associated narnavirus 24	QJT93756.1
Bremia lactucae associated narnavirus 3	QIP68021.1
Plasmopara viticola lesion associated narnavirus 2	UUW20990.1
Rhizoctonia solani narnavirus 4	UIW13846.1
Epirus cherry virus	YP_002019754.1
Ourmia melon virus	YP_002019757.1
Cassava virus C	YP_003104770.1
Hubei narna-like virus 3	YP_009337787.1
Beihai narna-like virus 9	YP_009333316.1
ssRNA phage Esthiorhiza.2_44	DAD51276.1
ssRNA phage Esthiorhiza.4_3	DAD50247.1
ssRNA phage Gerhypos.3_3	DAD50287.1

ssRNA phage Gerhypos.4_17	DAD51589.1
ssRNA phage Esthiorhiza.1_9	DAD52218.1

Endornaviridae	
Organism	**Accession number**
Ceratobasidium endornavirus A	YP_009310113.1
Alphaendornavirus sp.	UIA10509.1
Rhizoctonia solani endornavirus 7	QDW65434.1
Ceratobasidium endornavirus 2	QNN89174.1
Rhizoctonia solani endornavirus 4	QDW65431.1
Gyromitra esculenta endornavirus 1	AZT88615.1
Morchella importuna endornavirus 2	AZT88617.1
Rhizoctonia solani endornavirus 2	YP_010086587.1
Rhizoctonia solani endornavirus 6	QDW65433.1
Erysiphe cichoracearum alphaendornavirus	ALX17418.1
Grapevine endophyte alphaendornavirus	AFV91541.1
Helicobasidium mompa alphaendornavirus 1	BAE94538.1
Neofusicoccum parvum endornavirus 1	QTE76046.1
Helianthus annuus alphaendornavirus	UMQ74215.1
Agaricus bisporus endornavirus 1	AQM32768.1
Hot pepper alphaendornavirus	ALD49085.1
Bell pepper alphaendornavirus	YP_009513187.1
Phaseolus vulgaris alphaendornavirus 2	ALJ56098.1
Winged bean alphaendornavirus 1	BAV69339.1
Triticeae associated alphaendornavirus	QPF20438.1
Hordeum vulgare alphaendornavirus	ALT66307.1
Cluster bean endornavirus 1	AYA60157.1
Phytophthora alphaendornavirus 1	CAI47561.1
Rhizoctonia cerealis alphaendornavirus 1	YP_008719905.1
Lagenaria siceraria endornavirus-California	AHK22715.1
Cucumis melo alphaendornavirus	ALV83885.1
Yerba mate alphaendornavirus	AID67139.1
Persea americana alphaendornavirus 1	AEX28369.1
Oryza sativa alphaendornavirus	BAA06862.1
Phaseolus vulgaris alphaendornavirus 1	ALJ56097.1
Brown algae endornavirus 1	BBZ90073.1
Tvarminne alphaendornavirus	UYL94275.1
Sclerotinia sclerotiorum betaendornavirus 1	AHN50398.1
Botrytis cinerea betaendornavirus 1	AOZ66245.1
Gremmeniella abietina endornavirus 1	ABD73305.1
Sclerotinia minor betaendornavirus1	AXX39023.1
Tuber aestivum betaendornavirus	ADU64759.1
Alternaria brassicicola betaendornavirus 1	AJA41110.1
Rosellinia necatrix betaendornavirus1	BAT32944.1
Citrus sudden death-associated virus	YP_224218.1

Maize rayado fino virus	NP_115454.2
Grapevine fleck virus	NP_542612.1
Andean potato latent virus	AAC25015.1
Anagyris vein yellowing virus	YP_002308578.1

Totiviridae	
Organism	**Accession number**
Fusarium asiaticum victorivirus 2	UBS89881.1
Fusarium sambucinum victorivirus 1	UBR58457.1
Alternaria dianthicola victorivirus 1	UYZ32455.1
Totiviridae sp.	UHS72462.1
Magnaporthe oryzae virus 1	YP_122352.1
Botrytis cinerea victorivirus 2-BCS8	QLC37104.1
Chalara elegans RNA Virus 1	YP_024728.1
Rosellinia necatrix victorivirus 1	YP_008130308.1
Botryosphaeria dothidea victorivirus 1	YP_009072433.1
Beauveria bassiana victorivirus 1	YP_009508251.1
Ustilaginoidea virens RNA virus 1	AGO04407.1
Aspergillus foetidus slow virus 1	YP_009508249.1
Botryosphaeria dothidea victorivirus 2-like	UVZ34687.1
Epichloe festucae virus 1	YP_009508253.1
Coniothyrium minitans RNA virus	YP_392467.1
Gremmeniella abietina RNA virus L1	AAK11656.1
Leishmania RNA virus 1 1	NP_041191.1
Trichomonas vaginalis virus 1	AAA62868.1
Saccharomyces cerevisiae virus L-BC La	NP_042581.1
Giardia lamblia virus	AAB01579.1
Pseudomonas phage phiYY	YP_009618381.1
Pseudomonas phage phi13	NP_690817.1

Table S5: RNAi-related genes.

Class	*Nuerospora Crassa* protein ID	Identity (%)	*Ceratocystis fimbriata* protein ID	Tag	Transcript	TPM
DCL	Q7S8J7|DCL1_NEUCR	42.8	PHH50670.1	CFIMG_003413RA	APWK03000021.1_mrna_2919	0.292909
DCL	Q7SCC1|DCL2_NEUCR	34.4	PHH55345.1	CFIMG_001061RA	APWK03000012.1_mrna_993	12.476628
MRPL3	Q7SA88|Q7SA88_NEUCR	56.5	PHH51213.1	CFIMG_007246RA00001	APWK03000100.1_mrna_259	50.361821
QDE	Q7SDG4|Q7SDG4_NEUCR	44.3	PHH53977.1	CFIMG_000986RA	APWK03000033.1_mrna_4015	11.096152
QDE2/SMS2	V5IKU0|V5IKU0_NEUCR	36.5	PHH51584.1	CFIMG_008656RA00001	APWK03000090.1_mrna_6979	284.210272
QIP	U9W4S9|U9W4S9_NEUCR	32.3	PHH54443.1	CFIMG_003042RA	PWK03000026.1_mrna_3424	24.541323
SAD-1	Q7S3A3|Q7S3A3_NEUCR	34.7	PHH50118.1	CFIMG_005029RA	APWK03000147.1_mrna_1717	5.116523
SAD	Q1K907|Q1K907_NEUCR	42.3	PHH50567.1	CFIMG_004538RA	APWK03000124.1_mrna_1118	0.858896
SMS2	Q1K660|Q1K660_NEUCR	40.4	PHH49922.1	CFIMG_002952RA	APWK03000161.1_mrna_2066	1.363147

7. REFERÊNCIAS COMPLEMENTARES

- AGRIOS, George N., **Plant Pathology**, [s.l.]: Elsevier, 2005
- ANDERSON, Pamela K. *et al*, Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers, **Trends in Ecology & Evolution**, v. 19, n. 10, p. 535–544, 2004.
- ANDERSON, Robyn; BAYER, Philipp E; EDWARDS, David. Climate change and the need for agricultural adaptation. **Current Opinion in Plant Biology**, v. 56, p. 197–202, 2020. (Biotic interactions AGRI 2019).
- ANTON DE BARY. **Morphologie und Physiologie der Pilze, Flechten und Myxomyceten**. Leipzig: W. Engelmann, 1866. Disponível em: <https://www.biodiversitylibrary.org/bibliography/120970>.
- ALMEIDA, Rodrigo P. P.; NUNNEY, Leonard, How Do Plant Diseases Caused by Xylella fastidiosa Emerge?, **Plant Disease**, v. 99, n. 11, p. 1457–1467, 2015.
- BEBBER, Daniel P.; HOLMES, Timothy; GURR, Sarah J., The global spread of crop pests and pathogens, **Global Ecology and Biogeography**, v. 23, n. 12, p. 1398–1407, 2014.
- BEKELE, Frances; PHILLIPS-MORA, Wilbert. Cacao (Theobroma cacao L.) Breeding. *In*: AL-KHAYRI, Jameel M.; JAIN, Shri Mohan; JOHNSON, Dennis V. (Orgs.). Advances in Plant Breeding Strategies: Industrial and Food Crops: Volume 6. Cham: **Springer International Publishing,** 2019, p. 409–487. Disponível em: <https://doi.org/10.1007/978-3-030-23265-8_12>. Acesso em: 18 nov. 2022.
- CABRERA, Odalys García; MOLANO, Eddy Patricia López; JOSÉ, Juliana; *et al*. Ceratocystis Wilt Pathogens: History and Biology—Highlighting *C. cacaofunesta*, the Causal Agent of Wilt Disease of Cacao. *In*: BAILEY, Bryan A.; MEINHARDT, Lyndel W. (Orgs.). Cacao Diseases: A History of Old Enemies and New Encounters. **Cham: Springer International Publishing,** 2016, p. 383–428. Disponível em: <https://doi.org/10.1007/978-3-319-24789-2_12>. Acesso em: 29 set. 2022.
- CHAKRABORTY, S.; NEWTON, A. C., Climate change, plant diseases and food security: an overview, **Plant Pathology**, v. 60, n. 1, p. 2–14, 2011.
- CHALONER, Thomas M.; GURR, Sarah J.; BEBBER, Daniel P. Plant pathogen infection risk tracks global crop yields under climate change. **Nature Climate Change**, v. 11, n. 8, p. 710–715, 2021.
- CILAS, Christian; BASTIDE, Philippe. Challenges to Cocoa Production in the Face of Climate Change and the Spread of Pests and Diseases. **Agronomy**, v. 10, n. 9, p. 1232, 2020.
- DANG, Yunkun; YANG, Qiuying; XUE, Zhihong; *et al*. RNA Interference in Fungi: Pathways, Functions, and Applications. **Eukaryotic Cell**, v. 10, n. 9, p. 1148–1155, 2011.
- DEAN, Ralph *et al*, The Top 10 fungal pathogens in molecular plant pathology,

- **Molecular Plant Pathology**, v. 13, n. 4, p. 414–430, 2012.
- DE BEER, Z.W.; DUONG, T.A.; BARNES, I.; *et al*. Redefining *Ceratocystis* and allied genera. **Studies in Mycology**, v. 79, p. 187–219, 2014.
- DELGADO-OSPINA, Johannes; MOLINA-HERNÁNDEZ, Junior Bernardo; CHAVES-LÓPEZ, Clemencia; *et al*. The Role of Fungi in the Cocoa Production Chain and the Challenge of Climate Change. **Journal of Fungi**, v. 7, n. 3, p. 202, 2021.
- DOMINGO, E.; HOLLAND, J. J. RNA virus mutations and fitness for survival. **Annual Review of Microbiology**, v. 51, p. 151–178, 1997.
- ENGELBRECHT, C. J. B.; HARRINGTON, T. C.; ALFENAS, A. C.; *et al*. Genetic variation in populations of the cacao wilt pathogen, *Ceratocystis cacaofunesta*. **Plant Pathology**, v. 56, n. 6, p. 923–933, 2007.
- ENGELBRECHT, Christine J.; HARRINGTON, Thomas C.; ALFENAS, Acelino. Ceratocystis Wilt of Cacao—A Disease of Increasing Importance. **Phytopathology®**, v. 97, n. 12, p. 1648–1649, 2007.
- FERNANDES, Luciel Dos Santos; ROYAERT, Stefan; CORRÊA, Fábio M.; *et al*. Mapping of a Major QTL for *Ceratocystis* Wilt Disease in an F1 Population of Theobroma cacao. **Frontiers in Plant Science**, v. 9, p. 155, 2018.
- FISHER, Matthew C. *et al*, Emerging fungal threats to animal, plant and ecosystem health, **Nature**, v. 484, n. 7393, p. 186–194, 2012.
- GARRETT, K. A. *et al*, Climate change effects on plant disease: genomes to ecosystems, **Annual Review of Phytopathology**, v. 44, p. 489–509, 2006.
- GHABRIAL, Said A.; CASTÓN, José R.; JIANG, Daohong; *et al*. 50-plus years of fungal viruses. **Virology**, v. 479–480, p. 356–368, 2015.
- HALSTED, Byron David. **Some Fungous Diseases of the Sweet Potato**. [s.l.]: New Jersey Agricultural College Experiment Station, 1890.
- HARRINGTON, Thomas. The genus Ceratocystis. Where does the oak wilt fungus fit? **Proceedings of the 2nd National Oak Wilt Symposium**, p. 21–35, 2009.
- HAYASAKA, Daisuke; KUWAYAMA, Naoki; TAKEO, Azuma; *et al*. Different acute toxicity of fipronil baits on invasive Linepithema humile supercolonies and some non-target ground arthropods. **Ecotoxicology (London, England)**, v. 24, n. 6, p. 1221–1228, 2015.
- HOLLAND, L.A.; LAWRENCE, D.P.; NOURI, M.T.; *et al*. Taxonomic revision and multi-locus phylogeny of the North American clade of Ceratocystis. **Fungal Systematics and Evolution**, v. 3, p. 135–156, 2019.
- HUSSAIN, BABAR. Modernization in plant breeding approaches for improving biotic stress resistance in crop plants. **Turkish Journal of Agriculture and Forestry**, v. 39, n. 4, p. 515–530, 2015.
- ICCO. International Cocoa Organization - Statistics. Disponível em: <https://www.icco.org/statistics/>. Acesso em: 31 maio 2023.
- LIU, Hong; WANG, Hui; LIAO, Xiao Lan; *et al*. Mycoviral gene integration converts a plant pathogenic fungus into a biocontrol agent. **Proceedings of the National Academy of Sciences**, v. 119, n. 50, p. e2214096119, 2022.
- MARZANO, Shin-Yi Lee; DOMIER, Leslie L. Novel mycoviruses discovered from metatranscriptomics survey of soybean phyllosphere phytobiomes. **Virus Research**, v. 213, p. 332–342, 2016.
- MOKILI, John L; ROHWER, Forest; DUTILH, Bas E. Metagenomics and future perspectives in virus discovery. **Current Opinion in Virology**, v. 2, n. 1, p. 63–77, 2012.
- MU, Fan; XIE, Jiatao; CHENG, Shufen; *et al*. Virome Characterization of a Collection

of S. sclerotiorum from Australia. **Frontiers in Microbiology**, v. 8, p. 2540, 2017.

- MUNERET, Lucile; AURIOL, Arthur; THIÉRY, Denis; *et al*. Organic farming at local and landscape scales fosters biological pest control in vineyards. **Ecological Applications: A Publication of the Ecological Society of America**, v. 29, n. 1, p. e01818, 2019.
- NASUTION, A.; GLEN, M.; BEADLE, C.; *et al*. *Ceratocystis* wilt and canker – a disease that compromises the growing of commercial Acacia-based plantations in the tropics. **Australian Forestry**, v. 82, n. sup1, p. 80–93, 2019.
- NIBERT, Max L.; GHABRIAL, Said A.; MAISS, Edgar; *et al*. Taxonomic reorganization of family *Partitiviridae* and other recent progress in partitivirus research. **Virus Research**, v. 188, p. 128–141, 2014.
- NICOLÁS, Francisco E.; RUIZ-VÁZQUEZ, Rosa M. Functional Diversity of RNAi-Associated sRNAs in Fungi. **International Journal of Molecular Sciences**, v. 14, n. 8, p. 15348–15360, 2013.
- ORELLI, John L.; JONES, Alan L.; ALDWINCKLE, Herb S., Fire Blight Management in the Twenty-first Century: Using New Technologies that Enhance Host Resistance in Apple, **Plant Disease**, v. 87, n. 7, p. 756–765, 2003.
- NUSS, Donald L. Mycoviruses, RNA Silencing, and Viral RNA Recombination. **Advances in Virus Research**, v. 80, p. 25–48, 2011.
- PALADINES-REZABALA, Anderson; MOREIRA-MORRILLO, Anthony A.; MIELES, Alejandro E.; *et al*. Advances in understanding of the interaction between Ceratocystis cacaofunesta and *Xyleborus ferrugineus* (Coleoptera: Curculionidae: Scolytinae) on cocoa trees. **Scientia Agropecuaria**, v. 13, n. 1, p. 43–52, 2022.
- PAUTASSO, Marco *et al*, Plant health and global change – some implications for landscape management, **Biological Reviews**, v. 85, n. 4, p. 729–755, 2010.
- PLOETZ, Randy. The Impact of Diseases on Cacao Production: A Global Overview. *In*: BAILEY, Bryan A.; MEINHARDT, Lyndel W. (Orgs.). **Cacao Diseases: A History of Old Enemies and New Encounters**. Cham: Springer International Publishing, 2016, p. 33–59. Disponível em: <https://doi.org/10.1007/978-3-319-24789-2_2>. Acesso em: 18 nov. 2022.
- ROOSSINCK, Marilyn J. Metagenomics of plant and fungal viruses reveals an abundance of persistent lifestyles. **Frontiers in Microbiology**, v. 5, p. 767, 2015.
- SANTOS, Rogério Mercês Ferreira; SILVA, Stela Dalva Vieira Midlej; SENA, Kaleandra; *et al*. Kinetics and Histopathology of the Cacao-*Ceratocystis cacaofunesta* Interaction. **Tropical Plant Biology**, v. 6, n. 1, p. 37–45, 2013.
- SCHOCH, Conrad L; CIUFO, Stacy; DOMRACHEV, Mikhail; *et al*. NCBI Taxonomy: a comprehensive update on curation, resources and tools. **Database: The Journal of Biological Databases and Curation**, v. 2020, p. baaa062, 2020.
- SEGERS, Gert C.; ZHANG, Xuemin; DENG, Fuyou; *et al*. Evidence that RNA silencing functions as an antiviral defense mechanism in fungi. **Proceedings of the National Academy of Sciences**, v. 104, n. 31, p. 12902–12906, 2007.
- SHARMA, Mohit; GULERIA, Shiwani; SINGH, Kirti; *et al*. Mycovirus associated hypovirulence, a potential method for biological control of *Fusarium* species. **Virus Disease**, v. 29, n. 2, p. 134–140, 2018.
- STUKENBROCK, Eva H. *et al*, Origin and domestication of the fungal wheat pathogen Mycosphaerella graminicola via sympatric speciation, **Molecular Biology and Evolution**, v. 24, n. 2, p. 398–411, 2007.
- SUN, Yong; LI, Mengqiu; WANG, Yansu; *et al*. *Ceratocystis fimbriata* Employs a Unique Infection Strategy Targeting Peltate Glandular Trichomes of Sweetpotato

(*Ipomoea batatas*) Plants. **Phytopathology**, v. 110, n. 12, p. 1923–1933, 2020.

- SYAZWAN, Samsuddin Ahmad; MOHD-FARID, Ahmad; WAN-MUHD-AZRUL, Wan-Azhar; *et al*. Survey, Identification, and Pathogenicity of *Ceratocystis fimbriata* Complex Associated with Wilt Disease on *Acacia mangium* in Malaysia. **Forests**, v. 12, n. 12, p. 1782, 2021.
- VILLAN LARIOS, Diana Carolina; DIAZ REYES, Brayan Maudiel; PIROVANI, Carlos Priminho; *et al*. Exploring the Mycovirus Universe: Identification, Diversity, and Biotechnological Applications. **Journal of Fungi**, v. 9, n. 3, p. 361, 2023.
- WAGEMANS, Jeroen; HOLTAPPELS, Dominique; VAINIO, Eeva; *et al*. Going Viral: Virus-Based Biological Control Agents for Plant Protection. **Annual Review of Phytopathology**, v. 60, n. 1, p. 21–42, 2022.
- WEN, Caiyi; WAN, Xinru; ZHANG, Yuanyuan; *et al*. Molecular Characterization of the First Alternavirus Identified in *Fusarium oxysporum*. **Viruses**, v. 13, n. 10, p. 2026, 2021.
- WILKEN, P. Markus; STEENKAMP, Emma T.; WINGFIELD, Michael J.; *et al*. DNA loss at the *Ceratocystis fimbriata* mating locus results in self-sterility. **PloS One**, v. 9, n. 3, p. e92180, 2014.
- WILLIAMSON, Brian *et al*, Botrytis cinerea: the cause of grey mould disease, **Molecular Plant Pathology**, v. 8, n. 5, p. 561–580, 2007.
- XIAO, Xueqiong; CHENG, Jiasen; TANG, Jinghua; *et al*. A Novel Partitivirus That Confers Hypovirulence on Plant Pathogenic Fungi. **Journal of Virology**, v. 88, n. 17, p. 10120–10133, 2014.
- YU, Jisuk; LEE, Kyung-Mi; CHO, Won Kyong; *et al*. Differential Contribution of RNA Interference Components in Response to Distinct *Fusarium graminearum* Virus Infections. **Journal of Virology**, v. 92, n. 9, p. e01756-17, 2018.
- ZARRILLO, Sonia; GAIKWAD, Nilesh; LANAUD, Claire; *et al*. The use and domestication of *Theobroma cacao* during the mid-Holocene in the upper Amazon. **Nature Ecology & Evolution**, v. 2, n. 12, p. 1879–1888, 2018.
- ZHANG, Chao; WU, Zujian; LI, Yi; *et al*. Biogenesis, Function, and Applications of Virus-Derived Small RNAs in Plants. **Frontiers in Microbiology**, v. 6, p. 1237, 2015.
- ZHANG, Yong-Zhen; SHI, Mang; HOLMES, Edward C. Using Metagenomics to Characterize an Expanding Virosphere. **Cell**, v. 172, n. 6, p. 1168–1172, 2018.
- ZHU, Jun Zi; ZHU, Hong Jian; GAO, Bi Da; *et al*. Diverse, Novel Mycoviruses From the Virome of a Hypovirulent *Sclerotium rolfsii* Strain. **Frontiers in Plant Science**, v. 9, p. 1738, 2018.