

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL – UFRGS

**Síndrome anual da abelha mandaia (*Melipona quadrifasciata*) - o papel de simbiotes,
sistema imune e ambiente.**

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LISTA DE ABREVIATURAS E UNIDADES

+ssRNA: RNA fita simples de sentido positivo (*positive-sense single-stranded RNA*)

°C: graus Celsius

ABPV: vírus da paralisia aguda de abelha (*Acute bee paralysis virus*)

AMPs: peptídeos antimicrobianos

ANOVA: análise de variância

ARV-1: *Apis mellifera rhabdovirus-1*

BL: Boqueirão do Leão - RS

BLAST: ferramenta básica de busca de alinhamento local (*Basic Local Alignment Search Tool*)

BP: Bom princípio - RS

bp: pares de base (*base pairs*)

BQCV: vírus da célula de rainha negra (*Black queen cell virus*)

BU: teste incondicional de Barnard (*Barnard's Unconditional test*)

CBPV: vírus da paralisia crônica das abelhas (*Chronic bee paralysis virus*)

CCD: desordem do colapso das colônias (*Colony collapse disorder*)

CDS: sequência codificante (*Coding sequence*)

CMH: teste de Cochran-Mantel-Haenszel

CNRQ: quantidade relativa calibrada e normalizada (*Calibrated Normalized Relative Quantity*)

Cq: ciclo de quantificação (*quantification cycle*)

DEG: genes diferentemente expressos (*differentially expressed genes*)

dsDNA: DNA de fita dupla

DWV: vírus deformador da asa (*Deformed wing virus*)

E: eficiência do primer (*primer efficiency*)

fem: gene feminizador

g: gramas

GE: expressão gênica (*gene expression*)

GOX: glicose oxidase

H: saudável (*healthy*)

HDNA: amostra de DNA de abelhas saudáveis (*DNA sample from healthy bees*)

HJ: hormônio juvenil

HRNA: amostra de RNA de abelhas saudáveis (*RNA sample from healthy bees*)

IAPV: vírus da paralisia aguda israelense (*Israeli acute paralysis virus*)

Imd: via da imunodeficiência (*Immune deficiency*)

Jak/STAT: Janus quinase / transdutor de sinal e ativador da transcrição (*Janus kinase/Signal Transducer and Activator of Transcription*)

KBV: vírus de abelha da caxemira (*Kashmir bee virus*)

LSV: vírus do lago Sinai (*Lake Sinai virus*)

Ma: milhões de anos

min: minutos

mL: mililitros

mm: milímetros

mM: milimolar

MqC: *Melipona quadrifasciata cyclovirus*

MqC1: *Melipona quadrifasciata cyclovirus 1*

MqC2: *Melipona quadrifasciata cyclovirus 2*

MqD: *Melipona quadrifasciata densovirus*

MqV1a: *Melipona quadrifasciata virus 1a*

MqV1b: *Melipona quadrifasciata virus 1b*

MqV2: *Melipona quadrifasciata virus 2*

NF- κ B: fator nuclear kappa-B

NGE: expressão gênica normalizada (*normalized gene expression*)

nm: nanômetros

NMDS: escala multidimensional não métrica (*non-metric multidimensional scaling*)

nt: nucleotídeos

OcNV: *Osmia cornuta nudivírus*

OTUs: unidades taxonômicas operacionais (*Operational Taxonomic Units*)

p450: citocromo p450

PA: Porto Alegre - RS

PAMPs: Padrões Moleculares Associados a Patógenos

PCA: análise de componentes principais (*Principal Component Analysis*)

PCR: reação em cadeia da polimerase (*Polymerase Chain Reaction*)

PERMANOVA: análise multivariada permutacional de variância (*Permutational Multivariate Analysis of Variance*)

PO: fenoloxidase

r.p.m: rotações por minuto

RFU: unidade de fluorescência relativa (*Relative Fluorescence Unit*)

RL: Rolante – RS

RT-qPCR: PCR quantitativa de transcrição reversa (*quantitative reverse transcription PCR*)

SBPV: vírus da paralisia lenta das abelhas (*Slow bee paralysis virus*)

SBV: vírus de abelha *Sacbrood* (*Sacbrood bee virus*)

SGE: similaridade de expressão gênica (*gene expression similarity*)

ssDNA: DNA de fita simples

–ssRNA: RNA fita simples de sentido negativo (*negative-sense single-stranded RNA*)

U: doente (*unhealthy*)

UDNA: amostra de DNA de abelhas doente (*DNA sample from unhealthy bees*)

uL: microlitros

URNA: amostra de RNA de abelhas doentes (*RNA sample from unhealthy bees*)

VDV1: *Varroa destructor virus-1*

Vg: vitelogenina

Δ : delta

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RESUMO

Todos os anos ao final do verão, no sul do Brasil, colônias manejadas da abelha sem ferrão *Melipona quadrifasciata* manifestam uma síndrome que eventualmente leva-as ao colapso. Abelhas de colônias afetadas podem apresentar sintomas como tremores e paralisia, mas a principal característica da síndrome é a repentina morte de operárias, cujos corpos são encontrados em frente ou mesmo dentro da colméia. Nesta tese descrevemos nossos achados sobre as possíveis causas dessa mortalidade avaliando os diferentes níveis de complexidade de uma abelha social, desde o indivíduo e seus microrganismos associados até o nível da colônia. Mostramos que a microbiota de *M. quadrifasciata* é altamente variável, com grandes diferenças na composição bacteriana de abelhas de diferentes colônias. Bactérias patogênicas não foram detectadas em abelhas afetadas e o padrão na composição da microbiota não indicou uma correlação entre disbiose e a ocorrência da síndrome. Mostramos também que os sintomas observados em forrageiras doentes não são causados por vírus conhecidos como patogênicos em outras abelhas. Entretanto, novos vírus que nós identificamos no viroma da *M. quadrifasciata* são mais frequentemente diagnosticados em determinados meliponários durante a síndrome, e podem estar relacionados à intensidade dos sintomas e ao colapso de certas colméias. Por fim, num monitoramento temporal feito ao longo de cinco meses no verão de 2018/2019, mostramos que a síndrome foi resultado da incapacidade de expressar genes envolvidos na detoxificação de xenobióticos e resposta imune cerca de dois meses antes da síndrome, seguido da perda de peso das forrageiras, troca nos recursos polínicos utilizados e perda da capacidade de controle do clima interno da colônia. Além da divulgação e discussão no meio científico, elaboramos também, com base nestas investigações, um guia com recomendações de manejo aos meliponicultores que, se aplicadas, podem contribuir para a prevenção da síndrome anual de *M. quadrifasciata*, bem como da perda de outras abelhas nativas.

palavras-chave: abelha, abelha sem ferrão, simbiose, microbiota, vírus, sistema imune, comportamento, saúde das abelhas.

ABSTRACT

Every year at the end of summer, in southern Brazil, managed colonies of the stingless bee *Melipona quadrifasciata* manifest a syndrome that eventually leads to their collapse. Bees from affected colonies may show symptoms such as tremors and paralysis, but the main feature of the syndrome is the sudden death of workers, whose bodies are found in front or inside the hives. In this thesis we describe our discoveries on the possible causes of this mortality evaluating the different complexity levels of an eusocial bee, from the individual and its associated microorganisms until colony level. We show that the *M. quadrifasciata* microbiota is highly variable, with large differences in community composition among bees from different colonies. Pathogenic bacteria were not detected in affected bees, and the pattern of microbiota composition did not indicate a correlation between dysbiosis and syndrome occurrence. We have also shown that the symptoms observed in unhealthy foragers are not caused by viruses known to be pathogenic in other bees. However, novel viruses that we identified in the *M. quadrifasciata*-associated virome are more often diagnosed in some meliponaries during the outbreak, and may be related to the intensity of observed symptoms and to the collapse of some colonies. Finally, on a temporal survey performed for five months during the summer of 2018/2019, we show the syndrome resulted from the inability to express genes involved in the detoxification of xenobiotics and immune response about two months before the outbreak, followed by foragers weight loss, a shift in pollen resources used, and a decrease in performance to control the nest environment. In addition to reporting our results to the scientific community, we have also prepared, based on our investigations, a guide with recommendations for the management of stingless bees that may contribute to the prevention of the *M. quadrifasciata* annual syndrome, as well the loss of other native bees.

key words: bee, stingless bee, symbiosis, microbiota, virus, immune system, behavior, bee health.

CAPÍTULO 1

Introdução e objetivos

1. Introdução

1.1 Abelhas neotropicais

Polinizadores prestam um serviço importante na manutenção de ecossistemas naturais e também para a agricultura. As abelhas fazem parte das 100.000 espécies polinizadoras de mais de 200.000 espécies de plantas. Quanto às espécies de plantas cultivadas pelo homem, as abelhas são o grupo mais importante de polinizadores (Heard 1999; Klein et al. 2007; Giannini et al. 2015).

A região neotropical é reconhecida por suportar uma fauna rica em espécies de abelhas. Estima-se para a região um número total de mais de 15.000 espécies e para o Brasil a riqueza de espécies de abelhas pode chegar a 3.000 (Silveira et al. 2002). As abelhas fazem parte da ordem Hymenoptera e, dentre as sete famílias que a compõe, Apidae é a mais rica e abundante na Mata Atlântica. Espécies da tribo Meliponini são as mais registradas neste bioma (Gonçalves e Brandão 2008). A tribo agrupa as abelhas sociais encontradas nos trópicos e regiões subtropicais, conhecidas popularmente como abelhas sem ferrão por possuírem o ferrão atrofiado. Abelhas do gênero *Melipona*, reconhecidamente monofilético, fazem parte de um clado que inclui apenas abelhas sem ferrão neotropicais (Figura 1) (Ramírez et al. 2010; Rasmussen e Cameron 2010).

1.1.2 A abelha mandaçaia

A mandaçaia (*Melipona quadrifasciata*, Lepeletier 1836, Figura 2) é a segunda espécie de abelha sem ferrão mais cultivada por meliponicultores (aqueles que criam abelhas da tribo Meliponini) (Jaffé et al. 2015). As colônias de mandaçaia são compostas por 300 a 400 indivíduos (Tóth et al. 2003) e colônias selvagens costumam nidificar em troncos ocos. O período de desenvolvimento do ovo ao adulto é de em média 40 dias. Quando adultas, operárias medem 10 a 11 mm e são mais robustas que as abelhas melíferas. A determinação das castas em abelhas do gênero *Melipona* é diferente da maioria das abelhas sem ferrão, em que as larvas determinadas a desenvolverem-se como rainhas recebem e consomem uma quantidade maior de alimento comparado aos demais ovos depositados (Kerr 1969; Hartfelder et al. 2006).

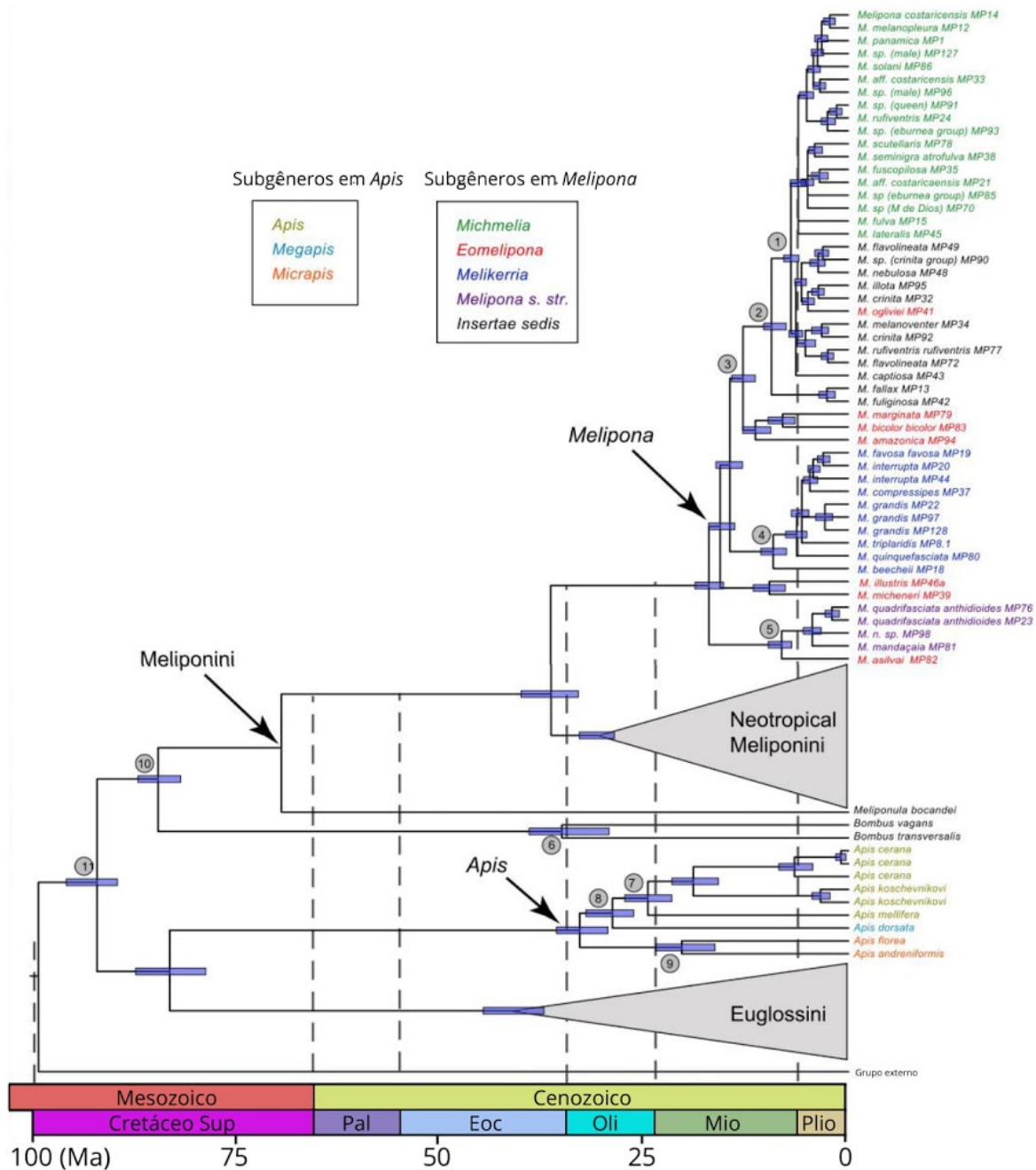


Figura 1. Análise filogenética Bayesiana baseada em cinco *loci* e cronograma com relógio-relaxado do gênero *Melipona* e grupos relacionados. Os tempos de divergência são apresentados em milhões de anos (Ma). *Melipona* compartilhou um ancestral comum durante o Mioceno, 14–17 milhões de anos atrás (adaptado de Ramírez et al. 2010).

Em *Melipona* a determinação de castas se dá por predisposição genética, seguindo um modelo de dois *loci*/dois alelos ainda não determinados (Kerr 1950; Hartfelder et al. 2006). Estudos mais recentes mostram que os níveis de transcritos do gene feminizador (*fem*) em abelhas fêmeas são provavelmente aumentados pelo hormônio juvenil (HJ), mais expresso em larvas

que irão se desenvolver como rainhas, sugerindo que *fem* atua como componente de interação entre vias de determinação do sexo e das castas (de Oliveira Campos et al. 1975; Bonetti et al. 1995; Brito et al. 2015).

As abelhas sem ferrão são consideradas polinizadoras generalistas da flora nativa e também fundamentais para polinização de cultivares agrícolas. Uma característica marcante das mandaiaias, assim como outras abelhas do gênero *Melipona*, é sua capacidade de coletar pólen por vibração especialmente em flores com anteras poricidas (onde o pólen sai da antera através de poros apicais), como da família de plantas Solanaceae. A abelha pousa sobre o ápice da antera, agarra-se ao estame e contrai os músculos torácicos de forma a vibrar e liberar o pólen (Nunes-Silva et al. 2010). Devido a este comportamento, a mandaiaia é uma importante polinizadora de cultivares como o de tomate, pimentão e berinjela (Cruz et al. 2005; dos Santos et al. 2009; Nunes-Silva et al. 2013; Bartelli e Nogueira-Ferreira 2014).

A mandaiaia ocorre na Argentina, Paraguai e Brasil. No Brasil a espécie *M. quadrifasciata* é dividida em duas subespécies. No nordeste, sudeste e centro-oeste do Brasil encontra-se a subespécie *M. q. anthidioides*, com bandas amarelas interrompidas no dorso do abdômen; enquanto no centro-oeste, sudeste e sul ocorre a subespécie *M. q. quadrifasciata*, na qual as bandas dorsais são contínuas (Batalha-Filho et al. 2009). Marcadores moleculares, como regiões polimórficas do DNA (Waldschmidt et al. 2000) e regiões do DNA mitocondrial com padrões diferentes de clivagem por enzimas de restrição (Souza et al. 2008; Batalha-Filho et al. 2010) já foram descritos para auxiliar na diferenciação das subespécies e para estudos de zonas híbridas, encontradas em São Paulo e Minas Gerais. As mandaiaias híbridas possuem um padrão de coloração intermediário, com bandas dorsais interrompidas e contínuas no abdômen. A subespécie *M. q. quadrifasciata* é classificada como em perigo na lista mais atual de espécies ameaçadas do Rio Grande do Sul, elaborada pela Fundação Zoobotânica (2014) e tentativas de encontrar ninhos selvagens na região não tiveram sucesso.

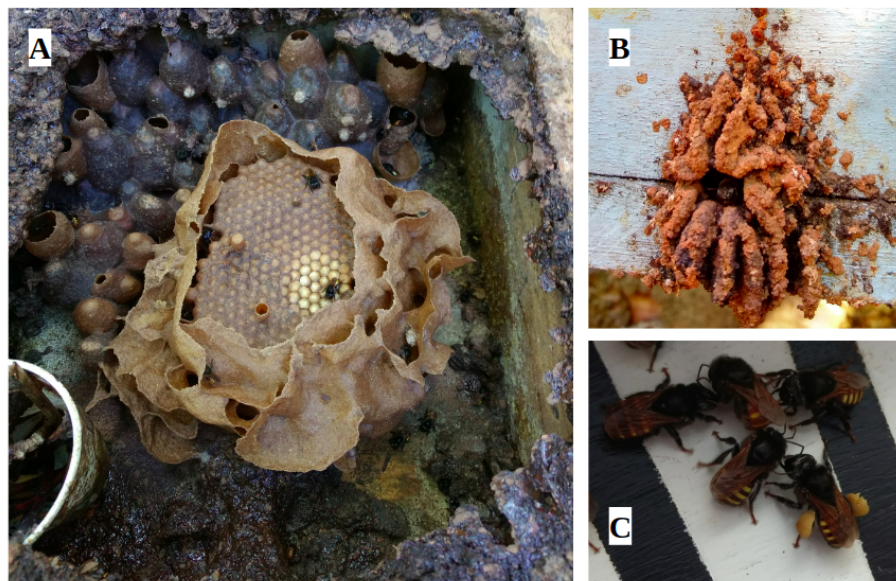


Figura 2. Características da abelha mandaçaia. (A) Parte interna de uma colônia manejada de mandaçaia, com discos de cria envoltos por cerume e no canto da caixa os potes de mel e pólen. (B) Entrada típica de um ninho de mandaçaia, feito de barro coletado por operárias. (C) Abelhas operárias da subespécie *M. q. quadrifasciata* do Rio Grande do Sul. (Fotos: Lílian Caesar)

1.2 Ameaças às abelhas e o colapso de colônias

Nas últimas décadas tem sido relatada a diminuição das populações de abelhas. Na América do Norte e Europa perdas anuais de populações de abelhas manejadas têm aumentado, podendo chegar até 40% do total de colônias (Genersch et al. 2010; Kulhanek et al. 2017). Em 2005 e 2006 apicultores dos Estados Unidos da América começaram a relatar o que passou-se a chamar de desordem do colapso de colônias (CCD, *colony collapse disorder*) (Stokstad 2007). A CCD é caracterizada pela repentina perda da população adulta de abelhas, sem que se encontrem indivíduos mortos no interior ou na proximidade das colônias. O grande número de colônias perdidas após o aparecimento da CCD levou a esforços de pesquisa principalmente voltados a gerar informações sobre cargas de patógenos em polinizadores, permitindo a identificação de possíveis ameaças emergentes à saúde das abelhas (Cox-Foster et al. 2007; Evans e Schwarz 2011; Schwarz, Huang, et al. 2015). Esse processo, no entanto, levou à conclusão de que as perdas de colônias são o resultado de vários estressores em interação, como aquecimento global e perda de habitat, má nutrição, ação de

patógenos e práticas agrícolas como a utilização de pesticidas (vanEngelsdorp e Meixner 2010).

O declínio de populações selvagens de abelhas é um fato (Koh et al. 2016; Meeus et al. 2018). Na região tropical, os fatores que têm influenciado o baixo *status* de conservação de abelhas selvagens incluem a disseminação de espécies de abelhas exóticas, que competem por recursos e podem transmitir patógenos às abelhas nativas, a perda de habitat e o uso de pesticidas (Freitas et al. 2009; Guzman-Novoa et al. 2015; Ueira-Vieira et al. 2015; Maggi et al. 2016; Santos et al. 2018). A meliponicultura, por sua vez, pode ser uma atividade benéfica ao criar e manejar abelhas nativas em risco de extinção, como a *M. q. quadrifasciata* (Jaffé et al. 2015). No entanto, meliponicultores do sul do Brasil relatam uma síndrome de ocorrência anual, geralmente no final do verão, que culmina com o colapso das colônias da espécie. A síndrome difere da conhecida CCD uma vez que são encontrados indivíduos mortos nas proximidades ou até dentro das colônias. A principal característica desta síndrome é a quantidade anormal de operárias mortas em frente a colônia (Figura 3). Em alguns meliponários indivíduos de colônias afetadas podem também apresentar um comportamento alterado e tremores, incapacidade de voo e a eversão da probóscide. O colapso da colônia ocorre devido à falta de operárias para a manutenção e proteção, de modo que a colônia acaba por fim sendo invadida por parasitas como fungos e moscas. A síndrome ocorre em meliponários com ambientes heterogêneos, situados em áreas urbanas à regiões cercadas por mata conservada. Há também diferenças na intensidade dos sintomas observados nos diferentes locais, assim como nem todas as colônias de mandaçaia ficam doentes. Portanto, assim como sugerido para o declínio das populações globais de abelhas e mesmo para a CCD, esta também pode ser uma síndrome multifatorial.

1.2.1 Bactérias

Diversas bactérias patogênicas são conhecidas por infectar principalmente abelhas melíferas, dentre elas as bactérias *Paenibacillus larvae* e *Melissococcus plutonius* são as mais bem caracterizadas (Fünfhaus et al. 2018). *P. larvae* é o agente causador da cria pútrida americana (*American Foulbrood*). O sintoma típico observado após a infecção das larvas por esporos da bactéria é a degradação da larva morta (J. Brødsgaard et al. 1998; Genersch 2010),

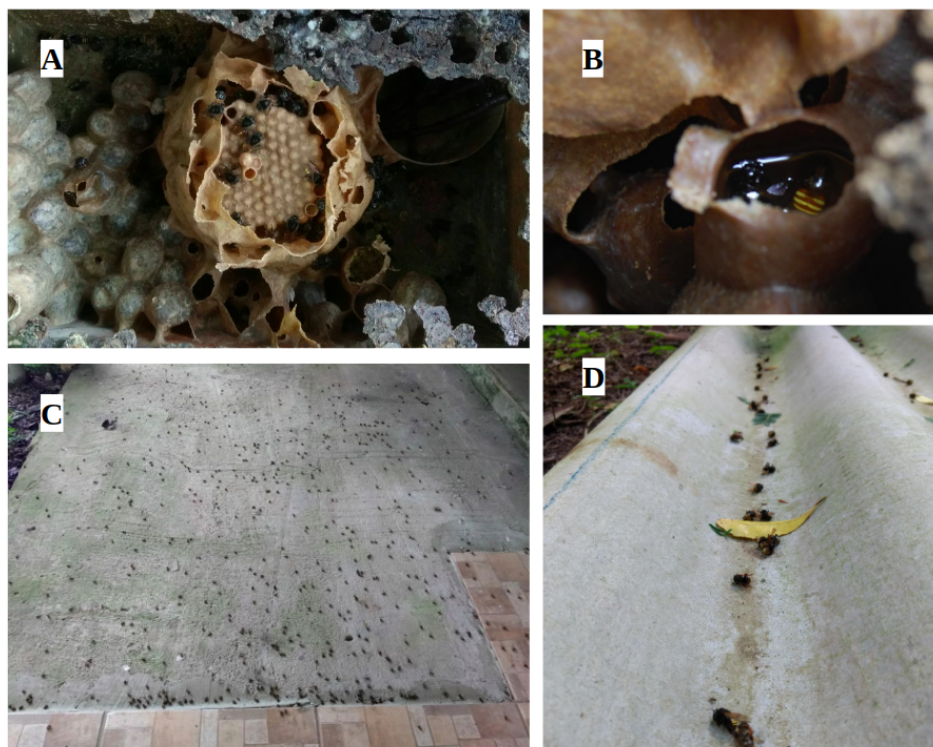


Figura 3. Imagens da síndrome anual das mandaçaias. (A) Parte interna de uma colônia manejada de mandaçaia no início da síndrome. Os discos de cria, potes de pólen e mel permanecem intactos, mas no fundo da colônia começam a acumular operárias mortas ou com sintomas da síndrome, como tremores e paralisia (Foto: Lílian Caesar). (B) Nos potes de mel também passam a ser observadas operárias afogadas que não são removidas por falta de operárias para desempenhar as atividades de limpeza dentro da colônia (Foto: Lucas Kehl). (C, D) A característica mais marcante da síndrome é o grande número de operárias mortas em frente às suas colônias (Foto C: Daniel Iochims; D: Lílian Caesar).

essencial para a transmissão de esporos para larvas saudáveis, bem como para a transmissão da doença dentro e entre colônias. Já *M. plutonius* é o agente causador da doença conhecida como cria pútrida européia (*European Foulbrood*). As larvas são infectadas ao ingerirem alimento larval contaminado e quando mortas são encontradas torcidas dentro da célula de cria aberta. Sua decomposição ocorre também pela presença de invasores secundários como *P. alvei* ou *Enterococcus faecalis* (Nakamura et al. 2016; Lewkowski e Erler 2018). Ambas bactérias já foram diagnosticadas em abelhas melíferas brasileiras em estudos de monitoramento (Maggi et al. 2016), mas não há detalhes sobre os sintomas da infecção. Recentemente *M. plutonius* foi também diagnosticado em abelhas sem ferrão do gênero *Melipona* que apresentavam os sintomas da cria pútrida européia e em alguns casos estava

levando ao colapso de colônias (Teixeira et al. 2020). No estudo mostram que a infecção ocorreu por meio da suplementação alimentar das colônias de abelhas sem ferrão com pólen de *Apis* contaminado.

A bactéria *Lysinibacillus sphaericus* é um conhecido patógeno de insetos, sendo inclusive usada em programas de controle de insetos vetores de doenças humanas (Lacey 2007). Apesar desta bactéria não ser, a princípio, patogênica à abelhas melíferas (West Davidson et al. 1977), ela é o agente causador da perda da prole de colônias manejadas da abelha sem ferrão australiana *Tetragonula carbonaria* (Shanks et al. 2017). Colônias afetadas parecem não ter rainhas, tem uma população reduzida de abelhas adultas e pouca atividade na entrada do ninho. As larvas infectadas apresentam descoloração ou aspecto apodrecido. No Brasil não há registros de abelhas sem ferrão infectadas por tal bactéria.

Outras bactérias patogênicas à abelhas são *Spiroplasma apis* e *S. melliferum*, que vivem intracelularmente nos tecidos do hospedeiro ou extracelularmente na hemolinfa. Abelhas infectadas por *S. apis* são incapazes de voar, rastejam no chão e tremem, além de ficarem com o abdômen inchado devido ao intestino cheio de pólen não digerido (Mouches et al. 1982; Mouches et al. 1983). A infecção por *S. melliferum* se dá na hemolinfa causando patologia em diversos tecidos e diminuindo a longevidade de abelhas adultas (Clark et al. 1985; Zheng e Chen 2014). Abelhas melíferas brasileiras, sem sintomas evidentes, já foram diagnosticadas para a infecção por ambas espécies de *Spiroplasma*, inclusive com infecções concomitantes de *S. apis* e *S. melliferum* (Schwarz et al. 2014).

A bactéria *Serratia marcescens*, encontrada tanto em plantas como animais, é também um patógeno oportunista de abelhas (Raymann et al. 2018). Está frequentemente presente em baixa densidade no intestino de abelhas adultas e já foi também isolada de ácaros parasitas e da hemolinfa de abelhas doentes ou mortas (El Sanousi et al. 1987; Burritt et al. 2016; Raymann et al. 2017). No Brasil não há relatos e falhas em colônias relacionado a infecção por esta bactérias.

1.2.2 Vírus

Até pouco tempo podíamos contar facilmente o número de vírus identificados em abelhas, principalmente em *A. mellifera* (Chen e Siede 2007; McMenamin e Genersch 2015).

Mas as recentes técnicas de sequenciamento de nova geração tem facilitado o sequenciamento de alto rendimento de transcriptomas, metagenomas e viromas, o que tem contribuído significativamente para a caracterização da diversidade de vírus associados às abelhas (Grozinger e Flenniken 2019). A maioria dos vírus de abelhas são de RNA fita simples de sentido positivo (+ssRNA). A família *Dicistroviridae* inclui vírus como: vírus da paralisia aguda israelense (IAPV, *Israeli acute paralysis virus*), vírus de abelha da caxemira (KBV, *Kashmir bee virus*), vírus da paralisia aguda de abelha (ABPV, *Acute bee paralysis virus*) e vírus da célula de rainha negra (BQCV, *Black queen cell virus*). Na família *Iflaviridae* fazem parte vírus como: vírus deformador da asa (DWV, *Deformed wing virus*), vírus *Varroa destructor*-1/DWV-B, vírus de abelha *Sacbrood* (SBV, *Sacbrood bee virus*) e vírus da paralisia lenta das abelhas (SBPV, *Slow bee paralysis virus*). Há também vírus +ssRNA taxonomicamente não classificados, como: vírus da paralisia crônica das abelhas (CBPV, *Chronic bee paralysis virus*) e vírus do lago Sinai (LSVs, *Lake Sinai virus*) (McMenamin e Flenniken 2018; Grozinger e Flenniken 2019).

Alguns destes vírus são conhecidos por causar paralisia em abelhas. O vírus SBPV acumula-se nos tecidos da parte anterior do corpo das abelhas causando a paralisia das pernas anteriores do inseto, sintoma observável apenas 12 dias após a inoculação do vírus, não sendo tão agressivo de imediato (Bailey e Woods 1974). O vírus CBPV causa sintomas semelhantes, podendo também ser observadas abelhas incapazes de voar, aglomeradas e rastejando próximas à colmeia, sem pelos e com o abdômen inchado (Bailey et al. 1983; Blanchard et al. 2007). Este vírus acumula-se principalmente no sistema nervoso da abelha melífera e infecta/aumenta sua carga viral particularmente no verão, podendo infectar e levar apenas algumas colônias de apiários ao colapso.

Em meliponários de Minas Gerais e Pernambuco, onde ocorreu uma alta mortalidade de *M. scutellaris*, o vírus ABPV foi detectado em abelhas operárias (Ueira-Vieira et al. 2015). Este vírus é considerado praticamente assintomático, mas leva rapidamente à morte de abelhas infectadas (Miranda et al. 2013). Análises filogenéticas mostraram que a linhagem de ABPV desta abelha sem ferrão derivou de sequências de ABPV de *A. mellifera* da Europa e da América do Sul (Ueira-Vieira et al. 2015). O vírus DWV e BQCV também já foi detectado em abelhas melíferas do Brasil, assim como em cinco diferentes espécies de abelhas sem ferrão (de Souza et al. 2019; Guimarães-Cestaro et al. 2020). Nestas espécies de abelha sem

ferrão em que o vírus foi detectado não há evidência de sintomas, mas quando a infecção é sintomática, como observado principalmente em abelhas melíferas, este vírus leva abelhas a desenvolverem-se com asas amassadas/vestigiais e abdômen inchado, levando à morte logo após o indivíduo emergir da pupa. A transmissão deste vírus por um vetor, como o ácaro *Varroa*, leva a infecções mais virulentas e com sintomas observáveis (Ryabov et al. 2014; Wilfert et al. 2016). O DWV também já foi detectado na abelha sem ferrão *Scaptotrigona mexicana*, espécie na qual também foi detectado o vírus BQCV (Guzman-Novoa et al. 2015), que costuma causar sintomas mais evidentes nos estágios iniciais do desenvolvimento, como pré-pupas mortas em células com paredes escurecidas ou larvas com aparência pálida e o tecido endurecido (Miranda et al. 2013). Devido ao compartilhamento do ecossistema com *A. mellifera*, o próprio contato físico entre as abelhas exóticas e nativas, ou o contato com pólen de plantas visitadas por ambas as espécies, é sugerido como possível via de transmissão destes vírus (Guzman-Novoa et al. 2015).

Outros vírus identificados em abelhas incluem vírus de RNA fita simples de sentido negativo (-ssRNA), como o *Apis mellifera* rhabdovirus-1 (ARV-1) e *Apis mellifera* rhabdovirus-2 (ARV-2) (Remnant et al. 2017). Quanto aos vírus de DNA, dois vírus de DNA de fita dupla (dsDNA) foram já descritos, o Vírus filamentososo de *Apis mellifera* (Gauthier et al. 2015) e o *Osmia cornuta* nudivírus (OcNV) (Schoonvaere et al. 2018). Também foram já identificados sequências virais correspondentes a vírus de DNA de fita simples (ssDNA) das famílias *Circoviridae* e *Parvoviridae* (Galbraith et al. 2018; Schoonvaere et al. 2018). A patogenicidades destes vírus em abelhas infectadas ainda precisa ser investigada.

1.2.3 Outros inimigos naturais

Além de bactérias e vírus, outros inimigos naturais das abelhas são os ácaros, fungos e tripanossomatídeos. O ácaro *Varroa destructor*, que foi transmitido da abelha *A. ceranae*, tolerante ao parasita, à *A. mellifera*, é hoje um dos mais importantes parasitas desta espécie de abelha (Rosenkranz et al. 2010; Evans e Cook 2018). Ele se beneficia da ampla distribuição da abelha melífera, juntamente com as práticas de manejo, como a alta densidade de colônias nos apiários, para ser transmitido horizontalmente entre colônias (Seeley e Smith 2015). *Varroa* causa danos pelos efeitos diretos do seu parasitismo, como malformações durante o desenvolvimento e diminuição da performance das colônias (Garedew et al. 2004; Dainat et

al. 2012). O ácaro também atua como vetor de vírus como KBV, IAPV e DWV (Chen et al. 2004; Shen et al. 2005; Di Prisco et al. 2011) e seu parasitismo tem contribuído para a emergência de estirpes virais, como do DWV, ao suprimir a resposta imune do hospedeiro desestabilizando a dinâmica de controle da infecção (Yang e Cox-Foster 2005; McMahon et al. 2016; Erban et al. 2019). Este ácaro já está espalhado por diferentes países da América Latina, incluindo o Brasil, mas até o momento não causa danos aparentes ou há evidências de sua atuação na transmissão de vírus (Strapazzon et al. 2009; de Mattos et al. 2016; Brettell e Martin 2017).

Nosema ceranae é um microsporídeo parasita intracelular obrigatório de abelhas melíferas (Fries et al. 1996; Fries 2010). Nosemoses afetam larvas e abelhas adultas, causando mortalidade de abelhas e podendo levar ao colapso de colônias (Higes et al. 2007; Higes et al. 2008; Higes et al. 2009; Bromenshenk et al. 2010; Paxton 2010), apesar de ser também diagnosticada em colônias assintomáticas (Cox-Foster et al. 2007; Fernández et al. 2012). A infecção por *Nosema* pode comprometer o sistema imune, forrageamento, produção de feromônios e hormônios e síntese lipídica (Kralj e Fuchs 2010; Goblirsch et al. 2013; Mayack et al. 2015; Badaoui et al. 2017; Li et al. 2018). O parasita é transmitido por via oral pelo mel, néctar, pólen e matéria fecal das abelhas (Smith 2012). Um estudo realizado em colônias de *A. mellifera* de 20 apiários do estado de São Paulo detectou esporos do parasita em indivíduos de todas colônias, mas nenhuma evidência clara de correlação da quantidade de esporos com o declínio das colônias foi observado (Teixeira et al. 2013). Esporos de *Nosema* já foram também detectados em abelhas sem ferrão, provavelmente adquiridos em flores também visitadas por *A. mellifera* parasitadas (Porrini et al. 2017; Purkiss e Lach 2019). Em um estudo recente o patógeno, no entanto, não foi encontrado no intestino das abelhas sem ferrão, sugerindo que essas abelhas não são afetadas (Guimarães-Cestaro et al. 2020).

Os tripanossomatídeos *Crithidia mellificae* e *Lotmaria passim* são parasitas obrigatórios unicelulares que costumam infectar abelhas melíferas (Schwarz et al. 2015). *L. passim* é atualmente a espécie de tripanossomatídeo predominante em *A. mellifera* de grande parte do mundo. Para a América do Sul as informações sobre a distribuição dos tripanossomatídeos permanecem escassas, apesar de já terem sido diagnosticados (Arismendi et al. 2016; Castelli et al. 2019). Apesar de *L. passim* ter sido associado à mortalidade de abelhas (Ravoet et al. 2013), os efeitos de sua infecção ainda são pouco conhecidos. Já *C.*

bombi é um tripanossoma parasita de *Bombus* sp., e sua infecção já foi melhor caracterizada. Ele reduz drasticamente a produção de machos, o sucesso na fundação de novas colônias e tamanho delas, além de prejudicar processos cognitivos nas abelhas parasitadas (Brown et al. 2003; Gegear et al. 2006). Também induz a expressão de genes relacionados ao sistema imunológico, como os codificadores de Relish e peptídeos antimicrobianos (AMPs) (Schlüns et al. 2010; Riddell et al. 2011; Riddell et al. 2014).

1.2.5 Agroquímicos

O Brasil é o maior consumidor mundial de agroquímicos, representando aproximadamente 20% do uso total (Albuquerque et al. 2016). Em um estudo de caso recente, 90% dos apicultores brasileiros entrevistados relataram perda de colônias que acreditam ser causadas por causa do uso de agroquímicos em culturas próximas a seus apiários (Cerqueira e Figueiredo 2017). Mas enquanto países desenvolvidos têm reduzido o uso de agroquímicos na agricultura, o Brasil tem ido na contramão, facilitando ainda mais o seu uso (Santos et al. 2018). Altas doses de agroquímicos podem ter efeito letal para as abelhas, levando colônias inteiras ao colapso. Mas o efeito subletal dos agroquímicos, aquele que não leva a morte imediata das abelhas, pode também ser muito prejudicial e contribuir para falha da colônias (Desneux et al. 2007).

Agroquímicos já foram detectados em níveis residuais na prole, pólen, mel e cera de colônias de abelhas melíferas (Wu et al. 2011; Calatayud-Vernich et al. 2018; Tosi et al. 2018). Estes resíduos influenciam negativamente no crescimento, força e sobrevivência das colônias de abelhas por comprometerem o retorno de forrageiras ao lar, habilidades cognitivas e de forrageamento (Mommaerts et al. 2010; Henry et al. 2012), a motilidade espermática em machos (Thompson et al. 2005; Ciereszko et al. 2017) e a emergência, sobrevivência e reprodução de rainhas (Thompson et al. 2005; Sandrock et al. 2014; dos Santos et al. 2016). O efeito de doses subletais de agroquímicos pode também ser acentuado ao agir de forma sinérgica com agroquímicos de outras classes (Spurgeon et al. 2016; Sgolastra et al. 2017), contribuir para o aumento da susceptibilidade a infecção por patógenos como *Nosema* sp. ou DWV (Alaux, Brunet, et al. 2010; Prisco et al. 2013; Aufauvre et al. 2014) e reduzir ainda mais a longevidade e sobrevivência de abelhas sob estresse nutricional (Tosi et al. 2017).

Diversos estudos experimentais têm mostrado os efeitos tóxicos de agroquímicos sob abelhas sem ferrão (Valdovinos-Núñez et al. 2009; Dorneles et al. 2017; Tomé et al. 2017; Padilha et al. 2020). Abelhas do gênero *Melipona* contaminadas sofrem uma diminuição na longevidade e mudanças no comportamento que influenciam a capacidade de forrageamento (Del Sarto et al. 2014; de Moraes et al. 2018; Nocelli et al. 2019). Um estudo que investigou a suscetibilidade de *M. quadrifasciata* e *A. mellifera* aos agroquímicos Deltametrina, Metamidofós e Abamectina mostrou que a abelha nativa é, inclusive, mais suscetível do que a abelha exótica comumente usada como parâmetro para estudos de toxicidade (Del Sarto et al. 2014). Um padrão similar foi observado para a abelha sem ferrão *Partamona helleri*, mais suscetível à ação sinérgica de misturas da Deltametrina e uma formulação combinada de fungicidas comparado à *A. mellifera* (Tomé et al. 2017)

1.2.6 Perda de habitat

Cerca de um terço do território nacional já foi convertido em produção agrícola, o que tem levado à perda de grandes áreas de vegetação natural (Ferreira et al. 2012). Essa expansão está relacionada principalmente à crescente quantidade de terra usada para o cultivo de monoculturas, como a da soja, observada nos últimos anos no Brasil e outros países da América do Sul (Pacheco 2012). A invasão das florestas pelo cultivo de monoculturas afeta a saúde das abelhas pelo aumento no uso de pesticida nestes cultivares, por fragmentar habitats de nidificação e estabelecimento das colônias, e por comprometer a nutrição das abelhas uma vez que leva à perda da flora nativa que fornece diversas fontes de néctar e pólen às abelhas. Uma boa nutrição é essencial para a saúde das abelhas (Brodschneider e Crailsheim 2010). O consumo de pólen diverso, polifloral, e de boa qualidade é fisiologicamente mais saudável, contribuindo para uma maior tolerância a infecções patogênicas por garantir a disponibilidade de micronutrientes necessários e estimular a expressão de genes do sistema imune inato da abelha (Alaux, Ducloz, et al. 2010; Perry et al. 2015; Pasquale et al. 2016; Filipiak et al. 2017). Por exemplo, a alimentação com pólen polifloral aumenta a longevidade de abelhas melíferas parasitadas por *Nosema* comparado a abelhas que se alimentaram de pólen monofloral (Pasquale et al. 2013). Esta resposta também é observadas na infecção por IAPV, em que uma dieta polifloral tem o potencial de reduzir a mortalidade de abelhas infectadas (Dolezal et al. 2019).

1.3 Sistema imune e saúde da abelha

A saúde da abelha não é um tópico trivial e quando se trata de abelhas sociais, como a mandaçaia, uma colônia saudável não pode ser reduzida à ausência de patógenos ou colônias populosas. Ao estudar a saúde das abelhas deve se respeitar os diferentes níveis de complexidade do superorganismo (Figura 4), a saúde deve ser medida e investigada a nível individual, da colônia e populacional (López-Urbe et al. 2020). É o resultado da interação destes diferentes níveis que possibilitará um estado de bem-estar onde a energia é adquirida, alocada e utilizada da maneira ideal, permitindo que os indivíduos vivam mais e/ou se reproduzam mais. Nesta tese investigamos a síndrome das mandaçaia sob uma perspectiva multinível, medindo aspectos individuais (microbioma intestinal, diagnóstico de patógenos, viroma e expressão gênica), assim como medindo características da colônias (peso dos indivíduos, temperatura e umidade dentro das colônias e comportamento imune social).

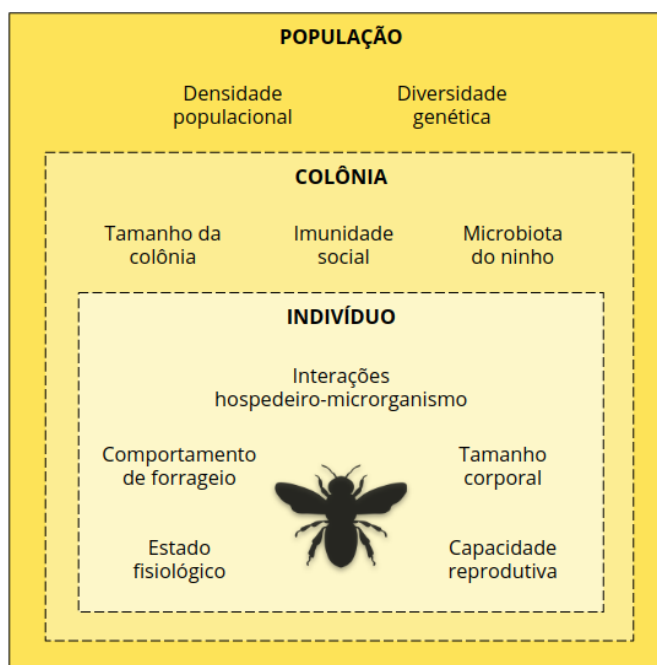


Figura 4. Os três níveis de organização biológica importantes para o estudo e discussões sobre a saúde das abelhas e, dentro de cada um, algumas das diferentes maneiras avaliá-los. As linhas pontilhadas entre os níveis representam o fluxo de informações de níveis mais específicos aos mais amplos. Por exemplo, avaliações a nível de indivíduo informam também sobre a saúde da colônia, e avaliações a nível de colônia informam também sobre a saúde a nível de população (adaptado de López-Urbe et al. 2020, ilustração da abelha de www.kindpng.com).

1.3.1 Imunidade social

A imunidade social é uma forma de defesa coletiva contra parasitas e patógenos, onde os indivíduos da colônia realizam pequenas tarefas que coletivamente têm um impacto na saúde da colônia (Cremer et al. 2007). A auto-higiene e higienização de outras abelhas da colônia é um comportamento imune social para remoção de pólen, partículas estranhas e parasitas, como ácaros, do corpo das abelhas (Jander 1976; Büchler et al. 1992; Danka e Villa 1998; Boecking e Spivak 1999). Comportamentos termorregulatórios, como a febre social gerada por abelhas operárias, são importantes no combate a patógenos sensíveis ao calor, como fungos (Simone-Finstrom et al. 2014). O comportamento higiênico envolve a detecção de células de cria com larvas mortas ou infectadas e sua remoção da colônia, evitando a disseminação de ácaros, vírus e fungos na colônia (Spivak e Gilliam 1998; Bigio et al. 2014; Toufailia et al. 2014; Al Toufailia et al. 2016). Quando a abelha adulta é quem está infectada, ela também pode abandonar a colônia para morrer fora dela, evitando a disseminação de doenças (Rueppell et al. 2010; Shorter e Rueppell 2012). Em abelhas sem ferrão podemos observar que as células de cria são preenchidas com alimento larval logo após a construção e antes da oviposição pela rainha, sendo seladas imediatamente após a oviposição (Lindauer 1976). Diferente das abelhas melíferas, em que o alimento é dado progressivamente às larvas em células de cria abertas, este comportamento impede que parasitas, como ácaros, tenham acesso à prole em desenvolvimento.

Outros comportamentos relacionados à imunidade social envolvem a auto-medicação, como o uso de resinas antimicrobianas coletadas nas plantas visitadas, que são misturadas à cera ou solo e então usadas na construção dos ninhos (Simone et al. 2009; Drescher et al. 2014; Popova et al. 2014; Drescher et al. 2017). Abelhas nutrizas também podem usar a glicose oxidase (GOX), produzida pelas abelhas que processam o néctar, no alimento larval e estoques de mel servindo como um agente antimicrobiano (Bucekova et al. 2014). A produção de GOX tem sido inclusive usada como um biomarcador de defesa imune social, apesar de sua expressão também poder variar devido a fatores genéticos e ambientais (Alaux, Ducloz, et al. 2010; López-Urbe et al. 2017; Jones et al. 2018).

1.3.2 Imunidade individual

Cada abelha de uma colônia possui resposta imune individual, podendo ser mecânica, fisiológica e imunológica. A cutícula e a membrana peritrófica revestindo o trato digestivo servem como uma primeira barreira mecânica impedindo a aderência ou entrada de microrganismos no corpo. Inibidores fisiológicos à invasão microbiana incluem alterações no pH e outras condições químicas do intestino de insetos tornando-o inóspito (Evans e Spivak 2010). Quando estas barreiras são ultrapassadas, as abelhas contam então com o sistema imune humoral (Evans et al. 2006; Wilson-Rich et al. 2008). Ele é ativado uma vez que reconhece motivos estruturais na superfície dos patógenos, os Padrões Moleculares Associados a Patógenos (PAMPs) (Brutscher et al. 2015). A sinalização desencadeada leva a ativação da resposta imune celular mediada por hemócitos, incluindo fagocitose, formação de nódulos e encapsulação da patógenos invasores, o início da cascata de fenoloxidase (PO) que regula a coagulação ou a melanização da hemolinfa, ou a síntese de AMPs (Casteels-Josson et al. 1994; Vizioli e Salzet 2002; Evans et al. 2006).

Algumas das vias da sinalização que controlam a expressão de AMPs e outros fatores antimicrobianos são Imd (*Immune Deficiency*), Jak/STAT (*Janus kinase/Signal Transducer and Activator of Transcription*) e Toll. Imd é uma via específica de resposta antimicrobiana que induz a produção de AMPs e outros genes envolvidos na defesa antimicrobiana através do fator de transcrição NF- κ B Relish. A via de sinalização JAK-STAT contribui também para o sistema imune inato induzindo transdutores de sinal e ativadores de transcrição a regular efetores antimicrobianos como TEP7 (*Thioester-containing protein 7*), TEPA, TEPB e o inibidor SOCS (*Suppressor of Cytokine Signaling*). Já a ativação do complexo Toll leva a degradação do inibidor de NF- κ B Cactus e então a translocação nuclear do fator de transcrição NF- κ B Dorsal que por sua vez aumenta a expressão de AMPs (Royet et al. 2005; Evans et al. 2006; Brutscher et al. 2015). Estas vias desempenham um papel importante para a saúde da abelhas e a desestabilização contribui para o colapso de colônias. Um exemplo vem do parasitismo pelo o ácaro *V. destructor*, que desestabiliza a resposta imune da abelha através da supressão de NF- κ B (Toll) possibilitando uma infecção mais virulenta pelo DWV (Nazzi et al. 2012).

Embora insetos não possuam anticorpos, que carregam a memória imunológica em vertebrados, eles possuem o que é chamado de imune *priming*, uma forma de imunidade transgeracional (Sadd et al. 2005; Freitak et al. 2014). Por exemplo, a prole de *A. mellifera* infectada experimentalmente por *P. larvae* tem maior resistência a uma nova infecção pelo patógeno (Hernández López et al. 2014). Posteriormente foi mostrado que a memória da infecção, que gerou resistência à prole, se dá através do transporte de fragmentos da parede celular do patógeno, contendo elicitores imunológicos, pela proteína vitelogenina (*Vg*) presente nos ovos (Salmela et al. 2015). A *Vg* também desempenha importante papel no controle do polietismo etário (Hartfelder et al. 2006; Dallacqua et al. 2007; Rodriguez Messan et al. 2018), agindo contra estresse oxidativo (Zhang et al. 2017; Park et al. 2018) e na resposta a patógenos (Alaux et al. 2011; Salmela et al. 2015; Steinmann et al. 2015). Uma menor expressão de *Vg* está associada à maturação comportamental acelerada e atividade de forrageamento em abelhas operárias, e a indução do forrageamento precoce pode também estar relacionada a uma resposta adaptativa contra transmissão de patógenos na colônia (Marco Antonio et al. 2008; Natsopoulou et al. 2016). As abelhas também possuem um sistema metabólico sofisticado que media a conversão de xenobióticos nocivos, como agroquímicos, em formas menos tóxicas. Uma das principais superfamílias enzimáticas responsáveis por esse mecanismo de desintoxicação são as monooxigenases do citocromo p450 (p450s), pertencentes à subfamília CYP9Q (Manjon et al. 2018). Já foi mostrado que p450s protegem as abelhas contra os efeitos tóxicos dos próprios inseticidas utilizados no controle de ácaros parasitas como *Varroa* (Mao et al. 2011). A expressão de p450s têm sido inclusive usada como um possível biomarcador em resposta a desafios por xenobióticos (Berenbaum e Johnson 2015; Manjon et al. 2018).

1.3.3 Microbioma de abelhas

As abelhas possuem relações com diversos simbiossiontes bacterianos, os quais desempenham um papel importante na saúde e homeostase do indivíduo, sendo mantidos nas populações por transmissão horizontal e vertical. A comunidade bacteriana melhor caracterizada até então é a de *A. mellifera*, que é composta por cinco grupos de bactérias: dois grupos de Proteobactérias (*Gilliamella* e *Snodgrassella*), dois grupos de Firmicutes (*Lactobacillus* Firm-4 e *Lactobacillus* Firm-5) e um de Actinobacteria (*Bifidobacterium asteroides*) (Kwong e Moran 2016). Estes grupos são também predominantes em *Bombus* e nas

poucas abelhas sem ferrão já caracterizadas, sugerindo que esses *taxa* compreendem o microbioma intestinal padrão de abelhas corbiculadas (Kwong et al. 2017). Outras bactérias em menores quantidades e com nichos específicos no intestino da abelha melífera também compõem o microbioma, como *Frischella perrara*, *Bartonella apis*, *Parasaccharibacter apium* e uma bactéria relacionada à *Gluconobacter*. Enquanto *B. apis* e *F. perrana* são filotipos específicos de abelhas melíferas, *Acetobacter*-like parece ser um filotipo específico de abelhas sem ferrão (Kwong et al. 2017).

A riqueza e a composição da microbiota padrão influencia diretamente na saúde da abelha. Estudos experimentais mostraram que a suscetibilidade de *Bombus terrestris* ao parasita *Crithidia bombi* é predominantemente determinada pela composição da microbiota e apenas secundariamente pelo genoma do hospedeiro (Koch e Schmid-Hempel 2012). O metagenoma de *A. mellifera* sugere que as bactérias como *Snodgrassella* e *Gilliamella* auxiliam na produção de biofilmes e na comunicação celular da abelha, o que poderia contribuir para a proteção contra patógenos. Bactérias dos grupos Gamaproteobacteria, Firmicutes e Bifidobacteria estão provavelmente envolvidas com o transporte e quebra de carboidratos (Engel et al. 2012). O microbioma core também é importante pois, com a presença dessas bactérias intestinais controladas, as abelhas regulam positivamente a expressão gênica de AMPs como apidaecina e himenoptaecina, o que por sua vez estimula o sistema imunológico contra futuras infecções patogênicas (Kwong et al. 2017). A disbiose, caracterizada por uma alteração na composição da microbiota padrão, pode ser causada por diversos fatores de estresse bióticos e abióticos (Hamdi et al. 2011). Pesticidas, por exemplo, podem alterar a abundância relativa de *taxa* microbianos no intestino das abelhas (Motta et al. 2018; Rouzé et al. 2019). A exposição de abelhas ao agroquímico Glifosato levou a uma diminuição na abundância relativa e absoluta de espécies dominantes da microbiota intestinal, como a bactéria *S. alvi*, e também aumentou a taxa de mortalidade de abelhas infectadas pela bactéria oportunista *S. marcescens* (Motta et al. 2018).

1.4 Objetivo

Investigar as causas da síndrome anual das mandaiaias (*Melipona quadrifasciata*) com um olhar para diferentes níveis de organização biológica da colônia e o impacto de estressores ambientais.

1.4.1 Objetivos específicos

- Caracterizar a microbiota intestinal de mandaçaias;
- Verificar a ocorrência de disbiose ou presença de bactérias patogênicas associadas a mandaçaias afetadas pela síndrome;
- Caracterizar o viroma de mandaçaias;
- Avaliar se existe uma relação entre a presença de vírus eucarióticos e a manifestação da síndrome;
- Investigar se mandaçaias adquiriram vírus da abelha exótica *A. mellifera*;
- Determinar a contribuição da genética das colônias e do ambiente para a ocorrência da síndrome;
- Mensurar a resposta imune e comportamental de mandaçaias, em nível de indivíduo e colônia, através de abordagem temporal;
- Investigar o papel de fatores ambientais no desencadeamento da síndrome anual.

CAPÍTULO 2

Report on the microbiota of *Melipona quadrifasciata* affected by a recurrent disease.

Este capítulo apresenta o seguinte artigo publicado:

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Short Communication

Report on the microbiota of *Melipona quadrifasciata* affected by a recurrent disease

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ABSTRACT

Melipona quadrifasciata is an eusocial stingless bee traditionally used for honey production in Brazil. In the last decades, the species disappeared from the wild in Southern Brazil, being kept exclusively in managed colonies for commercial and recreational purposes. Stingless beekeepers from this region report annual losses of their colonies due to a syndrome of yet unknown causes. We investigate whether it is associated to pathogenic microorganisms already known to cause disease in bees. These results provide a starting point for future studies aimed at clarifying the relationship between the microbial community of stingless bees and their colony collapses.

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1. Introduction

Stingless bees (Apidae, Meliponini) are the only highly social bees other than the true honeybees. In Brazil and other parts of South America stingless bees are by far the most abundant bee species, suggesting that they have a prominent role as pollinators in this region (Giannini et al., 2015; Heard, 1999). Before the introduction of honey bees in the 19th century, colonies of stingless bees and wasps were the only sources of honey used in Brazil (Nogueira-Neto, 1997), and stingless bee culture represents an old aboriginal tradition that helps to increase agricultural production by maintaining ecological interactions (Garibaldi et al., 2016). *Melipona quadrifasciata* is one of the most popular stingless bees cultivated in Brazil (Jaffé et al., 2015), where it is called “man daçaia”, which in the indigenous language means “beautiful vigilant”, referring to the guard that permanently protects the nest entrance. Two *M. quadrifasciata* subspecies that show significant

genetic divergence are recognized by their different abdomen coloration patterns, i.e., *M. q. anthidioides*, found in the southeast and northern Brazil, and *M. q. quadrifasciata*, which occurs in the south (Batalha-Filho et al., 2010; Tavares et al., 2013).

In the southernmost state of Brazil, Rio Grande do Sul, which corresponds to the southern limit of *M. quadrifasciata*'s geographic distribution, wild populations disappeared since more than 50 years, and the species is now regarded as endangered (Blochtein and Marques, 2003; Fundação Zoobotânica, 2014). Furthermore, numerous beekeepers from Rio Grande do Sul have been reporting annual losses of their *M. quadrifasciata* colonies. At the end of summertime, between February and April, workers become unable to fly and crawl with their proboscis everted, leading to massive deaths that ultimately end with the colony collapse. Although such collapses happen synchronously in many different localities, they can't be connected to a common environmental factor, such as a potentially toxic flower or pesticides. Deaths occur in colonies from very dissimilar habitats.

Symbiotic bacteria are known to play an important role in bee health (Hamdi et al., 2011; Vásquez et al., 2012). Pathogens, viruses or other factors may interfere with the normal composition of bacteria associated to the bee gut epithelium (Cariveau et al., 2014;

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Cox-Foster et al., 2007) and in turn the bacterial colonization interferes with subsequent susceptibility to infections (Koch and Schmid-Hempel, 2012; Schwarz et al., 2016). The aim of the present study is to investigate whether the syndrome that annually leads to *M. quadrifasciata* colony collapses in Southern Brazil is correlated to infection with pathogenic bacteria. We describe, for the first time, the bacterial symbiont communities of this stingless bee species based on high throughput sequencing of 16S rDNA.

2. Materials and methods

2.1. Sampling and DNA extraction

M. quadrifasciata adult individuals manifesting symptoms of disease, i.e., disorientation, flight incapacity or proboscis eversion (unhealthy; $n = 52$) as well as without any detectable symptom (healthy; $n = 24$) were removed from their colonies for DNA extraction using a clean forceps. Stingless bee colonies were sampled in two summers (February/March) of 2014 and 2015 in two localities of Rio Grande do Sul, i.e., Boqueirão do Leão (30°3'9.7"S; 51°11'6.03"W) and Porto Alegre (30°4'30.3"S; 51°8'4.5"W). DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) from individual abdomens, which were separated from bee bodies in aseptic conditions using sterile scalpels.

2.2. Metabarcoding of bacteria, and their possible link to disease

A segment of the bacterial 16S rDNA gene corresponding to the V1-V3 variable region was amplified from the DNA samples with modified barcoded versions of primers 27F (GAGTTTGATCCTGGCT-CAG) (Lane, 1991) and 519R (GTNTTACNGCGGCKGCTG) (Turner et al., 1999) and sequenced using Illumina MiSeq technology. Reads were processed with Mothur v. 1.36.1 (Schloss et al., 2009). After filtering out low quality sequences, chimeras were removed with UCHIME (Edgar et al., 2011), as well non-bacterial sequences, based on a preliminary classification using the SILVA v123 nr database (Quast et al., 2013). Only samples containing at least 500× coverage were retained in subsequent analyses ($n = 33$; see Table S1). Sequences showing $\geq 95\%$ identity were clustered in Operational Taxonomic Units (OTUs). A non-metric multidimensional scaling (NMDS) of Bray-Curtis dissimilarities among bacterial communities was performed in PAST version 3.13 (Hammer et al., 2001). To investigate the dynamics of stingless bee microbiota we also performed a Permutational Multivariate Analysis of Variance (PERMANOVA) of Bray-Curtis pairwise distances, in which the factors “sampling year”, “colony” and “health status”, as well as their interactions, were tested as sources of variation in bacterial community composition. The phylogenetic affinities among principal bacterial OTUs was inferred by maximum likelihood using a dataset of known bee symbiont sequences obtained from GenBank as reference. Alignment was made with MAFFT v. 7.187 (Katoh and Standley, 2013), and phylogenetic analysis was performed with PhyML (Guindon and Gascuel, 2003) using the GTR + G + Inv model ($\alpha = 0.5$), which showed the best AIC score in ModelTest (Darrriba et al., 2012). Local support values were estimated by nonparametric bootstrap based on 500 resamplings.

3. Results and discussion

The 16S rRNA amplicons from 33 *M. quadrifasciata* individuals belonging to 11 colonies (Table S1) yielded a total of 52,545 sequences (mean \pm SD = 1592 \pm 770 per sample) that were binned into 276 OTUs (mean \pm SD = 29 \pm 12 per sample). Rarefaction curves reach OTU saturation, indicating a good sampling (Fig. S1).

The 32 OTUs with ≥ 100 sequences (GenBank accession numbers KX021311-KX021342) that represent 93.8% of the dataset were used for further analyses. The *M. quadrifasciata* bacterial symbiont OTUs were classified in 11 clades (Fig. 1), seven of them belonging to Firmicutes, which correspond to 73.4% of the total sampling.

Overall, the seven clades of Firmicutes and 3 OTUs of Proteobacteria belonging to the family Acetobacteriaceae are the most representative bacteria; Firmicutes Group U, Firmicutes Group Z and Acetobacteraceae correspond respectively to 23%, 23% and 16% of the total sampling. Though OTU frequencies show extensive variation across *M. quadrifasciata* individuals (Fig. 2), the factor that best explains variations in microbiota composition is the colony ($F = 2.752$; $p = 0.0006$); however no significant associations are found between year of sampling ($F = 1.112$; $p = 0.3405$) or stingless bee health status ($F = 0.854$; $p = 0.4915$) and microbiota composition. Interestingly, although main effects of sampling year and health status are not statistically significant, their interaction effect is ($F = 1.146$; $p = 0.0218$), suggesting that the effect of bee health status on microbiota composition is not the same in both years. Overall, there is a crossover effect of sampling year and health status on microbiota composition, which is possibly caused by the higher abundance of Firmicutes group U and Z bacteria in unhealthy bees on 2014, but in healthy bees of 2015 (see Fig. 2). This conclusion is also supported by NMDS, where unhealthy bees of different sampling years fall on opposite sides of coordinate 1 (Fig. S2). Therefore, we tentatively suggest that the syndrome manifested by *M. quadrifasciata* colonies may have a link to its microbiota composition, but we haven't been able to detect it with this preliminary study. Nevertheless, no pathogenic bacteria known for the honeybee, such as *Spiroplasma*, *Melissococcus* and *Paenibacillus* that cause foulbrood disease, and mostly affect honeybee larvae (Bailey and Ball, 1991), were found in our samples.

The main bacterial OTUs identified in our study are phylogenetically related to other known bee symbionts (Fig. 1). Firmicutes group U is related to *Lactobacillus kunkeii*, and belongs to a clade of common symbionts of stingless bee species such as *Tetragonula carbonaria* and *Austroplebeia australis* (Fig. S3; Leonhardt and Kaltenpoth, 2014). Firmicutes group Z clusters with the so-called Firm-5 clade of lactic acid bacteria (Martinson et al., 2011). Different species from this clade were found in *Melipona panamica* (Koch et al., 2013), *Bombus* sp. (Praet et al., 2015) and *Apis mellifera* (Olofsson et al., 2014). The Lactobacillales and the Acetobacteraceae are mostly found in the honeybee stomach and rectum as well as in its hive products (Moran, 2015). Proteobacteria that are dominant in the honeybee ileum, such as *Gilliamella* and *Frischella* (Gammaproteobacteria) or *Snodgrassella* (Betaproteobacteria) are absent in our samples, concordant with previous studies of Meliponini microbiota (Koch et al., 2013). However, a clade of bacteria belonging to the Enterobacteriaceae (Gammaproteobacteria), which are common symbionts of the honeybee ileum, appears in low frequency in our dataset (2% of total sampling), but surprisingly in only four unhealthy individuals (Fig. 2; Table S1). The 16S sequence of this bacterium clusters with another found in the gut of *Eulaema* sp. (Euglossini) from Panama (Fig. 1), and is close to *Yokenella regensburgei* (Koch et al., 2013). Firmicutes group W, which represents 15% of our sampling, is phylogenetically related to *Streptococcus*, a bacterial genus known for causing opportunistic infections in larvae affected by foulbrood disease (Bailey et al., 1973). However, its previously reported presence in *M. panamica* (Koch et al., 2013), as well as the occurrence in healthy and unhealthy *M. quadrifasciata* individuals of the present study suggests a non-pathogenic interaction of *Streptococcus* and *Melipona*. Likewise, we haven't been able to detect other well-known eukaryotic bee pathogens (*Nosema* or *Crithidia*) by PCR (data not shown).

The lack of any direct evidence leaves the question of what is killing *M. quadrifasciata* colonies in Southern Brazil still open. Bee-

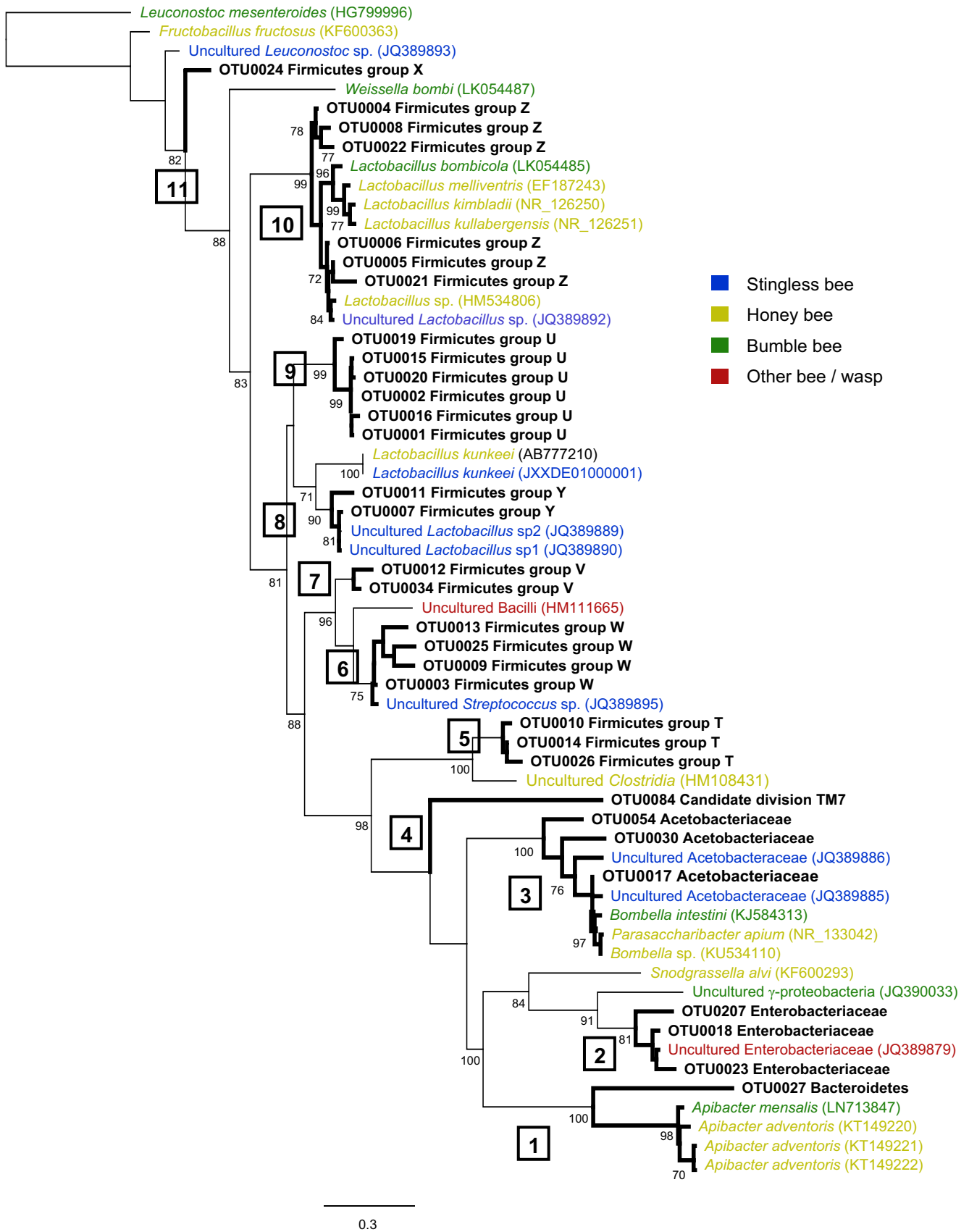


Fig. 1. Phylogenetic reconstruction based on bacterial 16S rRNA sequences. Thirty-two *Melipona quadrifasciata* symbiont OTUs belonging to 11 major clades (shown in bold) are compared to sequences from bacteria previously characterized for other bees, identified by their respective accession numbers. Bootstrap support values larger than 70% are shown at the respective branch nodes.

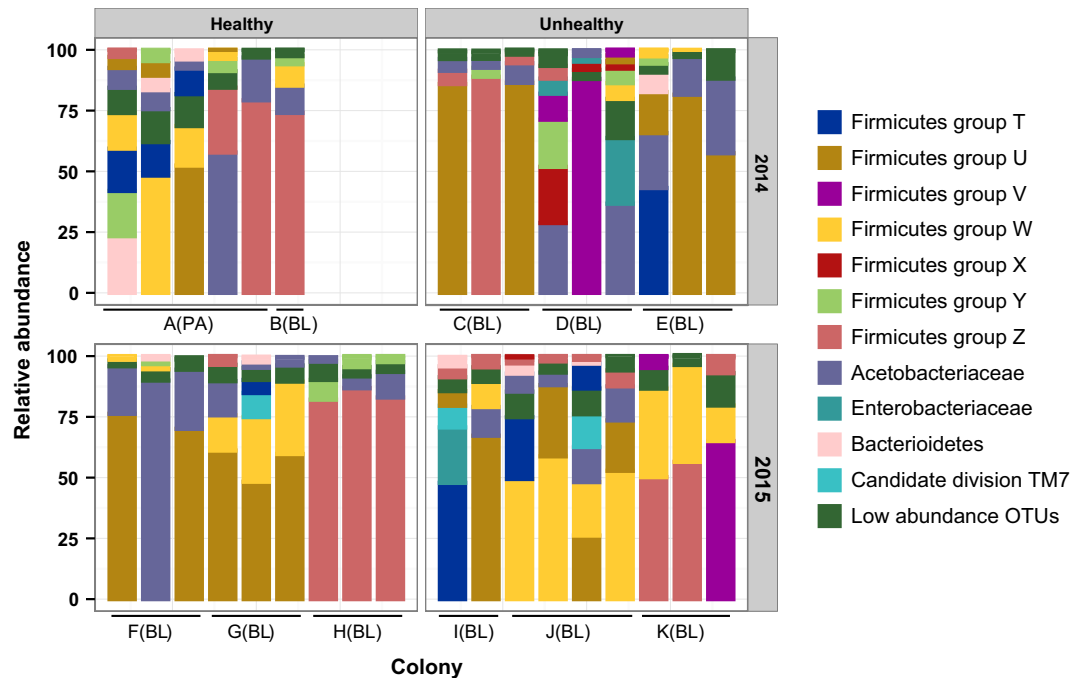


Fig. 2. Relative abundance of the 11 most representative bacterial taxa (clades) of healthy and unhealthy *Melipona quadrifasciata* sampled in two consecutive years. Letters indicate individuals from the same colony; PA and BL refer to the sampling localities, Porto Alegre and Boqueirão do Leão, respectively.

keepers learned that by preventing bees from leaving the colony, or by moving the colony to another locality, it is often possible to avoid the collapse, which makes them suspect that a toxic plant is poisoning their bees, but we observed that the pollen types in the crop of both healthy and unhealthy adults are indistinguishable (data not shown). *M. quadrifasciata* adults seem to forage mostly on *Eucalyptus* spp. – an observation corroborated by other studies on *Melipona* feeding habits in Southern Brazil (Hilgert-Moreira et al., 2014) – and on native Asteraceae, such as *Vernonanthura tweediana*, that bloom in the region in this time of the year. We think that, as already suggested for colony collapses of honeybees (Goulson et al., 2015; Nazzi et al., 2012; VanEngelsdorp et al., 2010), a synergistic effect of multiple factors, such as environmental stresses caused by climatic change, intensive management and the use of pesticides, as well as biological factors that haven't been assessed in the present study, such as viruses or other pathogens, may be at the heart of the problem.

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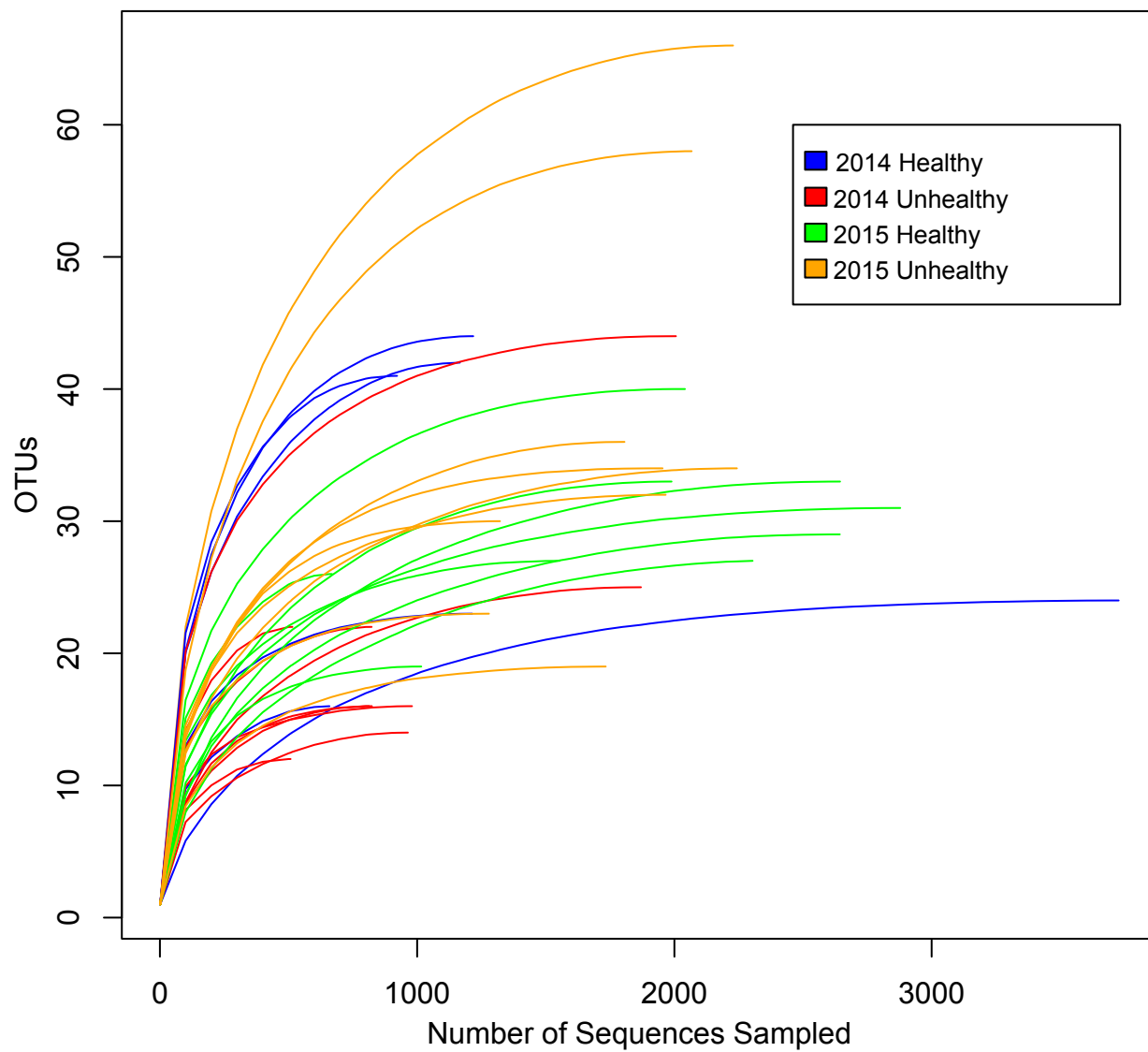
Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jip.2016.11.012>.

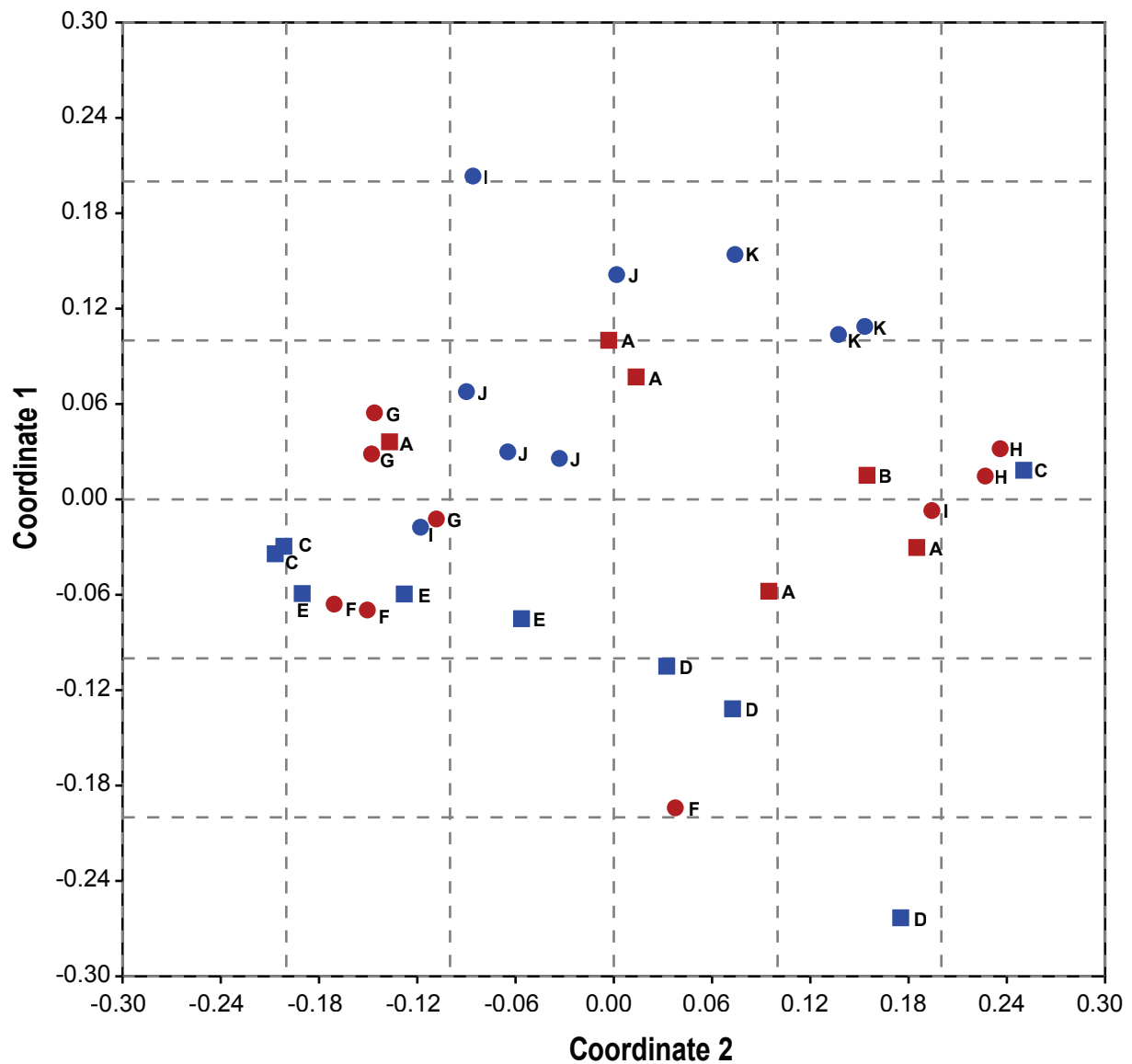
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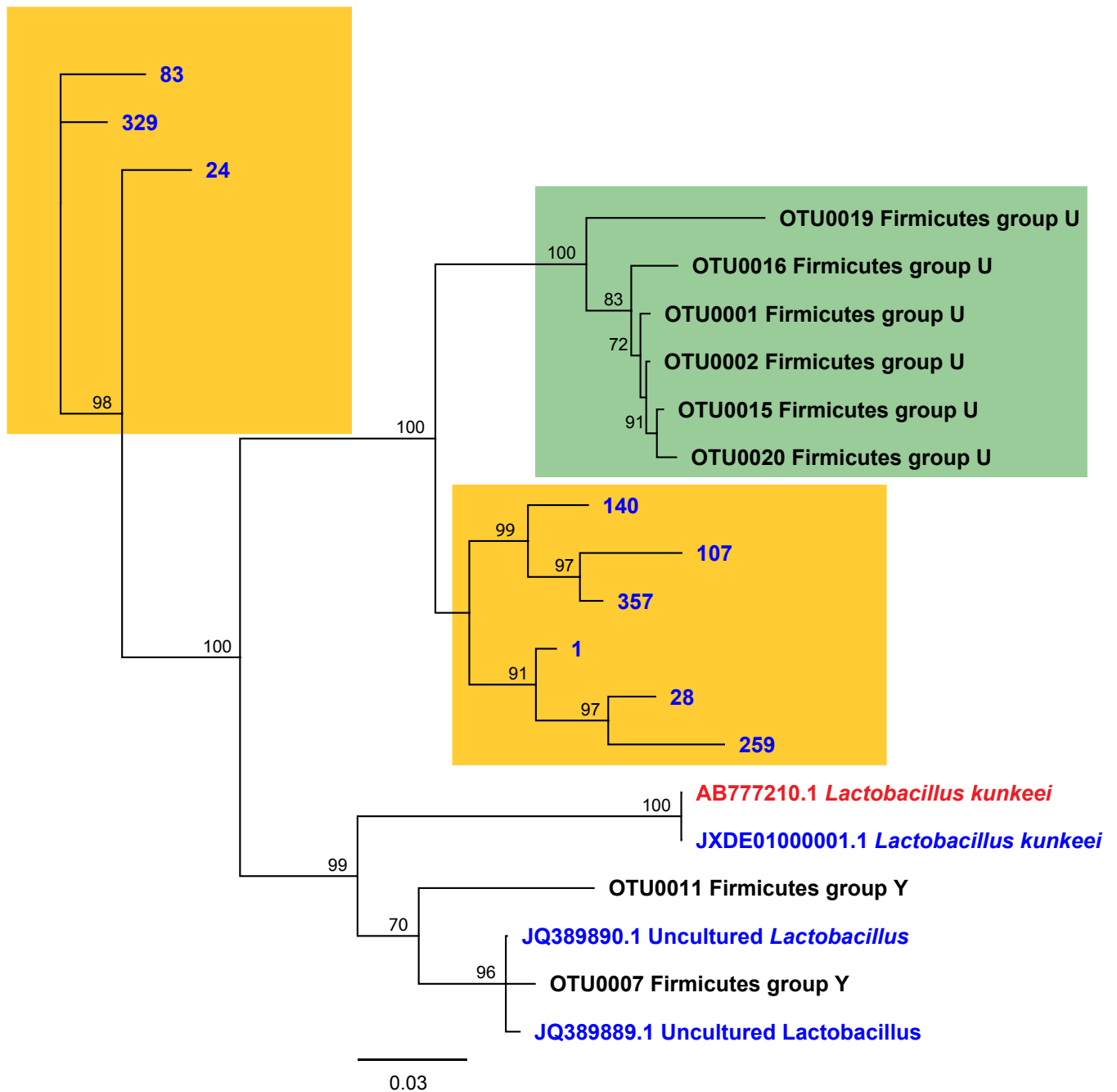
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Supplementary Figure 1. Microbiota rarefaction curves. Each line represents the cumulative fraction of bacterial community represented by the OTUs sampled for each *Melipona quadrifasciata* individual.



Supplementary Figure 2. NMDS ordination plot. Microbial communities are discriminated by Bray-Curtis dissimilarity on two coordinates (stress=0.19). Samples derived from unhealthy and healthy stingless bees are shown in blue and red respectively; squares represent samples from 2014 and circles correspond to samples from 2015. Letters indicate the respective colony of each stingless bee.



Supplementary Figure 3. Phylogenetic relationships of OTUs belonging to Firmicutes group U. Yellow boxes indicate Meliponini-associated OTUs previously identified by Leonhardt and Kaltenpoth (2014) in Australian stingless bees. These bacteria belong to a sister clade of Halictidae-associated Lactobacillales (see Leonhardt and Kaltenpoth for details). Sequences obtained in our study are shown in black, whereas blue and red represent sequences obtained in other studies from stingless bees and honey bees, respectively.

Table S1

Group	Year	Local	Healthy	Colony	# Sequences	# OTUs	Shannon	Chao1	Inverse Simpson	Clade Abundance (%)											
										Firmicutes group T	Firmicutes group U	Firmicutes group V	Firmicutes group W	Firmicutes group X	Firmicutes group Y	Firmicutes group Z	Acetobacteriaceae	Enterobacteriaceae	Bacteroidetes	Candidate division TM7	Low abundance OTUs
Agr10	2014	PA	Yes	A	1166	42	2.61	42.00	8.74	17.41	4.55	0.00	14.67	0.00	18.61	2.92	8.15	0.00	23.33	0.00	10.38
Agr13	2014	PA	Yes	A	922	41	2.37	41.00	4.59	13.77	5.10	0.00	48.26	0.00	4.77	0.76	7.81	0.00	5.97	0.00	13.56
Agr14	2014	PA	Yes	A	1218	44	2.45	44.00	6.54	10.51	52.38	0.00	16.26	0.00	0.49	0.33	3.78	0.00	3.12	0.00	13.14
Agr30	2014	PA	Yes	A	1214	23	1.68	23.00	2.83	0.00	1.57	0.00	2.14	0.00	5.11	26.69	57.74	0.00	0.00	0.00	6.75
Agr33	2014	PA	Yes	A	3727	24	0.76	24.00	1.57	0.00	0.00	0.00	0.13	0.00	0.21	79.23	17.63	0.00	0.00	0.00	2.79
Wal00	2014	PA	Yes	B	659	16	1.85	16.00	5.11	0.00	0.00	0.00	8.80	0.00	3.34	74.05	11.23	0.00	0.00	0.00	2.58
Mq021	2014	BL	No	C	824	16	1.36	16.00	2.75	0.00	85.92	0.00	0.85	0.00	0.00	5.46	4.85	0.00	0.00	0.00	2.91
Mq023	2014	BL	No	C	813	16	1.27	16.00	2.61	0.00	0.25	0.00	0.86	0.00	3.69	88.93	3.69	0.00	0.00	0.00	2.58
Mq024	2014	BL	No	C	508	12	1.32	12.00	2.63	0.00	86.42	0.00	0.59	0.00	0.00	3.54	8.07	0.00	0.00	0.00	1.38
Mq1A1	2014	BL	No	D	515	22	2.26	22.00	7.39	0.00	0.78	10.68	0.97	23.11	19.42	5.24	28.74	6.21	0.00	0.00	4.85
Mq1A3	2014	BL	No	D	1870	25	0.71	25.00	1.31	0.00	0.00	88.02	0.21	3.37	0.16	0.21	1.93	2.35	0.00	0.00	3.74
Mq1A4	2014	BL	No	D	2006	44	2.56	44.00	7.91	0.00	2.34	2.29	6.48	3.09	5.98	0.00	36.69	27.02	0.00	0.00	16.10
Mq1B1	2014	BL	No	E	823	22	2.12	22.00	6.05	43.13	16.89	0.24	2.43	0.00	3.28	0.00	22.60	0.00	8.02	0.00	3.40
Mq1B2	2014	BL	No	E	964	14	1.34	14.00	2.90	0.00	81.54	0.00	1.24	0.00	0.31	0.00	15.56	0.00	0.00	0.00	1.35
Mq1B3	2014	BL	No	E	980	16	1.67	16.00	4.10	0.00	57.45	0.00	0.61	0.00	0.00	0.00	30.71	0.00	0.00	0.00	11.22
MqVD1	2015	BL	Yes	F	2304	27	1.38	27.00	3.01	0.00	76.26	0.00	1.26	0.00	0.00	0.48	19.57	0.00	0.00	0.00	2.43
MqVD2	2015	BL	Yes	F	2644	33	0.73	33.00	1.30	0.00	0.15	0.00	2.12	0.00	1.97	1.17	89.94	0.00	0.00	0.00	4.65
MqVD3	2015	BL	Yes	F	2643	29	1.48	29.00	3.27	0.00	70.07	0.00	0.38	0.00	0.00	0.76	24.33	0.00	0.00	0.00	4.46
MqVF1	2015	BL	Yes	G	669	26	2.17	26.00	5.89	0.00	61.14	0.00	14.50	0.00	0.00	3.59	14.05	0.00	0.00	0.00	6.73
MqVF2	2015	BL	Yes	G	2041	40	2.28	40.00	6.20	5.39	48.31	0.00	26.60	0.00	0.00	0.78	2.20	0.00	1.81	9.90	5.00
MqVF3	2015	BL	Yes	G	1554	27	2.00	27.00	5.04	0.00	59.72	0.00	29.79	0.00	0.00	1.03	2.83	0.00	0.00	0.00	6.63
MqVG1	2015	BL	Yes	H	2878	31	1.55	31.00	2.78	0.00	0.00	0.07	0.45	0.00	8.13	82.07	1.70	0.00	0.00	0.07	7.51
MqVG2	2015	BL	Yes	H	1989	33	1.50	33.00	2.48	0.00	0.50	0.00	0.35	0.00	3.67	86.78	4.88	0.00	0.00	0.00	3.82
MqVG3	2015	BL	Yes	H	1016	19	1.54	19.00	3.00	0.00	0.00	0.00	0.79	0.00	1.67	82.97	10.53	0.00	0.00	0.00	4.04
MqVB4	2015	BL	No	I	1806	36	2.04	36.00	4.75	47.84	6.04	0.00	0.11	0.00	0.00	4.49	0.39	22.81	3.71	8.91	5.70
MqVB5	2015	BL	No	I	1322	30	1.95	30.00	4.53	0.00	67.25	0.00	10.36	0.00	0.00	4.61	11.80	0.00	0.00	0.00	5.98
MqVE1	2015	BL	No	J	2067	58	2.12	58.00	3.97	25.45	1.02	0.00	49.44	0.00	0.10	1.50	7.40	0.00	4.21	0.39	10.50
MqVE2	2015	BL	No	J	2243	34	1.77	34.00	3.46	0.00	29.29	0.00	58.76	0.00	0.00	2.18	5.17	0.00	0.00	0.00	4.59
MqVE3	2015	BL	No	J	2228	66	2.73	66.00	9.43	10.23	26.17	0.00	21.99	0.00	0.00	1.53	14.45	0.00	1.57	13.46	10.59
MqVE4	2015	BL	No	J	1954	34	1.93	34.00	3.89	0.00	20.73	0.00	52.81	0.00	0.00	6.55	14.02	0.00	0.00	0.00	5.89
MqVH1	2015	BL	No	K	1966	32	1.91	32.00	4.27	0.00	0.20	4.53	36.42	0.00	0.00	50.20	0.10	0.00	0.00	0.00	8.55
MqVH2	2015	BL	No	K	1733	19	1.54	19.00	3.64	0.00	0.29	0.00	39.87	0.00	0.00	56.49	0.12	0.00	0.00	0.00	3.23
MqVH3	2015	BL	No	K	1279	23	1.67	23.00	2.91	0.00	0.00	65.05	14.62	0.00	0.55	6.49	0.00	0.00	0.00	0.00	13.29

CAPÍTULO 3

The virome of an endangered stingless bee suffering from annual mortality in southern Brazil

Este capítulo apresenta o seguinte artigo publicado:

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The virome of an endangered stingless bee suffering from annual mortality in southern Brazil

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Abstract

Meliponiculture – the management of stingless bee colonies – is an expanding activity in Brazil with economic, social and environmental potential. However, unlike in apiculture, the pathogens that impact on meliponiculture remain largely unknown. In southern Brazil, every year at the end of the summer, managed colonies of the stingless bee *Melipona quadrifasciata* manifest a syndrome that eventually leads to collapse. Here we characterize the *M. quadrifasciata* virome using high-throughput sequencing, with the aim of identifying potentially pathogenic viruses, and test whether they are related to the syndrome outbreaks. Two paired viromes are explored, one from healthy bees and another from unhealthy ones. Each virome is built from metagenomes assembled from sequencing reads derived either from RNA or DNA. A total of 40621 reads map to viral contigs of the unhealthy bees' metagenomes, whereas only 11 reads map to contigs identified as viruses of healthy bees. The viruses showing the largest copy numbers in the virome of unhealthy bees belong to the family *Dicistroviridae* – common pathogenic honeybee viruses – as well as *Parvoviridae* and *Circoviridae*, which have never been reported as being pathogenic in insects. Our analyses indicate that they represent seven novel viruses associated with stingless bees. PCR-based detection of these viruses in individual bees (healthy or unhealthy) from three different localities revealed a statistically significant association between viral infection and symptom manifestation in one meliponary. We conclude that although viral infections may contribute to colony collapses in the annual syndrome in some meliponaries, viruses spread opportunistically during the outbreak, perhaps due to colony weakness.

INTRODUCTION

Stingless bee management in Brazil began with indigenous South Americans, who domesticated the non-aggressive native bees as a source for honey [1]. One of these bees, *Melipona quadrifasciata*, which is distributed from the state of Paraíba in the north of Brazil through to the southernmost state, Rio Grande do Sul [2], is the second most cultivated stingless bee in Brazil [3]. In spite of being widely cultivated, natural

populations of stingless bees in the neotropics have reduced drastically in recent years due to different factors, ranging from habitat fragmentation to the introduction of exotic bees [4] that not only compete for resources, but also bring new pathogens to which native bees might be susceptible. In most of southern Brazil, wild populations of the subspecies *M. q. quadrifasciata* have been disappearing for 50 years. The species is currently mostly maintained through management

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Keywords: *Melipona*; Apidae; virome; colony collapse; bee virus.

Abbreviations: ABPV, *Acute bee paralysis virus*; BLAST, Basic Local Alignment Search Tool; BQCV, *Black queen cell virus*; BU, Barnard's Unconditional test; CCD, Colony collapse disorder; CDS, Coding sequence; CMH, Cochran-Mantel-Haenszel; DWV, *Deformed wing virus*; IAPV, *Israeli acute paralysis virus*; KBV, *Kashmir bee virus*; MqC, *Melipona quadrifasciata cyclovirus*; MqC1, *Melipona quadrifasciata cyclovirus 1*; MqC2, *Melipona quadrifasciata cyclovirus 2*; MqD, *Melipona quadrifasciata densovirus*; MqV2, *Melipona quadrifasciata virus 2*; MqV1a, *Melipona quadrifasciata virus 1a*; MqV1b, *Melipona quadrifasciata virus 1a*; SBPV, *Slow bee paralysis virus*; VDV1, *Varroa destructor virus-1*.

MK190907 (MqC1), MK190906 (MqC2), MH340498 (MqD), MH340499 (MqV1a) and MH34500 (MqV1b), MK190905 (MqV2).

Two supplementary figures and four supplementary tables are available with the online version of this article.

in this region, and is therefore considered to be endangered [5, 6]. Managing stingless bees has been crucial for conservation, but on the other hand, practices that involve hive translocation may also spread pathogens [7]. Despite advances in beekeeping legislation and monitoring, there is no official bee surveillance in Brazil and, consequently, there are no regular records on bee mortality caused by pathogens. Nevertheless, it is a common knowledge among beekeepers from different localities in southern Brazil that every year at the end of the summer – normally during the first week of March – their *M. quadrifasciata* bees begin to manifest an unusual behaviour that may include tremors, crawling with everted proboscis and paralysis [8]. Although the disease symptoms are quite variable among stingless bees from different localities, during this period it is common to find large numbers of dead bees inside or in front of hives. Mortality, and the inability of bees to perform their regular activities, sometimes lead to colony collapse. Our previous studies on the *M. quadrifasciata* syndrome searched the associated microbiota for pathogenic bacteria or imbalances in bacterial community composition that could explain the symptoms [8]. However, no consistent differences in microbiota composition were found between healthy and unhealthy bees. While *Apis mellifera* shows a highly conserved core microbiota [9], *M. quadrifasciata*'s associated bacterial community is highly variable across colonies, independent of their health status. Furthermore, no pathogenic bacteria or other common bee pathogens such as *Nosema* and *Crithidia*, were detected in unhealthy bees.

Several viruses have already been identified in *A. mellifera* in Brazil [10], mainly in samples from areas where abnormal population declines have been observed [11]. These have included *Iflaviridae* such as the *Deformed wing virus* (DWV), and *Dicistroviridae* such as the *Black queen cell virus* (BQCV), *Acute bee paralysis virus* (ABPV) and *Israeli acute paralysis virus* (IAPV) [11, 12]. Characteristic symptoms of DWV infections are the death of pupae, or adult bees with deformed wings, sometimes associated with shortened and bloated abdomens, and miscolouring [13]. However, DWV may persist in covert infections with no apparent symptoms [14, 15], in spite of having strongly deleterious effects on honeybee foraging and survival [16]. Similarly, dicistroviruses may also persist with no obvious symptoms at the individual or colony level [17]. Nevertheless, it has been shown that an increase in BQCV titre kills developing queen larvae, whose necrotic remains result in a pupal cell with a darkened colour [18]. Other viruses of this family, such as ABPV and IAPV, are known to cause paralysis [19, 20]. They are extremely virulent when injected into pupae or adults, causing disease in the honeybee with fewer than 100 viral particles [21]. Honeybees experimentally infected with IAPV develop symptoms such as crawling, disorientation and trembling wings, which progress to paralysis and death within or outside the hive [22, 23]. Furthermore, viruses with RNA genomes such as the *Iflaviridae* and *Dicistroviridae*, which show higher mutation rates, quickly evolve into many variants, allowing them to switch between different bee hosts [24–26]. An example of viral host switching between bee species is the detection

of honeybee virus ABPV in managed colonies of *M. scutellaris* suffering from an unexpected increase in worker bee mortality in Brazil [27].

The main routes of virus transmission between bees are vectors such as mites or pollen resource sharing [28]. The most studied vector in honeybees is *Varroa destructor*, which transmits different lineages of DWV, *Varroa destructor virus-1* (VDV1) and IAPV [29–31]. These and many other viruses are also transmitted within and between species via floral sources [24, 32]. Bee species sharing habitats and resources tend to have similar associated micro-organisms. A study on viral distribution via pollen sources showed that in apiaries with colonies affected by colony collapse disorder (CCD), IAPV virus was associated with *A. mellifera* as well with non-*Apis* hymenopteran pollinators [32]. At the same time, in nearby apiaries not affected by CCD, the virus was present in neither *A. mellifera* nor non-*Apis* hymenopterans. The persistence of BQCV, DWV, ABPV and *Slow bee paralysis virus* (SBPV) in wild bumblebee populations in Europe, often at putatively low levels, may also result from indirect interactions with local *A. mellifera* through the shared use of floral resources [24].

Recent studies using high-throughput sequencing technologies expanded the list of known honeybee viruses, previously restricted to the Picornavirales [33]. Among the recently identified viruses are those from the family *Rhabdoviridae* with –ssRNA genomes, ssDNA viruses belonging to the families *Circoviridae* and *Parvoviridae*, and dsDNA viruses from the family *Nudiviridae* [34–36]. These viruses were identified in honeybees displaying variable health conditions. Hence, the viromes of bees have revealed that, in addition to the complex network of host-associated bacteria [9], a very diverse range of viruses are probably acting on and interacting in the bacterial network. Different bee viruses are frequently detected in healthy and unhealthy hosts [24, 34, 35, 37]. Chronic viral infections may become harmful by increasing their loads in genetically predisposed individuals, depending on the environmental conditions [37–39], or may become symbiotic [40].

Given that the symptoms observed in *M. quadrifasciata* affected by the annual syndrome resemble those of honeybee viral diseases such as IAPV and ABPV, and given that stingless bees probably host yet unidentified viruses, some of which may have been spread by honeybees due to beekeeping practices, we focus our investigation on viruses that might be implicated in the disease. Using high-throughput sequencing to characterize the virome of *M. quadrifasciata*, we identify seven novel stingless bee viruses.

RESULTS

Viromes of healthy and unhealthy *M. quadrifasciata*

In order to find viruses associated with the *M. quadrifasciata* syndrome, worker bees from a single colony showing disease symptoms such as tremor and paralysis (unhealthy bees), as well as workers from another colony where none of the individuals showed such symptoms (healthy bees), were

sampled from the same meliponary (place where stingless bees are reared). Each bee pool was macerated and underwent subsequent centrifugation steps, including an ultracentrifugation with a sucrose cushion for virus enrichment. The nucleic acids were then subjected to high-throughput sequencing, yielding the following number of paired-end reads: 640 953 (DNA sample from unhealthy bees=UDNA), 535 631 (DNA sample from healthy bees=HDNA), 582 603 (RNA sample from unhealthy bees=URNA) and 834 753 (RNA sample from healthy bees=HRNA). After assembly, viral contigs larger than 200 bp were classified based on the best BLASTX hit against the nr database, resulting in a total of 989 viral sequences. From these, only five were recovered from healthy bees (three from DNA and two from RNA), whereas all remaining 984 viral sequences were assembled from unhealthy bees.

From the total of 989 viral sequences (Table S1, available in the online version of this article), 901 were identified as bacteriophages and 17 were identified as viruses of unclassified or unknown hosts, and for the purpose of the present study, we focused on the remaining 71 sequences, which were identified as eukaryotic viruses (Fig. 1). The HRNA sample was left out of Fig. 1, since both viral contigs assembled from the RNA of healthy bees were identified as bacteriophages. Thus, the virome of healthy bees contained only three sequences from eukaryotic viruses that belonged to two different families. The virome of unhealthy bees, on the other hand, contained 38 sequences from viruses belonging to 11 different families, as well as 30 contigs showing similarity to unclassified viruses previously found in eukaryotes (Fig. 1). Using sequencing depth as a proxy for abundance, i.e. the number of copies of viruses within each virome, the most representative viruses in unhealthy bees (50–140 X depth) belonged to the families *Parvoviridae* (ssDNA genome) and *Circoviridae* (ssDNA genome). Viruses identified as *Dicistroviridae* (+ssRNA genome) that included potential honeybee pathogens showed lower sequencing depth, ranging from 8 to 15 X.

Characterization of novel *M. quadrifasciata* viruses

Seven eukaryotic viral sequences with significant length and depth were selected as candidates for further investigation: *Melipona quadrifasciata cyclovirus* (MqC), *Melipona quadrifasciata cyclovirus 1* (MqC1) and *Melipona quadrifasciata cyclovirus 2* (MqC2) from the family *Circoviridae*; *Melipona quadrifasciata densovirus* (MqD) from the family *Parvoviridae*; *Melipona quadrifasciata virus 1a* and *1b* (MqV1a and MqV1b) from the family *Dicistroviridae*; and *Melipona quadrifasciata virus 2* (MqV2), which is unclassified (Table S1). To evaluate whether these sequences represent already known or novel viruses, predicted proteins derived from their coding sequences (CDSs) were used for phylogenetic analyses. None of them showed sufficient similarity to other known viruses to be regarded as the same species.

The most abundant virus found in unhealthy stingless bees belonged to the family *Parvoviridae* (MqD; Table S1). The MqD sequence (4214 nt) probably represents a complete genome, encoding the non-structural (534 aa) and structural

(655 aa) proteins, containing conserved domains Parvo_NS1 (cl24009, e-value $4.57e^{-10}$) and Denso_VP4 (cl03545, e-value $4.83e^{-15}$), respectively. Phylogenetic inference based on their concatenated amino acid sequences groups MqD with other *Parvoviridae* found mainly in insects, within the genus *Densovirus* (Fig. S1), such as the recently described *Bombus cryptarum densovirus* [34].

Two *Circoviridae* sequences from unhealthy bees also contained recognizable CDSs: MqC1 and MqC2 (Table S1). MqC1 (1587 nt) is probably a complete genome as well, from which the non-structural protein (228 aa), with a P-loop_NTPase domain (cl21455, e-value $7.94e^{-18}$), and the structural protein (224 aa) are predicted. MqC2 (673 nt) is a partial virus genome bearing a partial non-structural protein (224 aa) with the P-loop_NTPase domain (e-value $3.34e^{-18}$). These two viruses cluster with viruses from the family *Circoviridae* within the genus *Cyclovirus* (Fig. 2), a sister clade of the genus *Circovirus*, that were found recently in *A. mellifera* (Circo-like 1 and Circo-like 2) [35].

Two other partial viral genomes show similarity to *Dicistroviridae* viruses: MqV1a (5913 nt) and MqV1b (1236 nt) (Table S1). A single protein is predicted from each, i.e. the structural protein (1888 aa), with RNA_dep_RNAP (cd01699, e-value $9.44e^{-83}$) and RNA_helicase (pfam00910, e-value $1.24e^{-31}$) domains, is encoded by MqV1a, whereas the partial non-structural protein (412 aa), with RHV_like (cd00205, e-value $5.94e^{-23}$) and Dicistro_VP4 (cl13011, e-value $4.36e^{-08}$) domains, is predicted from MqV1b. Both proteins group these viruses within *Dicistroviridae* known to infect bees and other arthropods (Fig. 3), such as ABPV and IAPV. However, the amino acid identities between MqV1a or MqV1b proteins and known ABPV and IAPV proteins are within the order of 28–15% and 29–16 %, respectively.

MqV2 (3730 nt) shows an unclassified virus as best hit (Table S1), and encodes a non-structural protein (479 aa), which contains RT_like (cl02808, e-value $8.87e^{-11}$) and RdRP_3 (cl24119, e-value $2.48e^{-05}$) domains. MqV2 is related to other unclassified RNA viruses that are found to be associated with a diversity of hosts, from plants to animals such as birds, molluscs and bees (Fig. S2) [41].

Virus detection in stingless bees and honeybees

Having characterized seven novel eukaryotic viruses found in high abundance in unhealthy stingless bees, we tested whether their presence is associated with the manifestation of disease symptoms (Table S2). We reasoned that if a virus is directly implicated in the annual syndrome, it should be detected more often in unhealthy than in healthy bees of different meliponaries. Virus detection by PCR was performed by testing individual worker bees to determine if the disease symptoms were present in three different meliponaries, using primers designed to amplify fragments of about 300 bp from each virus genome (Table 1). Table 2 summarizes the results of Cochran–Mantel–Haenszel (CMH) tests, which were used to verify the association between the detection of each candidate virus with the manifestation of symptoms, controlled

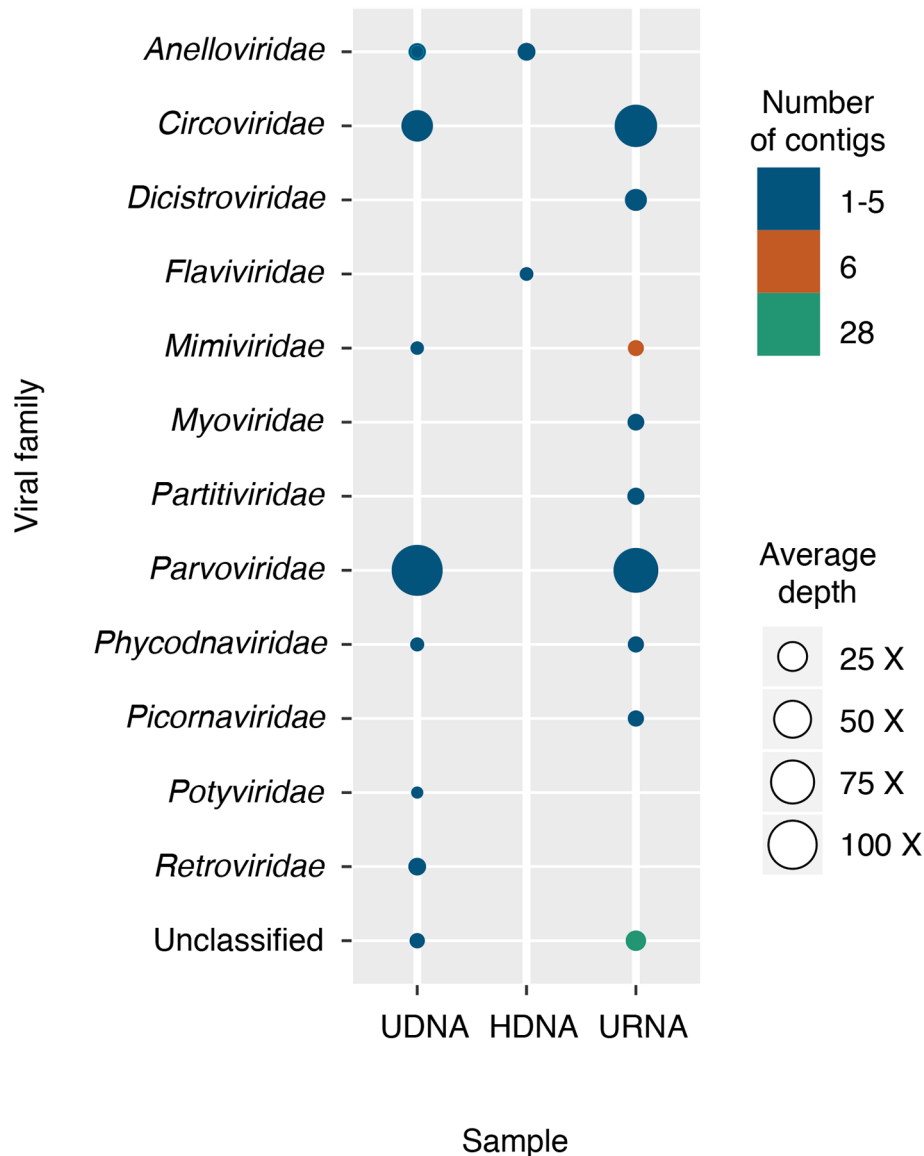
M. quadrifasciata-associated viruses

Fig. 1. Taxonomic distribution of *M. quadrifasciata* viral contigs in metagenomes of healthy (HDNA) and unhealthy bees (UDNA and URNA). Only contigs larger than 200 bp and showing best hits to eukaryotic viruses are considered in this plot. Sequences of viral families showing larger sequencing depth (larger circles) were chosen as candidates in subsequent analyses.

by locality, and the results of Barnard's unconditional (BU) tests applied to individual localities. All viruses were detected in both healthy and unhealthy bees, and only MqV1a was detected in bees from all meliponaries. None of the viruses were found to be significantly more associated with the syndrome manifestation in all localities in view of the CMH test. However, MqC, MqD and MqV1a viruses were more often associated with unhealthy bees in the Boqueirão do Leão (BL) meliponary, where *M. quadrifasciata* was sampled

to build the viromes. Interestingly, the three circoviruses (MqC, MqC1 and MqC2) and the unclassified virus MqV2 were also detected in *A. mellifera* colonies from one of the sampling localities [Bom Princípio (BP)] (see Table 2).

DISCUSSION

Bee populations have been declining for some years, leading to global concern over the plight of plant pollinators [42, 43].

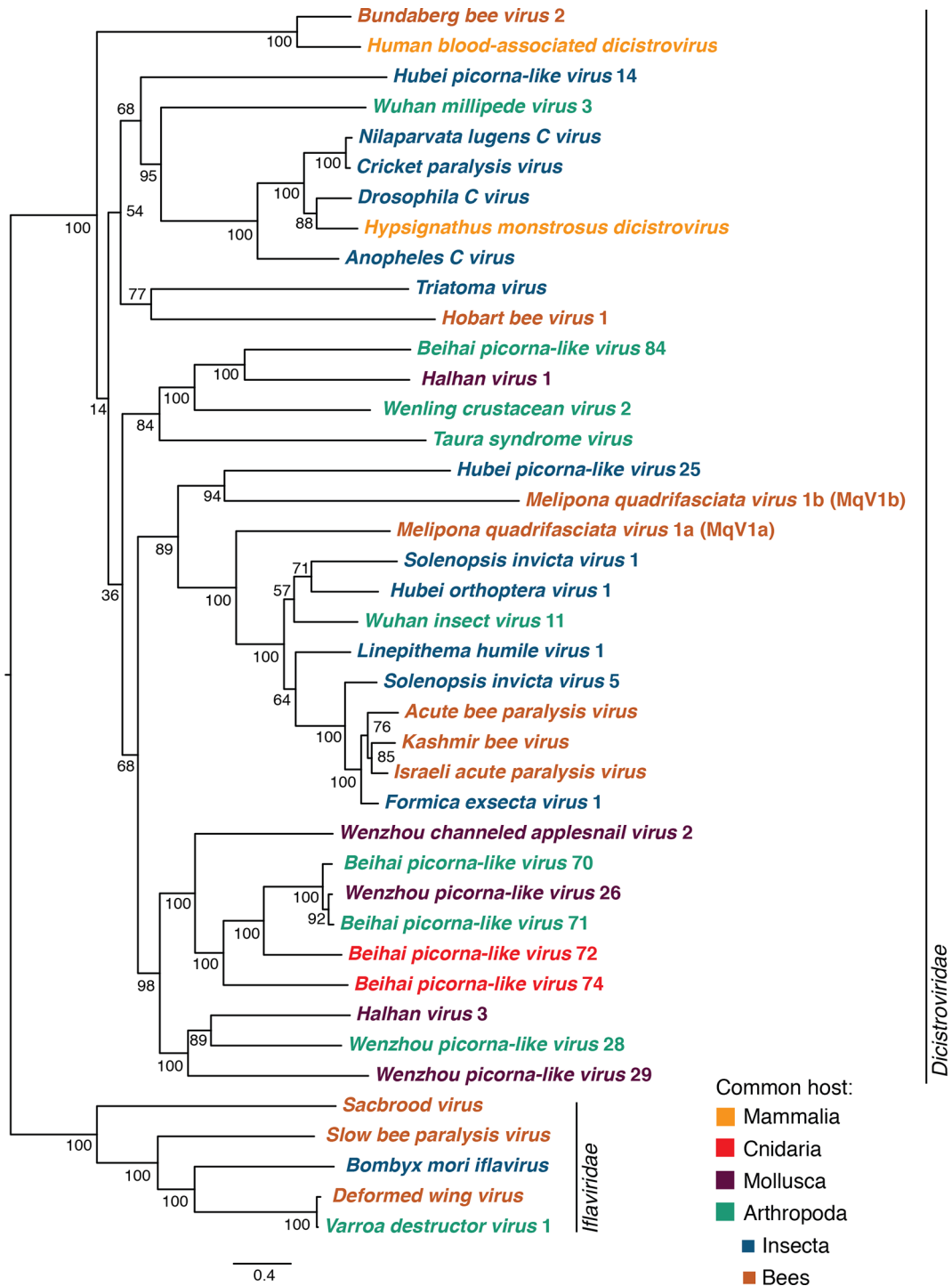


Fig. 2. Phylogenetic inference of MqC1 and MqC2 viruses (*Circoviridae*). The maximum-likelihood tree was inferred with 100 bootstrap resamples based on an alignment of 502 aa. The colours of the species names represent the viruses' common hosts (see lower right).

Viruses that are prevalent in weak colonies of honeybees are probably directly implicated in the population decline of different bee species [44–46]. For example, honeybee colonies affected by CCD, a syndrome characterized by the sudden disappearance of honeybees [47], often show higher

prevalence of IAPV, ABPV or *Kashmir bee virus* (KBV) [48, 49]. The apparent spread of CCD in the USA suggests that viruses, among numerous other factors such as pesticides, stress, climate and habitat change, and other natural enemies, could cause honeybee populations to drop so much

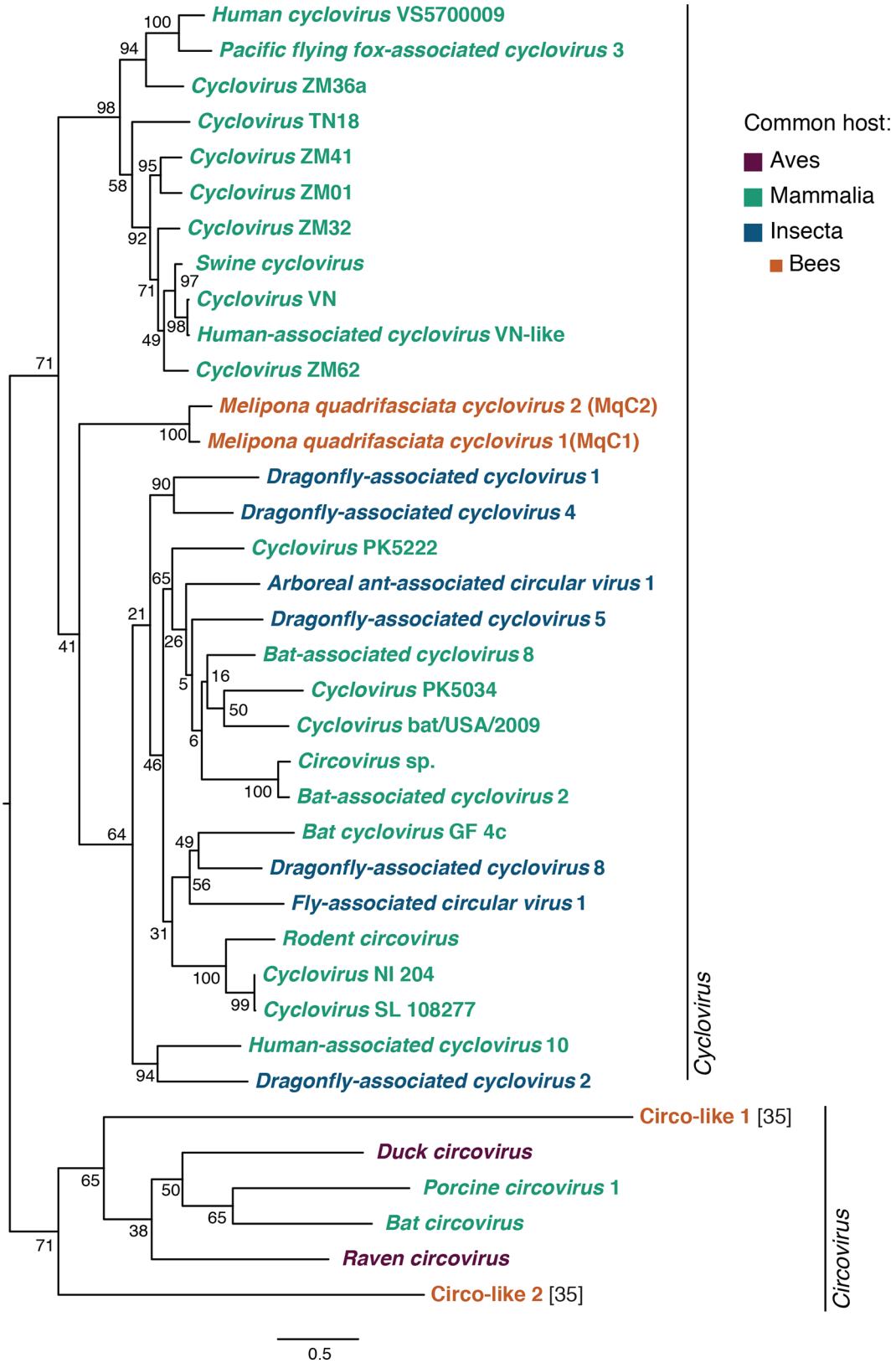


Fig. 3. Phylogenetic inference of MqV1a and MqV1b viruses (*Dicistroviridae*). The maximum-likelihood tree was inferred with 100 bootstrap resamples based on an alignment of 2748 aa. The colours of the species names represent the viruses' common hosts (see top right).

Table 1. Primer sequences designed for virus detection

Name	Sequence	Amplicon length (bp)
MqC F	5' ACTTCTGTGCCGTTGGTAGT 3'	300
MqC R	5' TCTCTCAAATTGCTTGCGCC 3'	
MqC1 F	5' GCCACTTCTGTGCCGATAT 3	304
MqC1 R	5' TCCTTCAAAGGTGCCAGCTC 3	
MqC2 F	5' ACTACTGGGAGCACGGAGAT 3	318
MqC2 R	5' ATCCGTCACCACATTCTCT 3	
MqD F	5' TCAAGCAGCGATTCTGGAGA 3'	300
MqD R	5' CCACACAGCGCTACTATTT 3'	
MqV1a F	5' CTCTTTTCCATCGACAAGCT 3'	300
MqV1a R	5' TCAGCCGCAAACCTCTTCGA 3'	
MqV1b F	5' TTAGTCTAAACGGGCCACT 3'	300
MqV1b R	5' TGGACATGCCAACACTACA 3	
MqV2 F	5' GCGCTGTATTGCATCTGGTG 3'	304
MqV2 R	5' CGACGCGCATTTCCTCAAT 3'	

that agricultural pollination will become unsustainable [50]. In spite of being less important with regard to crop pollination than honeybees, stingless bees and other native bees are fundamental to sustain nesting and alternative flower resources for crop pollinators, which are typically unavailable within intensively managed crop fields [51]. Moreover, the disappearance of *M. quadrifasciata* wild nests in southern Brazil is worrying because the management practices that are used to maintain bee populations in this region are not well

regulated, and the trade in stingless bee colonies across the country could potentially spread viruses and other pathogens.

The seven most abundant eukaryotic viruses found in the virome of unhealthy *M. quadrifasciata* are novel viruses. Two partial dicistrovirus genomes were obtained: MqV1a and MqV1b. Although each contains one of the two CDSs of a typical dicistrovirus, our attempts to bridge them by PCR failed (data not shown), suggesting that they may indeed represent related, but different, viruses. Both show low identity to other dicistroviruses causing similar pathological symptoms in honeybees (IAPV and ABPV). Their high divergence from honeybee dicistroviruses and the lack of detection of MqV1a and b sequences in honeybees indicate that the presence of dicistroviruses in *M. quadrifasciata* does not represent a horizontal transmission event from *A. mellifera* to stingless bees, as previously suggested for ABPV infections in *M. scutellaris* [27]. Rather, our phylogenetic analyses indicate that MqV1a and b belong to an evolutionary branch that diverged from the honeybee IAPV long before other dicistroviruses found in unrelated hosts such as ants and crustaceans. Similarly, a recent study on the prevalence of DWV in *Melipona subnitida* and *A. mellifera* colonies from a single island in Brazil showed that DWV strain C, which is typically found in the stingless bee, is virtually absent in the honeybee [52]. Such dissimilar scenarios concerning the simultaneous presence of dicistroviruses in stingless bees and honeybees in Brazil probably represent different stages of the evolutionary divergence process that follows a host-switching event.

Interestingly, the most abundant *M. quadrifasciata* viruses in our study have ssDNA genomes: MqD (genus *Densovirus*, family *Parvoviridae*), MqC, MqC1 and MqC2 (genus *Cyclovirus*, family *Circoviridae*). These viral families have not been

Table 2. Candidate virus detection in *M. quadrifasciata* and *A. mellifera*. The number of *M. quadrifasciata* individuals that were positive for each virus is shown in relation to the total number of tested bees. Differences in the proportion of positive samples between healthy (H) and unhealthy (U) bees were tested for each meliponary (BL, Boqueirão do Leão; BP, Bom Princípio; and RL, Rolante) using Barnard's unconditional (BU) test and for the total number of sampled bees controlled by locality using the Cochran–Mantel–Haenszel (CMH) test. Pools of bees from three colonies of *A. mellifera* from BP were tested for the same viruses

	BL			BP			RL			CMH	<i>A. mellifera</i>		
	H	U	p£	H	U	p£	H	U	p£	p	1	2	3
MqC	8/19	40/57	0.029	0/6	0/3	NT*	1/6	0/3	0.579	0.094	+	+	+
MqC1	13/19	49/57	0.107	2/6	2/3	0.492	0/6	0/3	NT*	0.106	+	–	+
MqC2	5/19	27/57	0.122	0/6	0/3	NT*	0/6	0/3	NT*	NT**	+	+	–
MqD	6/19	6/57	0.031	0/6	0/3	NT*	0/6	0/3	NT*	NT**	–	–	–
MqV1a	0/6	4/6	0.021	8/38	7/27	0.668	0/6	2/37	0.776	0.192	–	–	–
MqV1b	1/6	3/6	0.303	3/38	5/27	0.249	0/6	0/37	NT*	0.165	–	–	–
MqV2	0/6	2/6	0.197	1/38	0/27	0.569	0/6	0/37	NT*	0.919	+	–	+

£, BU test.

*, not tested; virus not detected.

** , not tested; virus detected in a single locality.

found to be associated with other bee species until recently. Using high-throughput sequencing technologies, viruses belonging to the *Nudiviridae* (dsDNA) and *Parvoviridae* (ssDNA) were found in *Bombus* sp. [34], and *Circoviridae* viruses (ssDNA) were identified in *A. mellifera* [35], with no apparent pathological consequences. Densoviruses are known to establish both mutualistic and pathogenic relationships with their arthropod hosts [53–55]. Viruses from the genus *Cyclovirus* are not known to cause pathology in insects, but have been detected in the cerebrospinal fluid of human patients with neurological conditions [56, 57], and in secretions from patients with respiratory disease [58]. Here, the three cycloviruses were detected in honeybees sharing their habitat with *M. quadrifasciata*. It is known that the pollen resources of honeybees and *Melipona* spp. overlap in different regions of Brazil [59–61], and by foraging on the same flowers they may potentially exchange viruses. Inter-species transmission of bee viruses such as DWV, IAPV, SBPV and BQCV through pollen sources is already well documented [32, 62]. It is not possible to confirm that the *M. quadrifasciata* cycloviruses are in fact replicating within the bee body, but the high loads of DNA viruses such as MqD (136 X), MqC (107 X) and MqC1 (83 X) in the metagenome derived from a RNA sample at least suggests that they are active.

Even though no direct association was found between the manifestation of the *M. quadrifasciata* annual syndrome and the detection of the seven candidate viruses in adult stingless bees from all localities, we do not rule out the possibility that these viruses indeed play a role in the outbreaks. Our viromes were built using pools of bees from BL, where they showed the most intense disease symptoms, and three viruses (MqC, MqD and MqV1a) were found significantly more often in unhealthy bees. This indicates that viruses apparently replicate opportunistically on a local basis, and that, given the regular timing of the outbreaks on different meliponaries, the common denominator might be a higher susceptibility of bees at the end of the summer. In the case of honeybee CCD, no single factor explains the manifestation of the disorder; rather, it seems that a combination of several factors may act synergistically [50]. Known examples of synergistic interactions of honeybee viruses with other factors include *Varroa* mites that destabilize DWV–host dynamics via suppression of the honeybee NF- κ B immune response [63] and pesticides that reduce honeybee resistance to viral infections [64]. Bee nutrition, which varies across landscapes and seasons, is also known to affect resistance to viruses and other pathogens [65].

In conclusion, there is no obvious answer to the question of whether or not viruses are implicated in the *M. quadrifasciata* annual syndrome. In spite of having shown a higher diversity and abundance of viruses in the virome of unhealthy bees in one of the studied meliponaries, our study suggests that viruses are not the direct cause of the syndrome, but may influence adult bee symptoms in certain localities. It is worth mentioning that eusocial bees differ from other animals in having an additional higher-level

immunity referred to as social immunity [66, 67]. Because social immunity is negatively correlated with host density [68], it is possible that the annual syndrome actually represents a mechanism of density control in *M. quadrifasciata* populations at the end of the summer. Nevertheless, our study calls attention to the presence of a diversity of as yet unknown bee viruses in stingless bees, and their ability to spread across species boundaries. Given their pathogenic potential, and global concern about the decline of bee populations, viruses must be taken into account when planning management practices and conservation strategies.

METHODS

Worker bee sampling

M. quadrifasciata worker bees were collected from four different meliponaries in southern Brazil: 76 bees were taken from BL (29°18'5.47"S/52°25'57.96"W), 43 bees were taken from Rolante (RL) (29°38'19.78"S/50°26'49.77"W) and 65 bees were taken from BP (29°31'2.30"S/51°17'29.00"W). Sampling was performed in February, March and September (i.e. before, during and after the syndrome outbreak, respectively) in different years. Worker bees with symptoms such as tremors and paralysis (unhealthy) and symptomless bees (healthy) were collected using an entomological pooter, stored temporarily in sample pots and brought alive or in RNAlater (Thermo Fisher Scientific, USA) to the laboratory where they were stored individually at –80 °C until nucleic acid extraction. Healthy individuals were only collected in colonies where none of the bees presented symptoms. During the 2017 syndrome outbreak at BL, an additional 25 healthy and 25 unhealthy worker bees were collected from a colony affected by the syndrome and one not affected by it, respectively, pooled and stored in 5 ml of RNAlater (Thermo Fisher Scientific, USA) at 4 °C until nucleic acid extraction. Additionally, three pools of ~100 *A. mellifera* workers were sampled in September 2018 from different colonies of the BP location and stored as described.

Nucleic acid extraction for metagenome sequencing

Nucleic acids (RNA and DNA) were extracted from samples enriched for viral particles following the protocol of de Sales Lima and collaborators [69]. Briefly, two pools of 25 worker bees (healthy and unhealthy) were individually ground with sterile sand in 3 ml of phosphate-buffered saline (PBS). The homogenate was filtered in a cell strainer to remove bee parts (i.e. wings, legs and cuticles) and then centrifuged at 3000 r.p.m. for 15 min at 4 °C. The supernatant was collected with a syringe and filtered through a 0.45 μ m filter (Sigma-Aldrich, USA) to remove bacteria-sized particles. The filtrate was transferred to a centrifuge tube in an SW-28 Ti rotor (Beckman Coulter, USA) already containing a sucrose cushion (25 % sucrose in Tris-Cl 10 mM, EDTA 1 mM) and ultracentrifuged at 27 000 r.p.m. for 4 h at 4 °C. The pellet, enriched for viral particles, was eluted in 200 μ l DEPC-treated water and split into two aliquots of 100 μ l each, one for DNA extraction with the universal phenol-chloroform

protocol [70] and another for RNA extraction with TRIzol (Ambion, USA), following the recommendations of the manufacturers. The yield and quality of nucleic acid purification were assessed with a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and a Qubit fluorometer (Invitrogen, USA). Aliquots of the purified RNA were used for cDNA synthesis with the High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, USA). The cDNA second strand was synthesized using Klenow fragment DNA polymerase (New England Biolabs, USA). Fragment libraries were further prepared with 1 ng of purified DNA or double-stranded cDNA using the Nextera XT DNA Library Preparation kit (Illumina, USA) and paired-end sequenced (2×150 nt) using the MiSeq Reagent kit v2 (Illumina, USA) on a MiSeq instrument (Illumina, USA).

De novo assembly and contig filtering

Paired-end reads from healthy and unhealthy bee DNA and RNA fragment libraries were debarcoded and used for *de novo* assembly. Datasets derived from DNA samples were assembled with SPAdes v3.10.1 [71], whereas RNA-derived reads were assembled with Trinity v2.2.0 [72]. Within SPAdes, reads were assembled with k-mer lengths 21, 33 and 55, without a filtering step before assembly. Within Trinity, a read quality trimming step (*--trimmomatic*) was added with the default settings [73]. Contaminations were removed by building subsets (bins) of the NCBI nucleotide (nt) database with the *blastdb_aliastool* command of BLAST [74]. Contigs with significant hits (e-value cutoff $1e^{-12}$) to bins including sequences from bacteria (taxid: 2), fungi (taxid: 4751) or plants (taxid: 33090) were filtered out. Another bin was used to filter out hits against the *M. quadrifasciata* genome (LIRP00000000.1) using the same parameters.

Virome sequence analysis and candidate virus selection

For taxonomic characterization, the remaining contigs were matched with BLASTX to a viral protein database created from the virus RefSeq database (taxid: 10239). This strategy provided consistent results independently of the BLAST algorithm (BLASTX or BLASTp) or database (nr or RefSeq) used in the analyses (Table S3). Only contigs matching the viral database were retained (e-value cutoff $1e^{-5}$). False positives were then filtered out by comparing the candidate viral dataset with the total protein database (nr) from the NCBI using BLASTX, and removing contigs showing best hits to non-viral sequences. Taxonomic information for retained contigs larger than 200 bp was retrieved with taxdb. Contig depth was estimated with the depth command from Samtools v1.3.1 [75] by mapping the trimmed reads onto the virome with Bowtie 2 v2.3.1 [76], and used as a proxy for abundance. The taxonomic and sequencing depth data were used as inputs for bubble plots drawn with the ggplot2 package [77] in R. Contigs that had a sequencing depth larger than 8 X, were longer than 500 bp and showed best BLASTX hits on eukaryotic viruses were chosen as candidates

for further investigation. Coding sequence prediction and annotation were performed with the prokaryotic genome annotator Prokka v1.12 [78] using the command options *--kingdom* (viruses) and *--metagenome*. To validate annotations with protein-conserved domains, predicted coding sequences were submitted to NCBI's conserved domain database (CDD) with the web application Batch CD-Search (www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi; e-value cutoff $1e^{-3}$).

Phylogenetic inferences

The predicted coding sequences of six candidate viruses (all candidate viruses, except MqC, which does not have predicted protein) were used to search for similar viral sequences in GenBank (e-value cutoff $1e^{-5}$), avoiding species redundancy. These sequences, plus relevant reference sequences (see Table S4), were used for phylogenetic inferences. Sequences were aligned using MAFFT as implemented in Geneious R11 [79], removing columns with more than 60 % gaps. For each alignment, the best-fitted model of amino acid substitution was selected using ProtTest v3.4.2 [80] under corrected Akaike information criteria (AIC). Maximum-likelihood phylogenies with 100 bootstrap resamples of the alignment datasets were generated with RaxML v8.2.9 [81] and then edited and visualized with FigTree v.1.4.2 [82].

Nucleic acid extraction for PCR and RT-PCR

For PCR-based detection of viruses, the extraction of DNA, RNA or simultaneous DNA/RNA from individual bees was performed with the DNeasy Blood and Tissue kit (Qiagen, Germany), TRIzol (Ambion, USA) and the QIAamp Cador Pathogen Mini kit (Qiagen, Germany), respectively, according to the manufacturers' protocols. The nucleic acid extractions tested for the presence of RNA had been treated previously with TURBO DNase (Thermo Fisher Scientific, USA) and were then used for cDNA synthesis with the High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, USA).

Virus detection by PCR and RT-PCR

Primers for virus detection were designed based on the seven candidate viral contig sequences (Table 1). An aliquot of 100–300 ng of nucleic acids purified from each of the tested samples was used as a template for PCR or RT-PCR, according to the sample type, mixed with 1 U of Platinum *Taq* DNA polymerase (Invitrogen, USA), 1× PCR buffer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.2 μM of each forward and reverse primer in a final reaction volume of 50 μl . For MqV1a, the temperature cycling protocol used was 94 °C for 5 min, 60 °C for 1 min and 72 °C for 2 min, followed by 20 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, with a touchdown of 0.5 °C per cycle, and another 20 cycles of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 2 min, and a final extension step of 72 °C for 10 min. For the remaining six viruses a similar touchdown procedure was used, with primer annealing temperatures

starting at 54 °C and ending at 44 °C. The amplicons were analysed by conventional agarose gel electrophoresis. To test for the association of virus detection in individual bees with the manifestation of disease symptoms we conducted CMH test with Compare2 v.3.85 from WinPep [83] and two-way Barnard's unconditional tests with the Barnard package [84] in R.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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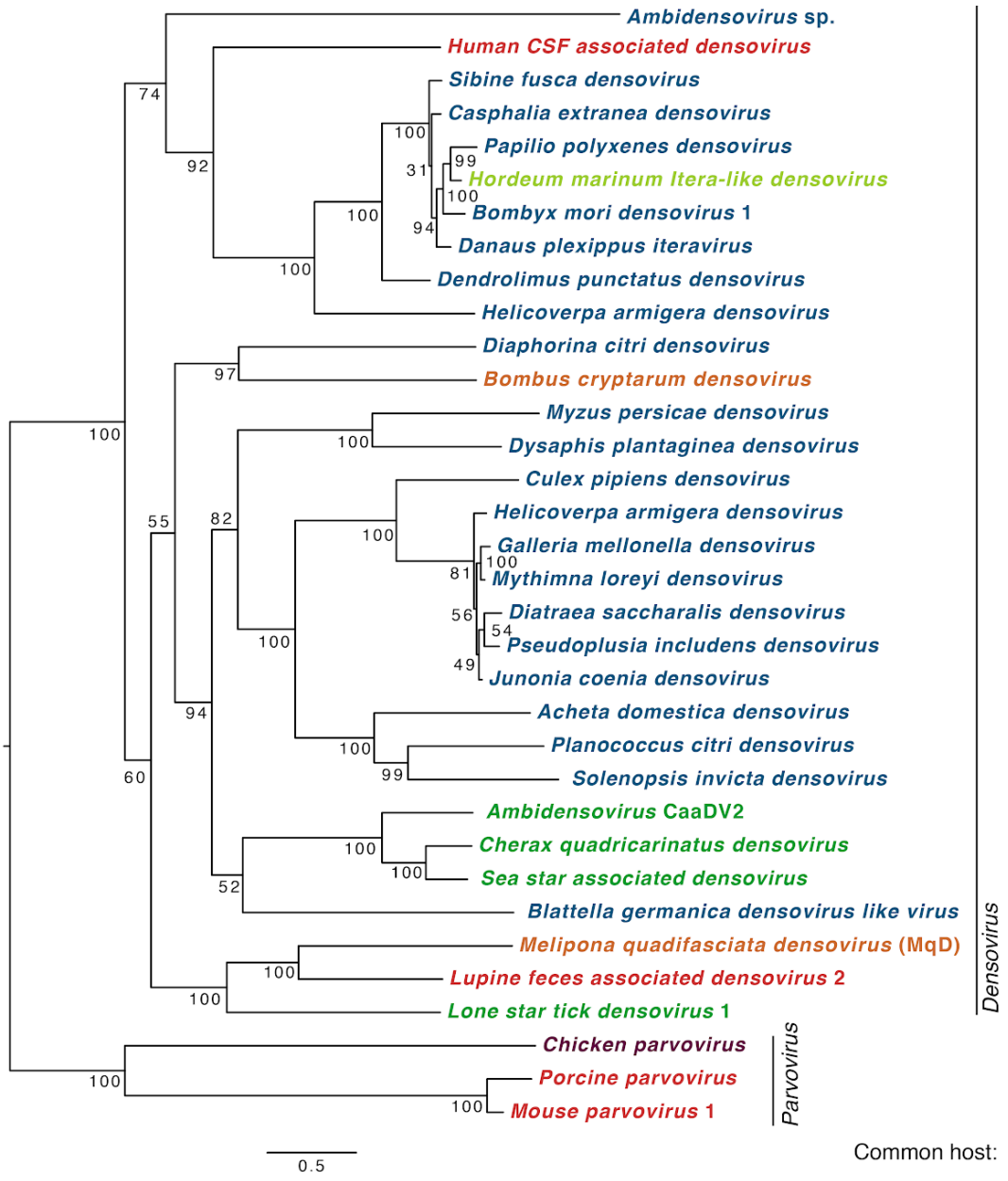
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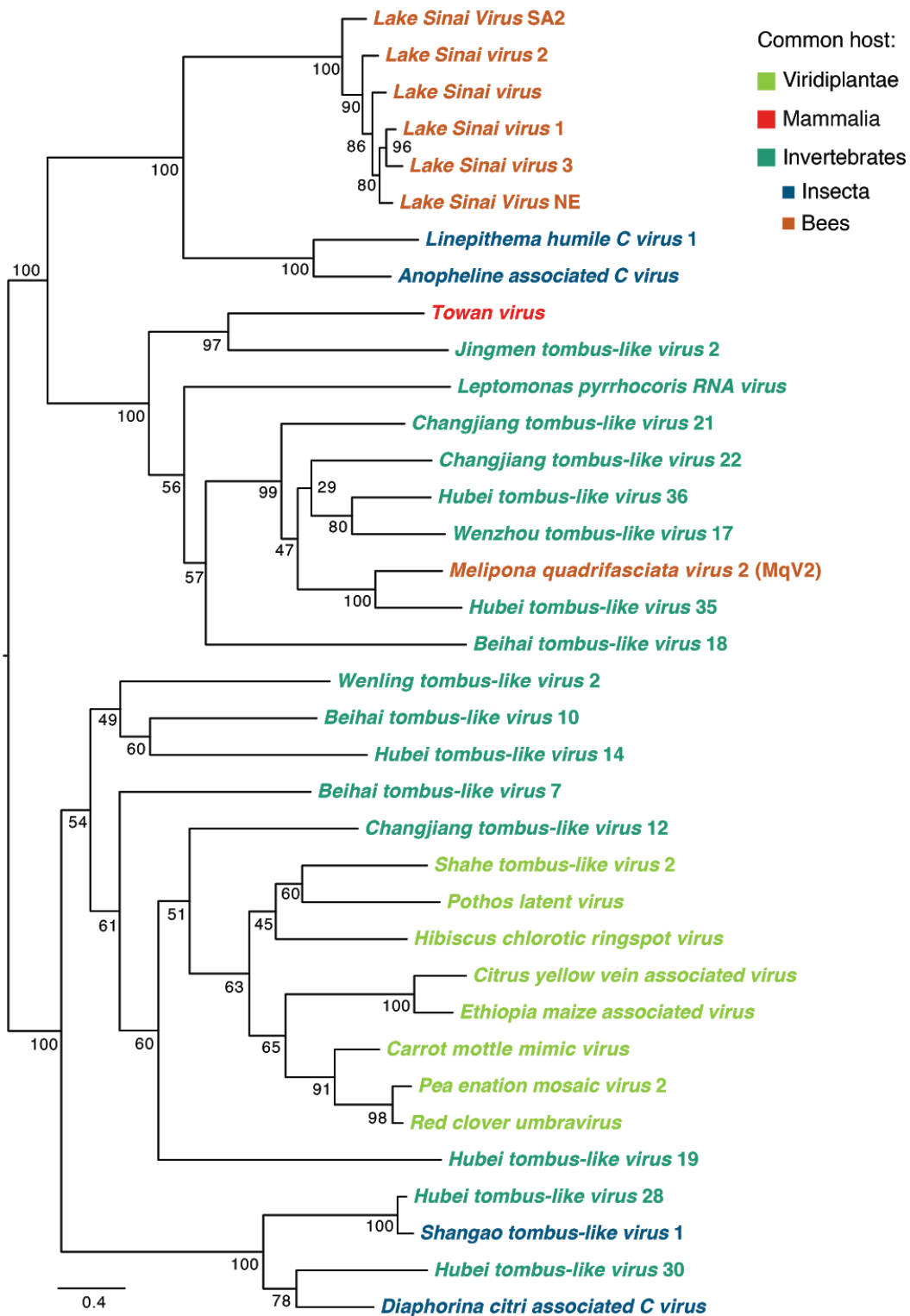
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Supplementary figure 1 - Phylogenetic inference of MqD virus (*Parvoviridae*). The maximum-likelihood tree was inferred with 100 bootstrap resamples based on an alignment of 1,310 aa. Species name colours represent the virus common host.

- Common host:
- Viridiplantae
 - Aves
 - Mammalia
 - Invertebrates
 - Insecta
 - Bees



Supplementary figure 2 - Phylogenetic inference of MqV2 virus. The maximum-likelihood tree was inferred with 100 bootstrap resamples based on an alignment of 598 aa. Species name colours represent the virus common host.

Supplementary Table 1 - Virome of unhealthy and healthy *M. quadrifasciata*: taxonomic classification of the best hit of each contig exceeding 200 bp in the four metagenomes (UDNA = DNA from unhealthy bees, HDNA = DNA from healthy bees, URNA = RNA from unhealthy bees, HRNA = RNA from healthy bees). Contigs are ordered according their coverages. Shading color indicates two major host groups bacteria (green) and eukaryotes (purple), highlighted in red are the contigs that were chosen to be investigated for their relation with the annual syndrome.

Name	Query (Contig name)	Length	Mean coverage	Subject	Bitscore	E-value	Staxids	Best hit species	Common host	Common host classification	Viral Order	Viral Family	Virus Molecule type
		Unhealthy bees DNA virome											
	NODE 1371 length 261 cov 5101.58	261	5937.31	gi 66395188 ref YP_239478.1	48.9	1.77E-07	320832	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 3218 length 202 cov 2485.69	202	4616.9	gi 658607919 ref YP_009044904.1	103	2.02E-28	1262519	<i>Leuconostoc phage phiLN34</i>	<i>Leuconostoc mesenteroides</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
	NODE 67 length 1399 cov 2196.09	1399	4396.57	gi 070100089 ref YP_009283993.1	179	1.45E-50	1543206	<i>Staphylococcus aureus</i> BP39	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 142 length 837 cov 2004.61	837	3448.91	gi 966201261 ref YP_009191338.1	112	4.28E-27	1747828	<i>Enterococcus phage vIME195</i>	<i>Enterococcus faecium</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 1097 length 523 cov 1537.21	523	2963.47	gi 107099421 ref YP_009278517.1	159	1.24E-38	182328	<i>Staphylococcus phage SLPV</i>	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 504 length 370 cov 1301.35	370	2793.61	gi 310702515 ref NP_852013.1	109	4.16E-28	230871	<i>Strepococcus phage CI</i>	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 257 length 556 cov 1608.69	556	2771.15	gi 29565740 ref NP_817314.1	180	8.89E-55	204086	<i>Staphylococcus phage 4AHLJD</i>	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 38 length 2005 cov 1102.3	2005	2141.64	gi 66395192 ref YP_239479.1	141	1.52E-35	320832	<i>Staphylococcus phage 66</i>	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 59 length 1601 cov 1305.56	1601	1969.64	gi 71291622 ref YP_009103964.1	55.8	2.64E-07	1161939	<i>Enterococcus phage vB_Efae230P-4</i>	<i>Enterococcus faecium</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 390 length 422 cov 1051.65	422	1902.38	gi 71291621 ref YP_009103960.1	101	6.56E-26	1161939	<i>Enterococcus phage vB_Efae230P-4</i>	<i>Enterococcus faecium</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 2661 length 219 cov 9609.49	219	1055.29	gi 327197601 ref YP_004301292.1	47.4	3.47E-07	764561	<i>Brochothrix phage NF5</i>	<i>Brochothrix thermosphacta</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
	NODE 1039 length 277 cov 6070.11	277	798.448	gi 327197601 ref YP_004301292.1	80.1	2.31E-18	764561	<i>Brochothrix phage NF5</i>	<i>Brochothrix thermosphacta</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
MaD	NODE 6 length 4214 cov 47.7802	4214	104.516	gi 56698740 ref YP_164339.1	173	2.00E-43	292208	<i>Dendrolimus punctatus densovirus</i>	<i>Dendrolimus punctatus</i>	Arthropoda; Metazoa; Eukaryota	unclassified	Parvoviridae	ssDNA
MaC2	NODE 181 length 673 cov 27.6828	673	59.263	gi 663809940 ref YP_009047065.1	260	7.66E-87	1348500	<i>Cyclovirus FN</i>	<i>Homo sapiens</i>	Chordata; Metazoa; Eukaryota	unclassified	Circoviridae	ssDNA
	NODE 1 length 14405 cov 25.8403	14405	55.6807	gi 151266296 ref YP_001333659.1	206	9.52E-55	338473	<i>Actinomyces phage Av-1</i>	<i>Actinomyces naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 482 length 379 cov 9.5957	379	19.7308	gi 007840595 ref YP_00021845.1	64.3	2.14E-12	1234882	<i>Dragonfly cyclovirus A3</i>	<i>Stomatochlora meridionalis</i>	Arthropoda; Metazoa; Eukaryota	unclassified	Circoviridae	ssDNA
	NODE 47 length 1772 cov 19.8427	1772	38.7472	gi 658310065 ref YP_009042257.1	227	2.90E-64	1327934	<i>Cronobacter phage CR8</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 12 length 1290 cov 15.8786	1290	32.3681	gi 966203645 ref YP_009188960.1	125	9.81E-31	1684115	<i>Cronobacter phage PBES 02</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 75 length 1310 cov 14.4797	1310	32.313	gi 363539710 ref YP_004893995.1	77	4.94E-17	889338	<i>Salmonella phage PVP-SE1</i>	<i>Salmonella enterica subsp. enterica serovar Enteritidis</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 23 length 2531 cov 15.0521	2531	31.1972	gi 593777390 ref YP_009015235.1	253	3.61E-73	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 16 length 2705 cov 15.2894	2705	28.9919	gi 730985156 ref YP_009111241.1	164	9.69E-45	1527519	<i>Escherichia phage As-05</i>	<i>Escherichia coli O157:H7</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 208 length 612 cov 13.7487	612	27.1373	gi 410492669 ref YP_006908871.1	48.9	2.55E-06	1604355	<i>Escherichia phage ECBP2</i>	<i>Escherichia coli</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 33 length 2212 cov 12.5535	2212	25.9118	gi 966203781 ref YP_009188906.1	60.5	6.08E-11	1684115	<i>Cronobacter phage PBES 02</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 27 length 2374 cov 11.8995	2374	22.9356	gi 363539688 ref YP_004893973.1	183	1.16E-50	889338	<i>Salmonella phage PVP-SE1</i>	<i>Salmonella enterica subsp. enterica serovar Enteritidis</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 19 length 2673 cov 11.95	2673	22.7565	gi 658310257 ref YP_009042449.1	374	2.27E-123	1327934	<i>Cronobacter phage CR8</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 7 length 3822 cov 11.8471	3822	22.5903	gi 658310123 ref YP_009042315.1	394	2.74E-121	1327934	<i>Cronobacter phage CR8</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 14 length 2710 cov 9.11455	2710	21.4587	gi 389059870 ref YP_006383023.1	410	2.03E-126	1162295	<i>Cronobacter phage CR3</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 91 length 1174 cov 9.15155	1174	19.7308	gi 389059880 ref YP_006383033.1	195	1.22E-60	1162295	<i>Cronobacter phage CR3</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 95 length 1355 cov 9.35	1355	18.3885	gi 593777139 ref YP_009014984.1	296	5.56E-98	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 36 length 2088 cov 9.15199	2088	17.8252	gi 389059955 ref YP_006383108.1	95.1	4.75E-22	1162295	<i>Cronobacter phage CR3</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 70 length 1325 cov 7.26457	1325	17.6581	gi 388570790 ref YP_006383773.1	51.2	6.96E-08	1187128	<i>Acinetobacter bacteriophage AP22</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 9 length 2927 cov 9.01358	2927	17.6194	gi 658310137 ref YP_009042329.1	208	2.21E-61	1327934	<i>Cronobacter phage CR8</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 110 length 998 cov 6.41569	998	17.5882	gi 682123183 ref YP_009055504.1	254	5.19E-81	1505225	<i>Acinetobacter phage YMC-13-01-C62</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 76 length 1310 cov 9.00717	1310	17.3725	gi 389060084 ref YP_006383237.1	207	3.80E-64	1162295	<i>Cronobacter phage CR3</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 34 length 2102 cov 8.80606	2102	17.0324	gi 593777352 ref YP_009015197.1	410	3.75E-138	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 44 length 1847 cov 8.88895	1847	16.7823	gi 593777363 ref YP_009015208.1	368	2.75E-122	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 3 length 5385 cov 8.33527	5385	16.5744	gi 448244744 ref YP_007392644.1	147	5.62E-37	1116482	<i>Pectobacterium phage phiTE</i>	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 86 length 1202 cov 8.1578	1202	16.3378	gi 389059887 ref YP_006383040.1	238	3.03E-77	1162295	<i>Cronobacter phage CR3</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 5 length 4317 cov 8.25059	4317	16.0644	gi 593777283 ref YP_009015128.1	88.6	1.29E-19	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 8 length 3348 cov 7.53325	3348	15.425	gi 563397615 ref YP_008857379.1	202	8.18E-57	1391428	<i>Enterobacteria phage 4MG</i>	<i>Escherichia coli K-12</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 24 length 2521 cov 8.03406	2521	15.386	gi 448246249 ref YP_007348345.1	53.9	5.33E-08	1264737	<i>Cronobacter phage vB_Cskp_GAP227</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 132 length 891 cov 6.60646	891	15.0236	gi 593777268 ref YP_009015113.1	124	3.89E-35	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 98 length 1104 cov 7.55005	1104	15.4516	gi 563397555 ref YP_008857319.1	158	7.26E-45	1391428	<i>Enterobacteria phage 4MG</i>	<i>Escherichia coli K-12</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 607 length 347 cov 7.86301	347	14.5504	gi 593777353 ref YP_009015198.1	61.2	3.75E-13	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 22 length 2568 cov 7.01074	2568	14.1347	gi 966203769 ref YP_009189084.1	68.9	2.43E-13	1684115	<i>Cronobacter phage PBES 02</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 488 length 376 cov 8.482866	376	14.0133	gi 388570783 ref YP_006383766.1	201	1.51E-63	1187128	<i>Acinetobacter bacteriophage AP22</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 163 length 751 cov 8.03679	751	13.7617	gi 563397604 ref YP_008857362.1	63.5	2.51E-12	1391428	<i>Enterobacteria phage 4MG</i>	<i>Escherichia coli K-12</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 1590 length 252 cov 9.17766	252	13.6667	gi 966203376 ref YP_009187688.1	134	3.27E-38	1791940	<i>Klebsiella phage vB_KpnM_KB57</i>	<i>Klebsiella pneumoniae</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 236 length 578 cov 7.26195	578	13.4931	gi 363539705 ref YP_004893990.1	49.7	9.71E-08	889338	<i>Salmonella phage PVP-SE1</i>	<i>Salmonella enterica subsp. enterica serovar Enteritidis</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 79 length 1265 cov 6.71157	1265	13.4324	gi 401723087 ref YP_006589997.1	84	3.02E-16	1136535	<i>Burkholderia phage DC1</i>	<i>Burkholderia cepacia</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
	NODE 28 length 2286 cov 6.76109	2286	13.0009	gi 563397519 ref YP_008857283.1	124	3.25E-28	1391428	<i>Enterobacteria phage 4MG</i>	<i>Escherichia coli K-12</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 315 length 496 cov 5.16327	496	12.8306	gi 448244797 ref YP_007392517.1	56.6	4.07E-09	1116482	<i>Pectobacterium phage phiTE</i>	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 2 length 1213 cov 6.22714	1213	12.597	gi 389059922 ref YP_009042676.1	52.8	4.31E-163	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 21 length 2596 cov 5.8717	2596	12.3617	gi 448244800 ref YP_007392520.1	51.4	2.06E-175	1116482	<i>Pectobacterium phage phiTE</i>	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 652 length 334 cov 5.73835	334	12.1048	gi 593777273 ref YP_009015118.1	63.9	8.15E-14	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 747 length 315 cov 7.04231	315	11.8286	gi 593777390 ref YP_009015235.1	86.7	1.85E-20	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 26 length 2424 cov 5.68763	2424	11.1993	gi 966203664 ref YP_009188979.1	84.3	3.17E-16	1684115	<i>Cronobacter phage PBES 02</i>	<i>Cronobacter sp.</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
	NODE 54 length 1661 cov 6.05168	1661	11.0319	gi 563397558 ref YP_008857322.1	155	3.51E-44	1391428	<i>Enterobacteria phage 4MG</i>	<i>Escherichia coli K-12</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses

NODE 415 length 413 cov 2.89106	413	5.54479	gii448244935 ref YP_007392655.1	126	1.16E-35	1116482	<i>Pectobacterium phase phiTE</i>	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1907 length 241 cov 3.22043	241	5.53112	gi 070106045 ref YP_009291896.1	71.2	1.18E-15	1792222	<i>Acinetobacter phase LZ35</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 703 length 323 cov 2.89179	323	5.45511	gi 917452437 ref YP_009199435.1	51.2	2.41E-08	1786606	<i>Escherichia phase phiL50SP1</i>	<i>Escherichia coli</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 530 length 365 cov 2.57742	365	5.44411	gi 157525617 ref YP_001468443.1	47.8	4.51E-07	40522	<i>Listeria phase Δ500</i>	<i>Listeria monocytogenes WSLC1042</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1759 length 246 cov 3.24607	246	5.26016	gi 414086874 ref YP_006987062.1	85.5	1.20E-20	1141135	<i>Cronobacter phase vB_CsaM_GAP31</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 514 length 368 cov 2.86262	368	5.18207	gi 327198375 ref YP_004306949.1	62	1.56E-11	59241	<i>Streptococcus phase Dp-1</i>	<i>Streptococcus pneumoniae</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1513 length 255 cov 3.845	255	5.14118	gi 448244960 ref YP_007392680.1	144	5.78E-45	1116482	<i>Pectobacterium phase phiTE</i>	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 301 length 514 cov 3.05229	514	5.11673	gi 389060077 ref YP_006383230.1	273	7.32E-92	1162295	<i>Cronobacter phase CR3</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1492 length 256 cov 2.34328	256	5.00781	gi 571798161 ref YP_008770540.1	80.5	1.31E-19	1406786	<i>Bacillus phase Page</i>	<i>Bacillus megaterium</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 1516 length 255 cov 2.6	255	5.00392	gi 514361130 ref YP_008130364.1	46.6	1.03E-06	1345637	<i>Human cyclovirus V55700009</i>	<i>Homio sapiens</i>	Chordata; Metazoa; Eukaryota	unclassified	Circoviridae	ssDNA
NODE 2341 length 227 cov 3.35465	227	5	gi 593777119 ref YP_009014964.1	129	1.49E-36	1162290	<i>Cronobacter phase CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 2208 length 231 cov 2.15909	231	4.98701	gi 682123139 ref YP_009055460.1	90.9	6.03E-24	1505225	<i>Acinetobacter phase YMC-13-01-C62</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 431 length 404 cov 1.60458	404	4.82673	gi 472340490 ref YP_007674015.1	142	4.55E-40	754049	<i>Vibrio phase vYD21-A</i>	<i>Vibrio sp. YD21</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1471 length 257 cov 2.9505	257	4.79377	gi 448244955 ref YP_007392675.1	124	2.27E-35	1116482	<i>Pectobacterium phase phiTE</i>	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 400 length 418 cov 2.00826	418	4.73684	gi 070106029 ref YP_009291880.1	66.2	7.89E-13	1792222	<i>Acinetobacter phase LZ35</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 608 length 347 cov 2.82534	347	4.69388	gi 939556927 ref YP_009168411.1	127	3.44E-36	1622234	<i>Citrobacter phase CIT22</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 1453 length 258 cov 2.12315	258	4.65504	gi 937456469 ref YP_009168394.1	123	1.24E-33	1622234	<i>Citrobacter phase CIT22</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 278 length 534 cov 2.40292	534	4.64794	gi 937456502 ref YP_009168428.1	54.7	5.62E-09	1622234	<i>Citrobacter phase CIT22</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 542 length 361 cov 2.5817	361	4.46814	gi 9626704 ref NP_040974.1	128	3.95E-37	11673	<i>Feline immunodeficiency virus</i>	<i>Felidae</i>	Chordata; Metazoa; Eukaryota	unclassified	Retroviridae	ssRNA RT
NODE 664 length 332 cov 2.11913	332	4.46386	gi 593777132 ref YP_009014977.1	132	3.70E-37	1162290	<i>Cronobacter phase CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1337 length 263 cov 3.02404	263	4.34221	gi 62327110 ref YP_022898.1	137	2.02E-38	235201	<i>Lactobacillus phase phiJL-1</i>	<i>Lactobacillus plantarum</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 249 length 562 cov 2.46351	562	4.30071	gi 930074400 ref YP_579204.1	123	1.30E-32	370623	<i>Streptomyces phase mu/1-6</i>	<i>Kitasatospora aureofaciens</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1142 length 272 cov 3.20737	272	4.28676	gi 388570790 ref YP_006383773.1	42	7.15E-06	1187128	<i>Acinetobacter bacteriophage AP22</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 374 length 433 cov 2.1746	433	4.27252	gi 658310265 ref YP_009042457.1	211	1.30E-64	1327934	<i>Cronobacter phase CR8</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 366 length 441 cov 2.41451	441	4.26757	gi 1068511077 ref YP_009274031.1	44.3	9.87E-06	1838072	<i>Gordonia phase McGonagall</i>	<i>Gordonia neofelaei</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 466 length 386 cov 1.84229	386	4.24611	gi 658310061 ref YP_009042253.1	152	1.47E-44	1327934	<i>Cronobacter phase CR8</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 611 length 345 cov 2.55172	345	4.23188	gi 448244889 ref YP_007392618.1	153	5.62E-47	1116482	<i>Pectobacterium phase phiTE</i>	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 412 length 415 cov 1.42222	415	4.15181	gi 971763872 ref YP_009216825.1	125	3.77E-36	1299760	<i>Acinetobacter phase phiAC-1</i>	<i>Acinetobacter soli</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 654 length 334 cov 2.48746	334	4.13772	gi 418489115 ref YP_007002969.1	140	1.64E-40	37105	<i>Lactobacillus phase JCL1032</i>	<i>Lactobacillus delbrueckii subsp. lactis</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 245 length 564 cov 2.41625	564	4.13121	gi 151266305 ref YP_001333668.1	112	3.90E-29	338473	<i>Actinomyces phase Av-1</i>	<i>Actinomyces naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 371 length 434 cov 2.53298	434	4.1129	gi 971748496 ref YP_009202413.1	62	3.23E-11	1698711	<i>Mycobacterium phase Loh9</i>	<i>Mycobacterium smegmatis str. MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 3036 length 210 cov 2.52903	210	3.9619	gi 151266296 ref YP_001333659.1	57.4	8.65E-11	338473	<i>Actinomyces phase Av-1</i>	<i>Actinomyces naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 874 length 295 cov 2.9125	295	3.95254	gi 971747642 ref YP_009201507.1	57.4	3.43E-10	1739609	<i>Lactobacillus phase Ia2</i>	<i>Lactobacillus paracasei</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 971 length 281 cov 2.73894	281	3.92171	gi 985757728 ref YP_009223104.1	128	6.05E-36	1567453	<i>Lactobacillus phase lifeSu</i>	<i>Lactobacillus fermentum</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 261 length 553 cov 2.07229	553	3.91139	gi 966203686 ref YP_009189001.1	86.7	2.90E-19	1684115	<i>Cronobacter phase PBES 02</i>	<i>Cronobacter sp.</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 3162 length 207 cov 2.63816	207	3.89855	gi 448244802 ref YP_007392522.1	88.6	8.57E-22	1116482	<i>Pectobacterium phase phiTE</i>	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 2491 length 223 cov 2.02381	223	3.88341	gi 070067156 ref YP_009289259.1	42.7	8.33E-06	1868596	<i>Pseudomonas phase MD8</i>	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 230 length 582 cov 2.26755	582	3.86254	gi 593777381 ref YP_009015226.1	325	8.25E-111	1162290	<i>Cronobacter phase CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 547 length 359 cov 2.11118	359	3.7883	gi 682123168 ref YP_009055489.1	115	2.51E-31	1505225	<i>Acinetobacter phase YMC-13-01-C62</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 314 length 388 cov 2.59077	388	3.78865	gi 102617795 ref YP_009321630.1	115	3.29E-06	1566993	<i>Artemis phase phiARI0746</i>	<i>Streptococcus pneumoniae</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 192 length 651 cov 2.01174	651	3.76651	gi 593777136 ref YP_009014981.1	191	1.65E-55	1162290	<i>Cronobacter phase CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 762 length 312 cov 2.31128	312	3.76603	gi 658310132 ref YP_009042324.1	147	3.21E-43	1327934	<i>Cronobacter phase CR8</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 302 length 514 cov 1.49455	514	3.76265	gi 971746169 ref YP_009200249.1	142	1.55E-39	1636200	<i>Pseudalteromonas phase HI03</i>	<i>Pseudalteromonas marina</i>	Proteobacteria; Bacteria	Caudovirales	unclassified	dsDNA viruses
NODE 701 length 324 cov 2.21933	324	3.75	gi 658310273 ref YP_009042465.1	175	1.13E-54	1327934	<i>Cronobacter phase CR8</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1004 length 279 cov 2.26786	279	3.65591	gi 937456472 ref YP_009168397.1	102	3.38E-26	1622234	<i>Citrobacter phase CIT22</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 1145 length 272 cov 2.17051	272	3.63971	gi 318069475 ref YP_004152328.1	66.2	5.33E-14	942033	<i>Cyclovirus PKoa21/1/PAK/2009</i>	<i>Capra hircus</i>	Chordata; Metazoa; Eukaryota	unclassified	Circoviridae	ssDNA
NODE 2826 length 215 cov 2.78125	215	3.60465	gi 971752673 ref YP_009206702.1	90.9	3.72E-23	1739607	<i>Lactobacillus phase CL1</i>	<i>Lactobacillus paracasei</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 2832 length 215 cov 1.99375	215	3.56279	gi 149408208 ref YP_001129464.1	87.4	2.51E-21	347327	<i>Pseudomonas phase PA11</i>	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	unclassified	dsDNA viruses
NODE 620 length 342 cov 2.31359	342	3.54971	gi 418489114 ref YP_007002968.1	179	4.48E-54	37105	<i>Lactobacillus phase JCL1032</i>	<i>Lactobacillus delbrueckii subsp. lactis</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 550 length 358 cov 1.72277	358	3.54749	gi 095666753 ref NP_818317.1	82.8	6.19E-19	205879	<i>Mycobacterium phase Vm3</i>	<i>Mycobacterium sp.</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 204 length 622 cov 2.00829	622	3.54502	gi 070106067 ref YP_009291948.1	355	3.14E-122	1792222	<i>Acinetobacter phase LZ35</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 645 length 336 cov 1.70107	336	3.54167	gi 764161525 ref YP_009126057.1	57.8	3.29E-09	1566993	<i>Artemis phase phiARI-7</i>	<i>Streptococcus pneumoniae</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 643 length 336 cov 1.88612	336	3.54167	gi 571797869 ref YP_008772035.1	145	3.88E-42	1399941	<i>Lactobacillus phase phiB</i>	<i>Lactobacillus delbrueckii</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 838 length 300 cov 2.43265	300	3.53667	gi 971751675 ref YP_009205624.1	97.1	3.92E-24	1175662	<i>Pseudomonas phase PAMs42</i>	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 440 length 400 cov 2.02319	400	3.5325	gi 203454733 ref YP_00225049.1	62.4	1.52E-11	546805	<i>Mycobacterium phase Myrna</i>	<i>Mycobacterium smegmatis str. MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 2919 length 213 cov 2.67089	213	3.53052	gi 937456472 ref YP_009168397.1	63.5	7.39E-13	1622234	<i>Citrobacter phase CIT22</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 2115 length 234 cov 1.97027	234	3.52991	gi 658310162 ref YP_009042354.1	111	9.13E-30	1327934	<i>Cronobacter phase CR8</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 2827 length 215 cov 2.475	215	3.52558	gi 160700666 ref YP_001552346.1	48.9	8.60E-08	480808	<i>Mycobacterium phase Giles</i>	<i>Mycobacterium smegmatis</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1205 length 269 cov 1.70561	269	3.51301	gi 509142291 ref YP_008061153.1	98.2	5.99E-25	1007869	<i>Rhodococcus phase E3</i>	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 307 length 511 cov 1.53289	511	3.45205	gi 410491660 ref YP_006906882.1	58.2	1.22E-09	1150869	<i>Escherichia phase P13374</i>	<i>Escherichia coli O104:H4 str. CB13374</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 2957 length 212 cov 1.84713	212	3.43868	gi 530545073 ref YP_008408677.1	67	3.86E-14	1340826	<i>Mycobacterium phase Whirlwind</i>	<i>Mycobacterium sp.</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 441 length 400 cov 1.93623	400	3.41175	gi 682123168 ref YP_009055489.1	11								

NODE 317 length 496 cov 1.39229	496	2.69758	gi 203460615 ref YP_002224349.1	98.6	1.32E-24	546808	<i>Mycobacterium phage Spud</i>	<i>Mycobacterium smegmatis</i> str. <i>MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 502 length 371 cov 1.63924	371	2.69003	gi 514051708 ref YP_008126134.1	46.6	4.52E-06	1262532	<i>Mycobacterium phage BTU-1</i>	<i>Mycobacterium smegmatis</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1019 length 278 cov 1.86547	278	2.68345	gi 59377132 ref YP_009014977.1	132	1.84E-37	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 2238 length 230 cov 2.06571	230	2.66957	gi 68693201 ref YP_009058944.1	73.2	3.46E-16	1543419	<i>Torque teno Tadarida brasiliensis virus</i>	<i>Tadarida brasiliensis</i>	Chordata; Metazoa; Eukaryota	unclassified	unclassified	ssDNA
NODE 284 length 531 cov 1.58193	531	2.60264	gi 388570812 ref YP_006383795.1	95.1	5.86E-24	181728	<i>Actinobacter bacteriophage AP22</i>	<i>Actinobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 812 length 304 cov 1.62499	304	2.59539	gi 151266296 ref YP_001333659.1	110	5.97E-29	338473	<i>Actinomyces phage Av-1</i>	<i>Actinomyces naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 2350 length 227 cov 1.51163	227	2.57269	gi 917571755 ref YP_009210688.1	44.7	3.84E-06	1701853	<i>Mycobacterium phage Serenity</i>	<i>Mycobacterium smegmatis</i> str. <i>MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 418 length 411 cov 1.70506	411	2.54745	gi 372449476 ref YP_005086792.1	58.9	2.96E-10	1109716	<i>Rhodococcus phage RGL3</i>	<i>Rhodococcus globularius</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 358 length 449 cov 1.63959	449	2.53229	gi 91767399 ref YP_009216797.1	164	2.39E-48	1229760	<i>Acinetobacter phage phiAC-1</i>	<i>Acinetobacter soli</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1962 length 239 cov 2.07065	239	2.51464	gi 985757333 ref YP_009222296.1	50.8	2.63E-08	1567484	<i>Lactobacillus phage LjefnJ</i>	<i>Lactobacillus fermentum</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 2788 length 216 cov 1.97516	216	2.49074	gi 1070103328 ref YP_009287971.1	83.6	6.68E-20	1891703	<i>Mycobacterium phage Tonenili</i>	<i>Mycobacterium smegmatis</i> str. <i>MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1730 length 247 cov 1.94792	247	2.44534	gi 371496158 ref YP_004957306.1	58.5	4.18E-11	994601	<i>Plankothrix phage PaV-LD</i>	<i>Plankothrix aqardhii HAB637</i>	Cyanobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 3088 length 209 cov 1.35065	209	2.43062	gi 589895959 ref YP_009004942.1	48.9	9.62E-08	1429768	<i>Erwinia phage Ea35-7</i>	<i>Erwinia amylovora</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 702 length 324 cov 1.64312	324	2.42901	gi 215401206 ref YP_002325211.1	162	6.65E-49	575608	<i>Staphylococcus phage phiSauS-IPLA88</i>	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 3169 length 207 cov 1.59211	207	2.40997	gi 682123182 ref YP_009055503.1	74.7	6.31E-17	1505225	<i>Acinetobacter phage YMC-13-01-C62</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 391 length 422 cov 2.06571	422	2.37915	gi 937456647 ref YP_009168397.1	156	2.14E-44	1622234	<i>Citrobacter phage CT22</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 2275 length 229 cov 1.68966	229	2.34934	gi 937456649 ref YP_009168394.1	115	6.40E-31	1622234	<i>Citrobacter phage CT122</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 1494 length 256 cov 1.83085	256	2.34766	gi 1070621526 ref YP_009302998.1	82.8	1.56E-19	1821562	<i>Gordonia phage SoilAssassin</i>	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 409 length 416 cov 1.45706	416	2.33654	gi 59377123 ref YP_009014968.1	176	1.01E-54	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1859 length 243 cov 1.83511	243	2.3251	gi 966203464 ref YP_009186755.1	133	5.86E-41	1719140	<i>Klebsiella phage vB_KpnM_KB57</i>	<i>Klebsiella pneumoniae</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 3043 length 210 cov 1.47742	210	2.30476	gi 937424920 ref YP_009165756.1	66.6	5.47E-14	1100043	<i>Apis mellifera filamentum virus</i>	<i>Apis mellifera</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	dsDNA
NODE 355 length 451 cov 1.2601	451	2.28603	gi 593771721 ref YP_009014966.1	103	3.39E-26	1162290	<i>Cronobacter phage CR9</i>	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1190 length 270 cov 1.7814	270	2.23333	gi 509142181 ref YP_008061043.1	110	4.10E-29	1007869	<i>Rhodococcus hoagii</i>	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 600 length 349 cov 1.10884	349	2.2235	gi 472340491 ref YP_007674016.1	52	3.46E-08	754049	<i>Vibrio phage vYD21-A</i>	<i>Vibrio sp. YD21</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1098 length 274 cov 2.02283	274	2.20438	gi 727142304 ref YP_009104703.1	86.3	1.47E-20	1527515	<i>Idiomarinaeace phage PhiM2-2</i>	<i>Idiomarinaeace bacterium M2-2</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1169 length 271 cov 1.73148	271	2.19188	gi 937456498 ref YP_009168424.1	61.6	7.37E-12	1622234	<i>Citrobacter phage CTY22</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 705 length 323 cov 1.21642	323	2.16718	gi 29566640 ref FNP_818206.1	78.6	1.31E-17	205787	<i>Mycobacterium phage Bx2</i>	<i>Mycobacterium sp.</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1006 length 279 cov 1.70982	279	2.16129	gi 1070103030 ref YP_009287678.1	64.7	5.52E-13	1838137	<i>Pseudomonas bacteriophage PFR1</i>	<i>Pseudomonas putida</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 881 length 295 cov 1.23333	295	2.14237	gi 106851191 ref YP_009274208.1	57	1.47E-11	1838071	<i>Gordonia phage Kvores</i>	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 980 length 281 cov 1.16372	281	2.14235	gi 326784531 ref YP_004324978.1	58.9	2.58E-11	445688	<i>Prochlorococcus phage P-SSM7</i>	<i>Prochlorococcus marinus</i> str. <i>NATL1A</i>	Cyanobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 2414 length 225 cov 1.51765	225	2.12444	gi 564292915 ref YP_008873609.1	52.8	7.26E-10	1007127	<i>Staphylococcus phage Sb-1</i>	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 954 length 284 cov 1.67249	284	2.12324	gi 593775732 ref YP_009013592.1	76.6	4.44E-17	1089121	<i>Mycobacterium phage Dori</i>	<i>Mycobacterium smegmatis</i> str. <i>MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1620 length 251 cov 1.58163	251	2.11155	gi 937456504 ref YP_009168430.1	114	1.72E-30	1622234	<i>Citrobacter phage CTY22</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 943 length 286 cov 1.40226	286	2.10839	gi 388684798 ref YP_006382678.1	65.5	3.70E-13	1161927	<i>Pseudomonas phage Lu11</i>	<i>Pseudomonas putida</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1460 length 258 cov 1.18227	258	2.10078	gi 589287688 ref YP_009010292.1	57.4	2.00E-10	1401669	<i>Erwinia phage PhiEai1</i>	<i>Erwinia amylovora</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 2243 length 230 cov 1.42286	230	2.1	gi 1070096285 ref YP_009277923.1	112	5.52E-31	1887651	<i>Gordonia phage Nycei1</i>	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 929 length 289 cov 0.884615	289	2.0692	gi 9626704 ref FNP_409474.1	61.2	5.57E-12	11673	<i>Feline immunodeficiency virus</i>	<i>Feline immunodeficiency virus</i>	Chordata; Metazoa; Eukaryota	unclassified	Retroviridae	ssRNA RT
NODE 1801 length 245 cov 1.49474	245	2.05714	gi 440876105 ref YP_006987800.1	49.7	8.18E-08	1141139	<i>Enterobacteria phage vB_EcoP_ACG-C91</i>	<i>Escherichia coli</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 2113 length 234 cov 2.27374	234	2.05128	gi 203454570 ref YP_002225066.1	79	2.56E-18	546805	<i>Mycobacterium phage Myria</i>	<i>Mycobacterium smegmatis</i> str. <i>MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 2056 length 247 cov 1.10426	247	2.05046	gi 106811846 ref YP_009275124.1	62.4	2.36E-12	1831554	<i>Gordonia phage E1toro</i>	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 846 length 299 cov 1.32377	299	2.01672	gi 939177065 ref YP_009172504.1	79.3	5.88E-18	1663280	<i>Ostreococcus lucimarinus virus 2</i>	<i>Ostreococcus lucimarinus'</i>	Chlorophyta; Viridiplantae; Eukaryota	unclassified	Phycodnaviridae	dsDNA
NODE 755 length 314 cov 1.40541	314	2.00637	gi 460042347 ref YP_007517785.1	68.2	5.74E-14	1283078	<i>Pelagibacter phage HTCC011P</i>	<i>Candidatus Pelagibacter ubique HTCC01062</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 1080 length 275 cov 1.37272	275	2	gi 1173244297 ref YP_009352305.1	60.1	2.32E-11	1220717	<i>Rhizobium phage RHEPh10</i>	<i>Rhizobium etli CFN 42</i>	Proteobacteria; Bacteria	Caudovirales	unclassified	unclassified
NODE 2211 length 231 cov 1.63636	231	1.96104	gi 937456465 ref YP_009168390.1	139	1.71E-41	1622234	<i>Citrobacter phage CTY22</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 1499 length 256 cov 1.22388	256	1.95312	gi 56693176 ref YP_164763.1	79.3	5.45E-19	298338	<i>Lactobacillus phage LP65</i>	<i>Lactobacillus plantarum</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 359 length 449 cov 1.02792	449	1.83964	gi 917748544 ref YP_009202464.1	47.4	3.88E-07	1698711	<i>Mycobacterium phage Loly9</i>	<i>Mycobacterium smegmatis</i> str. <i>MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 995 length 280 cov 1.25778	280	1.79643	gi 1070096446 ref YP_009278373.1	52	1.72E-08	1883372	<i>Erwinia phage vB_EamM_Ear/PhillipIV</i>	<i>Erwinia amylovora</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 2679 length 219 cov 1.16463	219	1.78539	gi 849250932 ref YP_009147766.1	85.9	1.16E-20	1560313	<i>Lactococcus phage WRP3</i>	<i>Lactococcus lactis</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1340 length 263 cov 1.36058	263	1.70342	gi 682123292 ref YP_009056237.1	48.9	1.73E-07	1529058	<i>Vibrio phage ICP2 2013 A Haiti</i>	<i>Vibrio cholerae</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 574 length 354 cov 1.08027	354	1.70056	gi 682123169 ref YP_009055490.1	68.2	9.78E-15	1505225	<i>Acinetobacter phage YMC-13-01-C62</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1480 length 257 cov 1.66931	257	1.6965	gi 1070064678 ref YP_009283092.1	74.3	1.77E-16	1815509	<i>Bacillus subtilis subsp. subtilis</i> str. <i>168</i>	<i>Bacillus subtilis subsp. subtilis</i> str. <i>168</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1736 length 247 cov 1.56625	247	1.68558	gi 1070064678 ref YP_009283092.1	75.1	5.03E-07	184477	<i>Pseudomonas phage NP1</i>	<i>Pseudomonas putida</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1152 length 272 cov 1.02765	272	1.62868	gi 481019400 ref YP_007877754.1	48.1	3.29E-07	356444	<i>Cyanophage P-RSM1</i>	<i>Prochlorococcus marinus</i> str. <i>MIT 9303</i>	Cyanobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1106 length 274 cov 0.940639	274	1.62774	gi 291349611 ref NP_803591.1	53.1	6.63E-09	196683	<i>Pseudomonas phage phiXZ</i>	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 493 length 374 cov 1.1348	374	1.6123	gi 514361054 ref YP_008130293.1	86.3	5.27E-20	1282995	<i>Serratia phage E1a</i>	<i>Serratia marcescens</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1370 length 262 cov 0.859903	262	1.60305	gi 526120194 ref YP_008320473.1	65.9	1.83E-13	1325731	<i>Flavobacterium phage pHX</i>	<i>Flavobacterium psychrophilum</i>	Bacteroidetes; Bacteria	unclassified	unclassified	dsDNA
NODE 481 length 381 cov 1.17791	381	1.5853	gi 363539900 ref YP_004894381.1	49.7	3.73E-07	1094892	<i>Megavirus chilensis</i>	<i>Acanthamoeba</i>	Longamoebia; Eukaryota	unclassified	Mimiviridae	dsDNA
NODE 1128 length 273 cov 0.958716	273	1.57143	gi 509142296 ref YP_008061158.1	89	1.89E-21	1007869	<i>Rhodococcus hoagii</i>	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 727 length 320 cov 0.977358	320	1.49687	gi 917753194 ref YP_009207149.1	98.2	2.13E-24	1690425	<i>Streptomyces phase Caliburn</i>	<i>Streptomyces eriseus</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 822 length 303 cov 1.39516	303	1.49175	gi 509142319 ref YP_008061181.1	102	3.41E-26	1007869	<i>Rhodococcus hoagii</i>	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 3176 length 207 cov 1.26316	207	1.45894	gi 151266296 ref YP_001333659.1	51.6	8.90E-09	338473	<i>Actinomyces phage Av-1</i>	<i>Actinomyces naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 3203 length 207 cov 1.19079	207	1.45894	gi 34610177 ref NP_919002.1	90.5	2.18E-22	242861	<i>Burkholderia phage BcepNasZul</i>	<i>Burkholderia cepacia</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 774 length 311 cov 1.07422	311	1.45659	gi 937456473 ref YP_009168398.1	102	3.42E-26	1622234	<i>Citrobacter phage CTY22</i>	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 334 length 208 cov 1.54922	208	1.45492	gi 571019892 ref YP_007446879.1	49.1	3.88E-19	1007869	<i>Caulophages P1201</i>	<i>Caulobacter glutamicum</i>	Alphaproteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 3212 length 207 cov 0.842105	207	1.44928	gi 327198317 ref YP_004306893.1	43.1	8.29E-06	59241	<i>Streptococcus phage Dp-1</i>	<i>Stre</i>				

NODE 1554 length 254 cov 0.959799	254	1.18504	gi 327198390 ref YP_004326978.1	44.7	9.45E-07	925986	<i>Erwinia phage phiEal04</i>	<i>Erwinia amylovora</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1528 length 255 cov 0.96	255	1.18431	gi 5669317 ref YP_164758.1	81.3	6.88E-19	298338	<i>Lactobacillus phage LP65</i>	<i>Lactobacillus plantarum</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1537 length 251 cov 0.954082	251	1.18327	gi 1070643172 ref YP_009300729.1	62	2.20E-12	1796997	<i>Arthrobacter phage Mudecat</i>	<i>Arthrobacter</i> sp. ATCC 21022	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1509 length 256 cov 0.955224	256	1.17969	gi 509142181 ref YP_008061043.1	57	2.01E-10	1007869	<i>Rhodococcus phage E3</i>	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1503 length 256 cov 0.955224	256	1.17969	gi 56693153 ref YP_164740.1	57.8	1.01E-10	998338	<i>Lactobacillus phage WRP3</i>	<i>Lactobacillus plantarum</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1504 length 256 cov 0.950249	256	1.17578	gi 849250941 ref YP_009147775.1	55.8	4.83E-10	1560313	<i>Lactococcus phage WLP5</i>	<i>Lactococcus lactis</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1485 length 257 cov 0.950495	257	1.1751	gi 966198453 ref YP_009188253.1	102	2.42E-26	1589733	<i>Cyanophage TMM40</i>	<i>Prochlorococcus marinus</i> str. NATL2A	Cyanobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA + ssRNA +
NODE 1481 length 257 cov 0.950495	257	1.1751	gi 803431957 ref YP_009129267.1	45.1	4.36E-06	46076	<i>Artichoke latent virus</i>	<i>Cynara cardunculus</i> var. <i>scolumbus</i>	Streptophyta; Viridiplantae; Eukaryota	unclassified	Potviridae	ssRNA +
NODE 1466 length 258 cov 0.945813	258	1.17054	gi 971767322 ref YP_009220192.1	54.3	1.08E-09	461711	<i>Synechococcus phage S-CBP42</i>	<i>Synechococcus</i> sp. WH 7803	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 1432 length 259 cov 0.941176	259	1.16602	gi 358356497 ref YP_004934248.1	105	4.14E-31	331278	<i>Yersinia phage phiR1-37</i>	<i>Yersinia enterocolitica</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1447 length 259 cov 0.926471	259	1.15444	gi 937456460 ref YP_009168385.1	80.9	4.32E-20	1622234	<i>Citrobacter phage CT122</i>	<i>Citrobacter</i> sp. TM1552	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 1362 length 262 cov 0.927536	262	1.15267	gi 971761177 ref YP_009214523.1	71.6	1.77E-15	1572704	<i>Staphylococcus phage phiPLA-C1C</i>	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1324 length 264 cov 0.918666	264	1.14394	gi 109302725 ref YP_009325851.1	103	1.47E-26	1917232	<i>Only Sengen Nebraska virus 5</i>	<i>Chlorella variabilis</i>	Chlorophyta; Viridiplantae; Eukaryota	unclassified	Phycodnaviridae	dsDNA
NODE 1369 length 262 cov 0.869565	262	1.14122	gi 985757332 ref YP_009222995.1	95.5	6.89E-24	1567484	<i>Lactobacillus phage L4e1f</i>	<i>Lactobacillus fermentum</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1333 length 264 cov 0.913876	264	1.14015	gi 906476370 ref YP_009160383.1	98.6	4.86E-26	1655656	<i>Gokushovirinae Fen672</i>	<i>Gokushovirinae Fen672</i>	na	unclassified	Microviridae	ssDNA viruses
NODE 1279 length 266 cov 0.905213	266	1.13758	gi 712914779 ref YP_009123168.1	47.4	7.00E-07	127373	<i>Escherichia phage BPEC0 4</i>	<i>Escherichia coli</i> O157	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 1254 length 267 cov 0.905666	267	1.13109	gi 1070065029 ref YP_009283143.1	55.5	8.19E-10	1815509	<i>Bacillus phage A97</i>	<i>Bacillus subtilis subsp. subtilis</i> str. 168	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1251 length 267 cov 0.905666	267	1.13109	gi 593774742 ref YP_009012610.1	50.8	3.97E-08	691964	<i>Rhodococcus phage ReaiPoc6</i>	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	unclassified	Siphoviridae	dsDNA viruses
NODE 1160 length 272 cov 0.875576	272	1.10294	gi 906476370 ref YP_009160383.1	90.1	1.11E-22	1655656	<i>Gokushovirinae Fen672</i>	<i>Gokushovirinae Fen672</i>	na	unclassified	Microviridae	ssDNA viruses
NODE 1113 length 274 cov 0.876712	274	1.10219	gi 971764128 ref YP_009217130.1	78.2	1.06E-17	998086	<i>Pseudomonas phage PhiP43</i>	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA viruses
NODE 1117 length 274 cov 0.872146	274	1.09854	gi 24371536 ref NP_720276.1	82	2.03E-19	173443	<i>Salmonella phage ST64</i>	<i>Salmonella enterica</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses
NODE 1083 length 275 cov 0.872727	275	1.09818	gi 944325910 ref YP_009174407.1	75.5	9.53E-17	1586713	<i>Yellowstone lake phycodnavirus 1</i>	na	na	unclassified	Phycodnaviridae	dsDNA viruses
NODE 1032 length 278 cov 0.860987	278	1.08633	gi 363540839 ref YP_004894409.1	75.5	9.51E-17	1094892	<i>Megavirus chilensis</i>	<i>Acanthamoeba</i>	na	unclassified	Mimiviridae	ssDNA
NODE 1036 length 278 cov 0.856502	278	1.08273	gi 1070643189 ref YP_009300747.1	135	6.92E-38	1796997	<i>Arthrobacter phage Mudecat</i>	<i>Arthrobacter</i> sp. ATCC 21022	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA viruses
NODE 860 length 297 cov 1.05372	297	1.0101	gi 422935909 ref YP_007005806.1	110	6.36E-31	1051675	<i>Erwinia phage vB_EamP-56</i>	<i>Erwinia amylovora</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA viruses

Healthy bees DNA virome

NODE 67 length 398 cov 1.82799	398	2.88442	gi 686983201 ref YP_009058944.1	60.8	4.18E-08	1543419	<i>Torque teno Tadarida brasiliensis virus</i>	<i>Tadarida brasiliensis</i>	Chordata; Metazoa; Eukaryota	unclassified	Anelloviridae	ssDNA
NODE 1120 length 225 cov 1.39412	225	2.69333	gi 353596680 ab AEF58760.1	66.6	4.63E-11	1034790	<i>Mosquito VEM Anellovirus SDBYL A</i>	<i>Culicidea</i>	Arthropoda; Metazoa; Eukaryota	unclassified	Anelloviridae	ssDNA
NODE 1058 length 227 cov 1.11628	227	1.3304	gi 557680986 db BAO04453.1	166	4.36E-46	381707	<i>Bovine viral diarrhoea virus 3</i>	<i>Bos taurus</i>	Chordata; Metazoa; Eukaryota	unclassified	Flaviviridae	ssRNA +

Unhealthy bees RNA virome

a29119.2756 total counts: 7460053 Seed: 2 K: 25 length: 2731	2731	1251.63	gi 589892981 ref YP_009004309.1	405	2.23E-132	1453367	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA
a145875.124 total counts: 517623 Seed: 5 K: 25 length: 4184	4184	136.13	gi 325852313 ref YP_164730.1	171	1.97E-03	925986	<i>Erwinia phage phiEal04</i>	<i>Erwinia amylovora</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a23.106 total counts: 858780 Seed: 2 K: 25 length: 835	835	107.419	gi 607840595 ref YP_009218453.1	64.3	4.47E-11	1234882	<i>Dendrolimus punctatus densovirus</i>	<i>Dendrolimus punctatus</i>	Arthropoda; Metazoa; Eukaryota	unclassified	Parvoviridae	ssDNA
a87654.83 total counts: 129393 Seed: 2 K: 25 length: 1587	1587	83.0183	gi 192699366 ref YP_009326252.1	276	4.64E-89	795331	<i>Bat cyclivirus GF-4c</i>	<i>Erythronium simplicifolius</i>	Chordata; Metazoa; Eukaryota	unclassified	Circoviridae	ssDNA
a116472.2 total counts: 909 Seed: 2 K: 25 length: 582	582	40.1554	gi 151266299 ref YP_001333662.1	90.9	8.08E-21	338473	<i>Actinomyces phage Av-1</i>	<i>Actinomyces naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a89036.2 total counts: 576 Seed: 2 K: 25 length: 260	260	27.1163	gi 151266305 ref YP_001333668.1	51.2	2.88E-08	338473	<i>Actinomyces phage Av-1</i>	<i>Actinomyces naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a356.26 total counts: 96963 Seed: 2 K: 25 length: 3730	3730	27.008	gi 132371839 ref YP_009376764.1	342	1.65E-104	1922816	<i>Changjiang tombus-like virus 22</i>	<i>Astovidea</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA
a148797.3 total counts: 838 Seed: 3 K: 25 length: 357	357	21.7183	gi 1068512414 ref YP_009276097.1	58.9	1.30E-10	1647473	<i>Gordonia malaquee</i>	<i>Gordonia</i> phage GRU3	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a29135.15 total counts: 37696 Seed: 18 K: 25 length: 2471	2471	20.1829	gi 388570783 ref YP_006383766.1	644	0.00E+00	1187128	<i>Acinetobacter bacteriophage AP22</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a145736.12 total counts: 14774 Seed: 6 K: 25 length: 1211	1211	17.6201	gi 1070106045 ref YP_009291896.1	268	5.96E-87	1792222	<i>Acinetobacter phage LZ35</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a145548.14 total counts: 225461 Seed: 2 K: 25 length: 16338	16338	14.2949	gi 593777809 ref YP_009015656.1	135	3.99E-30	1084719	<i>Bacillus phage G</i>	<i>Bacillus</i> sp.: <i>Geobacillus</i> sp.	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a115910.13 total counts: 77920 Seed: 2 K: 25 length: 5913	5913	14.2757	gi 134955725 ref YP_009342327.1	721	0.00E+00	1923715	<i>Wuhan insect virus 11</i>	<i>Astovidea</i>	Arthropoda; Metazoa; Eukaryota	Picornavirales	Dicistroviridae	ssRNA +
a3371.13 total counts: 6188 Seed: 8 K: 25 length: 488	488	14.2336	gi 44824486 ref YP_007392585.1	47	2.51E-06	1116482	<i>Pectobacterium phage phiTE</i>	<i>Pectobacterium atropenseum</i> SCRI1043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a58206.11 total counts: 43766 Seed: 5 K: 25 length: 3937	3937	13.4763	gi 966199285 ref YP_009189383.1	189	2.77E-48	691318	<i>Acinetobacter phage phiAB1</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a61384.15 total counts: 5982 Seed: 12 K: 25 length: 421	421	12.859	gi 23334610 ref NP_694833.1	47.8	2.68E-06	37138	<i>Galleria mellonella densovirus</i>	<i>Galleria mellonella</i>	Arthropoda; Metazoa; Eukaryota	unclassified	Parvoviridae	ssDNA
a29390.11 total counts: 22460 Seed: 8 K: 25 length: 1991	1991	12.807	gi 68123154 ref YP_00955475.1	375	4.00E-120	1505225	<i>Acinetobacter phage YMC-13-01-C62</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a30422.10 total counts: 7809 Seed: 16 K: 25 length: 836	836	12.3361	gi 97176381 ref YP_009216809.1	219	6.48E-68	1229760	<i>Acinetobacter phage PHAC-1</i>	<i>Acinetobacter soli</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a146893.11 total counts: 7131 Seed: 11 K: 25 length: 679	679	12.2548	gi 971765187 ref YP_009218283.1	46.6	5.96E-06	1597976	<i>Enterococcus phage EF6G1</i>	<i>Enterococcus faecalis</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a30349.8 total counts: 8570 Seed: 2 K: 25 length: 1075	1075	12.2239	gi 971746169 ref YP_009200249.1	243	1.48E-74	1636200	<i>Pseudoalteromonas phage H103</i>	<i>Pseudoalteromonas marina</i>	Proteobacteria; Bacteria	Caudovirales	unclassified	dsDNA
a29421.6 total counts: 2661 Seed: 7 K: 25 length: 474	474	11.6068	gi 764162207 ref YP_009126577.1	94.4	3.05E-23	1538804	<i>Vibrio phage VpKK5</i>	<i>Vibrio parahaemolyticus</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a134905.2 total counts: 525 Seed: 4 K: 25 length: 248	248	11.4022	gi 593778368 ref YP_009016208.1	70.9	2.72E-15	691963	<i>Rhodococcus phage ReaiPine5</i>	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a148892.2 total counts: 763 Seed: 2 K: 25 length: 492	492	11.3347	gi 22296540 ref NP_680500.1	58.2	8.31E-10	51369	<i>Lactobacillus phage A2</i>	<i>Lactobacillus casei</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a58544.8 total counts: 12547 Seed: 2 K: 25 length: 1519	1519	10.7031	gi 388570790 ref YP_006383773.1	52.4	3.45E-08	1187128	<i>Acinetobacter bacteriophage AP22</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a146684.12 total counts: 7542 Seed: 3 K: 25 length: 676	676	10.1553	gi 68123168 ref YP_00955489.1	93.2	1.31E-21	1505225	<i>Acinetobacter phage YMC-13-01-C62</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a14896.10 total counts: 1814 Seed: 5 K: 25 length: 212	212	9.5283	gi 388570830 ref YP_006383813.1	49.7	3.43E-08	1187128	<i>Acinetobacter bacteriophage AP22</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a146706.9 total counts: 7470 Seed: 3 K: 25 length: 826	826	9.40436	gi 593778368 ref YP_009016208.1	65.5	2.71E-11	114673	<i>Bacillus phage SHOU24</i>	<i>Bacillus megaterium</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a130327.2 total counts: 45 Seed: 4 K: 25 length: 283	283	8.97411	gi 589890583 ref YP_009006430.1	60.5	3.00E-12	1474379	<i>Vibrio phage SHOU24</i>	<i>Vibrio parahaemolyticus</i>	Firmicutes; Bacteria	unclassified	unclassified	dsDNA
a58401.11 total counts: 3538 Seed: 2 K: 25 length: 357	357	9.95238	gi 132371030 ref YP_009370119.1	79.3	1.12E-127	1923158	<i>Hubei picorna-like virus 74</i>	<i>Myriapoda</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA
a87374.9 total counts: 11851 Seed: 2 K: 25 length: 1293	1293	8.94122	gi 448244802 ref YP_007392522.1	101	3.19E-22	1116482	<i>Pectobacterium phage phiTE</i>	<i>Pectobacterium atropenseum</i> SCRI1043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a59767.7 total counts: 4925 Seed: 7 K: 25 length: 701	701	8.54066	gi 1070106029 ref YP_009291880.1	110	9.05E-28	1792222	<i>Acinetobacter phage LZ35</i>	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a29401.4 total counts: 887 Seed: 6 K: 25 length: 246	246	8.51717	gi 589893779 ref YP_009010488.1	44.3	5.46E-06	1458842	<i>Geobacillus phage GBK2</i>	<i>Geobacillus kaustophilus</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a89639.8 total counts: 4696 Seed: 6 K: 25 length: 631	631	8.50713	gi 448244960 ref YP_007392680.1	149	5.05E-45	1116482	<i>Pectobacterium phage phiTE</i>	<i>Pectobacterium atropenseum</i> SCRI1043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a92269.2 total counts: 813 Seed: 3 K: 25 length: 450	450	8.47983	gi 1070103030 ref YP_009287678.1	103	8.82E-26	1838137	<i>Propionibacterium phage PRP1</i>	<i>Propionibacterium freudenreichii</i>	Actinobacteria; Bacteria	unclassified	unclassified	dsDNA
a87907.9 total counts: 11161 Seed: 5 K: 25 length: 1236	1236	8.45793	gi 73747918 ref NP_851404.2	172	2.31E-46	68876	<i>Kashmir bee virus</i>	<i>Apis cerana</i>	Arthropoda; Metazoa; Eukaryota	Picornavirales	Dicistroviridae	ssRNA +
a90481.8 total counts: 2074 Seed: 2 K: 25 length: 295	295	8.39661	gi 966203611 ref YP_009188926.1	62	2.46E-12	1684115	<i>Cronobacter phage PBES 02</i>	<i>Cronobacter</i> sp.	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a152844.2 total counts: 557 Seed: 2 K: 25 length: 279	279	8.32605	gi 737450020 ref YP_005									

a146107.7	total counts: 6223	Seed: 2 K: 25	length: 980	980	6.25816	gii448244974ref Y007392694.1	275	8.43E-92	1116482	Pectobacterium phage phiTE	<i>Pectobacterium atrosepticum</i> SCRI1/043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a87678.6	total counts: 5258	Seed: 9 K: 25	length: 941	941	6.19497	gii1068358199ref Y009273940.1	86.7	4.83E-18	1838080	Gordonia phage Vendetta	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a65757.2	total counts: 3600	Seed: 2 K: 25	length: 257	257	6.16445	gii593536927ref Y009168411.2	101	7.99E-27	1622234	Citrobacter phage CVT122	<i>Citrobacter</i> sp. TM1552	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA	
a88229.6	total counts: 6692	Seed: 2 K: 25	length: 1024	1024	6.16309	gii593771253ref Y009014970.1	250	1.66E-74	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a58559.6	total counts: 3379	Seed: 5 K: 25	length: 610	610	6.14754	gii593777283ref Y009015128.1	88.6	7.29E-22	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a30198.7	total counts: 3618	Seed: 2 K: 25	length: 524	524	6.12977	gii1132327007ref Y009337788.1	58.2	1.62E-09	1923151	<i>Hubei picorna-like virus 68</i>	<i>Diplodopa</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA	
a148940.7	total counts: 2435	Seed: 12 K: 25	length: 372	372	6.12097	gii937456198ref Y009167808.1	77.8	1.65E-17	438780	Lactobacillus phage phiPBYB5	<i>Lactobacillus fermentum</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a116141.5	total counts: 2380	Seed: 2 K: 25	length: 475	475	6.05895	gii440789461ref Y007349171.1	56.6	6.21E-10	1262513	Serratia phage phiMIAM1	<i>Serratia</i> sp.	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA	
a147164.6	total counts: 5790	Seed: 8 K: 25	length: 1028	1028	6.04767	gii682123153ref Y009055474.1	112	9.36E-29	1505225	Acinetobacter phage YMC-13-01-C62	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a87623.6	total counts: 2023	Seed: 3 K: 25	length: 385	385	5.99481	gii448244881ref Y007392611.1	72.4	6.67E-17	1116482	Pectobacterium phage phiTE	<i>Pectobacterium atrosepticum</i> SCRI1/043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a116386.7	total counts: 5103	Seed: 11 K: 25	length: 785	785	5.9172	gii563397698ref Y008857462.1	65.9	2.20E-13	1391428	Enterobacteria phage 4MG	<i>Escherichia coli</i> K-12	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a88401.5	total counts: 4397	Seed: 4 K: 25	length: 967	967	5.88004	gii363539710ref Y008493995.1	77	2.17E-17	889338	Salmonella phage PVP-SE1	<i>Salmonella enterica</i> subsp. enterica serovar Enteritidis	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a61329.2	total counts: 582	Seed: 2 K: 25	length: 263	263	5.8669	gii764162033ref Y009126429.1	123	2.71E-35	1561065	Paracoccus phage vB_PmaS IMPEP1	<i>Paracoccus marcusii</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a87760.5	total counts: 3323	Seed: 8 K: 25	length: 633	633	5.85624	gii1132327007ref Y009337788.1	120	9.32E-31	1923151	<i>Hubei picorna-like virus 68</i>	<i>Diplodopa</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA	
a59531.6	total counts: 9726	Seed: 8 K: 25	length: 1704	1704	5.82453	gii658310294ref Y009042486.1	377	2.64E-123	1327934	Cronobacter phage CR8	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a117663.5	total counts: 2738	Seed: 6 K: 25	length: 1024	1024	5.8159	gii134598010ref Y009342254.1	105	9.30E-26	192362	<i>Wuhan arthropod virus 3</i>	<i>Hexapoda</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA	
a116657.5	total counts: 5007	Seed: 5 K: 25	length: 1025	1025	5.71512	gii563397489ref Y008857253.1	95.5	2.97E-21	1391428	Enterobacteria phage 4MG	<i>Escherichia coli</i> K-12	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a87688.7	total counts: 2137	Seed: 3 K: 25	length: 333	333	5.68769	gii422937549ref Y007007754.1	57.4	4.70E-10	1127514	Aeromonas phage vB_AsaM-56	<i>Aeromonas salmonicida</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a62989.8	total counts: 1709	Seed: 2 K: 25	length: 233	233	5.67811	gii593777355ref Y009015200.1	82.8	1.63E-20	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a120694.7	total counts: 1942	Seed: 6 K: 25	length: 286	286	5.66667	gii764162223ref Y009126593.1	133	3.49E-37	1538804	Vibrio phage VpKKS	<i>Vibrio parahaemolyticus</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a116247.6	total counts: 3553	Seed: 2 K: 25	length: 661	661	5.66263	gii1070106098ref Y009291949.1	180	1.62E-55	1792222	Acinetobacter phage LZ35	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a146679.6	total counts: 2317	Seed: 4 K: 25	length: 440	440	5.64773	gii1070099784ref Y009283489.1	44.7	2.08E-06	1647411	Salmonella phage NR01	<i>Salmonella enterica</i> subsp. enterica serovar Typhimurium	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a1808.3	total counts: 2394	Seed: 2 K: 25	length: 818	818	5.59889	gii971765014ref Y009218007.1	105	9.57E-25	1755682	Streptomyces phage phiSAJS1	<i>Streptomyces avermitilis</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a119732.5	total counts: 1999	Seed: 10 K: 25	length: 391	391	5.58568	gii127298679ref Y00933576.1	72.4	5.33E-15	1922550	<i>Beihai picorna-like virus 121</i>	<i>Lizixia exotica</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA	
a60775.5	total counts: 3496	Seed: 3 K: 25	length: 673	673	5.58247	gii971763828ref Y009216826.1	168	4.65E-51	1229760	Acinetobacter phage phiAC-1	<i>Acinetobacter soli</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a123068.6	total counts: 1164	Seed: 3 K: 25	length: 226	226	5.5708	gii712915520ref Y009103259.1	55.1	3.90E-10	1481187	Acinetobacter phage vB_AbaP Acibael007	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a147161.2	total counts: 758	Seed: 4 K: 25	length: 357	357	5.54862	gii712912873ref Y009097732.1	62.8	4.52E-12	1555208	Ruegeria phage DSS3-P1	<i>Ruegeria pomeroyi</i> DSS-3	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a8802.6	total counts: 2129	Seed: 2 K: 25	length: 384	384	5.53224	gii1070101651ref Y009285831.1	49.3	5.18E-07	1844477	Pseudomonas phage NP1	<i>Pseudomonas</i> sp.	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a157036.2	total counts: 541	Seed: 3 K: 25	length: 258	258	5.52753	gii1068511062ref Y009274016.1	50.8	3.35E-08	1838072	Gordonia phage McGonagall	<i>Gordonia neofluacis</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a150472.5	total counts: 1663	Seed: 2 K: 25	length: 344	344	5.50872	gii1132327029ref Y009336506.1	137	4.39E-38	1923011	<i>Hubei orthoherpes virus 3</i>	<i>Orthoherpes</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA	
a148545.5	total counts: 4407	Seed: 4 K: 25	length: 906	906	5.4713	gii593777136ref Y009014981.1	230	8.18E-69	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a17010.8	total counts: 10651	Seed: 7 K: 25	length: 1313	1313	5.46473	gii472342361ref Y007675869.1	181	8.37E-54	754058	Vibrio phage PWH13a-P1	<i>Vibrio natriegens</i>	Proteobacteria; Bacteria	Caudovirales	unclassified	unclassified	dsDNA
a91422.5	total counts: 7244	Seed: 6 K: 25	length: 1453	1453	5.46387	gii593777149ref Y009014994.1	116	7.15E-27	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a12310.3	total counts: 671	Seed: 5 K: 25	length: 285	285	5.44271	gii1070101678ref Y009285858.1	65.9	4.20E-14	1844477	Pseudomonas phage NP1	<i>Pseudomonas</i> sp.	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a147994.5	total counts: 2716	Seed: 2 K: 25	length: 623	623	5.40931	gii730985057ref Y009111142.1	86.3	4.28E-19	1527519	Escherichia phage Av-05	<i>Escherichia coli</i> O157:H7	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a89524.5	total counts: 5704	Seed: 5 K: 25	length: 1090	1090	5.40367	gii593777381ref Y009015226.1	268	2.17E-85	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a148424.6	total counts: 2706	Seed: 2 K: 25	length: 480	480	5.35113	gii1070101665ref Y009285845.1	68.2	2.26E-13	1844477	Pseudomonas phage NP1	<i>Pseudomonas</i> sp.	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a1179.5	total counts: 4904	Seed: 10 K: 25	length: 928	928	5.34914	gii389059974ref Y006383127.1	358	2.43E-122	1162295	Cronobacter phage CR3	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a10598.3	total counts: 614	Seed: 3 K: 25	length: 239	239	5.33197	gii1070099007ref Y009281736.1	85.6	9.35E-20	1874002	Bacillus phage Sitch	<i>Bacillus</i> sp.	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA	
a309.5	total counts: 3322	Seed: 2 K: 25	length: 601	601	5.32220	gii1070101651ref Y009285831.1	49.3	5.18E-07	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a146560.3	total counts: 818	Seed: 2 K: 25	length: 311	311	5.31379	gii1068357956ref Y009273286.1	63.1	4.55E-13	1647470	Gordonia phage GMA1	<i>Gordonia makuake</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a87786.4	total counts: 3868	Seed: 4 K: 25	length: 908	908	5.29846	gii363539684ref Y008493969.1	127	1.86E-35	889338	Salmonella phage PVP-SE1	<i>Salmonella enterica</i> subsp. enterica serovar Enteritidis	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a147329.4	total counts: 4586	Seed: 2 K: 25	length: 1094	1094	5.29616	gii971763920ref Y009216905.1	56.6	2.14E-08	1522092	Clostridium phage phiCDHM19	<i>Clostridium</i> sp.	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA	
a58707.5	total counts: 7658	Seed: 3 K: 25	length: 1538	1538	5.28088	gii389060077ref Y006383230.1	47.1	5.19E-164	1162295	Cronobacter phage CR3	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a29755.9	total counts: 7610	Seed: 2 K: 25	length: 902	902	5.26718	gii1132327050ref Y009336655.1	81.6	3.01E-16	1923160	<i>Hubei picorna-like virus 76</i>	<i>Mvriapoda</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA	
a148214.5	total counts: 1378	Seed: 5 K: 25	length: 322	322	5.26618	gii9633052ref INP_05160.1	48.1	8.03E-07	12417	Lactobacillus phage phiADT	<i>Lactobacillus gasseri</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a61287.2	total counts: 413	Seed: 2 K: 25	length: 214	214	5.25413	gii472339880ref Y007673409.1	46.6	5.33E-07	756282	Cellulophaga phage phiSH	<i>Cellulophaga</i> sp. MM#3	Bacteroidetes; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a87573.5	total counts: 8642	Seed: 3 K: 25	length: 1816	1816	5.25275	gii730985094ref Y009111179.1	192	9.27E-53	1527519	Escherichia phage Av-05	<i>Escherichia coli</i> O157:H9	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a146137.6	total counts: 2577	Seed: 8 K: 25	length: 483	483	5.21325	gii658310154ref Y009042486.1	68.2	7.59E-15	1327934	Cronobacter phage CR8	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a60893.5	total counts: 1556	Seed: 5 K: 25	length: 353	353	5.20963	gii593777377ref Y009015242.1	50.1	3.61E-08	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a1155.5	total counts: 2384	Seed: 2 K: 25	length: 494	494	5.19433	gii966203773ref Y009189092.1	214	4.67E-67	1684115	Cronobacter phage PBES 02	<i>Cronobacter</i> sp.	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a12005.2	total counts: 666	Seed: 2 K: 25	length: 353	353	5.18405	gii151266302ref Y009133665.1	58.5	5.24E-07	1162290	Acinetobacter phage CR9	<i>Acinetobacter naeclandii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a150305.6	total counts: 1803	Seed: 2 K: 25	length: 351	351	5.1453	gii389059922ref Y006383075.1	86.7	3.30E-20	1162295	Cronobacter phage CR3	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a147855.5	total counts: 1519	Seed: 6 K: 25	length: 339	339	5.06204	gii51797869ref Y008772035.1	77.8	2.56E-17	1399941	Lactobacillus phage phiJB	<i>Lactobacillus delbrueckii</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a58916.5	total counts: 4273	Seed: 4 K: 25	length: 899	899	5.05451	gii971762804ref Y009215890.1	53.1	6.32E-07	1296655	Bacillus phage JL	<i>Bacillus cereus</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA	
a58466.5	total counts: 1329	Seed: 2 K: 25	length: 293	293	5	gii389060116ref Y006383269.1	87.8	3.44E-23	1162295	Cronobacter phage CR3	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a150749.4	total counts: 1179	Seed: 6 K: 25	length: 342	342	4.99457	gii472340162ref Y007673409.1	45.4	5.94E-06	754067	Salicola phage CGh29	<i>Salicola</i> sp.	Proteobacteria; Bacteria	Caudovirales	unclassified	unclassified	RNA
a29359.5	total counts: 3883	Seed: 8 K: 25	length: 780	780	4.95897	gii1132327092ref Y009337064.1	54.3	1.91E-07	1923150	<i>Hubei picorna-like virus 67</i>	<i>Mvriapoda</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA	
a4746.5	total counts: 1034	Seed: 2 K: 25	length: 230	230	4.8913	gii966203360ref Y009187671.1	80.5	3.67E-19	1791940	Klebsiella phage vB_KpnM KB57	<i>Klebsiella pneumoniae</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a124153.2	total counts: 683	Seed														

a122019.3 total counts: 1194 Seed: 3 K: 25 length: 366	366	4.31842	gii1070620945 ref YP_009301454.1	64.7	7.17E-13	1821552	Gordonia phage Emalyn	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a149361.5 total counts: 1571 Seed: 9 K: 25 length: 310	310	4.3	gii9171763831 ref YP_009216829.1	76.6	5.48E-19	1229760	Acinetobacter phage phiAC-1	<i>Brevibacterium soli</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a147715.2 total counts: 378 Seed: 2 K: 25 length: 221	221	4.28602	gii157168388 ref YP_001456745.1	65.9	1.24E-13	28358	Corynebacterium phage BFK20	<i>[Brevibacterium] flavum</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a48405.4 total counts: 1813 Seed: 7 K: 25 length: 464	464	4.28017	gii593777292 ref YP_009015237.1	45.4	2.37E-06	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a15893.4 total counts: 1127 Seed: 6 K: 25 length: 436	436	4.27509	gii486997198 ref YP_0204928.1	177	5.81E-55	279280	Burkholderia phage BeePC6B	<i>Burkholderia cepacia</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a30965.4 total counts: 1194 Seed: 2 K: 25 length: 325	325	4.21231	gii848469552 ref YP_009145928.1	70.9	4.86E-15	1220714	Rhizobium phage RHtEP1	<i>Rhizobium cili GR56</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a375.4 total counts: 1247 Seed: 4 K: 25 length: 347	347	4.1902	gii966203437 ref YP_009187754.1	120	3.60E-35	1791940	Klebsiella phage vB_KpnM_KB57	<i>Klebsiella pneumoniae</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a121594.4 total counts: 629 Seed: 6 K: 25 length: 201	201	4.15423	gii593777201 ref YP_009015046.1	115	1.83E-31	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a97527.5 total counts: 2221 Seed: 2 K: 25 length: 436	436	4.15228	gii971762140 ref YP_009215331.1	119	5.01E-32	1636183	Streptomyces phage YDN12	<i>Streptomyces griseus subsp. griseus</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a62460.5 total counts: 972 Seed: 5 K: 25 length: 206	206	4.14563	gii389059866 ref YP_006383019.1	54.3	1.05E-09	1162295	Cronobacter phage CR3	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a148337.4 total counts: 1892 Seed: 2 K: 25 length: 460	460	4.12609	gii226377831 ref YP_002790879.1	59.7	2.93E-10	631345	<i>Solenopsis invicta virus 3</i>	<i>Solenopsis invicta</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	unclassified
a2658.5 total counts: 1125 Seed: 4 K: 25 length: 262	262	4.11832	gii472342218 ref YP_007675729.1	55.5	5.44E-10	756280	Cellulophaga phage phiSM	<i>Cellulophaga sp. MM#3</i>	Bacteroidetes; Bacteria	Caudovirales	Myoviridae	dsDNA
a147066.5 total counts: 1636 Seed: 3 K: 25 length: 324	324	4.11404	gii104911332 ref YP_006906554.1	122	5.20E-33	1229786	Propionibacterium phage P101A	<i>Cuitubacterium acnes</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146197.4 total counts: 3045 Seed: 2 K: 25 length: 808	808	4.10877	gii91751687 ref YP_009205612.1	80.9	1.74E-16	1175662	Pseudomonas phage PaMx42	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146959.3 total counts: 532 Seed: 4 K: 25 length: 207	207	4.06557	gii1070620973 ref YP_009301483.1	49.7	5.50E-08	1821552	Gordonia phage Emalyn	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a153042.2 total counts: 381 Seed: 3 K: 25 length: 239	239	4.04297	gii41179371 ref YP_00969679.1	62.5	1.94E-12	194529	Bondella phage BP1	<i>Bondella bronchiseptica</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a30547.1 total counts: 6700 Seed: 21 K: 25 length: 552	552	4.02958	gii72449802 ref YP_005087026.1	221	2.13E-70	1109717	Rhodococcus phage RHHI	<i>Rhodococcus rhodocensus</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a119223.2 total counts: 334 Seed: 2 K: 25 length: 221	221	4.01386	gii971762626 ref YP_009215748.1	93.6	2.37E-23	1718273	Pseudomonas phage PAE1	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a64277.5 total counts: 1172 Seed: 5 K: 25 length: 281	281	4.01068	gii448244878 ref YP_007920598.1	96.7	2.72E-24	1116482	Pectobacterium phage phiTE	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a29235.5 total counts: 2308 Seed: 2 K: 25 length: 522	522	4.00766	gii563397465 ref YP_008857229.1	188	5.09E-55	1391428	Enterobacteria phage 4MG	<i>Escherichia coli K-12</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a93068.2 total counts: 738 Seed: 3 K: 25 length: 357	357	4	gii151266305 ref YP_001133668.1	131	8.16E-38	338473	Actinomycetes phage Av-1	<i>Actinomycetes naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a4907.4 total counts: 1091 Seed: 5 K: 25 length: 303	303	3.9967	gii971765803 ref YP_009211802.1	119	2.35E-32	1655645	Parabacteroides phage YZ-2015b	<i>na</i>	unclassified	Microviridae	ssRNA +	
a115738.6 total counts: 2739 Seed: 8 K: 25 length: 448	448	3.97768	gii1132371030 ref YP_009337019.1	93.6	4.29E-22	1923158	<i>Hubei picorna-like virus 74</i>	<i>Myriapoda</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA
a2021.4 total counts: 1002 Seed: 5 K: 25 length: 266	266	3.97744	gii593777354 ref YP_009015199.1	100	2.88E-27	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a2748.4 total counts: 2112 Seed: 5 K: 25 length: 600	600	3.96667	gii389059910 ref YP_006383063.1	81.3	8.25E-20	1162295	Cronobacter phage CR3	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a150241.4 total counts: 1761 Seed: 6 K: 25 length: 519	519	3.95568	gii448244744 ref YP_007392464.1	55.5	1.25E-08	1116482	Pectobacterium phage phiTE	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a150393.3 total counts: 506 Seed: 4 K: 25 length: 202	202	3.94022	gii146529913 ref YP_001210221.1	46.6	4.79E-07	437329	Burkholderia phage BeePC6B	<i>Burkholderia cepacia</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a150158.4 total counts: 862 Seed: 6 K: 25 length: 226	226	3.93424	gii29663553 ref NP_059624.1	121	1.03E-35	10754	Enterobacteria phage P22	<i>Escherichia coli</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a147402.3 total counts: 683 Seed: 2 K: 25 length: 224	224	3.92994	gii937456460 ref YP_009168385.1	80.9	2.30E-20	1622234	Citrobacter phage CVT22	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a150827.3 total counts: 478 Seed: 2 K: 25 length: 211	211	3.89151	gii41179223 ref NP_958561.1	65.9	6.96E-15	139870	Lactobacillus prophage L965	<i>Lactobacillus johnsonii</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a148796.4 total counts: 1311 Seed: 2 K: 25 length: 335	335	3.88955	gii388570811 ref YP_006383794.1	136	8.44E-38	1187128	Acinetobacter bacteriophage AP22	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a146808.5 total counts: 2402 Seed: 2 K: 25 length: 488	488	3.87955	gii49060585 ref YP_009152819.1	52	1.86E-07	1540093	Bacillus phage Pookie	<i>Bacillus megaterium</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA
a29954.4 total counts: 4678 Seed: 6 K: 25 length: 1159	1159	3.86885	gii448244802 ref YP_007392522.1	179	1.16E-50	1116482	Pectobacterium phage phiTE	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a149052.3 total counts: 1216 Seed: 3 K: 25 length: 415	415	3.86024	gii593777266 ref YP_009015111.1	69.7	5.24E-16	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a59372.3 total counts: 1154 Seed: 3 K: 25 length: 355	355	3.84598	gii422935909 ref YP_007005806.1	84.3	1.90E-20	1051675	Erwinia phage vB_Eamp-S6	<i>Erwinia amylovora</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a149416.3 total counts: 822 Seed: 3 K: 25 length: 265	265	3.80755	gii410492122 ref YP_006907308.1	65.5	2.73E-13	57477	Bacillus phage Bastille	<i>Bacillus cereus</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a35779.4 total counts: 1247 Seed: 6 K: 25 length: 343	343	3.80466	gii36359688 ref YP_004893973.1	99	3.52E-25	889338	Salmonella phage PVP-SE1	<i>Salmonella enterica subsp. enterica serovar Enteritidis</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a147425.5 total counts: 2771 Seed: 5 K: 25 length: 661	661	3.79425	gii563397553 ref YP_008857317.1	121	1.46E-34	1391428	Enterobacteria phage 4MG	<i>Escherichia coli K-12</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a1848.3 total counts: 514 Seed: 4 K: 25 length: 200	200	3.785	gii448260870 ref YP_007349227.1	49.7	4.35E-08	1204533	Bacillus phage phiGATE	<i>Bacillus pumilus</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a1847149.9 total counts: 1293 Seed: 4 K: 25 length: 676	676	3.78214	gii148494281 ref YP_007003218.1	67.5	2.14E-12	194529	Bondella phage BP1	<i>Bondella bronchiseptica</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a35212.4 total counts: 971 Seed: 8 K: 25 length: 259	259	3.76062	gii593777120 ref YP_009014965.1	134	1.25E-40	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a117265.3 total counts: 661 Seed: 2 K: 25 length: 242	242	3.73554	gii906476396 ref YP_009160400.1	76.3	1.50E-17	1655659	<i>Gakushovirinae Fen7875 21</i>	<i>na</i>	unclassified	Microviridae	ssDNA	
a146821.5 total counts: 2458 Seed: 2 K: 25 length: 524	524	3.71545	gii589893779 ref YP_009010488.1	74.3	4.05E-15	1458842	Geobacillus phage GBBK2	<i>Geobacillus kaustophilus</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a146506.4 total counts: 3410 Seed: 3 K: 25 length: 814	814	3.71007	gii593777392 ref YP_009015237.1	49.3	3.60E-07	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a158898.2 total counts: 344 Seed: 2 K: 25 length: 206	206	3.68644	gii1070099269 ref YP_009282381.1	47	4.56E-07	1815511	Lactobacillus phage PLE2	<i>Lactobacillus casei BL23</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a65.6 total counts: 4013 Seed: 2 K: 25 length: 656	656	3.65753	gii62327110 ref YP_223898.1	151	2.75E-41	235201	Lactobacillus phage phiJL-1	<i>Lactobacillus plantarum</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a150391.2 total counts: 603 Seed: 3 K: 25 length: 285	285	3.63158	gii580177873 ref YP_008050946.1	55.5	1.20E-09	1325953	Mycobacterium phage PegL6	<i>Mycobacterium smegmatis str. MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a31857.4 total counts: 705 Seed: 2 K: 25 length: 224	224	3.62054	gii90592627 ref YP_529887.1	47.4	1.23E-07	363555	Lactobacillus phage KC5a	<i>Lactobacillus gasseri</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a152065.4 total counts: 2093 Seed: 6 K: 25 length: 561	561	3.61151	gii107096343 ref YP_009277980.1	125	4.45E-33	1654873	Propionibacterium phage QueenBey	<i>Cuitubacterium acnes</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a125574.4 total counts: 3571 Seed: 3 K: 25 length: 1017	1017	3.59095	gii65831013 ref YP_009042324.1	380	4.58E-130	1327934	Cronobacter phage CR8	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a156327.4 total counts: 1421 Seed: 2 K: 25 length: 394	394	3.59073	gii10491739 ref YP_009696961.1	60.5	7.29E-11	1204525	Streptomyces phage SV1	<i>Streptomyces venezuelae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a118987.6 total counts: 1591 Seed: 4 K: 25 length: 287	287	3.57491	gii132370375 ref YP_009336558.1	92.4	1.92E-30	1923009	<i>Hubei orbivirus</i>	<i>Orbivirus</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	RNA
a149082.2 total counts: 673 Seed: 5 K: 25 length: 316	316	3.56835	gii112913802 ref YP_009100909.1	69.7	1.41E-14	1536592	Idiomarinae phage IN2-2	<i>Idiomarinae bacterium IN2-2</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a7871.5 total counts: 1965 Seed: 6 K: 25 length: 443	443	3.56659	gii966203781 ref YP_009189096.1	58.9	5.91E-12	1684115	Cronobacter phage PBES02	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a1705.3 total counts: 715 Seed: 3 K: 25 length: 280	280	3.56429	gii448244951 ref YP_007392671.1	137	3.21E-39	1116482	Pectobacterium phage phiTE	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a30141.3 total counts: 903 Seed: 9 K: 25 length: 311	311	3.54019	gii593777348 ref YP_009015193.1	166	1.35E-48	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a62682.5 total counts: 1539 Seed: 2 K: 25 length: 321	321	3.51325	gii1068512754 ref YP_009276541.1	75.5	6.83E-17	1838064	Gordonia phage BritBrat	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a36095.3 total counts: 1669 Seed: 5 K: 25 length: 594	594	3.4899	gii593777396 ref YP_009015241.1	79.3	1.69E-18	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a31951.4 total counts: 1233 Seed: 3 K: 25 length: 360	360	3.48611	gii971848926 ref YP_009035505.1	101	1.28E-25	1416334	Lactobacillus phage phi Jlb1	<i>Lactobacillus gasseri ADH</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a146623.4 total counts: 2347 Seed: 5 K: 25 length: 639	639	3.46009	gii1070638430 ref YP_009302456.1	48.5	4.0							

a150976.4 total counts: 893 Seed: 3 K: 25 length: 229	229	3.11146	gi 219563209 ref YP_002455801.1	55.5	5.84E-10	578234	Lactobacillus phage Lv-1		Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a194817.4 total counts: 691 Seed: 4 K: 25 length: 200	200	3.1	gi 937456524 ref YP_009168450.1	62.4	1.31E-12	1622234	Citrobacter phage CVT22		Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA	
a45743.3 total counts: 1072 Seed: 4 K: 25 length: 418	418	3.09569	gi 593777349 ref YP_009015194.1	75.9	2.34E-18	1162290	Cronobacter phage CR9		Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a52821.2 total counts: 663 Seed: 2 K: 25 length: 372	372	3.091153	gi 070183030 ref YP_009287578.1	105	8.36E-27	183813	Prionomibacterium phage PRR1		Actinobacteria; Bacteria	unclassified	unclassified	dsDNA	
a35554.4 total counts: 1833 Seed: 2 K: 25 length: 435	435	3.08389	gi 122935909 ref YP_007005806.1	132	1.05E-38	1051675	Erwinia phage vB_Eamp-P56		Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA	
a31545.3 total counts: 713 Seed: 6 K: 25 length: 228	228	3.07895	gi 15809314 ref YP_001504127.1	67.4	2.32E-14	442493	Enterococcus phage phiEF24C		Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a154746.2 total counts: 656 Seed: 4 K: 25 length: 435	435	3.06872	gi 1070639841 ref YP_009304190.1	63.9	8.03E-13	1821553	Gordonia phage Guacamole		Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a150529.3 total counts: 678 Seed: 2 K: 25 length: 265	265	3.06415	gi 593777538 ref YP_009015381.1	111	9.90E-30	1084719	Bacillus phage G		Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA	
a154917.2 total counts: 285 Seed: 3 K: 25 length: 207	207	3.06093	gi 1102621404 ref YP_009322238.1	52.8	6.69E-10	1813769	Salmonella phage 64795_sal3		Proteobacteria; Bacteria	unclassified	unclassified	dsDNA	
a64192.2 total counts: 467 Seed: 2 K: 25 length: 263	263	3.05703	gi 1070106096 ref YP_009291947.1	93.6	2.66E-25	1792222	Acinetobacter phage LZ35		Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a87433.4 total counts: 1294 Seed: 2 K: 25 length: 343	343	3.05248	gi 682123168 ref YP_009055489.1	82.8	2.68E-19	1505225	Acinetobacter phage YMC-13-01-C62		Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a148132.4 total counts: 936 Seed: 4 K: 25 length: 269	269	3.04516	gi 148487735 ref YP_007002058.1	80.9	1.24E-18	1133292	Brucella phage Tb		Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA	
a148959.2 total counts: 508 Seed: 2 K: 25 length: 249	249	3.03571	gi 937456502 ref YP_009168428.1	43.5	7.66E-06	1622234	Citrobacter phage CVT22		Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA	
a162105.2 total counts: 849 Seed: 2 K: 25 length: 395	395	3.03139	gi 149408242 ref YP_001294521.1	131	3.54E-36	347328	Pseudomonas phage M6		Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a35477.3 total counts: 1262 Seed: 4 K: 25 length: 401	401	3.02993	gi 422937532 ref YP_007007737.1	86.7	1.01E-20	1127514	Aeromonas phage vB_Asam-56		Aeromonas salmonicida	Caudovirales	Myoviridae	dsDNA	
a149688.4 total counts: 1078 Seed: 2 K: 25 length: 265	265	3.01999	gi 179336582 ref YP_007859933.1	104	3.69E-27	1305510	Mycobacterium phage PhiM3		Mycobacterium avium subsp. paratuberculosis	Actinobacteria; Bacteria	unclassified	RNA	
a30180.2 total counts: 792 Seed: 2 K: 25 length: 351	351	3	gi 1132370297 ref YP_009336506.1	139	1.85E-38	1923011	Hubei orholister virus X		Orholister	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	
a60166.3 total counts: 965 Seed: 4 K: 25 length: 366	366	2.9871	gi 543171058 ref YP_008531119.1	75.1	3.68E-17	1354514	Mycobacterium phage Qanp		Mycobacterium smegmatis str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a58655.3 total counts: 818 Seed: 4 K: 25 length: 302	302	2.98344	gi 906476384 ref YP_009169392.1	103	5.69E-28	1655658	Microrividee Fen786_21		na	unclassified	Microriviridae	ssDNA	
a59367.6 total counts: 4260 Seed: 3 K: 25 length: 784	784	2.98333	gi 764162043 ref YP_009126439.1	59.3	8.04E-10	1561065	Paracoccus phage vB_PmaS IMPEI		Paracoccus carusii	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a119799.2 total counts: 1055 Seed: 6 K: 25 length: 453	453	2.97778	gi 1068359406 ref YP_009276409.1	248	9.59E-84	1647471	Gordonia phage GMA4		Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a147678.2 total counts: 645 Seed: 2 K: 25 length: 315	315	2.97143	gi 971848934 ref YP_009035513.1	82	4.63E-19	1416334	Lactobacillus phage phi1jfb1		Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA	
a124268.3 total counts: 568 Seed: 5 K: 25 length: 216	216	2.96928	gi 151266299 ref YP_001333662.1	56.6	1.95E-10	338473	Actinomycetes phage Av-1		Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA	
a62875.3 total counts: 1490 Seed: 3 K: 25 length: 454	454	2.96966	gi 1003725486 ref YP_009237509.1	73.9	1.69E-15	1685785	Lake Sarah-associated circular virus-8		na	unclassified	unclassified	unclassified	
a66836.2 total counts: 431 Seed: 3 K: 25 length: 201	201	2.96517	gi 209967989 ref YP_002296164.1	53.1	2.54E-09	181082	Emilitania huxleyi		Emilitania huxleyi	Haptophyta; Chromalveolata; Eukaryota	unclassified	Phycodnaviridae	dsDNA
a34995.2 total counts: 691 Seed: 2 K: 25 length: 327	327	2.94792	gi 1068511239 ref YP_009274344.1	164	4.47E-49	1647474	Tsakumurella phage TPA4		Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a68430.2 total counts: 500 Seed: 2 K: 25 length: 247	247	2.93927	gi 446730230 ref YP_007173600.1	49.7	8.89E-08	1206110	Lactobacillus helveticus		Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA	
a39562.2 total counts: 423 Seed: 2 K: 25 length: 210	210	2.93906	gi 151266299 ref YP_001333662.1	54.3	1.32E-09	338473	Actinomycetes phage Av-1		Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA	
a149738.3 total counts: 735 Seed: 3 K: 25 length: 268	268	2.9291	gi 1070620004 ref YP_009304800.1	59.7	1.14E-11	1605379	Acinetobacter phage vB_AbaM phiAbaA1		Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a149950.3 total counts: 649 Seed: 4 K: 25 length: 212	212	2.92777	gi 117921818 ref NP_958556.1	72	6.88E-16	139870	Lactobacillus prophage J1965		Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a120198.2 total counts: 774 Seed: 4 K: 25 length: 343	343	2.92711	gi 363540839 ref YP_004894409.1	51.6	6.09E-08	1094892	Megavirus chilensis		Longamoebia; Amoebozoa; Eukaryota	unclassified	Mimiviridae	dsDNA	
a128023.2 total counts: 427 Seed: 3 K: 25 length: 214	214	2.92203	gi 149408253 ref YP_001294532.1	57.4	3.20E-11	347328	Pseudomonas phage M6		Pseudomonas aeruginosa	Caudovirales	Siphoviridae	dsDNA	
a10175.3 total counts: 723 Seed: 3 K: 25 length: 281	281	2.92171	gi 363539649 ref YP_004893934.1	179	6.22E-57	889338	Salmonella phage PVP-SE3		Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a151953.6 total counts: 4135 Seed: 4 K: 25 length: 711	711	2.91603	gi 418489116 ref YP_007002970.1	157	4.63E-47	37105	Lactobacillus phage LJC1032		Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a120732.4 total counts: 839 Seed: 6 K: 25 length: 244	244	2.89855	gi 1070101675 ref YP_009285855.1	65.5	2.38E-13	1844477	Pseudomonas phage Np1		Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA	
a60776.2 total counts: 837 Seed: 3 K: 25 length: 364	364	2.88736	gi 966203396 ref YP_009187713.1	120	1.49E-34	1719140	Klebsiella phage vB_KpnM KB57		Klebsiella pneumoniae	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a149322.3 total counts: 1310 Seed: 4 K: 25 length: 469	469	2.8742	gi 226377764 ref YP_002790813.1	103	1.70E-25	632112	Lactobacillus phage Lb338-1		Lactobacillus paracasei	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a58672.3 total counts: 2088 Seed: 3 K: 25 length: 747	747	2.87015	gi 4482444883 ref YP_007392603.1	123	5.97E-33	1116482	Pectobacterium phage phiTE		Pectobacterium atropiscium SCR11043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a29228.9 total counts: 864 Seed: 2 K: 25 length: 947	947	2.86546	gi 971746169 ref YP_009200249.1	149	6.97E-40	1636200	Pseudotolerothomas phage H103		Pseudotolerothomas marina	Proteobacteria; Bacteria	Caudovirales	unclassified	dsDNA
a34270.1 total counts: 855 Seed: 3 K: 25 length: 323	323	2.86538	gi 109528236 ref YP_05524609.1	101	2.61E-09	130969	Myoviridae phiPBI1		Myoviridae	Caudovirales	Myoviridae	dsDNA	
a16753.1 total counts: 844 Seed: 4 K: 25 length: 302	302	2.86387	gi 1068512776 ref YP_009276563.1	73.6	5.16E-36	1838062	Gordonia phage BriflB4		Gordonia terrae	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a72710.2 total counts: 497 Seed: 3 K: 25 length: 327	327	2.85321	gi 1070638959 ref YP_009303093.1	54.7	3.34E-09	1796994	Arthrobacter phage Barret Lemon		Arthrobacter phage ATCC 21022	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a46325.2 total counts: 919 Seed: 4 K: 25 length: 220	220	2.85	gi 947835102 ref YP_009177523.1	73.2	1.47E-16	1720495	Escherichia phage slur16		Escherichia coli	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a59368.20 total counts: 48740 Seed: 7 K: 25 length: 2470	2470	2.83945	gi 401723087 ref YP_006589997.1	100	2.06E-20	1136535	Burkholderia phage DC1		Burkholderia cepacia	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a116340.3 total counts: 783 Seed: 7 K: 25 length: 265	265	2.83774	gi 593777364 ref YP_009015209.1	101	1.21E-28	1162290	Cronobacter phage CR9		Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA	
a146541.4 total counts: 1219 Seed: 2 K: 25 length: 343	343	2.83544	gi 985757694 ref YP_009222820.1	196	2.17E-61	1573458	Pseudomonas phage PS-1		Pseudomonas sp. 1-1-lb	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a147444.3 total counts: 542 Seed: 2 K: 25 length: 212	212	2.82075	gi 110492609 ref YP_006907795.1	47	4.56E-07	1136731	Bacillus phage BPS13		Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA	
a36038.2 total counts: 482 Seed: 5 K: 25 length: 245	245	2.81522	gi 609217172 ref YP_008772005.1	50.4	2.50E-08	1406790	Bacillus phage Slash		Bacillus megaterium	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a147745.2 total counts: 436 Seed: 2 K: 25 length: 222	222	2.81013	gi 16271799 ref NP_438136.1	43.9	3.67E-06	173707	Temperate phage phiNH1.1		Streptococcus pyogenes	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a1021.4 total counts: 2718 Seed: 2 K: 25 length: 670	670	2.80909	gi 130546184 ref YP_008409778.1	55.1	4.47E-18	1304825	Mycobacterium phage Trouble		Mycobacterium phage	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a149357.2 total counts: 900 Seed: 2 K: 25 length: 416	416	2.80529	gi 173244220 ref YP_009352241.1	70.5	3.55E-14	2190828	Diabrotica virgifera virus 2		Diabrotica virgifera virgifera	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	
a9077.3 total counts: 833 Seed: 3 K: 25 length: 312	312	2.80473	gi 103454733 ref YP_002250409.1	105	6.33E-27	256009	Mycobacterium phage Myrma		Mycobacterium smegmatis str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a154028.3 total counts: 851 Seed: 2 K: 25 length: 308	308	2.79221	gi 448244788 ref YP_007392508.1	55.1	1.57E-10	1116482	Pectobacterium phage phiTE		Pectobacterium atropiscium SCR11043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a29590.8 total counts: 488 Seed: 3 K: 25 length: 290	290	2.76543	gi 971821357 ref YP_009208706.1	75.5	1.57E-16	1664247	Achromobacter phage phiAxp-3		Achromobacter xylosoxidans	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a244.4 total counts: 3220 Seed: 9 K: 25 length: 772	772	2.75926	gi 1068512872 ref YP_009276644.1	72.8	1.03E-13	1838062	Gordonia phage Bluebruey		Gordonia terrae	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a12231.2 total counts: 723 Seed: 2 K: 25 length: 368	368	2.74728	gi 966203619 ref YP_009188934.1	191	2.98E-60	1684115	Cronobacter phage PBES 02		Cronobacter sp.	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a2166.3 total counts: 570 Seed: 4 K: 25 length: 243	243	2.7426	gi 206599597 ref YP_002242036.1	44.3	2.24E-06	561996	Mycobacterium phage Bruitia		Mycobacterium smegmatis str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a148860.2 total counts: 904 Seed: 2 K: 25 length: 419	419	2.74107	gi 971757354 ref YP_009210861.1	47	4.28E-06	1647301	Mycobacterium phage Vincenzo		Mycobacterium smegmatis str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146509.3 total counts: 878 Seed: 2 K: 25 length: 312	312	2.73944	gi 9632906 ref NP_049935.1	49.3	2.65E-07	72638	Streptococcus phage Sf19		Streptococcus thermophilus	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a6034.2 total counts: 325 Seed: 3 K: 25 length: 229	229	2.73684	gi 643654753 ref YP_009035933.1	62	2.75E-12	1458711	Mycobacterium phage Hawkwey		Mycobacterium smegmatis str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146213.5 total counts: 919 Seed: 2 K: 25 length: 215	215	2.72093	gi 682123142 ref YP_009055463.1	76.3	2.21E-18	1505225	Acinetobacter phage YMC-13-01-C62		Acinetobacter baumannii	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a65753.2 total counts: 359 Seed: 2 K: 25 length													

a150732.2 total counts: 730 Seed: 4 K: 25 length: 327	327	2.45051	gi 966198655 ref YP_009188488.1	61.6	1.29E-11	1589270	Streptococcus phase Str-PAP-1	<i>Streptococcus parauberis</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA
a30131.2 total counts: 500 Seed: 3 K: 25 length: 300	300	2.44	gi 14087049 ref YP_006987236.1	47.4	8.74E-07	1141136	Cronobacter phase vB CsAm GAP32	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a93248.2 total counts: 493 Seed: 4 K: 25 length: 248	248	2.43145	gi 313768216 ref YP_004681896.1	105	2.68E-27	880161	<i>Micromonas</i> sp. RCV119 virus Mp11	<i>Micromonas pusilla</i>	Chlorophyta; Viridiplantae; Eukaryota	unclassified	Phycodnaviridae	dsDNA
a7992.2 total counts: 506 Seed: 2 K: 25 length: 248	248	2.42742	gi 2088933 ref FNP_624351.1	95.1	4.89E-24	1511847	<i>Fusarium solani</i> var. <i>T</i>	<i>Fusarium solani</i>	Ascomycota; Fungi; Eukaryota	unclassified	Partitiviridae	dsRNA
a147754.4 total counts: 1356 Seed: 5 K: 25 length: 347	347	2.42604	gi 1068358200 ref YP_009273941.1	63.9	2.15E-12	1838080	Gordonia phase Vendetta	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a94533.4 total counts: 673 Seed: 7 K: 25 length: 200	200	2.42568	gi 937456498 ref YP_009168424.1	79.7	1.27E-18	1622234	Citrobacter phase CVT22	<i>Citrobacter</i> sp. TM1552	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA
a89575.3 total counts: 1059 Seed: 2 K: 25 length: 328	328	2.42378	gi 282599097 ref YP_003359014.1	48.5	1.36E-07	665032	Delftia phase pHW-14	<i>Delftia acidovorans</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	ssRNA
a95022.2 total counts: 512 Seed: 2 K: 25 length: 312	312	2.42308	gi 104332878 ref YP_009259743.1	64.7	6.18E-13	1676182	<i>Chimpanzee faeces associated microphage 2</i> na	na	unclassified	Microviridae	dsDNA	
a31208.2 total counts: 431 Seed: 3 K: 25 length: 225	225	2.42222	gi 971848665 ref YP_009042132.1	47	6.13E-07	1466539	<i>Podovirus Lau218</i>	na	Caudovirales	Podoviridae	dsDNA	
a87520.2 total counts: 530 Seed: 3 K: 25 length: 263	263	2.40928	gi 971748533 ref YP_009202450.1	52.8	1.39E-09	1698711	Mycobacterium phase Lolly9	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a32291.2 total counts: 651 Seed: 2 K: 25 length: 291	291	2.39437	gi 338826843 ref YP_004678757.1	125	4.69E-34	1041524	Enterobacteria phase K30	<i>Escherichia</i> sp.	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a35855.2 total counts: 537 Seed: 4 K: 25 length: 252	252	2.39286	gi 509142181 ref YP_008061043.1	54.7	1.69E-09	1007869	Rhodococcus phase E5	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a906.7 total counts: 14133 Seed: 6 K: 25 length: 1980	1980	2.3758	gi 589893782 ref YP_00910491.1	59.7	3.12E-08	1485842	Geobacillus phase GBK2	<i>Geobacillus kaustophilus</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a62006.4 total counts: 686 Seed: 2 K: 25 length: 214	214	2.37383	gi 282598836 ref YP_003359159.1	49.7	6.70E-09	686439	Clavibacter phase CMP1	<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a87978.3 total counts: 685 Seed: 5 K: 25 length: 251	251	2.36255	gi 29135075 ref FNP_803705.1	48.1	2.75E-07	169683	Pseudomonas phase pHZK	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a122566.2 total counts: 607 Seed: 3 K: 25 length: 320	320	2.35925	gi 4085949 ref FNP_009262385.1	46.2	3.52E-06	1679878	<i>Acetabobacter castellani</i>	<i>Acetabobacter castellani</i>	Longamoebia; Amoebozoa; Eukaryota	unclassified	unclassified	unclassified
a87947.2 total counts: 443 Seed: 3 K: 25 length: 230	230	2.33918	gi 937456514 ref YP_009168440.1	47.8	1.93E-07	1622234	Citrobacter phase CVT22	<i>Citrobacter</i> sp. TM1552	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a67842.3 total counts: 594 Seed: 4 K: 25 length: 251	251	2.32407	gi 1102618170 ref YP_009321910.1	73.2	3.93E-16	1792274	Flavobacterium phase Fvp20	<i>Flavobacterium psychrophilum</i>	Bacteroidetes; Bacteria	unclassified	unclassified	unclassified
a151216.3 total counts: 567 Seed: 2 K: 25 length: 243	243	2.32099	gi 203454799 ref YP_002225115.1	60.5	6.17E-12	546805	Mycobacterium phase Myrma	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a117809.3 total counts: 563 Seed: 2 K: 25 length: 225	225	2.31566	gi 658310061 ref YP_009042253.1	102	1.71E-26	1327934	Cronobacter phase CR8	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a88403.2 total counts: 486 Seed: 2 K: 25 length: 290	290	2.31529	gi 937456498 ref YP_009168424.1	112	1.79E-29	1622234	Citrobacter phase CVT22	<i>Citrobacter</i> sp. TM1552	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a58070.3 total counts: 2596 Seed: 4 K: 25 length: 848	848	2.31486	gi 448244948 ref YP_007392668.1	216	5.42E-71	1116482	Pectobacterium phase phiTE	<i>Pectobacterium atropenseum</i> SCRI043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a146862.3 total counts: 1436 Seed: 3 K: 25 length: 465	465	2.31111	gi 157168378 ref YP_001456735.1	144	8.02E-42	28358	Cornebacterium phase BFK20	<i>[Brevibacterium] flavum</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a35231.3 total counts: 562 Seed: 4 K: 25 length: 248	248	2.28629	gi 448244878 ref YP_007392598.1	71.6	1.49E-15	1116482	Pectobacterium phase phiTE	<i>Pectobacterium atropenseum</i> SCRI043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a91292.2 total counts: 562 Seed: 2 K: 25 length: 301	301	2.28571	gi 593771139 ref YP_009014984.1	104	7.99E-28	1162290	Cronobacter phase pHKZ	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a35475.2 total counts: 405 Seed: 2 K: 25 length: 230	230	2.28261	gi 29135075 ref FNP_803862.1	63.9	7.48E-14	169683	Pseudomonas phase pHZK	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a58842.3 total counts: 703 Seed: 2 K: 25 length: 227	227	2.28194	gi 147800662 ref FNP_009349663.1	59.7	2.18E-11	1929510	<i>Enterovirus AN12</i>	<i>Bos taurus</i>	Chordata; Metazoa; Eukaryota	unclassified	Microviridae	ssRNA + dsDNA
a63607.2 total counts: 825 Seed: 2 K: 25 length: 394	394	2.27919	gi 985760870 ref YP_009226385.1	49.7	1.01E-07	129659	Paenibacillus phase Jimmer1	<i>Paenibacillus phase Jimmer1</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146574.4 total counts: 2199 Seed: 2 K: 25 length: 626	626	2.27473	gi 148750838 ref YP_001285880.1	150	2.25E-42	12348	Lactobacillus phase LL-H	<i>Gardonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a90322.3 total counts: 622 Seed: 4 K: 25 length: 229	229	2.26894	gi 966198576 ref YP_009188410.1	44.3	2.00E-06	164774	Gordonia phase GT16E	<i>Gardonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a147979.2 total counts: 377 Seed: 4 K: 25 length: 204	204	2.2598	gi 310831534 ref YP_003970177.1	53.9	1.42E-09	693272	<i>Cafeteria roenbergensis</i> virus B-PW1	<i>Cafeteria roenbergensis</i>	Bicosocida; Eukaryota	unclassified	Mimiviridae	dsDNA
a148421.2 total counts: 718 Seed: 4 K: 25 length: 409	409	2.25428	gi 593777612 ref YP_009015458.1	81.6	3.33E-18	1084719	Bacillus phase G	<i>Bacillus</i> sp.; <i>Geobacillus</i> sp.	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a87756.2 total counts: 535 Seed: 2 K: 25 length: 294	294	2.2487	gi 372449823 ref YP_005087046.1	155	1.15E-45	1109713	Rhodococcus phase REO2	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a120792.3 total counts: 859 Seed: 3 K: 25 length: 338	338	2.2426	gi 658310047 ref YP_009042239.1	157	3.09E-46	1327934	Cronobacter phase CR8	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a90837.2 total counts: 749 Seed: 2 K: 25 length: 402	402	2.24129	gi 589898964 ref YP_009005029.1	45.1	4.97E-06	1429768	Erwinia phase Ea35-7	<i>Erwinia amylovora</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a5407.2 total counts: 487 Seed: 3 K: 25 length: 261	261	2.23372	gi 203454799 ref YP_002225113.1	62.4	3.40E-13	546805	Mycobacterium phase Myrma	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a73295.2 total counts: 394 Seed: 2 K: 25 length: 218	218	2.22936	gi 589898919 ref YP_009004802.1	53.9	1.79E-09	1429768	Erwinia phase Ea35-7	<i>Erwinia amylovora</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a97454.2 total counts: 421 Seed: 2 K: 25 length: 232	232	2.22845	gi 985757311 ref YP_009222774.1	46.2	9.92E-07	1567484	Lactobacillus phase L1efn	<i>Lactobacillus fermentum</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a155663.2 total counts: 740 Seed: 2 K: 25 length: 417	417	2.21343	gi 906476413 ref YP_009160408.1	140	2.11E-41	1655661	<i>Mycobacterium delbrueckii</i> Fen719 21	na	Actinobacteria; Bacteria	unclassified	Microviridae	dsDNA
a21517.2 total counts: 365 Seed: 4 K: 25 length: 397	397	2.21256	gi 72015791 ref FNP_00915374.1	207	1.01E-07	194699	Paenibacillus phase vB AbAm Acibel004	<i>Paenibacillus baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a90635.3 total counts: 848 Seed: 5 K: 25 length: 312	312	2.20502	gi 11793741 ref FNP_958682.1	87.8	6.96E-21	194699	Bordetella phase BPP-1	<i>Bordetella bronchiseptica</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a115758.3 total counts: 526 Seed: 3 K: 25 length: 229	229	2.17419	gi 1068512709 ref YP_009276510.1	63.5	8.02E-13	1838066	Gordonia phase Cozz	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a630.5 total counts: 1097 Seed: 5 K: 25 length: 264	264	2.17045	gi 428782719 ref YP_007112472.1	58.5	2.16E-11	1147158	Enterobacterial phase mEp390	<i>Escherichia coli</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a147453.6 total counts: 4131 Seed: 4 K: 25 length: 675	675	2.16846	gi 971741645 ref YP_009196166.1	244	1.01E-78	1632621	Paenibacillus phase Veas	<i>Paenibacillus larvae</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a149767.2 total counts: 429 Seed: 2 K: 25 length: 260	260	2.16154	gi 363540041 ref YP_004894551.1	107	3.90E-28	1094892	<i>Megavirus chilensis</i>	<i>Megavirus chilensis</i>	Longamoebia; Amoebozoa; Eukaryota	unclassified	Mimiviridae	dsDNA
a147905.2 total counts: 452 Seed: 2 K: 25 length: 280	280	2.15714	gi 46401632 ref YP_006476.1	59.7	3.82E-11	10678	Enterobacteria phase P1	<i>Escherichia coli</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a89668.2 total counts: 489 Seed: 2 K: 25 length: 273	273	2.14286	gi 29134961 ref FNP_803591.1	66.6	1.39E-13	169683	Pseudomonas phase pHZK	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a1231.2 total counts: 571 Seed: 2 K: 25 length: 341	341	2.13818	gi 1068357955 ref YP_009273285.1	47	1.66E-06	1647740	Gordonia phase GMA1	<i>Gordonia mалаquae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a4768.2 total counts: 352 Seed: 3 K: 25 length: 243	243	2.12346	gi 906476306 ref YP_009160347.1	99	2.84E-25	1655649	<i>Gokushovirinae</i> BG5712_52	na	unclassified	Microviridae	ssDNA	
a99443.2 total counts: 420 Seed: 2 K: 25 length: 256	256	2.12109	gi 363540068 ref YP_004894539.1	56.6	2.74E-10	1094892	<i>Megavirus chilensis</i>	<i>Megavirus chilensis</i>	Longamoebia; Amoebozoa; Eukaryota	unclassified	Mimiviridae	dsDNA
a33766.2 total counts: 736 Seed: 4 K: 25 length: 404	404	2.11881	gi 5831013 ref FNP_009042329.1	174	3.70E-56	1327934	Cronobacter phase CR8	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a3153.3 total counts: 626 Seed: 3 K: 25 length: 311	311	2.11526	gi 107016066 ref YP_00921977.1	44.3	9.81E-06	192922	Citrobacter phase LZ35	<i>Actinomyces naslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a94289.3 total counts: 739 Seed: 2 K: 25 length: 257	257	2.10644	gi 151266296 ref YP_001333659.1	62.8	2.05E-12	1338473	Actinomyces phase Av-1	<i>Actinomyces naslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a151167.2 total counts: 478 Seed: 3 K: 25 length: 273	273	2.10256	gi 607840598 ref YP_00921847.1	61.6	6.34E-12	1234881	<i>Dragonfly cylovirus 3</i>	<i>Erythremis simplicicollis</i>	Arthropoda; Metazoa; Eukaryota	unclassified	Circoviridae	ssDNA
a2130.2 total counts: 374 Seed: 2 K: 25 length: 217	217	2.09924	gi 593774692 ref YP_009012312.1	90.5	4.54E-23	1074309	Mycobacterium phase BioEz	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a151570.2 total counts: 914 Seed: 7 K: 25 length: 393	393	2.07143	gi 589287448 ref YP_009010939.1	48.5	9.21E-07	1465618	Salmonella phase vB Sen5-Ent2	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Enteritidis</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a116535.3 total counts: 1708 Seed: 2 K: 25 length: 582	582	2.06701	gi 448244963 ref YP_007392683.1	145	5.42E-43	1116482	Pectobacterium phase phiTE	<i>Pectobacterium atropenseum</i> SCRI043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a157881.2 total counts: 336 Seed: 3 K: 25 length: 209	209	2.06699	gi 363540510 ref YP_004894822.1	57.8	6.12E-11	1094892	<i>Megavirus chilensis</i>	<i>Megavirus chilensis</i>	Longamoebia; Amoebozoa; Eukaryota	unclassified	Mimiviridae	dsDNA
a59635.4 total counts: 790 Seed: 2 K: 25 length: 231	231	2.06494	gi 226377831 ref YP_002790879.1	58.5	4.65E-11	631345	<i>Solenopsis invicta</i> virus 3	<i>Solenopsis invicta</i>	Arthropoda; Metazoa; Eukaryota	unclassified	unclassified	unclassified
a88597.2 total counts: 485 Seed: 2 K: 25 length: 294	294	2.04422	gi 971764182 ref YP_009217184.1	62.4	4							

a150626.4 total counts: 2102 Seed: 2 K: 25 length: 586	586	2	gi 971748726 ref YP_009202615.1	88.6	6.66E-20	1698356	Mycobacterium phage Phatniss	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a30887.3 total counts: 2539 Seed: 2 K: 25 length: 774	774	2	gi 58989373 ref YP_009010023.1	89.4	2.32E-19	1458846	Mycobacterium phage Rshv	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146282.4 total counts: 5091 Seed: 2 K: 25 length: 1297	1297	2	gi 764160062 ref YP_009124741.1	274	1.24E-86	1567475	Mycobacterium phage Bbano	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a119119.3 total counts: 778 Seed: 4 K: 25 length: 286	286	2	gi 49366632 ref YP_00769980.1	60.8	1.35E-11	1205710	Mycobacterium phage B ManS FF47	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a151831.3 total counts: 578 Seed: 2 K: 25 length: 228	228	2	gi 530546937 ref YP_008410526.1	61.6	1.79E-12	1340713	Mycobacterium phage Wanda	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a87667.2 total counts: 336 Seed: 2 K: 25 length: 219	219	2	gi 509141053 ref YP_008059937.1	44.7	2.96E-06	1327036	Mycobacterium phage WIVsmall	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a31358.5 total counts: 1878 Seed: 2 K: 25 length: 404	404	2	gi 985760367 ref YP_009225887.1	76.6	8.27E-17	1556290	Streptomyces phage Jai2Jay	<i>Streptomyces lividans</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146790.4 total counts: 3112 Seed: 6 K: 25 length: 754	754	2	gi 971765016 ref YP_009218009.1	63.9	8.38E-11	1755682	Streptomyces phage phiSAJ51	<i>Streptomyces avermitilis</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a152986.2 total counts: 486 Seed: 2 K: 25 length: 304	304	2	gi 326425096 ref YP_004286319.1	7.9	7.82E-20	981330	Tsukamurella phage TPA2	<i>Tsukamurella paurometabola</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a116252.1 total counts: 45454 Seed: 6 K: 25 length: 4300	4300	2	gi 22296541 ref NP_680501.1	62	4.31E-09	51369	Lactobacillus phase A2	Lactobacillus sp.	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a29349.2 total counts: 802 Seed: 4 K: 25 length: 384	384	2	gi 418489408 ref YP_007003225.1	47.4	2.32E-06	947980	Lactobacillus phase LF1	Lactobacillus sp.	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a147077.5 total counts: 4358 Seed: 2 K: 25 length: 855	855	2	gi 195661221 ref YP_002117689.1	47.8	8.41E-06	496874	Lactobacillus phase LRm1	<i>Lactococcus rhamnosus</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146940.5 total counts: 1211 Seed: 3 K: 25 length: 274	274	2	gi 571797865 ref YP_008772031.1	68.9	1.83E-14	1399941	Lactobacillus phase phiJB	<i>Lactobacillus delbrueckii</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a154177.3 total counts: 506 Seed: 3 K: 25 length: 226	226	2	gi 557307973 ref YP_008767295.1	78.2	5.30E-20	39103	Lactobacillus phase PNL-1	<i>Lactobacillus paracasei</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a87642.7 total counts: 5502 Seed: 2 K: 25 length: 856	856	2	gi 557307959 ref YP_008767281.1	123	2.69E-33	39103	Lactobacillus phase PNL-1	<i>Lactobacillus paracasei</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a149756.3 total counts: 714 Seed: 4 K: 25 length: 245	245	2	gi 323498623 ref YP_008320190.1	122	1.63E-39	102539	Lactococcus phase BM12	<i>Lactococcus lactis</i> subsp. <i>lactis</i> III403	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a127697.2 total counts: 395 Seed: 4 K: 25 length: 226	226	2	gi 526244846 ref YP_008320190.1	74.5	8.01E-06	1229751	Lactococcus phase BM13	<i>Lactococcus lactis</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a89897.3 total counts: 1259 Seed: 2 K: 25 length: 396	396	2	gi 66395809 ref YP_2401178.1	46.6	5.02E-06	320841	Staphylococcus phage EM	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a30153.7 total counts: 2236 Seed: 7 K: 25 length: 362	362	2	gi 971766715 ref YP_009219657.1	100	2.28E-26	1610872	Staphylococcus phage IWE-SA4	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a153673.2 total counts: 398 Seed: 4 K: 25 length: 203	203	2	gi 66394700 ref YP_240855.1	42.4	8.81E-06	320850	Staphylococcus phage X2	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a151174.4 total counts: 983 Seed: 4 K: 25 length: 267	267	2	gi 9632896 ref NP_049925.1	68.2	3.27E-15	72638	Streptococcus phage Sfi19	<i>Streptococcus thermophilus</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a155772.2 total counts: 737 Seed: 2 K: 25 length: 368	368	2	gi 32469465 ref NP_862873.1	63.9	2.80E-12	157924	Streptococcus phage SM1	<i>Streptococcus mitis</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146417.4 total counts: 1476 Seed: 2 K: 25 length: 408	408	2	gi 764162029 ref YP_009126425.1	108	7.12E-28	1561065	Paracoccus phage P PaMS IMPEP1	<i>Paracoccus marcusii</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a116451.5 total counts: 4758 Seed: 5 K: 25 length: 1031	1031	2	gi 1070101634 ref YP_009285814.1	92	2.14E-21	1844477	Pseudomonas phase NP	<i>Pseudomonas</i> sp.	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a145642.3 total counts: 725 Seed: 2 K: 25 length: 263	263	2	gi 1070101664 ref YP_009285844.1	80.1	7.76E-19	1844477	Pseudomonas phase NP1	<i>Pseudomonas</i> sp.	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a30328.3 total counts: 1021 Seed: 4 K: 25 length: 408	408	2	gi 1070101679 ref YP_009285859.1	118	4.27E-33	1844477	Pseudomonas phase NP1	<i>Pseudomonas</i> sp.	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a7418.5 total counts: 4292 Seed: 8 K: 25 length: 853	853	2	gi 1070101667 ref YP_009285847.1	262	2.14E-86	1844477	Pseudomonas phase NP1	<i>Pseudomonas</i> sp.	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a118866.3 total counts: 1884 Seed: 4 K: 25 length: 577	577	2	gi 971762591 ref YP_009215714.1	87.4	2.35E-19	178273	Pseudomonas phage PAE1	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a122622.3 total counts: 1093 Seed: 5 K: 25 length: 344	344	2	gi 374531258 ref YP_005098041.1	56.6	6.11E-10	1129145	Pseudomonas phage phi297	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a67923.2 total counts: 681 Seed: 4 K: 25 length: 318	318	2	gi 162135148 ref YP_001595889.1	63.9	1.73E-12	462590	Pseudomonas phage YUa	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a29473.2 total counts: 964 Seed: 2 K: 25 length: 471	471	2	gi 162135088 ref YP_001595829.1	62.4	3.14E-11	462590	Pseudomonas phage YUa	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a145784.8 total counts: 4517 Seed: 6 K: 25 length: 590	590	2	gi 472340490 ref YP_00764015.1	133	1.06E-35	754049	Vibrio phage pYD21-A	<i>Vibrio</i> sp. YD21	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a58882.3 total counts: 799 Seed: 4 K: 25 length: 306	306	2	gi 764162223 ref YP_009126593.1	96.3	7.07E-24	1538804	Vibrio phage VnKK5	<i>Vibrio parahaemolyticus</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a20767.2 total counts: 277 Seed: 2 K: 25 length: 205	205	2	gi 102619166 ref YP_009323093.1	58.2	2.92E-11	1883368	Synechococcus phage S-CAM7	<i>Synechococcus</i> sp.	Cyanobacteria; Bacteria	unclassified	unclassified	unclassified
a70564.3 total counts: 651 Seed: 2 K: 25 length: 228	228	2	gi 509140424 ref YP_008059305.1	50.4	3.02E-08	1273749	Halovirus HTGV-1	<i>Halogramma</i> sp. SSS-1	Eurarchaeota; Archaea	unclassified	unclassified	dsDNA
a147494.5 total counts: 2823 Seed: 4 K: 25 length: 627	627	2	gi 1068513125 ref YP_009276859.1	116	2.33E-30	1567487	Bacillus phage Ba1Mu-1	<i>Bacillus alcalophilus</i> ATCC 27647 = CGMCC 1.3604	Firmicutes; Bacteria	unclassified	unclassified	dsDNA
a146566.4 total counts: 819 Seed: 6 K: 25 length: 210	210	2	gi 163932187 ref YP_001642377.1	55.1	9.19E-11	139871	Lactobacillus johnsonii prophage Lj771	<i>Lactobacillus johnsonii</i>	Firmicutes; Bacteria	unclassified	unclassified	unclassified
a9208.3 total counts: 823 Seed: 3 K: 25 length: 295	295	2	gi 163932195 ref YP_001642385.1	45.8	3.69E-06	139871	Lactobacillus johnsonii prophage Lj771	<i>Lactobacillus johnsonii</i>	Firmicutes; Bacteria	unclassified	unclassified	unclassified
a151847.2 total counts: 406 Seed: 2 K: 25 length: 216	216	2	gi 1070639738 ref YP_009304106.1	120	2.78E-33	1718278	Bruceella phage BiPB01	<i>Bruceella inopinata</i>	Proteobacteria; Bacteria	unclassified	unclassified	unclassified
a4117.4 total counts: 1746 Seed: 4 K: 25 length: 498	498	2	gi 17463469 ref YP_009204029.1	185	2.58E-55	1632600	Pseudomonas marina	<i>Pseudomonas marina</i>	Proteobacteria; Bacteria	unclassified	unclassified	dsDNA
a119264.6 total counts: 4099 Seed: 5 K: 25 length: 723	723	2	gi 48101892 ref YP_007872778.1	53.5	2.50E-08	373173	Vibrio phage helene 12B3	<i>Vibrio splendidus</i>	Proteobacteria; Bacteria	unclassified	unclassified	dsDNA
a89109.5 total counts: 2537 Seed: 4 K: 25 length: 541	541	1.99333	gi 93007440 ref YP_579204.1	103	2.09E-25	370623	Streptomyces phage mu11	<i>Kitatospora aureoflava</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a61764.3 total counts: 1169 Seed: 2 K: 25 length: 378	378	1.98684	gi 149882819 ref YP_001294798.1	107	2.50E-28	446529	Microbacterium phage MinI6	<i>Microbacterium nematophilum</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a116831.4 total counts: 1468 Seed: 5 K: 25 length: 420	420	1.98305	gi 3292972735 ref YP_006488695.1	47.4	2.63E-06	1118063	Staphylococcus phage SpaA1	<i>Staphylococcus pasteurii</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a34480.2 total counts: 589 Seed: 2 K: 25 length: 356	356	1.97753	gi 448244966 ref YP_007392686.1	89.4	4.71E-23	1116482	Pectobacterium phage phiTE	<i>Pectobacterium atrosepticum</i> SCR1043	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a150691.2 total counts: 408 Seed: 3 K: 25 length: 228	228	1.96789	gi 151266296 ref YP_001333659.1	49.7	6.78E-08	338473	Actinomyces phage Av-1	<i>Actinomyces naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a87571.2 total counts: 502 Seed: 2 K: 25 length: 232	232	1.96552	gi 906476306 ref YP_009160347.1	116	1.68E-31	1655649	<i>Gokushovirinae</i> Bog5712_52	na	unclassified	Microviridae	ssDNA	
a159187.2 total counts: 584 Seed: 4 K: 25 length: 366	366	1.96104	gi 298103488 ref YP_003714730.1	71.6	6.13E-15	747763	Streptomyces phage phiSASD1	<i>Streptomyces avermitilis</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a149541.4 total counts: 2097 Seed: 4 K: 25 length: 524	524	1.96026	gi 971762571 ref YP_009215694.1	80.1	3.04E-18	1718273	Pseudomonas phage PAE1	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a116854.3 total counts: 570 Seed: 3 K: 25 length: 201	201	1.9602	gi 422957534 ref YP_007007739.1	65.9	1.02E-13	1127514	Aeromonas phage vB_ASA-M56	<i>Aeromonas salmonicida</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a29426.4 total counts: 3393 Seed: 5 K: 25 length: 879	879	1.96	gi 17449802 ref YP_005087076.1	117	4.48E-29	1199717	Rhodococcus phage RRH1	<i>Rhodococcus rhodochrous</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a148540.2 total counts: 364 Seed: 2 K: 25 length: 265	265	1.94717	gi 17449802 ref YP_005087076.1	117	4.48E-29	1199717	Rhodococcus phage RRH1	<i>Rhodococcus rhodochrous</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a88247.2 total counts: 437 Seed: 3 K: 25 length: 264	264	1.94156	gi 966198577 ref YP_009193294.1	56.2	4.90E-10	1474887	Autonococcus phage Refokovirus	<i>Autonococcus amphigeniferens</i>	Actinobacteria; Bacteria	unclassified	Phycodnaviridae	dsDNA
a32896.3 total counts: 834 Seed: 6 K: 25 length: 310	310	1.93793	gi 192824184 ref YP_001994825.1	53.9	6.00E-09	540068	Mycobacterium phage Pukovnik	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a95332.2 total counts: 393 Seed: 3 K: 25 length: 216	216	1.93716	gi 971745058 ref YP_009199262.1	44.3	3.95E-06	690817	Streptomyces phage SFIH	<i>Streptomyces lividans</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a147319.5 total counts: 4372 Seed: 2 K: 25 length: 958	958	1.93293	gi 9633052 ref NP_050160.1	129	2.57E-32	12417	Lactobacillus phage phiAD	<i>Lactobacillus gasseri</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a58114.4 total counts: 1409 Seed: 5 K: 25 length: 385	385	1.92949	gi 764159561 ref YP_009124300.1	46.2	5.09E-06	1536603	Mycobacterium phage Estavel	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a148590.2 total counts: 421 Seed: 2 K: 25 length: 216	216	1.92949	gi 162135108 ref YP_001595849.1	125	1.04E-34	462590	Pseudomonas phage YUa	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a125126.3 total counts: 637 Seed: 3 K: 25 length: 218	218	1.92202	gi 985757280 ref YP_009222243.1	66.2	8.18E-14	1567484	Lactobacillus phase Lfelnf	<i>Lactobacillus fermentum</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a120378.2 total counts: 408 Seed: 3 K: 25 length: 232	232	1.91139	gi 937456462 ref YP_009168387.1	105	9.12E-28	1622234	Citrobacter phage CVT22	<i>Citrobacter</i> sp. TM1552	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a7292.3 total counts: 998 Seed: 5 K: 25 length: 352	352	1.91083	gi 1070098399 ref YP_009281132.1	62.8	6.93E-12	1887647	Gordonia phage Ghobe	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a147273.4 total counts: 2465 Seed: 4 K: 25 length: 618	618	1.91083	gi 1070101668 ref YP_009285848.1	191	6.84E-62	1844477	Pseudomonas phase NP1	<i>Pseudomonas</i> sp.	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a61414.6 total counts: 1937 Seed: 6 K: 25 length: 356	356	1.90351	gi 44826642 ref YP_007348345.1	53.7	1.09E-09	1264737	Cronobacter phage vB_GSK_CAP227	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a1047384.7 total counts: 368 Seed: 6 K: 25 length: 1012	1012	1.89937	gi 7479938 ref YP_009201029.1	86.2	2.43E-21	1674382	Citrobacter phage CVT22	<i>Mycobacterium smegmatis</i> str. MC2 155	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a62335.2 total counts: 1367 Seed: 2 K: 25 length: 245	245	1.89388	gi 556693138 ref YP_164725.1	63.6	9.00E-21	298338	Lactobacillus phage LP65	<i>Lactobacillus plantarum</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a8976.2 total counts: 428 Seed: 3 K: 25 length: 232												

a147032.4 total counts: 2138 Seed: 3 K: 25 length: 573	573	1.75194	gi 1068359285 ref YP_009275707.1	87	9.94E-20	1622193	Gordonia phage Gspu11	<i>Gordonia sputi</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a115875.2 total counts: 873 Seed: 2 K: 25 length: 442	442	1.75	gi 1498828405 ref YP_001294819.1	51.2	6.35E-08	446529	Microbacterium phage MinI	<i>Microbacterium nematophilum</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a88076.5 total counts: 2208 Seed: 6 K: 25 length: 460	460	1.75	gi 29566753 ref NP_818317.1	162	5.40E-08	205879	Mycobacterium phage Omega	<i>Mycobacterium sp.</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a88739.6 total counts: 2738 Seed: 12 K: 25 length: 457	457	1.75	gi 22258026 ref NP_690786.1	59.7	2.91E-11	10717	Bacillus phage phi105	<i>Bacillus subtilis</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a118768.3 total counts: 1047 Seed: 7 K: 25 length: 347	347	1.74419	gi 206600214 ref YP_002241820.1	90.9	3.44E-23	56123	Mycobacterium phage Ramsey	<i>Mycobacterium smegmatis str. MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a119050.10 total counts: 6075 Seed: 15 K: 25 length: 629	629	1.74419	gi 59378368 ref YP_009162028.1	84	5.17E-18	691963	Rhodococcus phage Rea1PinE5	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a61291.3 total counts: 674 Seed: 2 K: 25 length: 236	236	1.74153	gi 388570814 ref YP_006383797.1	45.4	2.00E-06	118128	Acinetobacter bacteriophage AP22	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a29416.1 total counts: 128119 Seed: 2 K: 25 length: 6724	6724	1.73988	gi 1068512423 ref YP_009276106.1	271	1.43E-78	1647473	Gordonia phage GRU3	<i>Gordonia malakae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a29962.5 total counts: 3288 Seed: 7 K: 25 length: 648	648	1.73563	gi 563398140 ref YP_008857900.1	77.4	6.86E-16	1414742	Arthrobacter phage vB ArS-ARv2	<i>Arthrobacter sp.</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a150174.3 total counts: 1110 Seed: 2 K: 25 length: 426	426	1.73563	gi 9635029 ref NP_056694.1	80.5	1.15E-17	78541	Streptococcus phage Sfi11	<i>Streptococcus sp.</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a116838.3 total counts: 618 Seed: 2 K: 25 length: 262	262	1.72519	gi 448244890 ref YP_007392610.1	45.1	1.73E-06	1116482	Pectobacterium phage phiTE	<i>Pectobacterium atrosepticum SCRI1043</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a88657.2 total counts: 472 Seed: 2 K: 25 length: 273	273	1.71759	gi 1068357954 ref YP_009273284.1	79.3	4.24E-18	1647470	Gordonia phage GMA1	<i>Gordonia malakae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146554.3 total counts: 1817 Seed: 3 K: 25 length: 623	623	1.71591	gi 11792888 ref NP_958506.1	85.1	1.20E-18	139872	Lactobacillus prophage IJ928	<i>Lactobacillus johnsonii</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a73400.2 total counts: 365 Seed: 2 K: 25 length: 206	206	1.71341	gi 971748040 ref NP_009201826.1	77	1.00E-19	1739608	Lactobacillus phage CL2	<i>Lactobacillus paracasei</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a116936.2 total counts: 396 Seed: 2 K: 25 length: 244	244	1.71311	gi 158079460 ref NP_001504273.1	51.6	8.20E-09	442495	Enterococcus phage phiEFT24C	<i>Enterococcus faecalis</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a149390.2 total counts: 192 Seed: 2 K: 25 length: 210	210	1.71191	gi 107015036 ref NP_009290193.1	57.8	2.85E-24	18376	Weissella phage phi230	<i>Weissella sp.</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a124699.3 total counts: 522 Seed: 3 K: 25 length: 220	220	1.70455	gi 74589294 ref YP_009035508.1	57.6	8.20E-11	1416334	Lactobacillus phage phi Jh1	<i>Lactobacillus gasseri ADH</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a15593.2 total counts: 633 Seed: 4 K: 25 length: 307	307	1.68404	gi 643217946 ref YP_009036156.1	98.2	9.92E-25	1486659	Bacillus phage Hakuna	<i>Bacillus thuringiensis</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a8778.2 total counts: 444 Seed: 3 K: 25 length: 266	266	1.67039	gi 589287594 ref YP_009010198.1	62	5.37E-12	1401669	Erwinia phage PhiEaH1	<i>Erwinia amylovora</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a118240.2 total counts: 479 Seed: 2 K: 25 length: 272	272	1.66544	gi 313768166 ref YP_004061597.1	45.1	5.39E-06	880159	<i>Bathyococcus sp. RCC1105 virus Bp17</i>	<i>Bathyococcus prasinos RCC1105</i>	Chlorophyta; Viridiplantae; Eukaryota	unclassified	Phycodnaviridae	dsDNA
a88367.5 total counts: 3217 Seed: 4 K: 25 length: 612	612	1.66013	gi 764162223 ref YP_009126593.1	200	5.15E-60	1538804	Vibrio phage VpKk5	<i>Vibrio parahaemolyticus</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a87757.8 total counts: 9851 Seed: 3 K: 25 length: 1279	1279	1.65934	gi 970580046 ref YP_009193590.1	118	6.89E-31	1572743	Mannheimia phage vB MhS_535AP2	<i>Mannheimia haemolytica</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a3464.2 total counts: 448 Seed: 4 K: 25 length: 321	321	1.65421	gi 9703777360 ref YP_009015205.1	60.8	1.17E-11	1162290	Cronobacter phage CR9	<i>Cronobacter sakazakii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a2900.3 total counts: 631 Seed: 2 K: 25 length: 257	257	1.65027	gi 151266300 ref YP_001333663.1	77.4	1.62E-17	338473	Actinomyces phage Av-1	<i>Actinomyces naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a119791.7 total counts: 4210 Seed: 2 K: 25 length: 595	595	1.64964	gi 157325017 ref YP_001468443.1	100	1.16E-26	40522	Listeria phage A500	<i>Listeria monocytogenes WSLC1042</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a31868.3 total counts: 2017 Seed: 5 K: 25 length: 703	703	1.64835	gi 1070619841 ref YP_009304275.1	74.7	1.34E-14	1821557	Gordonia phage LuckV10	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a64513.3 total counts: 687 Seed: 2 K: 25 length: 237	237	1.64481	gi 971748533 ref YP_009202450.1	48.1	5.41E-08	1698711	Mycobacterium phage LolyV9	<i>Mycobacterium smegmatis str. MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a149411.3 total counts: 766 Seed: 2 K: 25 length: 252	252	1.64286	gi 414090422 ref NP_006990535.1	44.3	5.88E-06	204534	Clostridium phage phiAMPQ2	<i>Clostridium difficile</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a66453.4 total counts: 996 Seed: 5 K: 25 length: 285	285	1.63243	gi 971751675 ref YP_009205624.1	93.6	4.30E-23	1175662	Pseudomonas phage PAmX42	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a155141.2 total counts: 477 Seed: 2 K: 25 length: 306	306	1.63072	gi 909592623 ref YP_529883.1	160	1.56E-46	363555	Lactobacillus phage KCS5a	<i>Lactobacillus gasseri</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a146813.3 total counts: 3069 Seed: 9 K: 25 length: 926	926	1.62703	gi 526177379 ref YP_008241215.1	185	1.04E-52	327983	Cellulophaga phage phi18.3	<i>Cellulophaga baltica</i>	Bacteroidetes; Bacteria	Caudovirales	Podoviridae	dsDNA
a96090.3 total counts: 507 Seed: 2 K: 25 length: 213	213	1.62385	gi 151266296 ref YP_001333659.1	64.3	3.94E-13	338473	Actinomyces phage AV-1	<i>Actinomyces naeslundii</i>	Actinobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a148252.4 total counts: 837 Seed: 3 K: 25 length: 245	245	1.61983	gi 1070096285 ref YP_009277923.1	59.7	2.12E-11	1887651	Gordonia phage Nvceira	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a119564.3 total counts: 930 Seed: 3 K: 25 length: 345	345	1.60963	gi 1070101647 ref YP_009285827.1	151	4.09E-44	1844477	Pseudomonas phage NP1	<i>Pseudomonas sp.</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a10171.2 total counts: 387 Seed: 2 K: 25 length: 213	213	1.60932	gi 338826838 ref YP_004678752.1	110	2.04E-29	1041524	Enterobacteria phage K30	<i>Escherichia sp.</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a14779.2 total counts: 342 Seed: 3 K: 25 length: 221	221	1.60633	gi 971763757 ref YP_009216768.1	45.4	1.56E-06	1701405	<i>Gokoshovirinae GNX39</i>	na	unclassified	Microviridae	ssDNA	
a1742.2 total counts: 564 Seed: 4 K: 25 length: 292	292	1.59788	gi 593779801 ref YP_009017628.1	119	4.07E-32	691965	Rhodococcus phage Rea1Pevp6	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146533.5 total counts: 4704 Seed: 2 K: 25 length: 977	977	1.59259	gi 372449804 ref YP_005087028.1	180	4.15E-53	1109717	Rhodococcus phage RRH1	<i>Rhodococcus rhodochrous</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a14624.4 total counts: 3208 Seed: 5 K: 25 length: 758	758	1.58421	gi 849250882 ref YP_009147716.1	125	4.80E-33	1560313	Lactococcus phage WRP2	<i>Lactococcus lactis</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a35573.3 total counts: 829 Seed: 3 K: 25 length: 285	285	1.58182	gi 14030219 ref NP_009297878.1	94.7	1.870E-25	183817	Propionibacterium phage PFR1	<i>Propionibacterium Freudenreichii</i>	Firmicutes; Bacteria	Caudovirales	unclassified	unclassified
a150311.2 total counts: 402 Seed: 2 K: 25 length: 220	220	1.57592	gi 163932155 ref YP_001462345.1	47.9	9.66E-08	139871	Lactobacillus johnsonii prophage Lj771	<i>Lactobacillus johnsonii</i>	Firmicutes; Bacteria	Caudovirales	unclassified	unclassified
a59363.5 total counts: 3352 Seed: 10 K: 25 length: 636	636	1.57447	gi 1068358199 ref YP_009273940.1	151	2.89E-42	1838080	Gordonia phage Vendetta	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a839.6 total counts: 1702 Seed: 2 K: 25 length: 318	318	1.55862	gi 48697428 ref YP_024813.1	72.4	2.26E-15	279303	Actinoplanes phage phiAsp2	<i>Actinoplanes sp. SN223/29</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a89492.2 total counts: 518 Seed: 4 K: 25 length: 241	241	1.54772	gi 563397579 ref YP_008857343.1	81.3	4.79E-20	1391428	Enterobacteria phage 4MG	<i>Escherichia coli K-12</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a88549.2 total counts: 594 Seed: 2 K: 25 length: 296	296	1.54138	gi 939536933 ref YP_009168408.1	61.2	1.82E-13	1622234	Citrobacter phage CVT22	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a98953.2 total counts: 444 Seed: 2 K: 25 length: 292	292	1.53082	gi 971752893 ref YP_009206096.1	48.5	3.81E-07	1690456	Bacillus phage TsrBomba	<i>Bacillus sp.</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a122345.2 total counts: 557 Seed: 3 K: 25 length: 260	260	1.53017	gi 937456522 ref YP_009168448.1	73.2	1.07E-16	1622234	Citrobacter phage CVT22	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a146785.2 total counts: 434 Seed: 2 K: 25 length: 234	234	1.52632	gi 418489112 ref YP_007002966.1	62.8	2.80E-13	37105	Lactobacillus phage JCL1032	<i>Lactobacillus delbrueckii subsp. lactis</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a2_3069 total counts: 35374508 Seed: 6 K: 25 length: 11549	11549	1.52282	gi 712916232 ref YP_009103975.1	271	2.98E-75	1161939	Enterococcus phage vB Efm30F-4	<i>Enterococcus faecium</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA
a159472.2 total counts: 356 Seed: 2 K: 25 length: 215	215	1.51256	gi 1068511238 ref YP_009273343.1	76.3	2.43E-17	164776	Tsuukamurella phage TP04	<i>Tsuukamurella paurometabola</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a90116.2 total counts: 419 Seed: 2 K: 25 length: 241	241	1.46829	gi 588592080 ref YP_009033431.1	44.3	5.98E-06	1392231	Streptococcus phage TAA6	<i>Streptococcus thermophilus DSM 20617</i>	Firmicutes; Bacteria	Caudovirales	unclassified	unclassified
a117786.2 total counts: 734 Seed: 3 K: 25 length: 328	328	1.46638	gi 29566753 ref NP_818317.1	115	1.02E-30	205879	Mycobacterium phage Omega	<i>Mycobacterium sp.</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a64062.2 total counts: 761 Seed: 2 K: 25 length: 345	345	1.43478	gi 107016098 ref YP_009291949.1	97.8	2.93E-24	1792222	Acinetobacter phage LZ35	<i>Acinetobacter baumannii</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a58823.3 total counts: 507 Seed: 3 K: 25 length: 211	211	1.43128	gi 56393176 ref YP_00164763.1	90.9	1.10E-23	298338	Lactobacillus phage LP65	<i>Lactobacillus plantarum</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a150645.4 total counts: 1468 Seed: 2 K: 25 length: 368	368	1.42587	gi 589286930 ref YP_009007125.1	47	1.62E-06	1494937	Pseudomonas phage vB PaeP_Tf60_Ah31	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	unclassified	unclassified
a30269.2 total counts: 378 Seed: 2 K: 25 length: 201	201	1.41294	gi 56693142 ref YP_009164729.1	64.7	2.48E-13	298338	Lactobacillus phage LP65	<i>Lactobacillus plantarum</i>	Firmicutes; Bacteria	Caudovirales	Myoviridae	dsDNA
a132959.2 total counts: 344 Seed: 3 K: 25 length: 214	214	1.41121	gi 363540839 ref YP_004894409.1	51.2	1.65E-08	1094892	<i>Megavirus chilensis</i>	na	Longaeomobilia; Amoebozoa; Eukaryota	unclassified	Mimiviridae	dsDNA
a100241.2 total counts: 326 Seed: 2 K: 25 length: 200	200	1.41	gi 906476402 ref YP_009160402.1	49.7	4.41E-08	1655660	<i>Microviridae Fen7895 21</i>	na	unclassified	Microviridae	ssDNA	
a152403.3 total counts: 578 Seed: 3 K: 25 length: 214	214	1.40476	gi 238801907 ref YP_009225110.1	60.1	1.35E-11	646413	Streptococcus phage 5093	<i>Streptococcus thermophilus</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a149773.2 total counts: 397 Seed: 2 K: 25 length: 215	215	1.40465	gi 848469534 ref YP_009145910.1	101	4.31E-27	1220714	Rhizobium phage RHEaP06	<i>Rhizobium etli GR56</i>	Proteobacteria; Bacteria	Caudovirales	Myoviridae	dsDNA
a31.6 total counts: 1996 Seed: 2 K: 25 length: 354	354	1.40464	gi 472340490 ref YP_00764015.1	91.3	6.52E-22	754049	Vibrio phage vYD21-A	<i>Vibrio sp. YD21</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a30650.2 total counts: 350 Seed: 4 K: 25 length: 214	214	1.39965	gi 973456504 ref YP_009168430.1	72.4	5.58E-16	222224	Citrobacter phage CVT22	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a147551.4 total counts: 934 Seed: 5 K: 25 length: 275	275	1.38406	gi 10707088374 ref YP_009281105.1	118	1.95E-33	1887467	Gordonia phage Ghobes	<i>Gordonia terrae</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a610264 total counts: 459 Seed: 2 K: 25 length: 201	201	1.38308	gi 29566753 ref NP_818317.1	115	1.02E-30	205879	Mycobacterium phage Omega	<i>Mycobacterium sp.</i>	Firmicutes; Bacteria	Caudovirales	unclassified	unclassified
a60343.2 total counts: 476 Seed: 3 K: 25 length: 215	215	1.381										

a58124:1283 total counts: 2159833 Seed: 5 K: 25 length: 1707	1707	1	gi 66395192 ref YP_239479.1	160	1.08E-42	320832	Staphylococcus phage 66	<i>Staphylococcus aureus</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA
a5806:2 total counts: 572 Seed: 3 K: 25 length: 293	293	1	gi 966198655 ref YP_009188488.1	57.4	3.10E-10	1589270	Streptococcus phage Str-PAP-1	<i>Streptococcus parauberis</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA
a145743:2 total counts: 471 Seed: 2 K: 25 length: 262	262	1	gi 937456458 ref YP_009168383.1	63.2	4.86E-13	1622234	Citrobacter phage CVT22	<i>Citrobacter sp. TM1552</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a154779:3 total counts: 942 Seed: 3 K: 25 length: 323	323	1	gi 41057303 ref NP_958202.1	62.8	7.36E-13	10761	Shiella phage Sif6	<i>Shiella fleisneri</i>	Proteobacteria; Bacteria	Caudovirales	Podoviridae	dsDNA
a62341:3 total counts: 1220 Seed: 4 K: 25 length: 422	422	1	gi 338826904 ref YP_004678817.1	50.1	4.09E-07	981323	Gordonia phage GTE2	<i>Nocardia; Rhodococcus; Gordonia</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a147877:3 total counts: 1313 Seed: 4 K: 25 length: 440	440	1	gi 530546920 ref YP_008410509.1	63.2	1.22E-12	1052673	Mycobacterium phage Thibault	<i>Mycobacterium smegmatis str. MC2 155</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a119828:3 total counts: 1364 Seed: 5 K: 25 length: 451	451	1	gi 372449813 ref YP_005087037.1	134	4.40E-37	1109717	Rhodococcus phage RRH1	<i>Rhodococcus rhodochrous</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a63661:2 total counts: 479 Seed: 2 K: 25 length: 301	301	1	gi 764162114 ref YP_009126495.1	62	1.20E-12	1552735	Lactobacillus phage Ldl1	<i>Lactobacillus delbrueckii subsp. lactis</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a151973:2 total counts: 631 Seed: 5 K: 25 length: 306	306	1	gi 327198366 ref YP_004306940.1	67.8	7.10E-14	59241	Streptococcus phage Dp-1	<i>Streptococcus pneumoniae</i>	Firmicutes; Bacteria	Caudovirales	Siphoviridae	dsDNA
a146202:6 total counts: 3699 Seed: 5 K: 25 length: 635	635	1	gi 146329916 ref YP_001210224.1	240	2.19E-76	437329	Burkholderia phage BcepGomr	<i>Burkholderia cepacia</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a62462:5 total counts: 1641 Seed: 3 K: 25 length: 339	339	1	gi 399528722 ref YP_006560759.1	92.8	1.33E-22	1132026	Burkholderia phage KL1	<i>Burkholderia cenocepacia</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a147376:5 total counts: 1753 Seed: 7 K: 25 length: 376	376	1	gi 62362234 ref YP_224159.1	118	2.71E-31	101570	Enterobacteria phage ES18	<i>Salmonella enterica subsp. enterica serovar Typhimurium</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a29643:2 total counts: 737 Seed: 2 K: 25 length: 321	321	1	gi 1070106097 ref YP_009291948.1	144	6.53E-42	1792222	Pseudomonas phage NP1	<i>Pseudomonas sp.</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a11418:2 total counts: 484 Seed: 3 K: 25 length: 227	227	1	gi 162135148 ref YP_001595889.1	114	5.43E-31	462590	Pseudomonas phage YuA	<i>Pseudomonas aeruginosa</i>	Proteobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a117147:6 total counts: 3442 Seed: 3 K: 25 length: 595	595	1	gi 372450021 ref YP_005087242.1	168	1.12E-48	1109714	Rhodococcus phage REO3	<i>Rhodococcus hoagii</i>	Actinobacteria; Bacteria	unclassified	unclassified	unclassified

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a332225:2 total counts: 397 Seed: 2 K: 25 length: 219	219	2.38813	gi 1068512422 ref YP_009276105.1	70.1	3.92E-15	1647473	<i>Gordonia phage GRU3</i>	<i>Gordonia rubripertincta</i>	Actinobacteria; Bacteria	Caudovirales	Siphoviridae	dsDNA
a18498:2 total counts: 429 Seed: 2 K: 25 length: 236	236	2	gi 712916216 ref YP_009103959.1	58.9	3.34E-11	1161939	<i>Enterococcus phage vB_Efae230P-4</i>	<i>Enterococcus faecium</i>	Firmicutes; Bacteria	Caudovirales	Podoviridae	dsDNA

Supplementary Table 2 - Detection of *M. quadrifasciata* viruses in stingless bee samples: samples of healthy (H) and unhealthy bees (U) of four different meliponaries in Southern of Brazil (BL = Boqueirão do Leão, BP = Bom Principio and RL = Rolante) were tested for virus presence/absence via PCR/RT-PCR. The + symbol means positive samples for the detection of the respective virus. Viruses with DNA genomes were only tested for detection in samples from which the DNA was extracted, the same for RNA viruses in samples from which the RNA was extracted (cells in gray were not tested).

Locality	Year	Syndrome period	Health	Sample type	MqC	MqC1	MqC2	MqD	MqV1a	MqV1b	MqV2
BL	2014	During	U	DNA whole body	+	+					
BL	2014	During	U	DNA whole body	+	+					
BL	2014	During	U	DNA whole body	+	+					
BL	2014	During	U	DNA whole body	+	+	+				
BL	2014	During	U	DNA whole body	+	+					
BL	2014	During	U	DNA whole body		+					
BL	2014	During	U	DNA whole body		+					
BL	2014	During	U	DNA whole body	+						
BL	2014	During	U	DNA whole body	+	+		+			
BL	2014	During	U	DNA whole body	+	+					
BL	2014	During	U	DNA whole body							
BL	2014	During	U	DNA whole body							
BL	2014	During	U	DNA whole body		+					
BL	2014	During	U	DNA whole body		+					
BL	2014	During	U	DNA whole body							
BL	2015	During	U	DNA whole body			+	+			
BL	2015	During	U	DNA whole body			+	+			
BL	2015	During	U	DNA whole body	+	+	+				
BL	2015	During	U	DNA whole body	+	+	+				
BL	2015	During	U	DNA whole body	+	+		+			
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body		+		+			
BL	2015	During	U	DNA whole body		+		+			
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+	+					
BL	2015	During	U	DNA whole body	+			+			
BL	2015	During	U	DNA whole body	+			+			
BL	2015	During	H	DNA whole body	+	+					
BL	2015	During	H	DNA whole body				+			
BL	2015	During	H	DNA whole body	+	+	+				
BL	2015	During	H	DNA whole body		+		+			
BL	2015	During	H	DNA whole body		+		+			
BL	2015	During	H	DNA whole body	+						
BL	2015	During	H	DNA whole body	+	+					
BL	2015	During	H	DNA whole body	+						
BL	2015	During	H	DNA whole body							
BL	2015	During	H	DNA whole body		+		+			
BL	2015	During	H	DNA whole body		+		+			
BL	2015	During	H	DNA whole body	+	+	+				

Supplementary Table 3 - Tests for the choice of BLAST type to be used for the retrieval and taxonomy of contigs. A sub-sample of contigs (49 contigs of unhealthy bees RNA viroma) was used in the blastP (tab 1) and blastX tests (tab 2), both against the nr database and against the viral RefSeq. In red are highlighted the candidate viral contigs used in the study.

Translated contig (amino acids) query acc.ver	BlastP against nr				BlastP against RefSeq							
	subject acc.ver	name	% identity	alignment length	evaluate	bit score	subject acc.ver	name	% identity	alignment length	evaluate	bit score
115738_6_total_counts: 2739.Seed: 8_K: 25.Length: 448.translasyon_frame_3_reversed	YP_009337019.1	hypothetical protein 1 [Hubei picorna-like virus 74]	36.4	143	3.64E-19	93.6	YP_009337019.1	hypothetical protein 1 [Hubei picorna-like virus 74]	36.4	143	4.19E-22	93.6
115755_8_total_counts: 8948.Seed: 6_K: 25.Length: 1124.translasyon_frame_1	YP_009342254.1	hypothetical protein 1 [Wuhan arthropod virus 3]	34.9	295	6.73E-43	170	YP_009342254.1	hypothetical protein 1 [Wuhan arthropod virus 3]	34.9	295	7.76E-46	170
115910_13_total_counts: 77920.Seed: 2_K: 25.Length: 5913.translasyon_frame_2	APG76667.1	hypothetical protein 1 [Wuhan insect virus 11]	45.7	870	0.00E+00	731	YP_009342327.1	hypothetical protein 1 [Wuhan insect virus 11]	45.9	870	0.00E+00	723
117633_5_total_counts: 2738.Seed: 6_K: 25.Length: 598.translasyon_frame_2	YP_009337788.1	hypothetical protein [Hubei picorna-like virus 68]	37.9	190	9.68E-24	108	YP_009337788.1	hypothetical protein [Hubei picorna-like virus 68]	37.9	190	1.12E-26	108
118987_6_total_counts: 1591.Seed: 4_K: 25.Length: 287.translasyon_frame_3	YP_009336558.1	hypothetical protein 2 [Hubei orthoptera virus 1]	28.7	87	4.70E-07	56.6	YP_009336558.1	hypothetical protein 2 [Hubei orthoptera virus 1]	28.7	87	5.42E-10	56.6
119065_2_total_counts: 518.Seed: 2_K: 25.Length: 276.translasyon_frame_3	BAW18990.1	hypothetical protein [Raltosmia phage RP12]	38.9	90	7.70E-16	79.7	YP_009207816.1	hypothetical protein [Raltosmia phage RSF1]	30.5	82	1.85E-06	46.2
119732_5_total_counts: 1999.Seed: 10_K: 25.Length: 391.translasyon_frame_1_reversed	YP_009333576.1	hypothetical protein 1 [Beihai picorna-like virus 121]	38.8	116	1.08E-15	82.8	YP_009333576.1	hypothetical protein 1 [Beihai picorna-like virus 121]	38.8	116	1.25E-18	82.8
121873_2_total_counts: 283.Seed: 2_K: 25.Length: 200.translasyon_frame_1	YP_002790880.2	putative structural polyprotein [Solenopsis invicta virus 3]	39.4	66	1.12E-05	51.2	YP_002790880.2	putative structural polyprotein [Solenopsis invicta virus 3]	39.4	66	1.29E-08	51.2
145575_124_total_counts: 517623.Seed: 5_K: 25.Length: 4184.translasyon_frame_1	ASM93489.1	NS1 [Lupine feces-associated densovirus 2]	37.1	510	2.32E-94	323	YP_009256211.1	putative nonstructural protein NS1 [Diaphorina citri densovirus]	31.8	453	1.66E-49	190
145575_124_total_counts: 517623.Seed: 5_K: 25.Length: 4184.translasyon_frame_2_reversed	ASM93488.1	VP1 [Lupine feces-associated densovirus 2]	34.5	548	7.41E-44	180	YP_009256212.1	putative structural protein [Diaphorina citri densovirus]	26.6	173	5.49E-06	53.1
147897_7_total_counts: 2386.Seed: 2_K: 25.Length: 366.translasyon_frame_1_reversed	SCDD_C	Chain C, Structural Polyprotein, VP2 [IAPV]	36.2	105	9.07E-24	100	NP_066242.1	capsid protein [Acute bee paralysis virus]	39.0	105	9.84E-26	102
149357_2_total_counts: 908.Seed: 2_K: 25.Length: 416.translasyon_frame_2	YP_009352233.1	polyprotein [Diabrotica virgifera virgifera virus 2]	29.3	133	3.93E-16	84.3	YP_009352233.1	polyprotein [Diabrotica virgifera virgifera virus 2]	29.3	133	4.53E-19	84.3
149871_4_total_counts: 1261.Seed: 2_K: 25.Length: 337.translasyon_frame_3_reversed	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	31.4	105	7.15E-06	53.9	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	31.4	105	8.24E-09	53.9
150472_5_total_counts: 1663.Seed: 2_K: 25.Length: 344.translasyon_frame_3_reversed	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	59.8	112	2.89E-35	137	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	59.8	112	3.33E-38	137
151167_2_total_counts: 478.Seed: 2_K: 25.Length: 273.translasyon_frame_1_reversed	ADFR80789.1	replication-association protein, partial [Cyclovirus PKbee25]	43.8	64	1.23E-09	60.5	YP_009021847.1	replication-associated protein [Dragonfly associated cyclovirus 3]	41.3	75	1.59E-11	60.5
23_106_total_counts: 85879.Seed: 2_K: 25.Length: 835.translasyon_frame_2_reversed	AEI28798.1	replication-associated protein [Bat circovirus ZS/Yunnan-China/2009]	61.2	49	1.31E-10	71.2	YP_009506302.1	replication-associated protein [Pacific flying fox associated cyclovirus-3]	56.3	48	5.42E-13	69.7
29359_5_total_counts: 3883.Seed: 8_K: 25.Length: 780.translasyon_frame_3_reversed	YP_009337064.1	hypothetical protein 1 [Hubei picorna-like virus 76]	29.9	131	9.52E-05	55.1	YP_009337064.1	hypothetical protein 1 [Hubei picorna-like virus 76]	29.9	131	1.10E-07	55.1
29755_9_total_counts: 7610.Seed: 2_K: 25.Length: 902.translasyon_frame_2	YP_009336655.1	hypothetical protein 1 [Hubei picorna-like virus 76]	29.9	281	2.55E-13	81.6	YP_009336655.1	hypothetical protein 1 [Hubei picorna-like virus 76]	29.9	281	2.94E-16	81.6
30180_2_total_counts: 792.Seed: 2_K: 25.Length: 351.translasyon_frame_3_reversed	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	56.6	113	8.88E-36	139	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	56.6	113	1.02E-38	139
30198_7_total_counts: 3618.Seed: 2_K: 25.Length: 524.translasyon_frame_1	YP_009337788.1	hypothetical protein 1 [Hubei picorna-like virus 68]	32.6	129	2.01E-06	57.8	YP_009337788.1	hypothetical protein 1 [Hubei picorna-like virus 68]	32.6	129	2.32E-09	57.8
30839_3_total_counts: 767.Seed: 2_K: 25.Length: 259.translasyon_frame_3	AXA52568.1	putative RdRp [Linpetheuma humile polycipivirus 1]	42.7	75	1.02E-07	57.8	NP_09407943.1	RNA-dependent RNA polymerase, partial [Lasius niger virus 1]	36.6	71	7.04E-09	52.8
356_26_total_counts: 96963.Seed: 2_K: 25.Length: 3730.translasyon_frame_2	APG76480.1	hypothetical protein 2, partial [Hubei tombsus-like virus 35]	51.3	517	7.89E-169	521	YP_009337674.1	hypothetical protein 2 [Changjiang tombsus-like virus 22]	39.8	487	1.40E-103	339
3942_5_total_counts: 1878.Seed: 8_K: 25.Length: 441.translasyon_frame_2_reversed	AZB48781.1	VP1, partial [Norovirus GII]	26.6	124	1.66E-05	52.4	YP_009237901.1	VP1 [Norovirus GII]	30.9	81	4.50E-06	47
58152_5_total_counts: 2250.Seed: 9_K: 25.Length: 516.translasyon_frame_2_reversed	YP_009345893.1	hypothetical protein [Wuhan insect virus 12]	50.0	164	9.85E-48	176	YP_009345893.1	hypothetical protein [Wuhan insect virus 12]	50.0	164	1.14E-50	176
58401_11_total_counts: 3538.Seed: 2_K: 25.Length: 357.translasyon_frame_1_reversed	YP_009337019.1	hypothetical protein 1 [Hubei picorna-like virus 74]	36.2	116	9.52E-15	79.3	YP_009337019.1	hypothetical protein 1 [Hubei picorna-like virus 74]	36.2	116	1.10E-17	79.3
58842_3_total_counts: 703.Seed: 2_K: 25.Length: 227.translasyon_frame_1_reversed	AEK82102.1	polyprotein [Human parechovirus 2]	43.5	69	3.19E-09	61.6	YP_009508969.1	protein 2C [Dromedary camel enterovirus 19CC]	37.1	70	5.19E-12	61.2
59635_4_total_counts: 790.Seed: 2_K: 25.Length: 231.translasyon_frame_1_reversed	YP_009337388.1	hypothetical protein 2 [Hubei picorna-like virus 64]	58.7	46	9.04E-10	63.2	YP_009337388.1	hypothetical protein 2 [Hubei picorna-like virus 64]	58.7	46	1.04E-12	63.2
60807_6_total_counts: 1537.Seed: 3_K: 25.Length: 303.translasyon_frame_2	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	34.8	92	7.09E-08	58.9	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	34.8	92	8.17E-11	58.9
61026_4_total_counts: 679.Seed: 2_K: 25.Length: 201.translasyon_frame_3	YP_00933392.1	hypothetical protein 1 [Beihai picorna-like virus 119]	38.6	44	1.49E-05	50.8	YP_00933392.1	hypothetical protein 1 [Beihai picorna-like virus 119]	38.6	44	1.71E-08	50.8
61384_15_total_counts: 5882.Seed: 12_K: 25.Length: 421.translasyon_frame_2_reversed	ASM93488.1	VP1 [Lupine feces-associated densovirus 2]	37.8	119	1.80E-19	93.6	NP_694830.1	structural protein VP4 [Galleria mellonella densovirus]	34.2	79	2.00E-06	48.1
62223_3_total_counts: 1272.Seed: 2_K: 25.Length: 407.translasyon_frame_2_reversed	NP_624351.1	capsid protein [Fusarium solani virus 1]	70.8	120	2.67E-52	179	NP_624351.1	capsid protein [Fusarium solani virus 1]	70.8	120	3.08E-55	179
6453_2_total_counts: 487.Seed: 2_K: 25.Length: 236.translasyon_frame_3	YP_009337387.1	hypothetical protein 1 [Hubei picorna-like virus 64]	53.3	60	2.82E-13	73.2	YP_009337387.1	hypothetical protein 1 [Hubei picorna-like virus 64]	53.3	60	3.25E-16	73.2
7992_2_total_counts: 506.Seed: 2_K: 25.Length: 248.translasyon_frame_3_reversed	NP_624351.1	capsid protein [Fusarium solani virus 1]	60.8	74	4.14E-21	95.1	NP_624351.1	capsid protein [Fusarium solani virus 1]	60.8	74	4.78E-24	95.1
87654_83_total_counts: 129393.Seed: 2_K: 25.Length: 1587.translasyon_frame_1	YP_004152328.1	Cap protein [Cyclovirus PKgoat21/PAK/2009]	37.5	168	6.00E-22	104	YP_004152328.1	Cap protein [Cyclovirus PKgoat21/PAK/2009]	37.5	168	6.92E-25	104
87654_83_total_counts: 129393.Seed: 2_K: 25.Length: 1587.translasyon_frame_2_reversed	YP_008130363.1	replication association protein [Human cyclovirus VSS700009]	57.1	226	6.48E-88	281	YP_008130363.1	replication association protein [Human cyclovirus VSS700009]	57.1	226	7.47E-91	281
87760_5_total_counts: 3323.Seed: 8_K: 25.Length: 633.translasyon_frame_2	YP_009337788.1	hypothetical protein [Hubei picorna-like virus 68]	36.1	208	7.90E-28	120	YP_009337788.1	hypothetical protein [Hubei picorna-like virus 68]	36.1	208	9.11E-31	120
87907_9_total_counts: 11161.Seed: 5_K: 25.Length: 1236.translasyon_frame_2_reversed	AA819088.1	structural polyprotein, partial [Kashmir bee virus]	32.2	404	3.53E-44	174	NP_851404.2	structural polyprotein, partial [Kashmir bee virus]	31.4	408	2.26E-46	172
87919_8_total_counts: 5141.Seed: 4_K: 25.Length: 682.translasyon_frame_3	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	36.5	208	4.19E-39	154	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	36.5	208	4.83E-42	154
Contig (nucleotide) query acc.ver		BLASTX against nr				BLASTX against RefSeq						
	subject acc.ver	name	% identity	alignment length	evaluate	bit score	name	% identity	alignment length	evaluate	bit score	
115738_6_total_counts: 2739.Seed: 8_K: 25.Length: 448	YP_009337019.1	hypothetical protein 1 [Hubei picorna-like virus 74]	36.4	143	4.04E-19	93.6	hypothetical protein 1 [Hubei picorna-like virus 74]	36.4	143	1.10E-23	93.6	
115755_8_total_counts: 8948.Seed: 6_K: 25.Length: 1124	YP_009342254.1	hypothetical protein 1 [Wuhan arthropod virus 3]	34.9	295	9.48E-43	170	hypothetical protein 1 [Wuhan arthropod virus 3]	34.9	295	2.58E-47	170	
115910_13_total_counts: 77920.Seed: 2_K: 25.Length: 5913	APG76667.1	hypothetical protein 1 [Wuhan insect virus 11]	45.7	870	0.00E+00	726	YP_009342327.1	hypothetical protein 1 [Wuhan insect virus 11]	45.9	870	0.00E+00	723
117633_5_total_counts: 2738.Seed: 6_K: 25.Length: 598	YP_009337788.1	hypothetical protein [Hubei picorna-like virus 68]	37.9	190	1.08E-23	108	YP_009337788.1	hypothetical protein [Hubei picorna-like virus 68]	37.9	190	2.93E-28	108
118987_6_total_counts: 1591.Seed: 4_K: 25.Length: 287	ATOS9710.1	structural polyprotein [Solenopsis invicta virus 5]	33.8	74	2.65E-07	57.4	YP_009337788.1	hypothetical protein 1 [Hubei orthoptera virus 1]	28.7	87	7.47E-12	57.4
119065_2_total_counts: 518.Seed: 2_K: 25.Length: 276	BAW18990.1	hypothetical protein [Raltosmia phage RP12]	38.9	90	8.56E-16	79.7	YP_009337788.1	hypothetical protein [Raltosmia phage RSF1]	30.5	82	2.06E-06	46.2
119732_5_total_counts: 1999.Seed: 10_K: 25.Length: 391	YP_009333576.1	hypothetical protein 1 [Beihai picorna-like virus 121]	41.8	98	5.02E-12	72.4	-1.0	hypothetical protein 1 [Beihai picorna-like virus 121]	41.8	98	1.36E-16	72.4
121873_2_total_counts: 283.Seed: 2_K: 25.Length: 200	YP_002790880.2	putative structural polyprotein [Solenopsis invicta virus 3]	39.4	66	1.25E-05	51.2	-1.0	putative structural polyprotein [Solenopsis invicta virus 3]	39.4	66	3.39E-10	51.2
145575_124_total_counts: 517623.Seed: 5_K: 25.Length: 4184	ASM93489.1	NS1 [Lupine feces-associated densovirus 2]	37.6	433	8.62E-94	321	-1.0	putative nonstructural protein NS1 [Diaphorina citri densovirus]	31.8	453	1.27E-53	198
147897_7_total_counts: 2386.Seed: 2_K: 25.Length: 366	SCDD_C	Chain C, Structural Polyprotein, VP2 [IAPV]	36.2	105	1.01E-23	100	ORF2	[Israeli acute paralysis virus]	36.2	105	3.67E-26	99.4
149357_2_total_counts: 908.Seed: 2_K: 25.Length: 416	YP_009352233.1	polyprotein [Diabrotica virgifera virgifera virus 2]	29.3	133	3.35E-11	70.5	YP_009352233.1	hypothetical protein 1 [Hubei orthoptera virus 3]	23.4	105	9.05E-14	64.7
149871_4_total_counts: 1261.Seed: 2_K: 25.Length: 337	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	31.4	105	7.94E-06	53.9	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	31.4	105	2.16E-10	53.9
150472_5_total_counts: 1663.Seed: 2_K: 25.Length: 344	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	59.8	112	4.13E-35	137	-3.0	hypothetical protein 1 [Hubei orthoptera virus 3]	59.8	112	1.12E-39	137
151167_2_total_counts: 478.Seed: 2_K: 25.Length: 273	ADFR80789.1	replication-association protein, partial [Cyclovirus PKbee25]	43.8	64	5.46E-10	61.6	-1.0	replication-associated protein [Dragonfly associated cyclovirus 3]	41.3	75	1.62E-13	61.6
23_106_total_counts: 85879.Seed: 2_K: 25.Length: 835	AEI28798.1	replication-associated protein [Bat circovirus ZS/Yunnan-China/2009]	61.2	49	4.30E-10	69.7	-2.0	rolling circle replication initiator protein [Cyclovirus ZM36fa]	56.3	48	2.63E-13	65.9
29359_5_total_counts: 3883.Seed: 8_K: 25.Length: 780	YP_009337064.1	hypothetical protein 1 [Hubei picorna-like virus 76]	29.9	281	2.84E-13	81.6	YP_009337064.1	hypothetical protein 1 [Hubei picorna-like virus 76]	29.9	131	4.89E-09	54.3
29755_9_total_counts: 7610.Seed: 2_K: 25.Length: 902	YP_009336655.1	hypothetical protein 1 [Hubei picorna-like virus 76]	29.9	281	2.55E-13	81.6	YP_009336655.1	hypothetical protein 1 [Hubei picorna-like virus 76]	29.9	281	7.72E-18	81.6
30180_2_total_counts: 792.Seed: 2_K: 25.Length: 351	YP_009336506.1	hypothetical protein 1 [Hubei orthoptera virus 3]	56.6	113	9.86E-36	139	-3.0	hypothetical protein 1 [Hubei orthoptera virus 3]	56.6	113	2.68E-40	139
30198_7_total_counts: 3618.Seed: 2_K: 25.Length: 524	YP_009337788.1	hypothetical protein [Hubei picorna-like virus 68]	32.6	129	1.52E-06	58.2	YP_009337788.1	hypothetical protein [Hubei picorna-like virus 68]	32.6	129	4.15E-11	58.2
30839_3_total_counts: 767.Seed: 2_K: 25.Length: 259	AXA52568.1	putative RdRp [Linpetheuma humile polycipivirus 1]	42.7	75	1.13E-07	57.8	YP_009337788.1	hypothetical protein 1 [Hubei picorna-like virus 68]	36.6	71	1.85E-10	52.8
356_26_total_counts: 96963.Seed: 2_K: 25.Length: 3730	APG76480.1	hypothetical protein 2, partial [Hubei tombsus-like virus 35]	51.3	517	1.33E-170	523	YP_009337674.1	hypothetical protein 2 [Changjiang tombsus-like virus 22]	39.8	487	4.23E-106	342
3942_5_total_counts: 1878.Seed: 8_K: 25.Length: 441	AMD33538.1	capsid protein [Norovirus GII]	30.6	111	2.55E-05	53.5	-2.0	VP1 [Norovirus GII]	30.9	81	1.30E-07	47
58152_5_total_counts: 2250.Seed: 9_K: 25.Length: 516	YP_009345893.1	hypothetical protein [Wuhan insect virus 12]	50.0	164	1.09E-47	176	-2.0	hypothetical protein [Wuhan insect virus 12]	50.0	164	2.98E-52	176
58401_11_total_counts: 3538.Seed: 2_K: 25.Length: 357	YP_009337019.1	hypothetical protein 1 [Hubei picorna-like virus 74]	36.2	116	1.06E-17	79.3	YP_009337019.1					

Supplementary Table 4 - Accession number (ID) of the sequences used in phylogenetic inferences.

IDs	Species
MqV2 phylogeny	
YP_009388491.1	<i>Lake Sinai Virus SA2</i>
ASS83296.1_	<i>Lake Sinai virus 2</i>
AJR19138.1	<i>Lake Sinai virus</i>
ASS83254.1	<i>Lake Sinai virus 1</i>
AYP65068.1	<i>Lake Sinai virus 3</i>
YP_009388495.1	<i>Lake Sinai Virus NE</i>
AXA52557.1	<i>Linepithema humile C virus 1</i>
YP_009011225.1	<i>Anopheline-associated C virus</i>
AOG30801.1	<i>Towan virus</i>
YP_009344965.1	<i>Jingmen tombus-like virus 2</i>
ASN64759.1	<i>Leptomonas pyrrhocoris RNA virus</i>
YP_009337040.1	<i>Changjiang tombus-like virus 21</i>
YP_009337674.1	<i>Changjiang tombus-like virus 22</i>
YP_009337712.1	<i>Hubei tombus-like virus 36</i>
YP_009342273.1	<i>Wenzhou tombus-like virus 17</i>
APG76480.1	<i>Hubei tombus-like virus 35</i>
YP_009336952.1	<i>Beihai tombus-like virus 18</i>
YP_009337190.1	<i>Wenling tombus-like virus 2</i>
YP_009336894.1	<i>Beihai tombus-like virus 10</i>
YP_009336965.1	<i>Hubei tombus-like virus 14</i>
YP_009337688.1	<i>Beihai tombus-like virus 7</i>
YP_009337155.1	<i>Changjiang tombus-like virus 12</i>
YP_009336861.1	<i>Shahe tombus-like virus 2</i>
BAU09527.1	<i>Pothos latent virus</i>
NP_619671.1	<i>Hibiscus chlorotic ringspot virus</i>
AGG23390.1	<i>Citrus yellow vein-associated virus</i>
AWS06679.1	<i>Ethiopia maize-associated virus</i>
ACJ03575.1	<i>Carrot mottle mimic virus</i>
NP_620846.3	<i>Pea enation mosaic virus 2</i>
AXN55932.1	<i>Red clover umbravirus</i>
YP_009336880.1	<i>Hubei tombus-like virus 19</i>
YP_009337113.1	<i>Hubei tombus-like virus 28</i>
APG76298.1	<i>Shangao tombus-like virus 1</i>
YP_009333257.1	<i>Hubei tombus-like virus 30</i>
ALV85423.1	<i>Diaphorina citri-associated C virus</i>
MqC1 and MqC2 phylogeny	
YP_008130364.1/YP_008130363.1	<i>Human cyclovirus VS5700009</i>
AMH87656.1/AMH87655.1	<i>Pacific flying fox-associated cyclovirus 3</i>
YP_009104365.1/YP_009104366.1	<i>Cyclovirus ZM36a</i>
ADD62479.1/ADD62480.1	<i>Cyclovirus TN18</i>
BAP81876.1/BAP81877.1	<i>Cyclovirus ZM41</i>
BAP81871.1/BAP81870.1	<i>Cyclovirus ZM01</i>
BAP81869.1/BAP81868.1	<i>Cyclovirus ZM32</i>
AIZ46815.1/AIZ46816.1	<i>Swine cyclovirus</i>
AGO59220.1/AGO59219.1	<i>Cyclovirus VN</i>
AIZ46821.1/AIZ46822.1	<i>Human cyclovirus VN-like</i>
BAP81882.1/BAP81883.1	<i>Cyclovirus ZM62</i>

ADY17984.1/ADY17985.1	<i>Dragonfly-associated cyclovirus 1</i>
YP_009513184.1/YP_009513183.1	<i>Dragonfly-associated cyclovirus 4</i>
YP_009506320.1/YP_009506319.1	<i>Cyclovirus PK5222</i>
AXL65887.1/AXL65888.1	<i>Arboreal ant-associated circular virus 1</i>
AFS65280.1/AFS65281.1	<i>Dragonfly-associated cyclovirus 5</i>
YP_009506293.1	<i>Bat-associated cyclovirus 8</i>
YP_009506324.1/YP_009506323.1	<i>Cyclovirus PK5034</i>
YP_004152332.1/YP_004152331.1	<i>Cyclovirus bat/USA/2009</i>
ARO38287.1/ARO38286.1	<i>Circovirus sp</i>
YP_009506285.1/YP_009506286.1	<i>Bat-associated cyclovirus 2</i>
YP_009362253.1/YP_009362252.1	<i>Bat cyclovirus GF 4c</i>
YP_009506313.1/YP_009506314.1	<i>Dragonfly-associated cyclovirus 8</i>
AXL65889.1/AXL65890.1	<i>Fly-associated circular virus 1</i>
ATP66708.1	<i>Rodent circovirus</i>
AKE49354.1/AKE49355.1	<i>Cyclovirus NI 204</i>
YP_009506329.1/YP_009506330.1	<i>Cyclovirus SL 108277</i>
YP_009021871.2/YP_009021870.2	<i>Human-associated cyclovirus 10</i>
AFS65290.1/AFS65289.1	<i>Dragonfly-associated cyclovirus 2</i>
Ref [35]	<i>Circo-like 1</i>
YP_271918.1/YP_271921.1	<i>Duck circovirus</i>
NP_065678.1/NP_065679	<i>Porcine circovirus</i>
YP_009423856.1/YP_009423857.1	<i>Bat circovirus</i>
YP_764455.1/YP_764456.1	<i>Raven circovirus</i>
Ref [35]	<i>Circo-like 2</i>

MqV1a and MqV1b phylogeny

AWK77852.1/AWK77853.1	<i>Bundaberg bee virus 2</i>
AWK23470.1/AWK23471.1	<i>Human blood-associated dicistrovirus</i>
YP_009337313.1/YP_009337314.1	<i>Hubei picorna-like virus 14</i>
YP_009345054.1/YP_009345055.1	<i>Wuhan millipede virus 3</i>
AIY53985.1/AIY53986.1	<i>Nilaparvata lugens C virus</i>
AKA63263.1/AKA63264.1	<i>Cricket paralysis virus</i>
NP_044945.1/NP_044946.1	<i>Drosophila C virus</i>
AZR39355.1/AZR39356.1	<i>Hypsignathus monstrosus dicistrovirus</i>
YP_009252204.1/YP_009252205.1	<i>Anopheles C virus</i>
NP_620562.1/NP_620563.1	<i>Triatoma virus</i>
AWK77884.1/AWK77885.1	<i>Hobart bee virus 1</i>
YP_009333496.1/YP_009333497.1	<i>Beihai picorna-like virus 84</i>
AYN75548.1/AYN75549.1	<i>Halhan virus 1</i>
YP_009336690.1/YP_009336691.1	<i>Wenling crustacean virus 2</i>
AFO53688.1/AFO53689.1	<i>Taura syndrome virus</i>
YP_009337654.1/YP_009337655.1	<i>Hubei picorna-like virus 25</i>
YP.164440.1/YP.164441.1	<i>Solenopsis invicta virus 1</i>
YP_009336557.1/YP_009336558.1	<i>Hubei orthoptera virus 1</i>
APG76667.1/APG76668.1	<i>Wuhan insect virus 11</i>
AXA52579.1/AXA52580.1	<i>Linepithema humile virus 1</i>
ATO59709.1/ATO59710.1	<i>Solenopsis invicta virus 5</i>
NP_066241.1/NP_066242.1	<i>Acute bee paralysis virus</i>
AHL83499.1/AHL83500.1	<i>Kashmir bee virus</i>
ABY57949.1/ABY57950.1	<i>Israeli acute paralysis virus</i>
YP_008888535.1/YP_008888536.1	<i>Formica exsecta virus 1</i>
YP_009336777.1/YP_009336778.1	<i>Wenzhou channeled applesnail virus 2</i>

APG76699.1/APG76700.1	<i>Beihai picorna-like virus 70</i>
YP_009337682.1/YP_009337683.1	<i>Wenzhou picorna-like virus 26</i>
YP_009333456.1/YP_009333457.1	<i>Beihai picorna-like virus 71</i>
APG76793.1/APG76794.1	<i>Beihai picorna-like virus 72</i>
APG78016.1/APG78017.1	<i>Beihai picorna-like virus 74</i>
AYN75554.1/AYN75555.1	<i>Halhan virus 3</i>
YP_009336994.1/YP_009336995.1	<i>Wenzhou picorna-like virus 28</i>
APG78539.1/APG78540.1	<i>Wenzhou picorna-like virus 29</i>
NP_049374.1	<i>Sacbrood virus</i>
YP_003622540.1	<i>Slow bee paralysis virus</i>
YP_009162630.1	<i>Bombyx mori iflavivirus</i>
NP_853560.2	<i>Deformed wing virus</i>
YP.145791.1/	<i>Varroa destructor virus 1</i>

MqD phylogeny

AWV66973.1/AWV66974.1	<i>Ambidensovirus sp.</i>
ANG55951.1/ANG55948.1	<i>Human CSF-associated densovirus</i>
YP_006576512.1/YP_006576514.1	<i>Sibine fusca densovirus</i>
NP_694838.1/NP_694840.1	<i>Casphalia extranea densovirus</i>
YP_006589928.1/YP_006589930.1	<i>Papilio polyxenes densovirus</i>
AIT71975.1/AIT71974	<i>Hordeum marinum Itera-like densovirus</i>
NP_542609.1/NP_542611.1	<i>Bombyx mori densovirus 1</i>
YP_009021036.1/YP_009021038.1	<i>Danaus plexippus plexippus iteravirus</i>
YP.164339.1/YP.164341.1	<i>Dendrolimus punctatus densovirus</i>
YP_004678720.1/YP_004678721.1	<i>Helicoverpa armigera densovirus</i>
YP_009256211.1/YP_009256212.1	<i>Diaphorina citri densovirus</i>
AVH76852.1/AVH76854.1	<i>Bombus cryptarum densovirus</i>
NP_874376.1/NP_874377.1	<i>Myzus persicae densovirus</i>
YP_009362129.1/YP_009362131.1	<i>Dysaphis plantaginea densovirus</i>
YP_002887625.1/YP_002887627.1	<i>Culex pipiens densovirus</i>
AFK91980.1/AFK91983.1	<i>Helicoverpa armigera densovirus</i>
NP_899650.1/NP_694830.1	<i>Galleria mellonella densovirus</i>
NP_958099.1/NP_958101.1	<i>Mythimna loreyi densovirus</i>
NP_046813.1/NP_046815.1	<i>Diatraea saccharalis densovirus</i>
YP_007003823.1/YP_007003825.1	<i>Pseudoplusia includens densovirus</i>
NP_694824.1/NP_694823.1	<i>Junonia coenia densovirus</i>
AGO32210.1/AGO32212.1	<i>Acheta domestica densovirus</i>
NP_694843.1/NP_694842.1	<i>Planococcus citri densovirus</i>
YP_008766862.1/YP_008766863.1	<i>Solenopsis invicta densovirus</i>
ARI46485.1/ARI46488.1	<i>Ambidensovirus CaaDV2</i>
YP_009134732.1/YP_009134734.1	<i>Cherax quadricarinatus densovirus</i>
YP_009507340.1/YP_009507339.1	<i>Sea star-associated densovirus</i>
AFC75858.1/AFC75860.1	<i>Blattella germanica densovirus-like virus</i>
ASM93489.1/ASM93488.1	<i>Lupine feces-associated densovirus 2</i>
ASU47551.1/ASU47552.1	<i>Lone star tick densovirus 1</i>
AYG77882.1/AYG77881.1/AYG77880.1	<i>Chicken parvovirus</i>
NP_757371.1/NP_757369.1	<i>Porcine parvovirus</i>
AAA61405.1/AAA61406.1	<i>Mouse parvovirus 1</i>

CAPÍTULO 4

Delayed effects of low gene expression, and behavioral changes associated with a stingless bee annual syndrome

Este capítulo apresenta o seguinte artigo em preparação para publicação:

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Delayed effects of low gene expression, and behavioral changes associated with a stingless bee annual syndrome

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Abstract

Bee populations are declining around the world and what appears to be at the root of colony failure is the combination of different sub-lethal factors, such as habitat loss, malnutrition and agrochemicals. In Brazil, an annual syndrome affects colonies of the stingless bee *Melipona quadrifasciata*, causing the sudden death of workers. In our previous studies, no pathogen was consistently associated with the disease. Here we conducted an integrative and temporal survey

on *M. quadrifasciata* managed colonies in order to further investigate the causes behind the syndrome by measuring individual- and colony-level traits. We suggest that both the lack of genetic variation in bee populations and environmental stressors contribute to disease outcomes. We have found that the incapacity to express important health-related genes (*cytochrome p450*, *vitellogenin*, *phenoloxidase*) two months before the outbreak is associated with symptom manifestation, suggesting poor metabolization of xenobiotics and weak immune responses in colonies that became unhealthy. Concomitantly, we observed a weight loss in foragers, change in pollen resources stored in bee colonies and a decrease in performance to control nest environment. Our findings corroborate theoretical studies proposing that bee collapses result from positive density dependence influenced by the combination of diverse sub-lethal factors, such as competition for resources, pathogens and agrochemicals.

Key-words: bee health, colony collapse, sub-lethal effects.

Introduction

The worldwide decrease in bee populations observed in the last decade is a matter of great concern. Colony collapses in North America and Europe, where numbers are better documented, are on average twice as high as historically (Genersch et al. 2010; Kulhanek et al. 2017). Some of these losses can be explained by the presence of pathogens or infectious agents (Evans and Schwarz 2011; Schwarz et al. 2015). Additionally, several other interacting stressors, such as climate change, habitat loss, malnutrition, agrochemicals and colony management practices were shown to reduce the fitness of bee populations (vanEngelsdorp and Meixner 2010; Goulson et al. 2015). Theoretical studies indicate that although multifactorial stresses could cause colony failure, this failure is most likely a result of a critical level of stress from the accumulation of sub-lethal factors, even without synergistic effects (Bryden et al. 2013). Mathematical models demonstrate that the reason why some colonies collapse while others do not, despite suffering from the same sub-lethal stresses, involves colony density (Khoury et al. 2011; Bryden et al. 2013).

Sub-lethal factors are those that do not cause the immediate death of individuals, but

reduce individual fitness by cumulative or synergistic effects, eventually causing death later on. Such delayed effects are reported for many bee populations. Colonies of *Apis mellifera* parasitized by *Varroa destructor* and infected by *Deformed wing virus* (DWV) during summer and autumn are more susceptible to collapse in winter, probably due to immunosuppression caused by pathogens (Dainat et al. 2012; Barroso-Arévalo et al. 2019). Honey bee workers reared in pollen-stressed colonies develop completely, but as adults they are less likely to waggle dance, and, if they dance, the information about food location is less precise (Scofield and Mattila 2015). Bumblebee workers have impaired learning performance when exposed to sub-lethal doses of agrochemicals during any developmental stage (Smith et al. 2020). Delayed effects of gene expression may as well represent sub-lethal factors that contribute to the collapse of bee colonies. In *A. mellifera* the decrease in the expression of *defensin*, which encodes an antimicrobial peptide, was correlated to colony collapse months after (Barroso-Arévalo et al. 2019). Furthermore, the combination of low *defensin* expression with a decrease in the expression of *relish*, a gene that belongs to the immune deficiency (IMD) signalling pathway, results in a reduced chance of bee colony survival in a future infection with DWV and *Varroa* (Barroso-Arévalo et al. 2019). Honey bee colonies are also more likely to successfully overwinter if workers express more vitellogenin (*Vg*), which regulates immune function and longevity, in fall (Dainat et al. 2012; Steinmann et al. 2015).

In southern Brazil, beekeepers report an annual syndrome that usually occurs in late summer (frequently in March), often leading to the collapse of managed colonies of the stingless bee *Melipona quadrifasciata* (Díaz et al. 2017; Caesar et al. 2019). The main feature of this syndrome is the sudden death of worker bees. In some localities affected workers show a number of neurologic symptoms, such as tremors and paralysis, suggesting the implication of viruses. However, although the virome of unhealthy bees includes novel viruses related to those causing similar symptoms in *A. mellifera*, such as viruses from the family *Dicistroviridae* (Genersch and Aubert 2010; Caesar et al. 2019), they were not found consistently associated to unhealthy bees (Caesar et al. 2019). No pathogenic bacteria, *Nosema* or *Crithidia* were identified as causative agents of the syndrome either (Díaz et al. 2017).

Pollinator health is a complex trait. The absence of pathogens is not a sufficient condition for bee health, and social bees show an additional level of epidemiological complexity besides the individual and the population, i.e., the colony (López-Urbe et al. 2020). The use of biomarkers for each of these levels of complexity contributes to investigations on bee health and disorders. At the individual level, gene expression indicates the effect of certain factors, as

for example the expression of cytochrome p450 genes, which indicates a response to xenobiotics. At the colony level, conventional measures, such as colony size, reveal about colony performance (López-Urbe et al. 2020).

Here we report an integrative and temporal study designed to investigate the factors underlying the annual syndrome of *M. quadrifasciata*. Our survey was conducted on three pairs of sister colonies kept in two separate localities, in order to determine the contribution of both the colony genetic background as well as the environment to the syndrome manifestation, measuring individual- and colony-level traits.

Materials and Methods

(a) Searching for genes differentially expressed during the syndrome

Initially, three *M. quadrifasciata* colonies were selected for transcriptomic analyses aiming to identify genes with differential gene expression during the syndrome. Two were sampled during the outbreak in March 2018 in meliponaries from Bom Princípio (BP; 29°31'2.30"S/51°17'29.00"W) and Estância Velha (EV; 29°38'50.316"S/51°10'23.592"W). Both colonies were manifesting syndrome symptoms, such as dead bees in front of the colony, or bees presenting tremors and paralysis. A third colony showed no symptoms during the outbreak, and was sampled in April (after the outbreak) in BP. Three bees from each colony (labeled U1, U2 and H, respectively) were collected and used for RNA extraction and transcriptome sequencing.

Total RNA of individual bees was extracted with TRIzol™ Reagent (Thermo Fisher Scientific, USA), following the manufacturer's recommendations. RNA yield was assessed with Qubit fluorometer (Invitrogen, USA) and the integrity was checked on a 1% agarose gel. For sequencing, aliquots of 2 µg RNA treated with TURBO DNase (Thermo Fisher Scientific, USA) from three foragers were pooled with respective colony samples and stored at -80 °C until use. Transcripts were purified by polyA-tail selection, followed by library construction using TruSeq Stranded mRNA Library Prep Kit (Illumina, EUA). Single-end sequencing (read length = 150 nt) was performed on an Illumina NextSeq instrument, producing about 100 million reads per sample.

Trimmomatic v.0.36 was run with default parameters to remove low quality reads

(Bolger et al. 2014). Gene expression (*GE*) was estimated with *depth* command from Samtools v.1.3.1 (Li et al. 2009) by mapping the trimmed reads onto *M. quadrifasciata* genome (GenBank assembly accession: GCA_001276565.1) with GSNAP v. 2018-07-04 (Wu et al. 2016) and recovering the number of reads per gene. To normalize gene expression (*NGE*) among the three transcriptomes the following formula was used:

$$NGE = (100,000,000/n^{\circ} \text{ of mapped reads}) * GE$$

Next, the similarity in gene expression (*SGE*) between transcriptomes was estimated by comparing *NGE* from healthy and unhealthy samples with the following formula:

$$SGE = (H + U)/(2 * max)$$

Where *H* is the *NGE* of healthy transcriptome, *U* is the *NGE* of one unhealthy transcriptome, and *max* is the largest *NGE* value between *H* and *U* being compared. The result ranges between 0.5 and 1, and genes were regarded as differentially expressed (DEGs) when the *SGE* was equal or lower than 0.7 in both comparisons of *H* vs. *U* transcriptomes. Hypothetical genes among DEGs were re-annotated with BLASTp versus nr database (cut-off 1e-5) (Altschul et al. 1990). DEGs with putative roles in bee health were selected for relative quantification with RT-qPCR or by enzymatic assay (see below).

(b) Monitoring bee colonies before, during and after an outbreak

In order to monitor key changes in several bee biological features, observations were made in colonies under semi-controlled conditions during six months in two localities, *i.e.*, Bom Princípio (BP; 29°31'2.30"S/51°17'29.00"W) and Porto Alegre (PA; 30°2'4.7292"S/51°13'3.5724"W). The BP meliponary is located inside a small agricultural property where agrochemicals are regularly used. PA colonies were kept in the vicinity of a secondary forest located inside the University Campus. To control for genetic factors contributing to the syndrome, three colonies from BP (named BP1, BP2 and BP3) were divided in February 2018, resulting in three pairs of sister colonies. After six months, when both paired colonies became mature, the three sister colonies (named PA1, PA2 and PA3) were translocated to PA, and all colonies were then monitored monthly from December 2018 (Summer) to May 2019 (Autumn). Each colony was equipped with a datalogger device (model U23-001 HOBO Pro v2, version 3.6.2, ONSET, Brazil) to record within-hive temperature and humidity. Records were made daily every six hours during the experiment and downstream analyses were conducted with the daily

lowest temperature (6 a.m.) and the highest humidity (12 p.m.).

(c) Pollen resources used by stingless bees

Aliquots of pollen stored by worker bees were collected with tweezers every month and stored in the laboratory at 4 °C. Because there was sometimes no stored pollen within hives, or storage inaccessible for sampling, a few other colonies from both meliponaries were sampled as well. Pollen was chemically processed by acetolysis (Erdtman 1952). Four slides were mounted for each sample with glycerin gelatine (Salgado-Labouriau 2007) and around 500 pollen grains were identified at the family, genus or species level. The number of pollen grains per plant family was quantified for each sample.

(d) Bee weight and glucose oxidase assay

With an entomological sucker, five foragers were collected monthly from each of the six colonies between December 2018 and April 2019. Bees were brought to the laboratory alive, weighted, decapitated and heads stored separately from the remaining body at -80 °C. SpectraMax M2e fluorescence microplate reader was used to quantify glucose oxidase (GOX) activity using the Amplex® Red Glucose/Glucose Oxidase Assay Kit (Thermo Fisher Scientific, USA). Five heads per colony were pooled and macerated in 400µL of phosphate-buffered saline, centrifuged for 1 min at 5000 rpm and two separated aliquots of 10µL from the homogenate was used to estimate GOX activity. Fluorescence was measured with 544 nm excitation and 590 nm emission every minute and the linear slope of the reaction was calculated up to 20 min. The results are expressed as Relative Fluorescence Unit (RFU)/min/µL and normalized for body weight.

(e) Relative quantification of gene expression

Other five foragers were removed monthly from the experimental colonies between January and April 2019. They were transferred individually to separate vials containing 200 µL of RNAlater (Thermo Fisher Scientific, USA) and stored at -80 °C for RNA extraction and gene expression analysis by RT-qPCR. Total RNA was extracted from the whole body and quantified as previously explained. An aliquot of 1 µg RNA from each forager was used as input for first strand cDNA synthesis with the High-Capacity Reverse Transcription kit (Thermo Fisher Scientific, USA).

Five genes found differentially expressed in the initial transcriptomic analyses, and with

a putative role in bee health were selected for monitoring worker bee expression patterns during the experiment: two vitellogenin domain-containing proteins (*Vg1* and *Vg2*), RHD domain-containing protein (putative nuclear factor kappa-B; *NF-kB*), putative cytochrome p450 (*p450*) and phenoloxidase subunit 1 (*PO*; supplementary material, Table S1). Primer3 implemented in Geneious R11 (Kearse et al. 2012) was used to design primers based on the sequence of each of these genes in *M. quadrifasciata* genome (supplementary material, Table S2). Actin (*act*) and 40S ribosomal protein S5 (*rps5*) were used as reference for gene expression normalization (Evans et al. 2006; Brito et al. 2015; Freitas et al. 2019). StepOnePlus™ Real-Time PCR System (Applied Biosystems) was used for the RT-qPCR assays. Amplifications were carried out in 25 µL reaction solutions containing 12.5 µL cDNA (diluted to 1:30), 0.2 X SYBR™ Green I Nucleic Acid Gel Stain (Thermo Fisher Scientific, USA), 0.25 U of Platinum Taq DNA polymerase (Invitrogen, USA), 1 X PCR buffer (Tris-HCl 200 mM, pH 8.4, KCl 500 mM), 3 mM MgCl₂, 0.1 mM of each dNTP, 0.2 µM of each specific primer.

Primer amplification efficiency (*E*) was calculated with qBASE+ software (Hellemans et al. 2007) from the slope of a five-point 1:10 serial dilution of calibrator cDNA samples, as follows: $E = 10^{-1/\text{slope}}$ (supplementary material, Table S2). Experimental setup of qPCR involved the sample maximization method (Hellemans et al. 2007), with three technical replicates for each sample, and inter-run calibrator samples were considered in calculations for run-to-run variation effects. For template quantification the qBASE+ pipeline was used (Hellemans et al. 2007), by first calculating the means and standard deviations of quantification cycle (C_q) values of technical replicates and relativizing C_q values based on the gene specific amplification efficiency. Next, the sample specific normalization factors were calculated by taking the geometric mean of the relative quantities of the two reference genes (*act* and *rps5*). The normalized C_q values were finally rescaled in relation to the sample with the lowest relative quantity (Hellemans et al. 2007), expressed in the form of calibrated normalized relative quantities (CNRQs), and used for statistical analyses.

(f) Statistical analyses

To identify differences in weight measures during the course of the monitoring survey, one-way ANOVA using “month” as factor was performed. In order to identify whether temperature and humidity were different among colonies during the outbreak period, specifically in those that became unhealthy, a t-test was conducted with data from March. Pearson’s correlation coefficient between foragers weight and nest temperature and humidity

was calculated with PAST v.4.0 (Hammer et al. 2001). To access monthly extremes of temperature (t) and humidity (h) suffered by colonies, which also reflect the performance of worker bees in controlling their nest environment, a variable called delta (Δ = maximum value - minimum value) was calculated. The Δ s were tested for their fit to normality and variance homogeneity using Shapiro's ($P < 0.05$) and Bartlett's ($P < 0.05$) tests, respectively. Box-Cox transformation with package MASS (Venables and Ripley 2002) in R (R Core Team 2019) was used for non-normal and non-homogeneous data. Differences in Δt and Δh were tested with one-way ANOVA using "month" and "colony" as factors. T-test and variance analyses were performed in R (R core team 2019), and the laercio package was used for mean comparisons with Tukey's test (Silva 2015).

Expression data of the selected genes was also tested for normality distribution and variance, with Box-Cox transformation used for non-normal and non-homogeneous data as mentioned above. To identify differences in our measures during the course of the monitoring survey, one-way ANOVA using "month" and "colony" as factors was performed for each variable. Similarly, a one-way ANOVA was used with each variable, to check for the differences within the "health status" of colonies (whether or not the syndrome manifested itself in March) and within the "intensity" of syndrome, since two of the affected colonies showed only mild effects of the syndrome (see below). Finally, a one-way ANOVA was performed for variables using "sister colonies" as factors to assess putative genetic effects on all variables, assuming that sister colonies, *i.e.*, obtained by division, are more related genetically than non-sister colonies. Considering that the syndrome occurs always in March, and that colonies have been monitored from January until April, a two-way ANOVA using either "colony", "sister colony", "health status" or "intensity" combined with "month" as factors was also performed for each variable, to identify interactions in specific periods during the course of our survey. Variance analyses and Tukey's test were performed in R (R core team 2019) as mentioned above. We used PAST v. 4.0 (Hammer et al. 2001) to evaluate combined variations in expression patterns of the five genes with one-way and two-way PERMANOVA, using Euclidean distances and 9999 permutations, in a procedure similar as described above for each gene individually.

Principal component analysis (PCA) was performed with PAST v. 4.0 (Hammer et al. 2001) to identify possible temporal patterns from combined variables measured from stingless bee colonies that eventually culminate with the syndrome. Two PCAs were run, discriminating variables that represent traits expressed at the level of individual bees, such as gene expression,

from those that are manifested at the colony level, such as social immunity, stored pollen, weight, temperature and humidity. Raw data used in our statistical analyses are provided as supplementary material (Table S3).

Results

(a) Transcriptomic overview of differentially expressed genes

Transcriptome sequencing yielded 99,766,936, 102,034,731, 135,982,124, single-end high quality-trimmed reads for U1, U2 and H, respectively. From all reads, 87-99% mapped against the *M. quadrifasciata* genome. The comparison between transcriptomes of healthy and unhealthy bees revealed 558 DEGs, from which 212 were hypothetical genes. Based on BLAST hits of the hypothetical genes to characterized genes from the *nr* database, 108/212 *M. quadrifasciata* DEGs were re-annotated (supplementary material, Table S1).

From total DEGs, 493 were down-regulated and 44 were up-regulated in unhealthy bees transcriptomes. Interestingly, among the most differentially expressed genes are genes involved in developmental processes, such as sensory organ development, methylation and behavior (supplementary material, Table S1). Only 21 DEGs showed inconsistent expression patterns. Looking for genes known for their role in bee health, five DEGs (*Vg1*: WN51_06142, *Vg2*: WN51_14077, *NF-kB*: WN51_13409, *p450*: WN51_04136 and *PO*: WN51_02761) were chosen for gene expression quantification using RT-qPCR and since five other DEGs (WN51_09191, WN51_04932, WN51_04931, WN51_07421, WN51_04930) that encode glucose dehydrogenase/oxidase are putatively involved in social immunity, the enzyme was quantified at the protein level.

(b) Behavioral changes of worker bees

Among the six colonies monitored in our study, four manifested symptoms of the syndrome in March 2019. Colonies BP2 and BP3 manifested the strongest symptoms, with some bees showing tremors and often paralysis, and high mortality of workers (~ 100 dead bees found in front of the hives). Their respective sister colonies PA2 and PA3 maintained in a separate locality became slightly unhealthy, since fewer deaths were observed in front of the hives (~ 10 dead bees) and bees did not manifest abnormal behavior. We rule out the possibility that the unhealthy bees died due to lethal doses of agrochemicals, since residue analyses

conducted by NSF International Laboratories (Porto Alegre, Brazil) did not indicate contamination by agrochemical compounds in three pools of ~20 bees from unhealthy colonies (supplementary material, Table S4). Sister colonies BP1 and PA1 did not manifest the syndrome, suggesting that the genetic component predisposing to disease. We found a marked reduction in forager weight from January until March ($P < 0.0005$), when the syndrome occurred (Figure 1). The lowest average weight was reached in March, and was higher in colonies that remained healthy during the outbreak (0.064 g), as compared to slightly unhealthy (0.063 g) and unhealthy (0.060 g) colonies, although the differences are not statistically significant. From January to February we observed a sudden change in the pollen stored by worker bees, *i.e.*, its composition shifted from mostly *Eucalyptus* sp. to mostly *Mimosa bimucronata* (Figure 1).

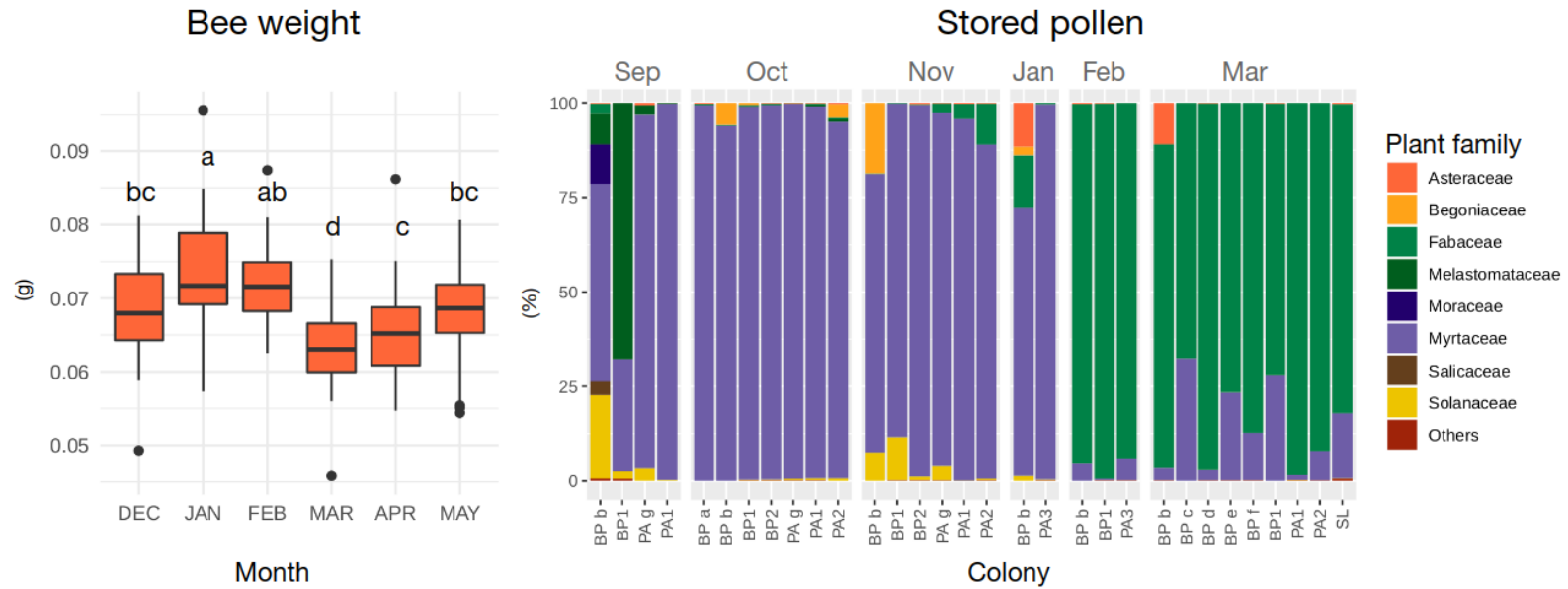


Figure 1: Changes in forager weight and stored pollen. (a) On the left a boxplot showing the temporal reduction in average forager weight. Lower case letters above bars represent significant differences ($P < 0.05$). (b) Barplot showing temporal variation in the percentage of pollen stored by workers, evidencing the change between January and February.

Bee weight reduction was accompanied by a similar reduction in the internal temperature of colonies ($r = 0.63$, $P = 0.0083$), and increase in humidity ($r = -0.51$, $P = 0.0431$). This pattern was more pronounced in colonies that became unhealthy during syndrome outbreak, such as sister colonies 2 (BP2 and PA2; $P < 0.0005$). Furthermore, temperature was lower ($P = 0.0006$) and humidity was higher ($P < 0.0005$) inside unhealthy colonies when the syndrome symptoms were first observed (Figure 2). Higher differences in daily temperature within colonies occurred in December and March ($P < 0.0005$), and BP colonies, which showed the most intense syndrome symptoms, had the highest differences in daily humidity ($P = 0.0022$, Figure 2).

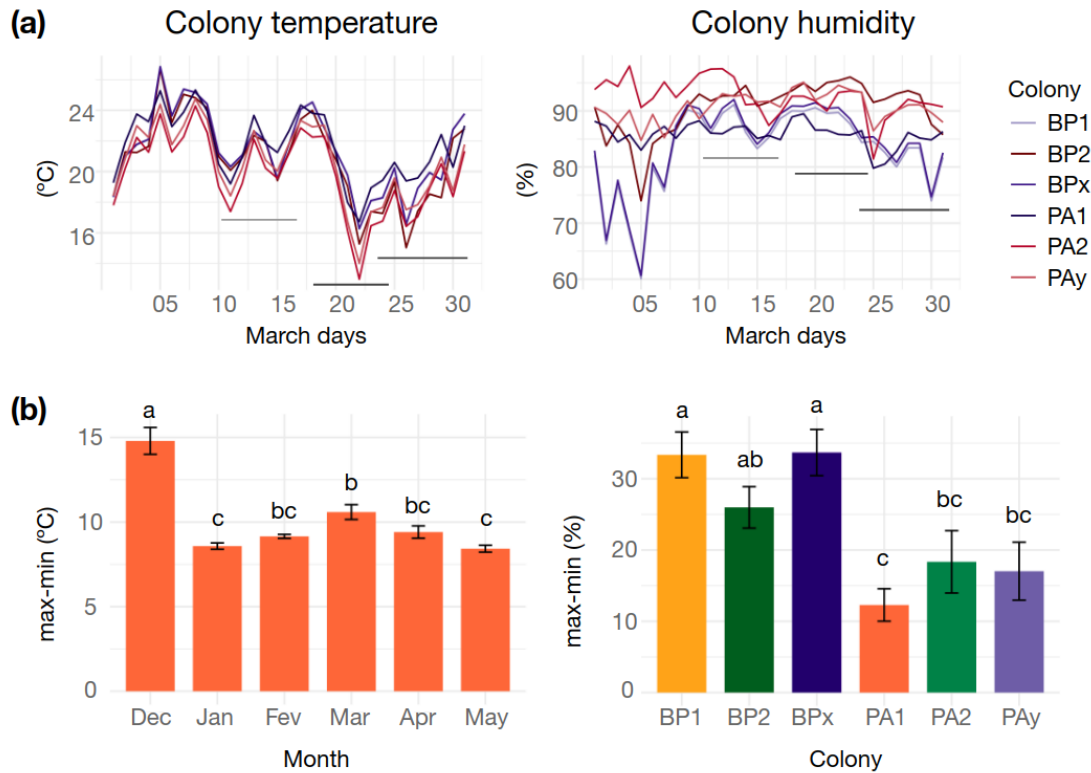


Figure 2: Temperature and humidity fluctuation. (a) Plot of the minimum daily temperature and maximum daily humidity in March. Horizontal bars indicate the days of syndrome manifestation, with higher (dark gray) or lower (light gray) intensity. Colonies affected by the syndrome (shades of red) show the lowest temperatures and highest humidities, especially during the outbreak period. (b) Barplot of the average monthly difference in maximum vs. minimum temperature (left) and average monthly difference in maximum vs. minimum humidity in different colonies (right). Bars indicate the standard error, and distinct lower case letters represent significant differences ($P < 0.05$).

(c)Temporal variation in forager gene expression

Transcriptome analysis revealed a large number of DEGs in unhealthy foragers, some of which are already known for their role in immunity and health of other bee species. Table 1 summarizes the genes selected for expression quantification in foragers sampled from the six colonies monitored in our study, their putative biological roles and the hypotheses behind their use as biomarkers for different aspects of colony health.

Table 1: Bee health-related genes differentially expressed in transcriptomes (*SGE*), comparing both unhealthy transcriptomes (U1 and U2) to the healthy (H). ANOVA results on data from the temporal survey comparing gene expression between colonies (C), months (M), sister colonies (SC), health status (HS) and syndrome intensity (I). Temporal variation in effects of C, SC, HS and I are shown as well (Interactions). For more details on the statistically significant comparisons, see electronic supplementary material (Figure S1).

Gene	Role	Hypothesis	References	<i>SGE</i>		Effect					Interactions			
				U1	U2	C	M	SC	HS	I	CxM	SCxM	HSxM	IxM
<i>p450</i>	Metabolism/detoxification of xenobiotics.	Increase in <i>p450</i> expression may indicate response to agrochemicals exposure.	Berenbaum and Johnson 2015.	0.67 (H > U)	0.59 (H > U)	ns	$P = 0.0243$	ns	ns	ns	$P = 0.0103$	ns	$P = 0.0213$	$P = 0.0205$
<i>Vg1</i>	Increase life span, antioxidant and immune function. Involved also in caste and task determination.	Increase in <i>Vg</i> expression may indicate better colony nutrition and performance.	Seehuus et al. 2006; Alaux et al. 2011.	0.57 (H < U)	0.63 (H > U)	ns	ns	ns	ns	ns	$P < 0.0005$	ns	ns	$P = 0.0023$
<i>Vg2</i>				0.60 (H > U)	0.53 (H > U)	$P = 0.0386$	ns	ns	ns	ns	$P < 0.0005$	$P = 0.0392$	$P = 0.0182$	$P = 0.0114$
<i>PO</i>	Activates melanogenesis.	Increase in <i>PO</i> expression may indicate a response to wound healing and defense against parasites.	Schmid-Hempel 2005.	0.69 (H < U)	0.65 (H > U)	$P = 0.0577$	ns	ns	ns	ns	ns	$P = 0.0149$	$P = 0.0163$	ns
<i>NF-κB</i>	Regulates humoral immune response (<i>e.g.</i> AMPs synthesis), but also cell survival and proliferation.	Increase in <i>NF-κB</i> expression may indicate a response to viral infection.	Nazzi et al. 2012; Ganesan et al. 2011.	0.62 (H > U)	0.56 (H > U)	ns	ns	$P = 0.022$	$P = 0.0303$	ns	$P = 0.0345$	$P = 0.0151$	ns	ns
<i>GOX</i>	Antimicrobial agent secreted by eusocial bees in larval food and honey stocks.	Colonies that secrete more <i>GOX</i> may indicate a stronger social immunity.	López-Uribe et al. 2017	0.59 – 0.66 (H > U)	0.50 – 0.62 (H > U)	ns	ns	ns	ns	ns	ns	ns	ns	ns
Combined expression pattern						$P = 0.0202$	ns	ns	ns	ns	$P < 0.0005$	$P = 0.008$	$P = 0.0541$	$P < 0.0005$

ns = not significant

Significant variation in *p450* expression was found across months, with peaks in March in both healthy and unhealthy colonies, and in January only in healthy colonies. In March foragers from healthy colonies expressed more *p450* compared to unhealthy colonies, similarly to what was found in our transcriptome analyses (Figure 3).

Vg2 and *PO* had similar patterns of expression during the course of the survey. Both showed significant differences between colonies (Table 1), with BP1 and BP2 showing the highest and lowest expression, respectively, on average (supplementary material, Figure S1). Furthermore, similarly to *p450*, bees that remained healthy during the outbreak period showed the highest expression in January (Figure 3). *Vg1* did not show association with the factors analyzed in our study (Table 1). Taking into account the hypotheses raised on Table 1, the lower expression of *p450*, *Vg2* and *PO* in bees that later became unhealthy suggests delayed effects in the response to xenobiotics, poor nutrition and weak immunity.

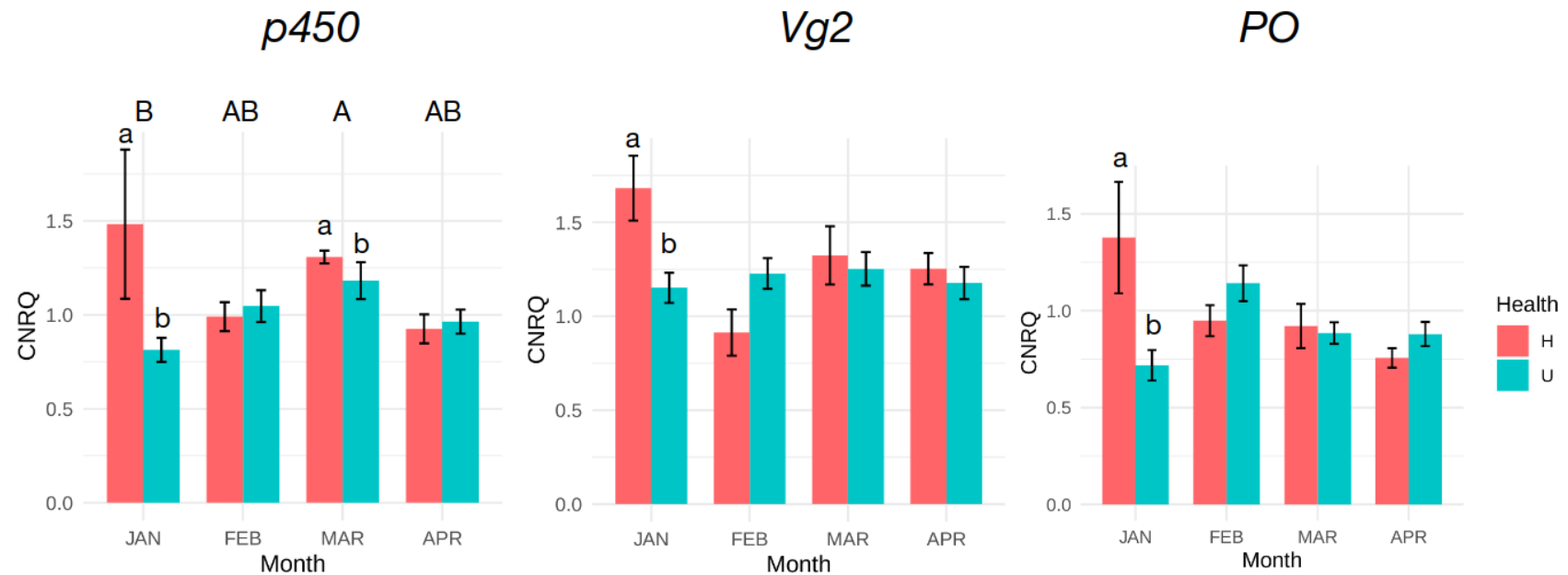


Figure 3: Relative expression of *p450*, *Vg2* and *PO*. Barplot comparing gene expression in foragers from healthy colonies (H), with those from colonies that showed signs of disease during syndrome outbreak (U). Bars indicate the standard error; distinct uppercase letters designate differences among months and distinct lowercase letters indicate differences between healthy and unhealthy colonies within each month, according to Tukey's test ($P < 0.05$). Only significant differences are indicated by letters.

Among the five genes analyzed in our study, *NF-kB* was unique for showing significant differences in expression between pairs of sister colonies, suggesting a strong genetic influence on expression (Table 1). Although *NF-kB* is more expressed in healthy foragers on average (supplementary material, Figure S1), this effect is not consistent across months. The amount of GOX secreted by worker bees as part of their social immunity remained stable over the months, suggesting a constitutive expression pattern (with one outlier, see supplementary material, Figure S2).

(d) Combined analyses of traits

Considering that all measures performed in our longitudinal survey represent phenotypes, we ran PCA analyses in order to identify differences between months and colony health status. Therefore, we discriminated traits expressed at the individual bee from those expressed at the level of the whole colony. We considered that while gene expression patterns are observed at the individual level, stored pollen composition, as well as within-colony temperature and humidity represent colony-level traits. Similarly, although GOX is expressed at the level of the individual, it is part of a social behavior used to fight against potentially pathogenic microorganisms. Forager weight was also regarded as a colony level trait, considering that a positive correlation exists between bee weight and colony density (Jones et al. 2018). Overall, variation in individual-level traits does not explain the temporal changes that eventually culminate with the syndrome, even though *p450*, *Vg2* and *PO* are among genes that most contribute to data our distribution (Figure 4). Colony-level traits behave as better indicators of health, with factors daily highest humidity, daily humidity difference and daily lowest temperature explaining most of the data distribution (Figure 4).

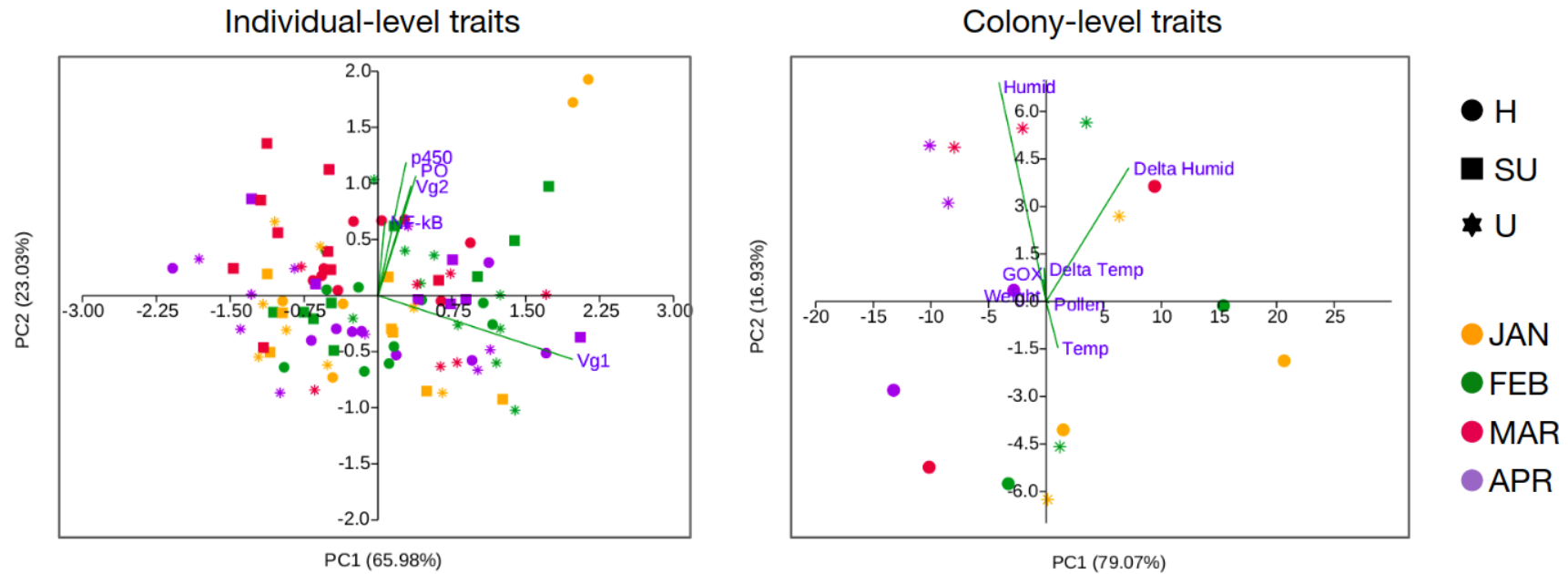


Figure 4: Principal component analysis (PCA) with individual- and colony-level traits. On the left, the plot shows the distribution of individuals according to the expression of five genes quantified by real time PCR. On the right the plot shows the distribution of colonies according to characteristics measured at the colony level. Symbols refer to healthy (H), slightly unhealthy (SU) and unhealthy (U) colonies. Samples from different months are plotted with distinct colors.

Discussion

Our temporal survey on six *M. quadrifasciata* managed colonies revealed that both genetic and environmental factors underlie the annual syndrome reported by beekeepers in Southern Brazil. Sister colonies (genetic relatedness of 0.375; see (Oliveira et al. 2015)) presented a similar health status during the 2019 syndrome outbreak, even though they have been kept in different localities. Though, the symptoms of worker bees were stronger in two affected colonies kept nearby an agricultural setting.

(a) Bee domestication and responses to environmental factors

Domestication leads to the loss of genetic variation and gene expression diversity (Liu et al. 2019). The stingless bee *M. quadrifasciata* is a highly domesticated species, whose wild nests virtually disappeared in Southern Brazil (Fundação Zoobotânica 2014). The species is intensively managed for colony trading in the region, with some meliponaries maintaining up to 50 colonies. The practice of colony division is being performed since decades, and became more common with recent trade intensification (Jaffé et al. 2015). We suspect that the general use of this management practice, associated with the concomitant loss of wild nests, may have reduced the genetic diversity of *M. quadrifasciata* populations in Southern Brazil.

Reduced genetic variation may have affected stingless bee populations to properly respond to environmental stresses, such as pesticides, pathogens and competition for food resources with other bees, especially *A. mellifera*. By analyzing the differential gene expression of healthy vs. unhealthy *M. quadrifasciata* worker bees, and focusing on a subset of key genes, we suggest that the syndrome is manifested by bees' incapacity to respond to environmental stresses. We show that the expression of *M. quadrifasciata* genes homologous to *A. mellifera* genes related to health and immunity is significantly lower in foragers from colonies that became unhealthy two months before the outbreak period. In *A. mellifera* cytochrome p450 acts in detoxification of xenobiotics (Berenbaum and Johnson 2015), vitellogenin, besides being involved in caste and task determination, also protects against oxidative stress, enhances longevity and is a biomarker for good nutrition (Hartfelder et al. 2006; Seehuus et al. 2006; Alaux et al. 2011); and phenoloxidase activates melanogenesis needed for parasites defense and response to wound healing (Schmid-Hempel 2005).

Melipona development takes around 40 days from egg to adult (Alves et al. 2009), thus the *M. quadrifasciata* foragers sampled in March were juveniles in January. This may explain

the observed delayed effect of gene expression. Considering the whole period, *PO* and *Vg2* showed similar patterns of gene expression with significant differences between colonies, and sister colonies responding similarly to the different environmental factors (*PO* interaction $P = 0.0149$, *Vg2* interaction $P = 0.0392$; supplementary material, Figure S1). It may also indicate a functional relationship between the *Vg2* and *PO*. *Vg2* encodes a secreted protein, with a putative signal peptide (Piulachs et al. 2003). The functional relationship between *Vg2* and *PO* suggests its role as a regulator of immune function.

We did not see any consistent relationship of *NF-kB* expression and the syndrome outbreak. In *A. mellifera* this gene was shown to be involved in the response against viral infections (Nazzi et al. 2012), and was used in our study to test the hypothesis that colonies fail for not being able to control viral proliferation. We have previously shown that although viruses did not seem to determine the outcome of the syndrome in all meliponaries, the relationship between the presence of viruses and the syndrome manifestation varies across localities (Caesar et al. 2019). In our transcriptome analyses we found an increase in the expression of *NF-kB* in healthy foragers, but the temporal survey using real time PCR quantification revealed the opposite result (Table 1; supplementary material, Figure S1). Our survey suggests that there might be a stronger genetic component in the variation of *NF-kB* expression (Table 1; supplementary material, Figure S1), which could mean that this gene is less responsive to the stressors faced by our bees. Furthermore, the *M. quadrifasciata* genome contains another *NF-kB*-related gene (WN51_01536), bringing into question whether the gene chosen in our study indeed represents the *Apis* ortholog.

(b) Sub-lethal effects

Among the five genes analyzed in our survey, only *p450* showed a significantly higher level of expression during the outbreak period (Table 1, Figure 3). In honey bees, bumblebees and solitary bees, cytochrome p450 functions as a detoxifying agent and determine the sensitivity to agrochemicals (Manjon et al. 2018; Beadle et al. 2019). Even though we did not detect lethal doses of agrochemicals in colonies affected by the syndrome, Southern Brazil is the region where they are most used in agriculture (Pignati et al. 2017). Furthermore, the significant interaction of *p450* expression levels and month, with healthy foragers sampled in January showing the highest levels of expression, suggests that a poor metabolism of xenobiotics in January possibly results in sub-lethal effects that contribute to forager impairment later on. Our transcriptome analysis also suggests neurological and behavioral

changes in bees affected by the syndrome, such as lower expressions of ADP/ATP translocase (WN51_03592), pheromone-binding protein (WN51_03399), chaoptin (WN51_11844) and neuropeptide (WN51_06770; supplementary material, Table S2). It was already shown that *M. q. anthidioides* and bumblebees, that had contact with neonicotinoids at juvenile stages, showed reduced brain development resulting in compromised mobility and learning performance in adults, thus affecting major colony activities such as foraging (Tomé et al. 2012; Smith et al. 2020).

Another sub-lethal effect suggested by our study is the exposure to pathogens. Similarly to *p450*, we detected a significantly reduced expression of *PO* in January in foragers from colonies that later manifest the syndrome. January might be characterized by a higher density of pollinators in general, including *A. mellifera*, creating opportunities for getting in contact with a larger diversity of pathogens. Stingless bees from Brazil sharing foraging areas with honey bees were found contaminated with viruses and the microsporidium *N. ceranae*, suggesting the occurrence of spillover from *A. mellifera* (Guimarães-Cestaro et al. 2020). To fight against pathogens *PO* was found up-regulated in honey bee larvae infected with *Paenibacillus larvae* (Chan et al. 2009), in pupae infested with *Varroa* (Tesovnik et al. 2017) and in *Apis* infected by *Deformed wing virus* (Mazzei et al. 2016). Considering that *Vg2* expression pattern at the colony level was similar to *PO*, we think that its function is also related to immunity. *Vg1*, on the other hand, was the gene with the highest relative expression in our study (*Vg1* CNRQ: 0.8 - 4.9; *Vg2* CNRQ: 0.4 - 2.3), but with no clear connection to the syndrome. Vitellogenin (*Vg*) plays an important role promoting longevity in honey bees (Münch et al. 2013), but homologous genes that diverged from *Vg* have specialized functions. In *A. mellifera*, *Vg-like-A* responds more to inflammatory and oxidative conditions and *vg-like-B* responds more to oxidative stress (Salmela et al. 2016). There are at least 3 paralogous vitellogenin genes in *M. quadrifasciata* genome (WN51_13813, WN51_04373, WN51_14077) and they might correspond to *Vgs* with specialized functions too.

Finally, pollen stress might have been an additional factor with sub-lethal effects. We detected weight loss of foragers from January to March (Figure 1), suggesting poor nutrition and/or developmental acceleration. Furthermore, we detected an abrupt change in pollen stored by worker bees, which shifted from mostly *Eucalyptus* to mostly *Mimosa* between January and February. Such a shift may result from competitive exclusion, since *M. quadrifasciata* apparently competes with *A. mellifera* for flowers (Wilms and Wiechers 1997). Similarly to agrochemicals, pollen stress can also lead to neurological impairment, as suggested by the

compromised foraging and recruitment performance of honey bee workers if they were reared as larvae in a pollen-limited colony environment (Scofield and Mattila 2015). Moreover, pollen-stressed workers are known to become lighter, dying sooner, and some of them do not even perform the forage activity (Scofield and Mattila 2015). Two months after *M. quadrifasciata* starts to use *Mimosa*, which begins to bloom in February, we noticed a gradual recovery in foragers weight (Figure 1). In *A. mellifera*, which suffered reduction in head mass, immune system depletion and increase in DWV titres caused by antibiotics, the supply with pollen helped to recover body mass and immune response as by increasing *Vg* expression (Li et al. 2019).

(c) Individual level vs. colony level traits

It is now well established that combined sub-lethal effects are at the root of colony failures (Bryden et al. 2013; Lu et al. 2020). PCA of the factors evaluated in our study suggest that colony-level traits provide a better description of differences between health and disease in the *M. quadrifasciata* syndrome (figure 4), but such traits are themselves consequences of biological processes that occur at the individual level. Bee weight is a known proxy for colony density (Jones et al. 2018). Similarly, nest temperature and humidity are controlled by worker bees through specialized behaviors (Jones and Oldroyd 2006; Ayton et al. 2016), which are influenced by different sub-lethal factors that we inferred from our gene expression survey at the individual level. Surprisingly, social immunity evaluated in our study by the amount of glucose oxidase in the heads of worker bees was not affected by the factors that stimulate other immune responses assessed in our study such as *PO*. Nevertheless, this result is in agreement with previous findings showing that GOX is constitutively secreted by *A. mellifera* workers (López-Urbe et al. 2017). Honey bee nurses secrete it into larval jelly and foragers into honey (Bucekova et al. 2014).

Although four of the six colonies surveyed in our study manifested some degree of syndrome symptoms, none of them collapsed during the outbreak period. We think that the annual collapses reported for *M. quadrifasciata* colonies in Southern Brazil result from positive density dependence influenced by the combination of diverse sub-lethal factors, as suggested by mathematical modeling studies (Khoury et al. 2011; Bryden et al. 2013). Unfortunately, the complexity of causes behind colony collapses worldwide entangle efforts to sustain pollination services. Based on our findings, actions such as limiting the use of agrochemicals in the vicinity of managed colonies and providing natural polyfloral resources through the conservation of

native forests, could help to prevent the annual *M. quadrifasciata* syndrome, and the loss of other native bee species.

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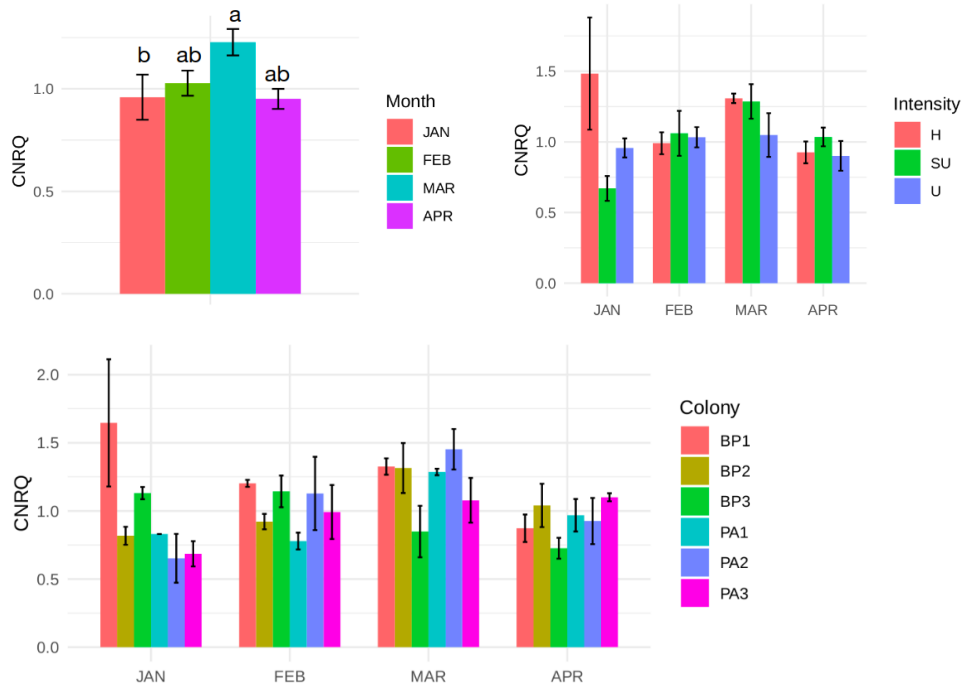
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p450



Vg1

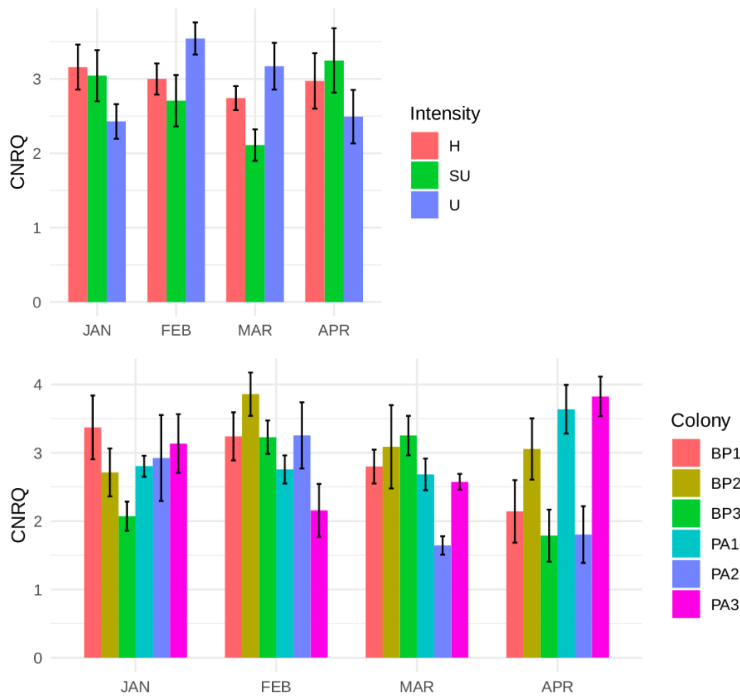
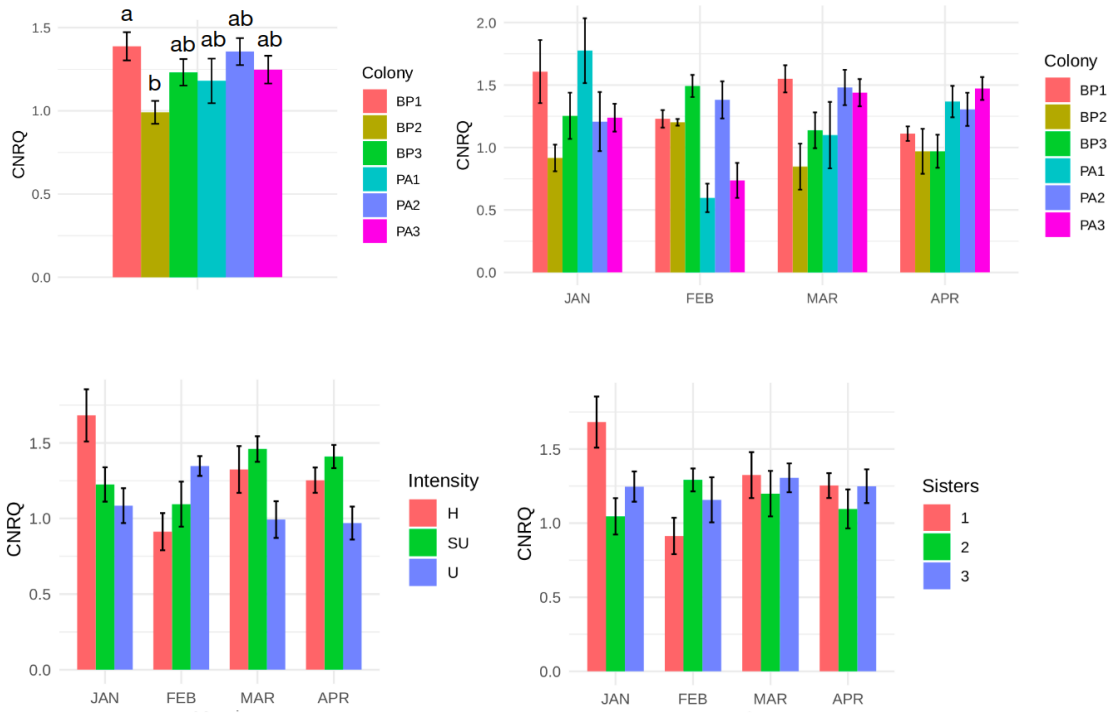


Figure S1: Relative gene expression of *p450*, *Vg1*, *Vg2*, *PO* and *NF-kB*. Barplots refer to comparisons that revealed statistical significance in variance analyses ($P < 0.05$) listed on Table 1.

Vg2



PO

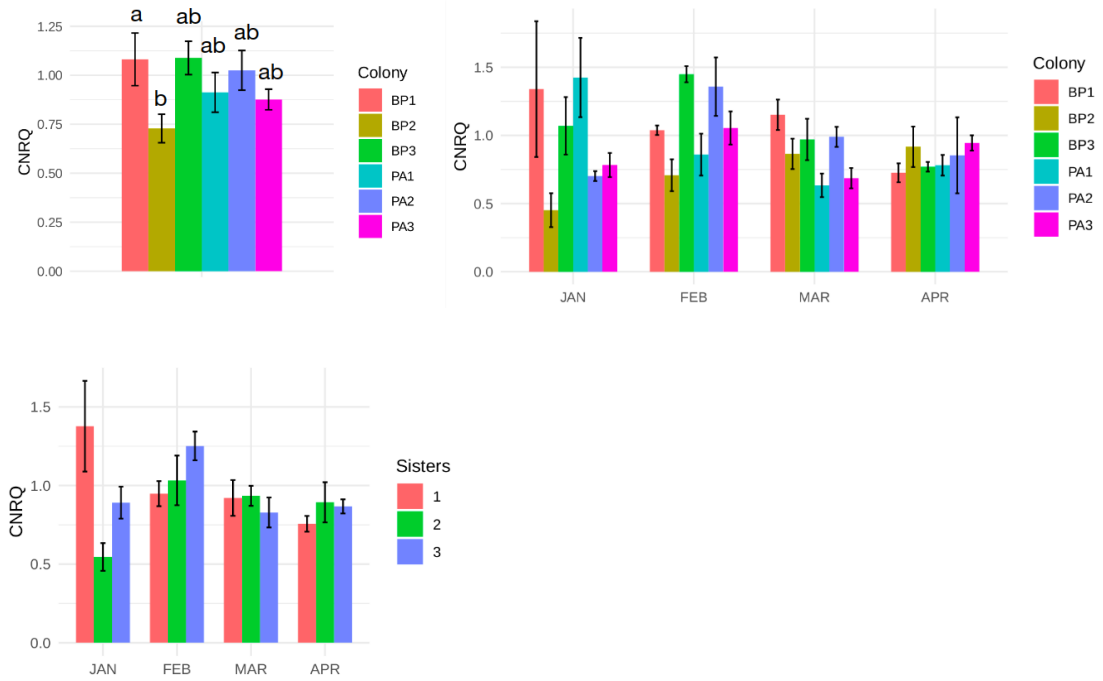


Figure S1: (Continuation).

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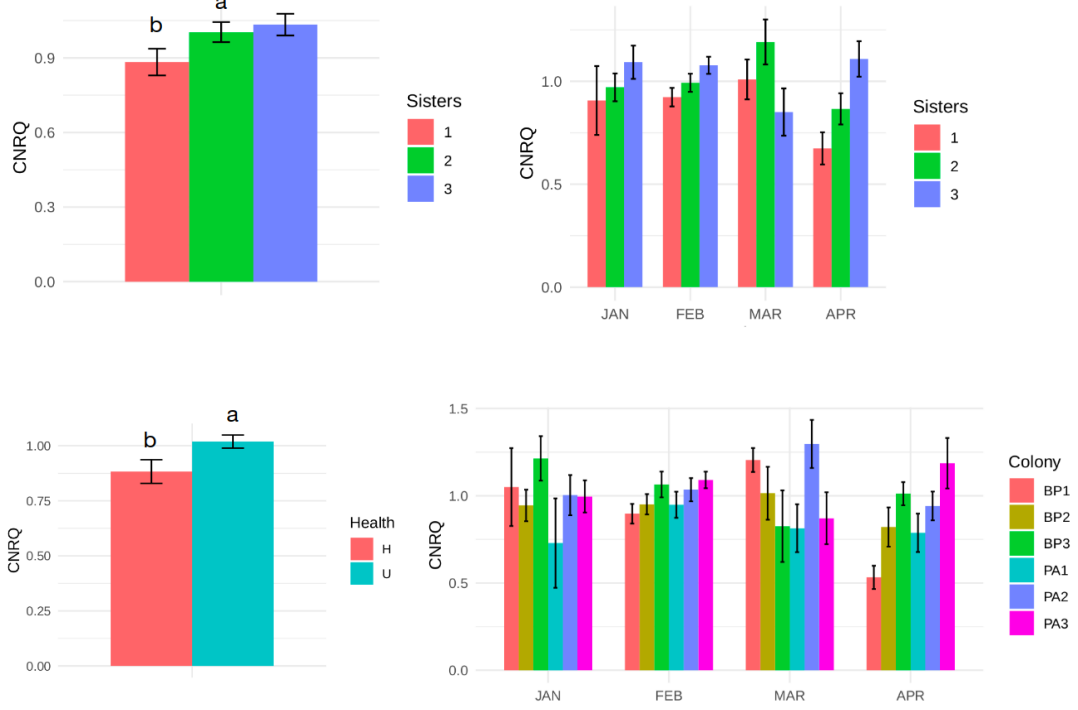


Figure S1: (Continuation).

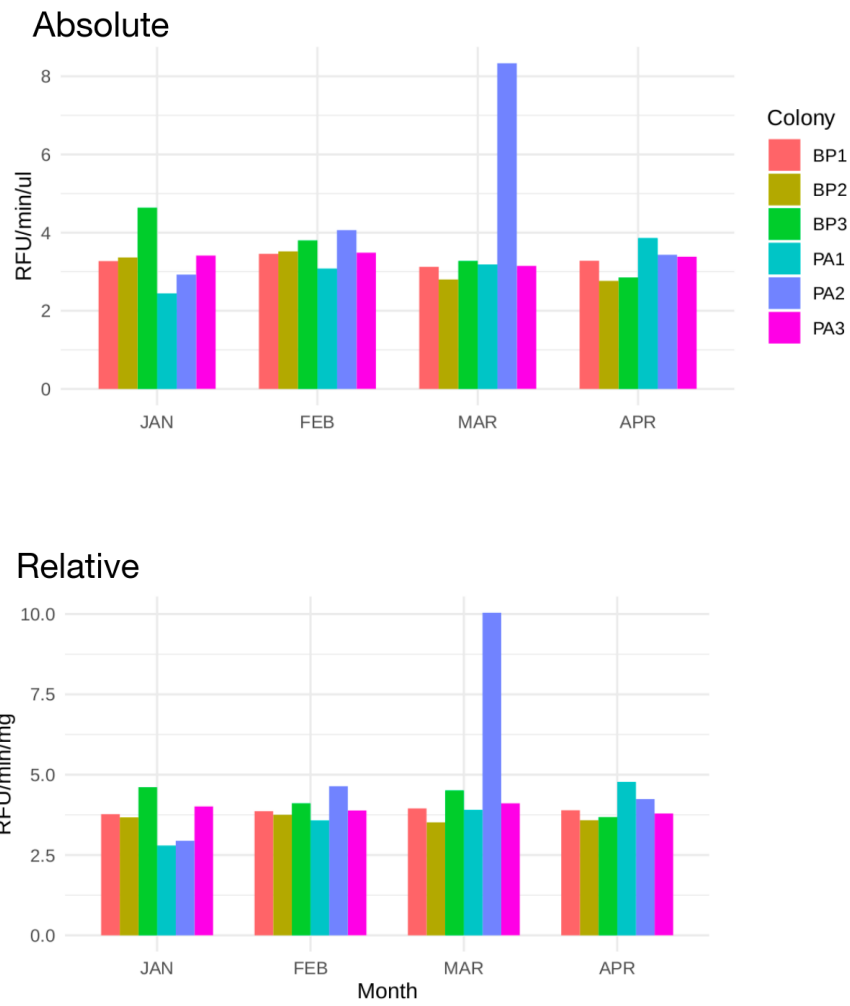


Figure S2: Glucose oxidase (GOX) activity in each colony and month. Above the absolute quantification and below the quantification relative to foragers weight of respective colony.

Table S1(a): list of differently expressed genes comparing U1 (unhealthy bees from Bom Princípio), U2 (unhealthy bees from Estância Velha) e H (healthy bees from Bom Princípio) transcriptomes and their respective normalized gene expression (NGE) and similarity in gene expression (SGE). Most differently expressed genes are above (light red) and genes chosen for quantification by RT-qPCR or enzymatic assay are also highlighted (green).

Protein	NGE U1	NGE U2	NGE H	SGE U1	SGE U2	Biological process
WN51_11205_hypothetical_protein	0.04	0.05	28.69	0.50	0.50	
WN51_05670_hypothetical_protein	0.13	104.14	27.59	0.50	0.63	
WN51_04193_jmjC_domain-containing_protein_4_isoform_X2	0.51	15.14	54.81	0.50	0.64	cellular process, metabolic process, viral RNA genome replication, localization, regulation of cell cycle.
WN51_07902_hypothetical_protein	0.27	0.12	23.85	0.51	0.50	
WN51_03592_ADP/ATP_translocase_4	0.45	0.02	22.13	0.51	0.50	cellular process, biological regulation, cellular component organization, localization, response to stimulus, developmental process, chemical synaptic transmission, determination of adult lifespan, reproductive process, locomotion, synaptic growth at neuromuscular junction, viral process, metabolic process.
WN51_12208_hypothetical_protein	0.98	0.00	47.54	0.51	0.50	
WN51_03399_Pheromone-binding_protein-related_protein_3	0.68	6.93	32.98	0.51	0.60	multicellular organismal process, behavior, response to pheromone, courtship behavior, multicellular organism reproduction, cellular process, biological regulation, copper ion transport, metabolic process, cellular component organization.
WN51_04637_protein_amnionless-like	2.66	0.04	114.01	0.51	0.50	cellular process, metabolic process, developmental process, regulation of biological process, actin cytoskeleton reorganization, multicellular organism development.
WN51_11844_Chaoptin	1.23	10.03	49.75	0.51	0.60	cellular process, cellular component organization, locomotion, sensory organ development, regulation of biological process, response to stimulus, homophilic cell adhesion via plasma membrane adhesion molecules, visual perception, metabolic process.
WN51_06770_Neuropeptide_FF_receptor_2	0.93	0.04	34.41	0.51	0.50	biological regulation, cellular process, response to stimulus, mating behavior and sex discrimination, multicellular organismal process, methylation.
WN51_13573_hypothetical_protein	0.56	0.56	20.61	0.51	0.51	
WN51_04190_putative_cytochrome_P450_12a5_mitochondrial	0.78	0.22	21.50	0.52	0.51	
WN51_12311_Papilin	1.57	2.78	42.73	0.52	0.53	
WN51_12392_hypothetical_protein	1.16	78.75	30.63	0.52	0.69	
WN51_06061_Cubilin	4.54	1.76	107.10	0.52	0.51	
WN51_00353_Pituitary_homeobox_like_protein_Ptx1	0.93	0.08	21.51	0.52	0.50	
WN51_11275_probable_JmjC_domain-containing_histone_demethylation_protein_2C	1.78	4.33	41.11	0.52	0.55	
WN51_06580_hypothetical_protein	1.04	67.82	24.02	0.52	0.68	
WN51_09379_hypothetical_protein	1.65	0.00	37.90	0.52	0.50	
WN51_03763_Myogenic_factor_5	3.18	0.49	70.28	0.52	0.50	
WN51_03684_Reticulocyte-binding_protein_2_like_protein_a	1.11	81.06	24.05	0.52	0.65	
WN51_07801_Protein_yellow	0.98	2.02	20.75	0.52	0.55	
WN51_09070_orcokinin_peptides-like_isoform_X2	1.24	184.57	25.89	0.52	0.57	
WN51_09378_Homeobox_protein_araucan	1.17	4.03	24.22	0.52	0.58	
WN51_00177_hypothetical_protein	3.13	19.87	63.77	0.52	0.66	
WN51_05941_Knirps-related_protein	1.41	7.42	28.08	0.53	0.63	
WN51_10836_hypothetical_protein	1.26	9.49	24.47	0.53	0.69	
WN51_00173_hypothetical_protein	3.35	5.85	64.50	0.53	0.55	
WN51_05104_Fatty_acid_synthase	3.41	1.09	64.72	0.53	0.51	
WN51_08196_Protein_trachealeas	1.32	8.65	24.75	0.53	0.67	
WN51_14534_cAMP-specific_3',5'-cyclic_phosphodiesterase	1.96	5.10	36.03	0.53	0.57	
WN51_12044_hypothetical_protein	2.18	2.44	36.45	0.53	0.53	
WN51_12673_1-phosphatidylinositol_4,5-bisphosphate_phosphodiesterase_epsilon-1	1.90	106.74	31.38	0.53	0.65	
WN51_09419_hypothetical_protein	1.96	6.70	32.32	0.53	0.60	
WN51_07675_hypothetical_protein	2.92	1.56	45.12	0.53	0.52	
WN51_00523_hypothetical_protein	2.48	5.38	38.08	0.53	0.57	
WN51_02326_Sodium_channel_protein_para	4.35	13.22	60.89	0.54	0.61	
WN51_05779_Chitin_deacetylase-like_5	4.32	10.79	59.95	0.54	0.59	
WN51_00399_transposase	3.00	0.00	40.19	0.54	0.50	
WN51_07143_hypothetical_protein	6.93	32.47	90.06	0.54	0.68	
WN51_07303_HEAT_repeat-containing_protein_2	3.43	16.48	42.44	0.54	0.69	
WN51_03377_Cytochrome_c_oxidase_subunit_6B1	2.56	0.00	31.21	0.54	0.50	
WN51_13754_hypothetical_protein	4.02	13.99	48.40	0.54	0.64	
WN51_12010_hypothetical_protein	2.44	10.23	29.30	0.54	0.67	
WN51_12072_X-linked_retinitis_pigmentosa_GTPase_regulator	1.88	2.37	22.53	0.54	0.55	
WN51_04441_chloride_channel_CLIC-like_protein_1_isoform_X6	6.40	0.00	74.93	0.54	0.50	
WN51_14585_B1_protein	11.74	8.52	135.64	0.54	0.53	
WN51_07674_thyrotroph_embryonic_factor_isoform_X2	2.43	7.82	28.10	0.54	0.64	
WN51_08604_hypothetical_protein	10.00	21.85	113.07	0.54	0.60	
WN51_12846_hypothetical_protein	6.77	10.72	76.10	0.54	0.57	
WN51_00042_NADH-ubiquinone_oxidoreductase_chain_5	85.99	149.61	7.68	0.54	0.53	
WN51_00468_BBSome-interacting_protein_1	10.89	12.99	120.37	0.55	0.55	
WN51_07556_hypothetical_protein	7.63	1.22	83.97	0.55	0.51	
WN51_14222_hypothetical_protein	5.16	180.11	56.28	0.55	0.66	

WN51_00859_hypothetical_protein	10.13	1.14	109.13	0.55	0.51
WN51_11173_Torso-like_protein	7.70	5.86	82.73	0.55	0.54
WN51_01027_Organic_cation_transporter_1	6.16	12.35	64.87	0.55	0.60
WN51_02439_hypothetical_protein	31.11	24.67	2.96	0.55	0.56
WN51_09281_mucin-5AC-like_isoform_X3	8.25	20.91	84.93	0.55	0.62
WN51_03560_Synaptotagmin-14	2.09	83.75	21.38	0.55	0.63
WN51_01741_low_density_lipoprotein_receptor_adapter_protein_1-like_isoform_X3	3.76	5.24	38.38	0.55	0.57
WN51_03220_DnaJ_like_protein_subfamily_C_member_14	5.54	0.17	56.53	0.55	0.50
WN51_02823_adoMet-dependent_rRNA_methyltransferase_SPB1_isoform_X1	2.73	6.07	27.43	0.55	0.61
WN51_05305_Sensory_neuron_membrane_protein_1	8.23	9.69	82.36	0.55	0.56
WN51_00428_F-box/LRR-repeat_protein_14	2.95	3.60	29.41	0.55	0.56
WN51_04933_titin	6.26	12.60	61.91	0.55	0.60
WN51_04269_Tensin	8.21	17.65	80.43	0.55	0.61
WN51_12588_calphotin-like	7.78	12.78	75.68	0.55	0.58
WN51_14147_hypothetical_protein	2.12	0.94	20.15	0.55	0.52
WN51_12744_patched_domain-containing_protein_3_isoform_X2	5.99	1.80	56.55	0.55	0.52
WN51_01239_hypothetical_protein	10.12	0.35	95.05	0.55	0.50
WN51_14148_hypothetical_protein	3.30	0.00	30.93	0.55	0.50
WN51_14599_Protein_brown	14.08	7.18	128.92	0.55	0.53
WN51_08948_Mitochondrial_thiamine_pyrophosphate_carrier	9.19	25.08	80.90	0.56	0.66
WN51_06500_Ras-specific_guanine_nucleotide-releasing_factor_1	4.18	138.51	36.77	0.56	0.63
WN51_14628_RNA_pseudouridylate_synthase_domain-containing_protein_2	8.48	17.95	72.02	0.56	0.62
WN51_01490_hypothetical_protein	4.38	7.71	36.65	0.56	0.61
WN51_03687_Putative_polypeptide_N-acetylgalactosaminyltransferase_9	2.94	0.14	24.26	0.56	0.50
WN51_03909_hypothetical_protein	12.59	29.79	103.68	0.56	0.64
WN51_11144_Carboxypeptidase_D	8.93	14.59	72.54	0.56	0.60
WN51_05679_MAM_domain-containing_glycosylphosphatidylinositol_anchor_protein_1	13.33	8.55	106.02	0.56	0.54
WN51_08080_Glutamate_synthase_2_[NADH]_chloroplastic	15.92	5.12	126.16	0.56	0.52
WN51_07276_SCO-spondin	2.96	6.77	23.24	0.56	0.65
WN51_06649_Retinol_dehydrogenase_10-A	15.93	5.61	124.64	0.56	0.52
WN51_04261_Protein_abrupt	4.23	9.74	33.08	0.56	0.65
WN51_14382_Low-density_lipoprotein_receptor-related_protein_2	7.78	9.57	60.48	0.56	0.58
WN51_04327_Putative_zinc_metalloproteinase_T16A9.4	5.97	15.53	46.18	0.56	0.67
WN51_01357_Methylenetetrahydrofolate_reductase	6.59	12.99	50.08	0.57	0.63
WN51_01017_high_molecular_weight_rhoptry_protein_2_putative	11.25	1.92	85.31	0.57	0.51
WN51_10562_Heart_and_neural_crest_derivatives-expressed_protein_2	4.48	4.81	33.86	0.57	0.57
WN51_11848_Molybdenum_cofactor_sulfurase	12.33	6.32	92.34	0.57	0.53
WN51_09653_Histone_H2A	112.79	67.19	15.09	0.57	0.61
WN51_12862_ribonuclease_P/MRP_protein_subunit_POP5	3.52	0.00	26.09	0.57	0.50
WN51_01295_serine-rich_adhesin_for_platelets-like	15.78	1.25	116.69	0.57	0.51
WN51_02793_Cuticlin-1	7.88	4.74	58.11	0.57	0.54
WN51_14278_Histone-lysine_N-methyltransferase_SETMAR	3.77	0.01	27.82	0.57	0.50
WN51_14055_Vitellogenin-6	8.82	4.39	64.19	0.57	0.53
WN51_13358_Cytochrome_P450_6a2	13.20	30.87	95.14	0.57	0.66
WN51_09750_Homeobox_protein_MOX-2	60.47	23.50	8.40	0.57	0.68
WN51_06720_UPF0193_protein_EVG1	2.98	0.03	21.34	0.57	0.50
WN51_07515_Elongation_of_very_long_chain_fatty_acids_protein_7	8.06	8.06	57.56	0.57	0.57
WN51_02336_Multiple_PDZ_domain_protein	7.36	10.24	52.20	0.57	0.60
WN51_06361_Neuron_navigator_2	4.29	9.06	30.43	0.57	0.65
WN51_03256_Branched-chain-amino-acid_aminotransferase_cytosolic	15.14	12.66	107.10	0.57	0.56
WN51_00618_Solute_carrier_organic_anion_transporter_family_member_3A1	3.90	3.13	27.42	0.57	0.56
WN51_14090_G/T_mismatch-specific_thymine_DNA_glycosylase	8.83	15.25	61.88	0.57	0.62
WN51_04406_division_abnormally_delayed_protein	12.68	11.47	88.76	0.57	0.56
WN51_07086_Receptor-type_tyrosine-protein_phosphatase_kappa	15.16	17.69	104.79	0.57	0.58
WN51_10823_Acyl-CoA_Delta(11)_desaturase	93.84	56.96	13.59	0.57	0.62
WN51_11601_MATH_and_LRR_domain-containing_protein_PFE0570w-like	5.36	0.05	36.85	0.57	0.50
WN51_06142_Vitellogenin [Vg1]	167.60	6.83	24.40	0.57	0.64
WN51_00299_Microtubule-associatedA	5.53	6.00	37.73	0.57	0.58
WN51_01963_ABC_transporter_G_family_member_23	17.99	3.18	122.40	0.57	0.51
WN51_09925_1-acyl-sn-glycerol-3-phosphate_acyltransferase_alpha	14.54	38.20	97.22	0.57	0.70
WN51_07017_Gamma-aminobutyric_acid_type_B_receptor_subunit_1	3.09	64.49	20.67	0.57	0.66
WN51_12667_Glycoprotein_3-alpha-L-fucosyltransferase_A	7.73	16.67	51.61	0.57	0.66
WN51_00306_hypothetical_protein	6.09	2.93	40.68	0.57	0.54
WN51_10563_Nose_resistant_to_fluoxetine_protein_6	13.71	1.68	91.48	0.57	0.51
WN51_02144_Utrophin	6.99	4.92	46.53	0.58	0.55
WN51_03774_UBX_domain-containing_protein_6	16.56	32.51	110.32	0.58	0.65
WN51_13725_hypothetical_protein	33.10	26.27	4.98	0.58	0.59
WN51_06050_dirigent_protein_10-like	35.03	51.30	5.29	0.58	0.55
WN51_12041_Tyrosine_aminotransferase	9.65	21.92	63.69	0.58	0.67
WN51_13753_tRNA_2-thiocytidine_biosynthesis_protein_TtcA	16.60	30.92	109.46	0.58	0.64
WN51_02659_hypothetical_protein	11.74	8.38	77.37	0.58	0.55
WN51_02937_Galanin_receptor_type_1	7.24	6.30	47.45	0.58	0.57
WN51_13283_hypothetical_protein	7.24	16.12	47.41	0.58	0.67
WN51_02934_pyroglutamylated_Rfamide_peptide_receptor-like_isoform_X2	5.80	13.09	37.74	0.58	0.67
WN51_13311_Tolloid-like_protein_2	18.69	3.22	121.31	0.58	0.51
WN51_08474_Xin_actin-binding_repeat-containing_protein_2	51.44	20.37	7.99	0.58	0.70
WN51_08417_skin_secretory_protein_xp2-like	3.46	1.41	22.17	0.58	0.53
WN51_12032_Spermine_oxidase	20.43	29.54	3.21	0.58	0.55
WN51_06482_hypothetical_protein	6.87	17.25	43.64	0.58	0.70
WN51_09251_Choline_O-acetyltransferase	3.28	66.20	20.83	0.58	0.66
WN51_04902_Spondin-1	19.01	23.42	120.10	0.58	0.60
WN51_01650_zinc_finger_protein_395	4.36	3.47	27.55	0.58	0.56
WN51_14190_Protein_trapped_in_endoderm-1	5.46	2.24	34.40	0.58	0.53
WN51_11332_hypothetical_protein	4.84	0.00	30.35	0.58	0.50
WN51_03729_Glutamate_receptor_ionotropic_kainate_1	7.06	2.81	44.15	0.58	0.53
WN51_12996_Phospholipase_A2	4.37	7.05	27.35	0.58	0.63

WN51_08056_Protein_SDA1_like_protein	7.83	8.55	48.65	0.58	0.59
WN51_01347_Sn1-specific_diacylglycerol_lipase_alpha	10.37	24.29	64.18	0.58	0.69
WN51_04639_hypothetical_protein	4.67	1.58	28.75	0.58	0.53
WN51_12447_Regulator_of_microtubule_dynamics_protein_1	9.94	7.85	61.13	0.58	0.56
WN51_09769_myb-like_protein_M	10.98	16.46	67.48	0.58	0.62
WN51_11769_Krueppel-like_factor_15	11.63	5.90	70.16	0.58	0.54
WN51_11350_Pupal_cuticle_protein_C1B	27.16	114.58	4.53	0.58	0.52
WN51_11255_SPRY_domain-containing_SOCS_box_protein_1	11.95	19.22	71.44	0.58	0.63
WN51_11040_Transmembrane_protein_60	16.98	33.54	101.52	0.58	0.67
WN51_14249_Neuroparsin-A	8.75	137.62	52.16	0.58	0.69
WN51_06495_Insulin_enhancer_protein_ISL-1	3.76	3.57	22.38	0.58	0.58
WN51_06607_Anoctamin-4	3.88	0.19	23.08	0.58	0.50
WN51_12146_Fibrillin-2	8.58	19.42	50.62	0.58	0.69
WN51_10745_DmX-like_protein_2	6.81	14.23	39.95	0.59	0.68
WN51_02141_Dystrophin_isoforms_A/C/F/G/H	5.39	8.06	31.55	0.59	0.63
WN51_02729_tRNA_pseudouridine(38/39)_synthase	10.61	20.10	61.52	0.59	0.66
WN51_03453_Thyrotropin-releasing_hormone-degrading_ectoenzyme	5.40	9.17	31.22	0.59	0.65
WN51_01652_zinc_finger_protein_395_isoform_X2	8.79	14.66	50.76	0.59	0.64
WN51_09085_MIEF1_upstream_open_reading_frame_protein	13.80	6.76	79.35	0.59	0.54
WN51_06599_hypothetical_protein	4.24	0.06	23.91	0.59	0.50
WN51_10599_Steroid_receptor_seven-up_isoform_A	12.02	11.10	67.46	0.59	0.58
WN51_13136_hypothetical_protein	4.03	5.48	22.58	0.59	0.62
WN51_10737_Neurotrimin	4.04	74.49	22.61	0.59	0.65
WN51_06946_Outer_row_dynein_assembly_protein_16_like_protein	5.05	6.72	28.22	0.59	0.62
WN51_11278_hypothetical_protein	4.64	0.94	25.80	0.59	0.52
WN51_13943_hypothetical_protein	7.77	6.47	43.08	0.59	0.58
WN51_13564_Histone_H2A	37.88	27.71	6.84	0.59	0.62
WN51_09191_Glucose_dehydrogenase [acceptor]	13.12	0.22	72.50	0.59	0.50
WN51_08603_hypothetical_protein	7.67	8.74	41.97	0.59	0.60
WN51_00378_Fatty-acid_amide_hydrolase_2-A	3.82	2.38	20.92	0.59	0.56
WN51_06963_Cytochrome_P450_4C1	20.77	0.35	113.44	0.59	0.50
WN51_04629_Insulin-like_peptide	55.13	39.55	10.09	0.59	0.63
WN51_07629_TWIK_family_of_potassium_channels_protein_7	7.39	8.86	40.26	0.59	0.61
WN51_02660_hypothetical_protein	15.95	9.42	86.21	0.59	0.55
WN51_00316_extracellular_serine/threonine_protein_CG31145	19.25	29.60	103.94	0.59	0.64
WN51_05693_Protein_slit	5.57	4.72	30.05	0.59	0.58
WN51_08457_Glycerol-3-phosphate_acyltransferase_1_mitochondrial	23.07	22.86	123.67	0.59	0.59
WN51_09416_hypothetical_protein	14.51	18.26	77.79	0.59	0.62
WN51_10023_Inositol-pentakisphosphate_2-kinase	6.00	12.37	32.10	0.59	0.69
WN51_04694_Leucine-rich_repeats_and_immunoglobulin-like_domains_protein_3	6.14	6.82	32.80	0.59	0.60
WN51_12743_Patched_domain-containing_protein_3	12.65	6.91	67.39	0.59	0.55
WN51_06247_Synaptotagmin-like_protein_3	12.85	6.92	68.28	0.59	0.55
WN51_01489_Mushroom_body_large-type_Kenyon_cell-specific_protein_1	15.36	28.60	81.40	0.59	0.68
WN51_10135_Prostacyclin_receptor	8.01	7.53	42.26	0.59	0.59
WN51_07094_putative_cytochrome_P450_6a14	11.15	18.31	58.66	0.60	0.66
WN51_05958_Frizzled-5	10.45	19.42	54.92	0.60	0.68
WN51_01966_max_dimerization_protein_1-like	4.65	8.66	24.27	0.60	0.68
WN51_01099_C-myc_promoter-binding_protein	8.09	2.84	42.05	0.60	0.53
WN51_10147_hypothetical_protein	15.36	4.67	79.31	0.60	0.53
WN51_00156_Leucine-rich_repeat-containing_protein_15	14.11	12.14	72.74	0.60	0.58
WN51_14508_Endothelin-converting_enzyme_2	5.34	79.69	27.50	0.60	0.67
WN51_05738_thyroid_adenoma-associated_protein	6.44	7.41	33.14	0.60	0.61
WN51_07866_Krueppel-like_factor_5	5.22	6.95	26.83	0.60	0.63
WN51_14274_Elongation_of_very_long_chain_fatty_acids_protein_6	8.78	0.75	45.01	0.60	0.51
WN51_13329_hypothetical_protein	5.92	6.67	30.26	0.60	0.61
WN51_08594_Monocarboxylate_transporter_12	5.20	3.45	26.55	0.60	0.56
WN51_07552_hypothetical_protein	12.08	5.56	61.45	0.60	0.55
WN51_12174_Heparan-sulfate_6-O-sulfotransferase_2	22.40	31.81	113.85	0.60	0.64
WN51_07501_serine/threonine-protein_kinase_KIPK1	4.52	1.55	22.83	0.60	0.53
WN51_07944_hypothetical_protein	42.12	36.04	8.37	0.60	0.62
WN51_10602_COUP_transcription_factor_2	16.54	24.79	83.28	0.60	0.65
WN51_07615_Calcitonin_receptor	12.82	5.86	63.58	0.60	0.55
WN51_08079_Putative_glutamate_synthase [NADPH]	26.55	17.72	131.49	0.60	0.57
WN51_13883_Dual_specificity_tyrosine-phosphorylation-regulated_kinase_2	11.91	9.97	58.80	0.60	0.58
WN51_14102_hypothetical_protein	5.31	10.39	26.06	0.60	0.70
WN51_02106_Protein_bowel	104.53	0.01	21.34	0.60	0.50
WN51_09518_POU_domain_protein_CF1A	16.72	13.90	81.51	0.60	0.59
WN51_07261_Sphingomyelin_phosphodiesterase	13.44	8.32	64.88	0.60	0.56
WN51_09744_glutamyl-tRNA(Gln)_amidotransferase_subunit_C_mitochondrial	14.79	19.63	71.35	0.60	0.64
WN51_02228_Phosphatidylinositol_3-kinase_regulatory_subunit_alpha	12.74	2.88	61.43	0.60	0.52
WN51_14077_Vitellogenin [Vg2]	13.35	3.95	64.27	0.60	0.53
WN51_13921_Low_molecular_weight_phosphotyrosine_protein_phosphatase	16.64	27.43	79.95	0.60	0.67
WN51_14597_ABC_transporter_G_family_member_15	10.40	1.52	49.73	0.60	0.52
WN51_00058_hypothetical_protein	16.20	0.35	76.73	0.61	0.50
WN51_06535_hypothetical_protein	30.99	0.07	146.68	0.61	0.50
WN51_08002_Tetratricopeptide_repeat_protein	5.60	0.04	26.52	0.61	0.50
WN51_06308_kinase_protein	8.11	6.87	38.30	0.61	0.59
WN51_10892_DC-STAMP_domain-containing_protein_1	13.74	10.16	64.80	0.61	0.58
WN51_12982_Beta-1,3-galactosyltransferase_1	20.35	36.12	95.37	0.61	0.69
WN51_04941_otolith_matrix_protein_OMM-64_isoform_X5	6.04	7.70	28.11	0.61	0.64
WN51_12498_Tyrosine-protein_phosphatase_69D	16.84	18.95	78.06	0.61	0.62
WN51_03604_neuroendocrine_convertase_2	4.39	6.16	20.22	0.61	0.65
WN51_02029_E3_ubiquitin-protein_ligase_MARCH3	121.71	0.00	26.59	0.61	0.50
WN51_06851_Vacuolar_protein_sorting-associated_protein_13A	12.76	20.67	58.13	0.61	0.68
WN51_02945_DNA_primase_small_subunit	24.69	5.98	112.46	0.61	0.53
WN51_10011_hypothetical_protein	61.58	100.51	13.54	0.61	0.57

WN51_13647_Down_syndrome_cell_adhesion_molecule-like_protein_Dscam2	4.63	55.28	21.00	0.61	0.69
WN51_00860_Ferrochelatase_mitochondrial	25.71	45.28	116.37	0.61	0.69
WN51_08505_digestive_organ_expansion_factor_homolog	12.66	10.99	57.11	0.61	0.60
WN51_03686_Putative_polypeptide_N-acetylgalactosaminyltransferase_9	8.28	10.08	37.25	0.61	0.64
WN51_05974_hypothetical_protein	12.59	5.77	56.51	0.61	0.55
WN51_10495_DNA_ligase_4	5.69	2.25	25.55	0.61	0.54
WN51_09974_probable_endochitinase	8.79	1.83	39.36	0.61	0.52
WN51_12753_Hemocytin	15.77	10.89	70.37	0.61	0.58
WN51_12215_Ectopic_P_granules_protein_5_like_protein	6.22	11.03	27.72	0.61	0.70
WN51_02497_Esterase_FE4	27.07	7.35	120.15	0.61	0.53
WN51_04222_putative_ATP-dependent_RNA_helicase_DHX36	11.02	13.81	48.88	0.61	0.64
WN51_07555_Scavenger_receptor_class_B_member_1	19.65	3.02	86.73	0.61	0.52
WN51_01391_MMS19_nucleotide_excision_repair_protein_like_protein	16.83	28.57	73.42	0.61	0.69
WN51_07212_hypothetical_protein	4.73	68.48	20.64	0.61	0.65
WN51_05976_hypothetical_protein	15.16	11.25	65.95	0.61	0.59
WN51_01238_hypothetical_protein	15.89	11.42	68.69	0.62	0.58
WN51_04932_Glucose_dehydrogenase [acceptor]	14.49	15.73	62.44	0.62	0.63
WN51_10858_nucleolar_protein_4-like_isoform_X3	4.96	1.23	21.35	0.62	0.53
WN51_09212_Folypolyglutamate_synthase_mitochondrial	19.60	14.18	84.15	0.62	0.58
WN51_07030_Brain-specific_angiogenesis_inhibitor_1-associated_protein_2	10.26	5.45	43.54	0.62	0.56
WN51_01857_hypothetical_protein	45.13	29.85	10.70	0.62	0.68
WN51_08963_putative_palmitoyltransferase_ZDHHC24	14.61	24.31	61.61	0.62	0.70
WN51_10802_Transcription_factor_Sox-6	6.16	3.11	25.96	0.62	0.56
WN51_00609_Protein_artemis	5.25	8.37	22.11	0.62	0.69
WN51_02802_Vacuolar_protein_sorting-associated_protein_41_like_protein	15.96	17.61	67.10	0.62	0.63
WN51_00902_GTP-binding_protein_RAD	6.65	89.89	27.91	0.62	0.66
WN51_07978_Bifunctional_ATP-dependent_dihydroxyacetone_kinase/FAD-AMP_lyase_(cyclizing)	34.14	44.68	143.10	0.62	0.66
WN51_09876_Nudix_hydrolase_8	22.09	21.63	91.61	0.62	0.62
WN51_03061_SIFamide-related_peptide	13.13	157.38	54.41	0.62	0.67
WN51_13409_nuclear_factor_NF-kappa-B_p100_subunit_isoform_X1 [NF-kB]	24.47	12.17	100.80	0.62	0.56
WN51_08329_protein_arginine_n-methyltransferase_7-like_protein	12.82	20.57	52.80	0.62	0.69
WN51_04168_flagellar_attachment_zone_protein_1-like	11.25	3.10	46.29	0.62	0.53
WN51_06228_Rhodopsin	7.73	3.28	31.70	0.62	0.55
WN51_11791_probable_JmjC_domain-containing_histone_demethylation_protein_2C_isoform_X7	8.33	0.00	34.04	0.62	0.50
WN51_12324_hypothetical_protein	10.58	15.55	43.22	0.62	0.68
WN51_00877_NADH-ubiquinone_oxidoreductase_chain_5	136.79	144.31	33.55	0.62	0.62
WN51_01656_Diuretic_hormone_receptor	5.99	0.04	24.38	0.62	0.50
WN51_05041_Tubulin_polyglutamylase_ttl6	14.14	14.95	57.25	0.62	0.63
WN51_14507_Ecdysone_receptor	12.17	17.15	49.23	0.62	0.67
WN51_07502_Dual_specificity_testis-specific_protein_kinase_2	11.29	7.79	45.45	0.62	0.59
WN51_12622_hypothetical_protein	7.17	0.01	28.84	0.62	0.50
WN51_04103_cytochrome_b5_reductase_4	17.05	25.86	68.60	0.62	0.69
WN51_03842_Fibrous_sheath-interacting_protein_2	7.52	3.52	30.17	0.62	0.56
WN51_12413_Growth_arrest-specific_protein_1	18.96	14.12	75.44	0.63	0.59
WN51_08607_Solute_carrier_family_35_member_F5	17.64	13.74	70.07	0.63	0.60
WN51_12661_hypothetical_protein	9.62	12.16	38.18	0.63	0.66
WN51_13012_hypothetical_protein	23.24	0.10	91.74	0.63	0.50
WN51_00352_Pituitary_homeobox_2	7.43	2.81	29.22	0.63	0.55
WN51_06480_hypothetical_protein	13.18	10.28	51.67	0.63	0.60
WN51_01744_DNA_replication_licensing_factor_MCM8	13.13	15.61	51.35	0.63	0.65
WN51_05564_Importin-4	28.42	13.82	110.64	0.63	0.56
WN51_07206_Sperm-associated_antigen_6	19.70	6.78	76.44	0.63	0.54
WN51_03196_Scavenger_receptor_class_B_member_1	28.79	3.96	110.96	0.63	0.52
WN51_12043_Accumulation-associated_protein	34.58	19.85	133.25	0.63	0.57
WN51_06534_mitochondrial_sodium/hydrogen_exchanger_9B2_isoform_X1	27.03	8.58	103.52	0.63	0.54
WN51_13316_hypothetical_protein	19.76	15.81	75.44	0.63	0.60
WN51_10025_Neurofibromin	12.69	13.96	48.34	0.63	0.64
WN51_09908_Protein_vav	24.03	29.20	91.39	0.63	0.66
WN51_04065_Proton-coupled_amino_acid_transporter_4	102.95	4.81	27.11	0.63	0.59
WN51_08463_Exportin-7	19.76	11.58	75.01	0.63	0.58
WN51_07270_Alpha-(1,3)-fucosyltransferase_11	23.36	32.18	88.18	0.63	0.68
WN51_10270_Alpha-tocopherol_transfer_protein-like	140.66	2.72	37.27	0.63	0.54
WN51_07734_Organic_cation_transporter_protein	12.85	9.62	48.47	0.63	0.60
WN51_08597_Lamin-B_receptor	19.25	23.58	72.63	0.63	0.66
WN51_07643_Vacuolar_protein_sorting-associated_protein_18_like_protein	12.18	13.84	45.92	0.63	0.65
WN51_00365_Mothers_against_decapentaplegic_like_protein_6	16.87	8.62	63.62	0.63	0.57
WN51_00789_Sperm-associated_antigen_1	9.22	4.13	34.73	0.63	0.56
WN51_06011_Ankyrin_repeat_SAM_and_basic_leucine_zipper_domain-containing_protein_1	20.89	26.94	78.61	0.63	0.67
WN51_14401_putative_uncharacterized_protein_DDB_G0291812	17.26	0.00	64.88	0.63	0.50
WN51_08046_Slowpoke-binding_protein	97.38	77.86	26.00	0.63	0.67
WN51_00948_PR_domain_zinc_finger_protein_10	13.59	13.16	50.89	0.63	0.63
WN51_05301_leucine-rich_repeat-containing_protein	31.98	41.16	119.53	0.63	0.67
WN51_04062_Protein_mago_nashi	35.24	50.91	131.59	0.63	0.69
WN51_06987_coilin_isoform_X1	6.90	4.46	25.67	0.63	0.59
WN51_09651_Glycerate_kinase	30.51	15.46	113.55	0.63	0.57
WN51_00300_rho-associated_protein_kinase_2-like	7.90	5.95	29.38	0.63	0.60
WN51_05259_Putative_odorant_receptor_13a	10.59	9.26	39.27	0.63	0.62
WN51_08169_UDP-galactose_translocator	18.19	10.64	67.43	0.63	0.58
WN51_04148_Protein_hid-1_like_protein	18.09	26.57	66.76	0.64	0.70
WN51_14702_Talin-1	11.99	16.90	44.22	0.64	0.69
WN51_00025_Fatty_acyl-CoA_reductase_1	19.38	8.38	71.49	0.64	0.56
WN51_09162_transmembrane_protein_164	24.75	35.11	90.88	0.64	0.69
WN51_11210_Regulator_of_microtubule_dynamics_protein_1	22.48	22.26	82.45	0.64	0.63
WN51_09516_Major_facilitator_superfamily_domain-containing_protein_6-A	16.38	14.41	60.08	0.64	0.62
WN51_00890_unpaired-like_protein	115.34	5.56	31.59	0.64	0.59
WN51_08716_33_kDa_inner_dynein_arm_light_chain_axonemal	8.55	6.58	31.13	0.64	0.61

WN51_00669_Protein_bride_of_sevenless	24.82	21.82	90.33	0.64	0.62
WN51_07079_hypothetical_protein	39.38	14.69	142.61	0.64	0.55
WN51_01275_BCL-6_corepressor	8.66	6.99	31.27	0.64	0.61
WN51_11927_Sodium/potassium/calcium_exchanger_5	17.16	9.71	61.90	0.64	0.58
WN51_03071_BarH-like_1_homeobox_protein	20.55	16.97	73.89	0.64	0.61
WN51_12051_nuclear_receptor_coactivator_1-like_isoform_X8	7.21	2.86	25.86	0.64	0.56
WN51_07273_Protein_singed	56.64	53.43	15.79	0.64	0.65
WN51_03199_transmembrane_protein_KIAA1109_isoform_X1	9.20	7.60	32.80	0.64	0.62
WN51_05254_hypothetical_protein	26.90	30.77	95.30	0.64	0.66
WN51_07771_hypothetical_protein	9.19	5.43	32.54	0.64	0.58
WN51_06276_Cytosolic_endo-beta-N-acetylglucosaminidase	26.60	14.58	94.16	0.64	0.58
WN51_03699_Arginine_kinase	54.57	41.67	15.42	0.64	0.69
WN51_10378_Alpha-1,2-mannosyltransferase_ALG9	10.81	12.22	38.21	0.64	0.66
WN51_04513_Gamma-secretase_subunit_PEN-2	38.56	44.53	135.97	0.64	0.66
WN51_08272_Histone-lysine_N-methyltransferase_SETD2	6.39	0.03	22.50	0.64	0.50
WN51_14209_Zinc_finger_MYND_domain-containing_protein	9.31	6.90	32.62	0.64	0.61
WN51_00014_hypothetical_protein	22.27	2.76	78.01	0.64	0.52
WN51_00684_hypothetical_protein	27.24	26.26	95.16	0.64	0.64
WN51_10242_Phosphatidylinositol_4-phosphate_3-kinase_C2_domain-containing_subunit_beta	9.30	11.82	32.43	0.64	0.68
WN51_09964_Inhibitor_of_nuclear_factor_kappa-B_kinase_subunit_beta	11.50	8.21	40.10	0.64	0.60
WN51_00554_Rap1_GTPase-GDP_dissociation_stimulator_1-B	30.55	36.67	106.45	0.64	0.67
WN51_07725_Ras-related_protein_Rab-8A	8.59	11.60	29.91	0.64	0.69
WN51_06307_kinase_protein	9.06	6.41	31.49	0.64	0.60
WN51_03811_apomucin_isoform_X2	25.41	8.01	88.24	0.64	0.55
WN51_13085_zinc_finger_protein_37-like	15.70	20.42	54.52	0.64	0.69
WN51_01803_putative_maltase_H	154.00	9.62	44.45	0.64	0.61
WN51_05900_Bifunctional_heparan_sulfate_N-deacetylase/N-sulfotransferase	26.14	9.78	90.31	0.64	0.55
WN51_00583_3-hydroxyisobutyryl-CoA_hydrolase_mitochondrial	18.00	13.85	62.02	0.65	0.61
WN51_14659_RB1-inducible_coiled-coil_protein_1	16.41	13.45	56.42	0.65	0.62
WN51_11231_DNA_repair_protein_RAD51_like_protein_4	25.26	20.57	86.62	0.65	0.62
WN51_03215_zinc_finger_protein_2_homolog	15.85	20.01	54.27	0.65	0.68
WN51_00356_TELO2-interacting_protein_1_like_protein	16.33	11.29	55.89	0.65	0.60
WN51_12869_CUGBP_Elav-like_family_member_2_isoform_X2	7.34	0.49	25.09	0.65	0.51
WN51_07982_Protein_doublesex	17.42	10.75	59.56	0.65	0.59
WN51_10105_Coiled-coil_domain-containing_protein	25.17	22.83	85.81	0.65	0.63
WN51_06479_hypothetical_protein	12.04	13.35	41.02	0.65	0.66
WN51_02785_Serine/threonine-protein_phosphatase_6_regulatory_ankyrin_repeat_subunit_A	14.36	8.12	48.77	0.65	0.58
WN51_06099_GPI_mannosyltransferase_3	22.71	28.07	77.01	0.65	0.68
WN51_13417_Stearoyl-CoA_desaturase_5	32.59	3.01	110.54	0.65	0.51
WN51_09166_hypothetical_protein	6.11	0.00	20.72	0.65	0.50
WN51_12346_hypothetical_protein	7.61	10.30	25.77	0.65	0.70
WN51_07600_Ecdysone-inducible_protein_E75	21.26	20.63	72.03	0.65	0.64
WN51_00588_Protocadherin-15	7.26	9.05	24.57	0.65	0.68
WN51_00693_E3_ubiquitin-protein_ligase_RFWD2	35.65	47.73	120.25	0.65	0.70
WN51_02967_probable_serine/threonine-protein_kinase_clkA	6.66	6.55	22.47	0.65	0.65
WN51_14679_Zinc_finger_protein_ush	16.47	10.56	55.53	0.65	0.60
WN51_08468_Kinesin-like_protein_KIF9	12.30	15.31	41.35	0.65	0.69
WN51_09665_transmembrane_protein_104_homolog_isoform_X2	27.12	8.01	91.14	0.65	0.54
WN51_05288_Arf-GAP_with_SH3_domain_ANK_repeat_and_PH_domain-containing_protein_1	13.31	3.04	44.68	0.65	0.53
WN51_03839_hypothetical_protein	11.89	15.75	39.91	0.65	0.70
WN51_08541_Myosin-VIIa	11.55	2.64	38.68	0.65	0.53
WN51_09365_B(0,+)-type_amino_acid_transporter_1	29.12	5.54	97.10	0.65	0.53
WN51_12472_Division_abnormally_delayed_protein	19.44	5.30	64.78	0.65	0.54
WN51_14201_Inhibin_beta_A_chain	40.80	33.61	135.72	0.65	0.62
WN51_11674_Eyes_absent_like_protein_4	63.60	73.29	19.15	0.65	0.63
WN51_06311_kinase_protein	23.69	9.06	78.61	0.65	0.56
WN51_11527_Putative_amino_acid_permease_F13H10.3	18.16	5.74	60.27	0.65	0.55
WN51_13112_hypothetical_protein	29.50	28.57	97.85	0.65	0.65
WN51_00138_tRNA_(guanine-N(7)-)-methyltransferase	20.25	22.22	67.13	0.65	0.67
WN51_07249_Ubiquitin-conjugating_enzyme_E2-24_kDa	11.02	5.86	36.44	0.65	0.58
WN51_04931_Glucose_dehydrogenase_[acceptor]	38.38	11.63	126.88	0.65	0.55
WN51_08964_Sulfhydryl_oxidase_1	18.77	23.68	62.01	0.65	0.69
WN51_02307_Alpha-1,3/1,6-mannosyltransferase_ALG2	30.22	29.90	99.48	0.65	0.65
WN51_13282_Sorbin_and_SH3_domain-containing_protein_1	19.50	23.20	63.93	0.65	0.68
WN51_13404_Protein_ST7_like_protein	29.62	32.15	96.73	0.65	0.67
WN51_06451_Beta-1,4-N-acetylgalactosaminyltransferase_bre-4	37.09	15.32	121.07	0.65	0.56
WN51_00307_inward_rectifier_potassium_channel_4-like_isoform_X3	25.30	30.17	82.53	0.65	0.68
WN51_09503_Metallo-beta-lactamase_domain-containing_protein_1	15.82	12.25	51.61	0.65	0.62
WN51_12172_Atrial_natriuretic_peptide-converting_enzyme	13.93	4.40	45.26	0.65	0.55
WN51_11225_Protein-tyrosine_sulfotransferase	17.84	17.25	57.82	0.65	0.65
WN51_06068_POU_domain_class_2_transcription_factor_1	72.88	2.84	22.52	0.65	0.56
WN51_08229_pancreatic_triacylglycerol_lipase-like	11.07	6.90	35.77	0.65	0.60
WN51_00146_Inositol_hexakisphosphate_and_diphosphoinositol-pentakisphosphate_kinase	12.98	16.06	41.78	0.66	0.69
WN51_12257_Putative_aldehyde_dehydrogenase_family_7_member_A1_like_protein	41.28	21.63	132.55	0.66	0.58
WN51_06938_hypothetical_protein	8.10	6.18	25.99	0.66	0.62
WN51_11156_Patatin-like_phospholipase_domain-containing_protein_2	36.81	15.92	118.01	0.66	0.57
WN51_06644_Neuronal_acetylcholine_receptor_subunit_alpha-6	41.02	34.28	131.49	0.66	0.63
WN51_07421_Glucose_dehydrogenase_[acceptor]	42.85	29.98	137.22	0.66	0.61
WN51_10727_Argininosuccinate_lyase	16.82	9.38	53.80	0.66	0.59
WN51_07235_Gamma-tubulin_complex_component_6	12.94	16.01	41.31	0.66	0.69
WN51_13046_hypothetical_protein	16.11	3.28	51.43	0.66	0.53
WN51_13561_Integral_membrane_protein	9.49	5.82	30.17	0.66	0.60
WN51_07527_Nuclear_RNA_export_factor_2	15.84	18.20	50.37	0.66	0.68
WN51_06344_hypothetical_protein	21.06	53.67	6.62	0.66	0.56
WN51_09224_Ecdysone-induced_protein_74EF_isoform_A	9.69	0.17	30.75	0.66	0.50
WN51_12089_Neuroguidin-A	40.00	11.03	126.15	0.66	0.54

WN51_00427_Putative_phosphatidate_phosphatase	18.32	6.55	57.71	0.66	0.56
WN51_07512_Elongation_of_very_long_chain_fatty_acids_protein_7	42.52	6.64	133.80	0.66	0.52
WN51_10732_Calpain-7	29.38	36.45	91.97	0.66	0.70
WN51_01317_hypothetical_protein	22.63	22.28	70.77	0.66	0.66
WN51_03184_Leucine-rich_repeat-containing_protein_34	7.64	8.41	23.84	0.66	0.68
WN51_03412_hypothetical_protein	8.12	6.29	25.34	0.66	0.62
WN51_05716_DNA_polymerase_epsilon_catalytic_subunit_A	6.52	8.07	20.30	0.66	0.70
WN51_08546_Membrane_magnesium_transporter_1	36.35	40.66	113.10	0.66	0.68
WN51_09852_Receptor_expression-enhancing_protein_6	26.65	9.71	82.90	0.66	0.56
WN51_11642_E3_ubiquitin-protein_ligase_MARCH5	38.69	18.68	120.09	0.66	0.58
WN51_04930_Glucose_dehydrogenase [acceptor]	31.41	24.08	97.42	0.66	0.62
WN51_04468_Tyrosine_kinase_receptor_Cad96Ca	8.47	4.87	26.24	0.66	0.59
WN51_13882_Dual_specificity_tyrosine-phosphorylation-regulated_kinase_2	28.28	28.08	9.15	0.66	0.66
WN51_13027_hypothetical_protein	27.44	20.38	84.71	0.66	0.62
WN51_04474_nucleoporin_GLE1	12.93	105.87	39.87	0.66	0.69
WN51_07790_N-lysine_methyltransferase_SMYD2-A	7.35	5.68	22.61	0.66	0.63
WN51_02781_Dymeclin	18.53	4.91	56.90	0.66	0.54
WN51_02076_putative_cytochrome_P450_6a20	67.82	7.08	22.16	0.66	0.66
WN51_04016_COMM_domain-containing_protein_5	24.68	10.78	75.49	0.66	0.57
WN51_10552_putative_cytochrome_P450_49a1	7.75	2.87	23.66	0.66	0.56
WN51_12624_hypothetical_protein	24.41	0.00	74.24	0.66	0.50
WN51_12632_UDP-N-acetylglucosamine_transferase_subunit_ALG14_like_protein	24.24	25.78	73.65	0.66	0.68
WN51_08499_hypothetical_protein	10.09	9.66	30.55	0.67	0.66
WN51_11935_Cytochrome_b-c1_complex_subunit_Rieske_mitochondrial	72.87	74.42	24.10	0.67	0.66
WN51_02250_Cytokine_receptor	25.22	12.62	75.98	0.67	0.58
WN51_03117_Ring_canal_kelch_like_protein	20.87	9.26	62.63	0.67	0.57
WN51_00145_Dolichyldiphosphatase_1	41.65	14.21	124.56	0.67	0.56
WN51_00106_hypothetical_protein	39.60	26.31	118.36	0.67	0.61
WN51_08427_hypothetical_protein	148.23	141.69	49.66	0.67	0.68
WN51_14285_Putative_odorant_receptor_9a	27.85	18.24	83.06	0.67	0.61
WN51_02321_F-box/WD_repeat-containing_protein_5	27.58	18.71	82.17	0.67	0.61
WN51_06908_hypothetical_protein	23.13	21.64	68.59	0.67	0.66
WN51_00309_hypothetical_protein	15.07	2.12	44.64	0.67	0.52
WN51_08334_Putative_E3_ubiquitin-protein_ligase_SINAT1	24.32	14.99	71.99	0.67	0.60
WN51_12596_E3_ubiquitin-protein_ligase_HUWE1	7.76	6.01	22.96	0.67	0.63
WN51_10241_hypothetical_protein	30.87	3.32	91.21	0.67	0.52
WN51_02961_WD_repeat-containing_protein_6	14.09	13.40	41.57	0.67	0.66
WN51_04074_Longitudinals_lacking_protein_isoforms_A/B/D/L	135.65	17.20	45.99	0.67	0.69
WN51_08370_bromodomain-containing_protein_DDB_G0270170_isoform_X1	16.50	9.02	48.64	0.67	0.59
WN51_04160_hypothetical_protein	24.32	0.06	71.54	0.67	0.50
WN51_02742_Methyltransferase-like_protein_13	33.80	35.92	99.09	0.67	0.68
WN51_11236_hypothetical_protein	9.13	0.61	26.72	0.67	0.51
WN51_03211_Protein_spaetzle	21.85	17.59	63.88	0.67	0.64
WN51_04908_Putative_homeodomain_transcription_factor	26.81	15.76	77.86	0.67	0.60
WN51_02301_cell_wall_protein_DAN4-like	19.16	21.86	55.57	0.67	0.70
WN51_03434_Neuronal_PAS_domain-containing_protein_2	35.45	28.39	102.70	0.67	0.64
WN51_10123_hypothetical_protein	75.65	10.09	26.15	0.67	0.69
WN51_09894_GDP-D-glucose_phosphorylase_1	10.38	0.04	29.95	0.67	0.50
WN51_04638_Cyclic_AMP-responsive_element-binding_protein_3-like_protein_1	21.11	4.09	60.77	0.67	0.53
WN51_04203_myoneurin-like	24.62	2.75	70.79	0.67	0.52
WN51_07823_Serine/threonine-protein_kinase-transforming_protein_mos	58.48	8.09	20.34	0.67	0.70
WN51_13065_Chitinoglycosaminidase	35.26	16.63	101.24	0.67	0.58
WN51_03396_Mucosa-associated_lymphoid_tissue_lymphoma_translocation_protein_1	35.44	35.96	101.42	0.67	0.68
WN51_06460_Phosphatidylinositol_3,4,5-trisphosphate_3-phosphatase_and_dual-specificity_protein_phosphatase_1	14.02	12.04	40.10	0.67	0.65
WN51_14217_putative_ATP-dependent_RNA_helicase_DHX35	25.61	19.38	73.13	0.68	0.63
WN51_12003_hypothetical_protein	43.38	46.35	123.87	0.68	0.69
WN51_07268_Tubulin-specific_chaperone_D	31.55	17.96	90.09	0.68	0.60
WN51_12063_Endothelin-converting_enzyme_1	33.77	11.33	96.28	0.68	0.56
WN51_10698_Protein_flightless-1	18.55	3.19	52.89	0.68	0.53
WN51_09671_zinc_finger_protein_616-like	35.45	31.57	100.84	0.68	0.66
WN51_07632_Ceramide_synthase_6	37.95	1.97	107.89	0.68	0.51
WN51_08967_5-formyltetrahydrofolate_cyclo-ligase	42.32	15.14	120.09	0.68	0.56
WN51_02692_Integrator_complex_subunit_2	11.71	10.41	33.15	0.68	0.66
WN51_04136_cytochrome_p450_315a1 [p450]	37.19	19.59	105.14	0.68	0.59
WN51_06378_Peritrophin-1	23.37	46.97	8.28	0.68	0.59
WN51_04370_Insulin-like_growth_factor-binding_protein_complex_acid_labile_subunit	10.82	3.43	30.52	0.68	0.56
WN51_12698_Methyl-CpG-binding_domain_protein_5	17.89	6.51	50.41	0.68	0.56
WN51_08557_hypothetical_protein	14.77	1.95	41.61	0.68	0.52
WN51_08178_Regulator_of_telomere_elongation_helicase_1_like_protein	13.14	8.56	37.00	0.68	0.62
WN51_02202_F-box_only_protein_28	34.43	35.62	96.86	0.68	0.68
WN51_02453_1-phosphatidylinositol_4,5-bisphosphate_phosphodiesterase_gamma-1	21.30	18.64	59.91	0.68	0.66
WN51_07179_Histone-lysine_N-methyltransferase_ash1	19.29	9.24	54.14	0.68	0.59
WN51_05801_PDZ_and_LIM_domain_protein_Zasp	24.39	24.58	67.55	0.68	0.68
WN51_05255_hypothetical_protein	27.57	13.32	76.24	0.68	0.59
WN51_01442_Kv_channel-interacting_protein_2	11.08	84.04	30.62	0.68	0.68
WN51_08788_Exocyst_complex_component_1	24.68	26.77	68.01	0.68	0.70
WN51_01303_putative_beta-hexosaminidase_fdl	39.15	30.57	107.82	0.68	0.64
WN51_04675_Endoplasmic_reticulum_metallopeptidase_1	34.81	20.01	95.82	0.68	0.60
WN51_02872_Chloride_channel_protein_2	40.84	33.52	112.04	0.68	0.65
WN51_12294_early_endosome_antigen_1-like	21.03	16.23	57.67	0.68	0.64
WN51_12218_Werner_Syndrome-like_exonuclease	17.32	15.29	47.48	0.68	0.66
WN51_05287_Arf-GAP_with_SH3_domain_ANK_repeat_and_PH_domain-containing_protein_1	15.72	11.80	43.04	0.68	0.64
WN51_05068_hypothetical_protein	188.54	192.83	68.96	0.68	0.68
WN51_01056_Chaoptin	10.72	8.30	29.18	0.68	0.64
WN51_00121_gag-pol_polyprotein	12.79	10.00	34.74	0.68	0.64
WN51_12893_putative_cation-transporting_ATPase_13A1	36.72	9.43	99.72	0.68	0.55

WN51_11015_putative_Ufm1-specific_protease_2	35.69	29.61	96.75	0.68	0.65
WN51_03038_serine/arginine_repetitive_matrix_protein_1	18.26	18.49	49.45	0.68	0.69
WN51_04527_hypothetical_protein	10.93	0.13	29.52	0.69	0.50
WN51_03497_DNA-directed_RNA_polymerase_I_subunit_RPA1	18.95	12.04	51.11	0.69	0.62
WN51_09473_Thiamine_transporter_2	37.31	38.60	100.39	0.69	0.69
WN51_01666_E3_ubiquitin-protein_ligase_UBR3	18.45	16.08	49.65	0.69	0.66
WN51_08602_hypothetical_protein	25.45	26.81	68.47	0.69	0.70
WN51_09392_Codanin-1	37.24	35.64	100.19	0.69	0.68
WN51_12623_hypothetical_protein	20.32	10.85	54.59	0.69	0.60
WN51_12395_Cytochrome_b561	51.70	25.62	138.70	0.69	0.59
WN51_11680_Leucine-rich_repeat-containing_protein_47	36.21	12.62	96.83	0.69	0.57
WN51_13123_Protein_bric-a-brac_1	14.98	12.98	40.05	0.69	0.66
WN51_11182_Zinc_finger_protein_32	27.40	7.64	73.24	0.69	0.55
WN51_10480_Oxidation_resistance_protein_1	31.09	32.10	82.88	0.69	0.69
WN51_06007_Polypeptide_N-acetylgalactosaminyltransferase_35A	25.05	22.71	66.77	0.69	0.67
WN51_03293_WD_repeat-containing_protein_CG11141	42.97	30.45	114.42	0.69	0.63
WN51_00448_Anoctamin-1	36.16	23.37	96.25	0.69	0.62
WN51_10240_Homeobox_protein_CD_X-4	25.72	1.09	68.46	0.69	0.51
WN51_10725_histone-lysine_N-methyltransferase_PRDM9-like_isoform_X2	14.97	9.56	39.83	0.69	0.62
WN51_02791_Neogenin	14.42	10.18	38.32	0.69	0.63
WN51_05589_hypothetical_protein	10.93	141.85	28.99	0.69	0.60
WN51_09295_General_receptor_for_phosphoinositides_1-associated_scaffold_protein	47.34	77.58	17.95	0.69	0.62
WN51_05803_Phospholipase_A2	40.84	25.97	107.72	0.69	0.62
WN51_01359_EKC/KEOPS_complex_subunit_LAGE3-like_isoform_X2	31.96	24.45	84.31	0.69	0.64
WN51_07807_Optomotor-blind_protein	24.74	60.22	9.40	0.69	0.58
WN51_06835_Transient_receptor_potential_cation_channel_trpm	20.88	20.76	54.89	0.69	0.69
WN51_14065_hypothetical_protein	68.10	155.97	25.93	0.69	0.58
WN51_04172_tRNA_pseudouridine_synthase-like_1	25.13	10.54	65.97	0.69	0.58
WN51_00537_ubiquitin_carboxyl-terminal_hydrolase_MINDY-3_homolog	48.19	32.11	126.35	0.69	0.63
WN51_09658_Broad-complex_core_protein_isoforms_1/2/3/4/5	29.10	23.26	76.23	0.69	0.65
WN51_02055_Hemicentin-2	9.28	3.45	24.29	0.69	0.57
WN51_02351_Xanthine_dehydrogenase	44.35	14.89	115.83	0.69	0.56
WN51_12047_Collagen_alpha-1(IV)_chain	41.93	38.68	109.38	0.69	0.68
WN51_09547_Chromodomain_Y_protein	15.47	9.63	40.30	0.69	0.62
WN51_02353_protein_PFC0760c-like_isoform_X1	33.64	29.60	87.64	0.69	0.67
WN51_05459_Rap_guanine_nucleotide_exchange_factor_2	25.14	14.04	65.36	0.69	0.61
WN51_11677_Trafficking_protein_particle_complex_subunit_2-like_protein	45.24	41.47	117.37	0.69	0.68
WN51_03129_Peroxisomal_membrane_protein_PEX16	14.36	6.93	37.24	0.69	0.59
WN51_02679_Facilitated_trehalose_transporter_Tret1	53.48	8.28	138.57	0.69	0.53
WN51_05977_hypothetical_protein	36.71	18.10	95.02	0.69	0.60
WN51_01286_Low-density_lipoprotein_receptor-related_protein_11	9.50	9.47	24.55	0.69	0.69
WN51_05425_FGFR1_oncogene_partner	10.58	2.47	27.35	0.69	0.55
WN51_02096_Protein_bowel	10.98	4.26	28.35	0.69	0.58
WN51_14616_Ubiquitin_carboxyl-terminal_hydrolase_CYLD	41.92	23.79	108.19	0.69	0.61
WN51_14191_Laminin_subunit_beta-1	25.20	17.94	64.94	0.69	0.64
WN51_10265_RING_finger_protein_121	38.55	17.58	99.32	0.69	0.59
WN51_06788_Glycine_dehydrogenase_[decarboxylating]_mitochondrial	37.86	29.66	97.46	0.69	0.65
WN51_11575_Protein_polybromo-1	13.95	2.80	35.90	0.69	0.54
WN51_03972_Ubiquitin-protein_ligase_E3B	33.26	28.86	85.59	0.69	0.67
WN51_02317_Alanine--tRNA_ligase_cytoplasmic	29.07	23.27	74.71	0.69	0.66
WN51_08164_Histone-lysine_N-methyltransferase_SETMAR	12.54	11.00	32.21	0.69	0.67
WN51_09678_DNA_polymerase_delta_small_subunit	41.45	42.37	106.46	0.69	0.70
WN51_09813_hypothetical_protein	23.31	15.63	59.64	0.70	0.63
WN51_07208_dynactin_150_kDa_isoform_isoform_X1	10.06	3.36	25.73	0.70	0.57
WN51_06102_RNA-binding_protein_fusilli_isoform_X1	47.64	12.76	121.86	0.70	0.55
WN51_10127_hypothetical_protein	15.40	7.58	39.40	0.70	0.60
WN51_06891_Transcription_factor_GATA-4	32.49	12.66	82.98	0.70	0.58
WN51_02455_Membralin	47.99	11.40	122.53	0.70	0.55
WN51_14372_Vacuolar_protein_sorting-associated_protein_72_like_protein	36.04	12.81	91.94	0.70	0.57
WN51_05212_Alpha-1-macroglobulin	50.91	40.57	129.86	0.70	0.66
WN51_11422_hypothetical_protein	16.56	15.22	42.15	0.70	0.68
WN51_07209_Cullin-1	37.08	21.18	94.31	0.70	0.61
WN51_03418_hypothetical_protein	11.55	3.02	29.36	0.70	0.55
WN51_14303_Putative_ATP-dependent_RNA_helicase_DHX33	17.39	11.06	44.21	0.70	0.63
WN51_13651_Tyrosine-protein_phosphatase_99A	26.10	6.25	66.33	0.70	0.55
WN51_02113_cGMP-dependent_protein_kinase_1	27.49	12.85	69.85	0.70	0.59
WN51_12587_hypothetical_protein	20.75	20.45	8.17	0.70	0.70
WN51_07601_hypothetical_protein	21.40	19.73	54.14	0.70	0.68
WN51_07159_proline-rich_protein_12-like	64.77	82.98	25.62	0.70	0.65
WN51_11543_Prestin	45.06	35.50	113.62	0.70	0.66
WN51_13233_Leucine-rich_repeat-containing_protein_26	52.87	12.06	133.18	0.70	0.55
WN51_02761_Phenoloxidase_subunit_1 [PO]	69.09	8.65	27.56	0.70	0.66
WN51_10202_Calcium-binding_mitochondrial_carrier_protein_Aralar1	41.57	35.61	104.20	0.70	0.67
WN51_00999_E3_ubiquitin-protein_ligase_MYLIP	36.20	11.37	90.61	0.70	0.56

Table S1(b): annotation of hypothetical genes according to the best BLASTp hit.

Query	WNS1	Accession	Description	E-value	Query coverage	Bit-Score	% Pairwise Identity	Grade	Hit end	Hit start	Query end	Query start	Max Sequence Length	Min Sequence Length	Sequence Length
gi 925682931 gb KOX79557.1	WNS1_02823	XP_020722090	adoMet-dependent rRNA methyltransferase SPB1 isoform X1	3.87E-74	59.67%	242	69.2%	64.4%	311	105	284	69	216	207	221
gi 925674026 gb KOX71504.1	WNS1_03811	XP_011638228	apomucin isoform X2	0.00E+00	100.00%	868	47.2%	75.0%	1328	54	1293	1	1293	1275	1373
gi 925674026 gb KOX71504.1	WNS1_03699	PBC31636	Arginine kinase	0.00E+00	83.00%	839	57.3%	80.2%	555	20	547	1	547	536	551
gi 925683615 gb KOX80193.1	WNS1_08370	XP_006561066	bromodomain-containing protein DDB G0270170 isoform X1	0.00E+00	53.35%	1181	61.9%	57.6%	1179	1	1188	1	1188	1179	1268
gi 925678801 gb KOX75800.1	WNS1_12588	XP_023709364	calphostin-like	3.72E-87	83.25%	285	44.7%	66.6%	537	149	411	59	389	353	407
gi 925676235 gb KOX73488.1	WNS1_14534	PBC26964	CAMP-specific 3',5'-cyclic phosphodiesterase	3.65E-151	76.89%	444	72.8%	74.8%	380	42	389	54	339	339	349
gi 925674286 gb KOX71732.1	WNS1_02301	XP_025993928	cell wall protein DAN4-like	0.00E+00	92.24%	935	58.6%	75.4%	935	22	980	77	974	904	950
gi 925681137 gb KOX77893.1	WNS1_05779	KOB79386	Chitin deacetylase-like 5	0.00E+00	43.51%	743	76.1%	59.8%	2227	1817	951	513	419	411	439
gi 925672223 gb KOX69927.1	WNS1_04441	XP_012243282	chloride channel CLIC-like protein 1 isoform X6	6.53E-18	96.83%	86	67.2%	57.0%	78	18	61	1	61	61	61
gi 925681443 gb KOX78188.1	WNS1_09547	PBC29602	Chromodomain Y protein	0.00E+00	99.59%	1398	67.1%	83.3%	1264	1	1226	1	1226	1226	1297
gi 925682391 gb KOX79057.1	WNS1_10105	EZA61334	Coiled-coil domain-containing protein	0.00E+00	100.00%	1478	79.2%	89.6%	939	6	908	1	934	908	937
gi 925670942 gb KOX68819.1	WNS1_06987	XP_012176075	coilin isoform X1	1.21E-64	42.58%	232	42.9%	46.3%	325	5	336	4	333	321	347
gi 925678384 gb KOX75419.1	WNS1_12869	XP_025154382	CUGBP Elav-like family member 2 isoform X2	2.11E-16	28.07%	89	59.8%	18.9%	91	7	193	130	85	64	87
gi 925670708 gb KOX68617.1	WNS1_04103	XP_017755415	cytochrome b5 reductase 4	5.83E-16	65.85%	83	61.0%	38.4%	77	1	81	1	81	77	82
gi 925670741 gb KOX68650.1	WNS1_04136	KMQ90601	cytochrome p450 315a1	0.00E+00	81.07%	527	57.4%	69.2%	439	1	522	99	439	424	439
gi 925670835 gb KOX68726.1	WNS1_06963	OAD62263	Cytochrome P450 4C1	2.31E-30	87.13%	122	59.1%	73.1%	88	1	88	1	88	88	88
gi 925682056 gb KOX78746.1	WNS1_08505	XP_017792183	digestive organ expansion factor homolog	8.67E-63	95.06%	216	69.9%	82.5%	155	1	154	1	155	154	156
gi 92568156.1	WNS1_06050	XP_015190994	dirigent protein 10-like	1.16E-16	25.54%	85	84.5%	30.0%	124	54	268	198	81	71	71
gi 925672190 gb KOX69894.1	WNS1_04406	XP_017759273	division abnormally delayed protein	4.17E-39	58.97%	150	77.3%	68.1%	619	523	92	1	97	92	97
gi 925683053 gb KOX79679.1	WNS1_02945	PBC29248	DNA primase small subunit	6.40E-116	89.66%	365	67.0%	78.3%	836	540	290	5	297	286	300
gi 925674510 gb KOX71943.1	WNS1_03220	OAD59427	DnaJ like protein subfamily C member 14	7.05E-23	53.95%	202	53.7%	53.6%	78	1	109	28	82	78	82
gi 925676602 gb KOX73805.1	WNS1_13883	OAD53296	Dual specificity tyrosine-phosphorylation-regulated kinase 2	0.00E+00	88.22%	541	79.8%	84.0%	596	276	363	42	322	321	322
gi 925682549 gb KOX79206.1	WNS1_07208	XP_003400043	dynactin, 150 kDa isoform isoform X1	1.24E-139	88.12%	406	78.4%	83.3%	264	1	267	1	267	262	269
gi 925678523 gb KOX75550.1	WNS1_12294	XP_006620391	early endosome antigen 1-like	7.88E-49	100.00%	176	65.7%	82.9%	935	796	115	1	140	115	140
gi 925675681 gb KOX72991.1	WNS1_01359	XP_024272411	EKC/KEOPS complex subunit LAGE3-like isoform X2	4.12E-19	100.00%	82	92.9%	71.4%	90	49	42	1	42	42	42
gi 925677246 gb KOX74413.1	WNS1_00316	XP_015439231	extracellular serine/threonine protein CG31145	1.98E-90	37.77%	309	52.0%	44.9%	345	6	778	484	340	295	342
gi 925677757 gb KOX74866.1	WNS1_00378	KOX74867	Fatty-acid amide hydrolase 2-A	6.18E-49	35.54%	169	98.8%	67.2%	109	24	90	5	86	86	86
gi 925684448 gb KOX80942.1	WNS1_00860	KYN39468	Ferrochelatase, mitochondrial	3.78E-25	85.22%	109	60.0%	72.6%	103	4	98	1	100	98	100
gi 925670773 gb KOX68682.1	WNS1_04168	XP_024226552	flagellar attachment zone protein 1-like	3.29E-178	74.11%	531	63.4%	68.8%	725	239	587	153	487	435	487
gi 925684763 gb KOX81213.1	WNS1_00121	PIK41367	gag-pol polyprotein	8.64E-14	71.43%	72	64.4%	42.9%	92	20	45	1	45	45	45
gi 925683045 gb KOX79671.1	WNS1_02937	OAD54970	Galanin receptor type 1	1.09E-22	93.24%	95	63.8%	78.2%	69	1	69	1	69	69	69
gi 925680615 gb KOX77420.1	WNS1_09744	XP_012237451	glutamyl-tRNA(Gln) amidotransferase subunit C, mitochondrial	6.35E-20	100.00%	86	79.6%	68.8%	135	87	49	1	49	49	49
gi 925674743 gb KOX72155.1	WNS1_01017	XP_028860879	high molecular weight thoftry protein 2, putative	1.22E-13	92.31%	74	58.1%	50.2%	659	598	64	5	62	60	62
gi 925684952 gb KOX81390.1	WNS1_10725	XP_014482497	histone-lysine N-methyltransferase PRDM9-like isoform X2	0.00E+00	100.00%	616	56.3%	78.2%	647	1	735	1	735	647	751
gi 925668660 gb KOX67365.1	WNS1_08272	KYM93355	Histone-lysine N-methyltransferase SETD2	0.00E+00	92.25%	594	49.4%	71.1%	790	1	786	61	790	726	806
gi 925669866 gb KOX67927.1	WNS1_08164	KOX70315	Histone-lysine N-methyltransferase SETMAR	1.09E-27	78.13%	106	96.0%	87.1%	50	1	50	1	50	50	50
gi 925677026 gb KOX74210.1	WNS1_13561	PBC33385	Integral membrane protein	0.00E+00	96.32%	951	96.8%	96.6%	493	25	489	19	471	469	471
gi 925677237 gb KOX74404.1	WNS1_00307	XP_006611024	inward rectifier potassium channel 4-like isoform X3	4.30E-35	100.00%	136	70.3%	85.2%	117	1	104	1	117	104	118
gi 925673645 gb KOX71170.1	WNS1_04193	XP_015182352	jmjC domain-containing protein 4 isoform X2	5.14E-58	82.61%	202	48.2%	69.3%	518	297	236	27	222	210	222
gi 925671345 gb KOX69154.1	WNS1_06307	PBC29048	kinase protein	7.45E-157	97.28%	458	54.6%	76.0%	398	10	405	12	394	389	399
gi 925671346 gb KOX69155.1	WNS1_06308	PBC29048	kinase protein	5.38E-82	93.87%	261	50.4%	72.1%	240	1	245	1	245	240	246
gi 925671349 gb KOX69158.1	WNS1_06311	PBC29048	kinase protein	3.55E-167	61.60%	493	60.0%	60.8%	398	10	638	246	393	389	395
gi 925684379 gb KOX80881.1	WNS1_05301	KMQ96186	leucine-rich repeat-containing protein	0.00E+00	95.32%	619	42.3%	72.7%	1022	9	1068	51	1018	1014	1100
gi 925673972 gb KOX71467.1	WNS1_01741	XP_003486183	low density lipoprotein receptor adapter protein 1-like isoform X3	7.83E-50	27.05%	184	70.4%	48.7%	117	15	523	382	142	103	142
gi 925676272 gb KOX73513.1	WNS1_01286	OAD62934	Low-density lipoprotein receptor-related protein 11	0.00E+00	99.51%	4336	66.9%	83.2%	3324	1	3252	1	3324	3252	3389
gi 925679334 gb KOX76270.1	WNS1_11601	XP_028522768	MATH and LRR domain-containing protein PFE0570w-like	8.42E-134	73.92%	406	54.7%	64.3%	409	2	579	152	428	408	437
gi 925684156 gb KOX80678.1	WNS1_01966	XP_020289855	max dimerization protein 1-like	4.11E-15	37.40%	79	89.1%	38.3%	248	205	123	78	46	44	46
gi 925677229 gb KOX74396.1	WNS1_00299	PBC28359	Microtubule-associatedA	1.19E-57	97.27%	195	82.4%	89.8%	108	1	107	1	107	107	108
gi 925682637 gb KOX79283.1	WNS1_09085	XP_012168616	MIEF1 upstream open reading frame protein	2.86E-22	69.33%	91	84.6%	76.3%	52	1	52	1	52	52	52
gi 925671907 gb KOX69648.1	WNS1_06534	XP_017760200	mitochondrial sodium/hydrogen exchanger 9B2 isoform X1	1.99E-137	92.25%	411	81.7%	87.0%	234	1	262	1	262	234	263
gi 925680830 gb KOX77616.1	WNS1_09281	XP_014613093	mucin-5AC-like isoform X3	2.36E-164	100.00%	473	76.3%	88.1%	394	26	327	1	369	327	371
gi 925680640 gb KOX77445.1	WNS1_09769	XP_017759425	myb-like protein M	0.00E+00	100.00%	774	66.3%	83.1%	674	1	650	1	674	650	724
gi 925673655 gb KOX71180.1	WNS1_04203	XP_011146613	myoneurin-like	0.00E+00	93.57%	802	51.9%	72.7%	913	57	945	59	887	857	933
gi 925673520 gb KOX71063.1	WNS1_03604	XP_017757624	neuroendocrine convertase 2	1.42E-64	81.87%	220	80.3%	81.1%	142	1	140	1	140	140	142
gi 925683345 gb KOX79949.1	WNS1_06361	KOC60556	Neuron navigator 2	0.00E+00	80.81%	781	62.4%	71.6%	718	1	907	154	754	718	774
gi 925678013 gb KOX75102.1	WNS1_13409	XP_020721409	nuclear factor NF-kappa-B p100 subunit isoform X1	7.06E-81	99.46%	267	68.2%	83.8%	221	21	183	1	201	183	201
gi 925678716 gb KOX75724.1	WNS1_12051	XP_017886597	nuclear receptor coactivator 1-like isoform X8	2.71E-15	38.54%	80	100.0%	44.3%	42	6	51	15	37	37	37
gi 925680436 gb KOX77252.1	WNS1_10858	XP_017761695	nucleolar protein 4-like isoform X3	1.82E-119	38.96%	371	84.3%	61.6%	256	74	220	11	210	183	210
gi 925672255 gb KOX69959.1	WNS1_04474	XP_014222438	nucleoporin GLE1	6.89E-34	100.00%	129	42.6%	75.0%	217	1	183	1	217	183	223
gi 925678400.1	WNS1_07807	KOX69571	Optomotor-blind protein	7.02E-14	28.57%	77	90.0%	34.3%	246	207	140	101	40	40	40
gi 925679268.1	WNS1_09070	XP_016908608	orpokinin peptides-like isoform X2	1.80E-21	98.44%	91	74.6%	82.0%	151	90	63	1	63	62	63
gi 925671926 gb KOX69659.1	WNS1_04941	XP_016920258	otolith matrix protein OMM-64 isoform X5	6.01E-34	59.69%	138	63.2%	61.5%	154	52	166	50	117	103	117
gi 925668618 gb KOX67323.1															

gi 925670220 gb KOX68208.1	WN51_06102	XP_012249361	RNA-binding protein fusilli isoform X1	2.67E-63	71.59%	217	95.3%	83.4%	709	583	164	39	127	126
gi 925676281 gb KOX73522.1	WN51_01295	XP_017795855	serine-rich adhesin for platelets-like	0.00E+00	98.63%	1146	55.7%	77.2%	1294	133	1162	13	1162	1150
gi 925674453 gb KOX71893.1	WN51_03038	XP_012287305	serine/arginine repetitive matrix protein 1	2.64E-25	71.84%	109	63.2%	67.5%	793	718	101	28	76	74
gi 925681936 gb KOX78640.1	WN51_07501	XP_003402214	serine/threonine-protein kinase KIPK1	0.00E+00	92.93%	645	65.2%	79.1%	570	1	486	1	570	486
gi 925675160 gb KOX72529.1	WN51_03061	XP_016909675	SIFamide-related peptide	3.57E-41	80.65%	139	84.0%	82.3%	75	1	93	19	75	75
gi 925683662 gb KOX80240.1	WN51_08417	XP_017764944	skin secretory protein xp2-like	1.68E-110	93.58%	339	80.4%	87.0%	388	4	405	27	385	379
gi 925672473 gb KOX70162.1	WN51_04902	KOC65037	Spondin-1	5.83E-38	94.32%	144	81.9%	88.1%	115	33	88	6	83	83
gi 925669918 gb KOX67966.1	WN51_08002	EZA56707	Tetratricopeptide repeat protein	3.23E-45	87.77%	167	66.4%	77.1%	496	360	127	6	137	122
gi 925681096 gb KOX77852.1	WN51_05738	XP_012167972	thyroid adenoma-associated protein	0.00E+00	97.79%	592	80.0%	88.9%	1301	947	354	1	355	354
gi 925669744 gb KOX67821.1	WN51_07674	XP_012347802	thyrotroph embryonic factor isoform X2	2.06E-54	76.00%	183	94.7%	85.4%	96	2	120	26	95	95
gi 925671918 gb KOX69651.1	WN51_04933	XP_025074166	titin	0.00E+00	74.10%	617	41.4%	62.1%	1161	17	1066	17	1145	1050
gi 925680538 gb KOX77343.1	WN51_09665	XP_012166150	transmembrane protein I04 homolog isoform X2	1.60E-156	68.35%	460	76.9%	72.7%	502	198	395	126	305	270
gi 925668978 gb KOX67439.1	WN51_09162	XP_017753925	transmembrane protein 164	0.00E+00	90.03%	548	84.9%	87.5%	298	1	331	34	298	298
gi 925674489 gb KOX71922.1	WN51_03199	XP_011343539	transmembrane protein KIAA1109 isoform X1	0.00E+00	99.98%	9001	86.5%	93.2%	5035	1	4992	1	5035	4992
gi 925677668 gb KOX74792.1	WN51_00399	AAB49410	transposase	4.19E-20	98.31%	85	67.2%	57.8%	60	3	58	1	58	58
gi 925679603 gb KOX76518.1	WN51_12498	OAD62167	Tyrosine-protein phosphatase 69D	2.45E-89	64.04%	301	76.3%	70.2%	1712	1525	202	16	188	187
gi 925677440 gb KOX74582.1	WN51_00537	XP_003490264	ubiquitin carboxyl-terminal hydrolase MINDY-3 homolog	3.88E-123	98.35%	365	96.1%	97.2%	179	1	179	1	179	179
gi 925674198 gb KOX71660.1	WN51_03774	KYM85472	UBX domain-containing protein 6	1.54E-26	83.74%	113	56.5%	70.1%	108	1	103	1	108	103
gi 925684478 gb KOX80972.1	WN51_00890	AFC60675	unpaired-like protein	5.75E-97	97.31%	293	64.0%	80.6%	274	54	217	1	221	217
gi 925673312 gb KOX70866.1	WN51_03293	XP_011067560	WD repeat-containing protein CG11141	0.00E+00	99.45%	1017	71.3%	85.4%	736	1	728	1	736	728
gi 925676926 gb KOX74129.1	WN51_14209	PBC25517	Zinc finger MYND domain-containing protein	1.52E-89	44.44%	290	73.4%	58.9%	501	322	413	230	184	180
gi 925674505 gb KOX71938.1	WN51_03215	XP_015435743	zinc finger protein 2 homolog	0.00E+00	94.58%	1157	79.4%	87.0%	718	1	716	1	718	716
gi 925677514 gb KOX74651.1	WN51_13085	XP_011639980	zinc finger protein 37-like	0.00E+00	99.29%	829	53.5%	76.4%	756	6	851	7	845	751
gi 925673882 gb KOX71377.1	WN51_01650	XP_017754344	zinc finger protein 395	4.25E-114	82.59%	347	90.7%	86.7%	194	1	185	1	194	185
gi 925673884 gb KOX71379.1	WN51_01652	XP_020724307	zinc finger protein 395 isoform X2	5.40E-133	93.24%	395	89.4%	91.3%	556	353	222	16	207	204
gi 925680544 gb KOX77349.1	WN51_09671	XP_003693701	zinc finger protein 616-like	0.00E+00	100.00%	513	89.3%	94.6%	289	1	287	1	289	287

Table S2: list of primers used for RT-qPCR assays.

Primer	Sequence	ID	Fragment size (pb)	Annealing temperature (°C) *	Efficiency (E)
Vg1 F	ACGACAAGACACCATCCACC	KOX68248.1 /	136	60	2.114±0.062
Vg1 R	CTTCCTACGCGCAGATCCAT				
Vg2 F	CAGGCTGAAACTAGACGGCA	KOX73997.1 /	137	60	2.104±0.109
Vg2 R	TGTCATGCACGGTACTGCTT				
NF-kB F	TCTGGATCCTGCAGTAATACGA	KOX75102.1 /	103	50	1.988±0.021
NF-kB R	ATCTTGACCAGCATCTCTACGG				
P450 F	TTGGAGGAAATTCGTTGCGTC	KOX68650.1 /	129	50	2.055±0.025
P450 R	TCCTCAGCTCGGATACCTTCAT				
PO F	TTTCCAAGCACGTTGATACCC	KOX79496.1 /	120	50	2.057±0.125
PO R	TCAAGATCTGGAAAGATGGCG				
rps5 F	ATAATTCACCTGTTGACCGGG	KOX78800.1 /	148	60	1.897±0.035
rps5 R	TTACTCGTCGTAGCGGAGAAA				
Act F	CTGCTGGAAGGTGGACAGAG	Brito et al. 2015.	138	50	1.884±0.059
Act R	GCCGATCGTATGCAGAAGGA				

***qPCR conditions:** 95 °C for 3 min followed by 40 cycles of 95 °C for 15 sec, 60 °C (for rps5, Vg1 and Vg2) or 50 °C (for act, PO, NF-kB and P450) for 30 sec and 72 °C for 30 sec. The amplification specificity was confirmed by melting curve analysis (95 °C for 15 sec, 60 °C for 1 min and a continuous raise in temperature to 95 °C at 0.3 °C/sec ramp rate followed by 95 °C for 15 sec).

Table S3(a): calibrated normalized relative quantities (CNRQs) of gene expression for five health-related bee genes. For each sample there is information from the Month, Health in March (H = healthy, U = unhealthy, Intensity (H = healthy, SU = slightly unhealthy, U = unhealthy, Locality (BP = meliponary in Bom Princípio, PA = meliponary in Porto Alegre), Sisters (1 = colonies BP1 and PA1, 2 = colonies BP2 and PA2, 3 = colonies BP3 and PA3) and Colony.

Month	Health in March	Intensity	Locality	Sisters	Colony	NF-kB	p450	PO	Vg1	Vg2
APR	H	H	BP	1	BP1	0.40393	0.68156	0.62291	2.30409	1.21100
APR	H	H	BP	1	BP1	0.49486	0.91759	0.75866	2.78038	1.16520
APR	H	H	BP	1	BP1	0.51081	0.75520	0.90834	2.68193	1.12127
APR	H	H	BP	1	BP1	0.71951	1.13653	0.61239	0.80512	0.94431
APR	U	U	BP	2	BP2	0.49571	0.54405	0.57382	2.15483	0.29769
APR	U	U	BP	2	BP2	0.73196	0.92746	0.83528	4.02957	0.90546
APR	U	U	BP	2	BP2	0.81841	1.12291	0.95468	1.98528	1.20335
APR	U	U	BP	2	BP2	0.86493	1.07878	0.76386	4.11033	1.14243
APR	U	U	BP	2	BP2	1.19265	1.52828	1.45653	3.00167	1.29949
APR	U	U	BP	3	BP3	0.84810	0.82940	0.73560	1.63140	1.08661
APR	U	U	BP	3	BP3	0.97722	0.50283	0.87123	1.61803	0.58382
APR	U	U	BP	3	BP3	1.06376	0.74974	0.71002	2.85523	1.02761
APR	U	U	BP	3	BP3	1.15887	0.82268	0.76193	1.04493	1.18115
APR	H	H	PA	1	PA1	0.60806	1.00171	0.94383	4.63445	1.45137
APR	H	H	PA	1	PA1	0.61272	0.78342	0.58713	3.18476	1.22451
APR	H	H	PA	1	PA1	0.70325	0.79097	0.80850	3.93723	1.25030
APR	H	H	PA	1	PA1	0.81130	0.84617	0.62507	2.54957	1.09589
APR	H	H	PA	1	PA1	1.20330	1.41889	0.94163	3.88383	1.81578
APR	U	SU	PA	2	PA2	0.78643	0.92944	0.97721	2.21866	1.28203
APR	U	SU	PA	2	PA2	0.96854	0.63022	0.32152	NaN	1.08747
APR	U	SU	PA	2	PA2	1.06952	1.21748	1.26286	1.38856	1.54639
APR	U	SU	PA	3	PA3	0.80914	1.11853	1.14126	4.93630	1.43340
APR	U	SU	PA	3	PA3	0.90577	1.03001	0.99472	3.26563	1.37499
APR	U	SU	PA	3	PA3	1.21623	1.13177	0.84885	3.62170	1.32607
APR	U	SU	PA	3	PA3	1.47475	1.03625	0.88811	3.76915	1.39518
APR	U	SU	PA	3	PA3	1.52514	1.18454	0.85083	3.53806	1.83276
FEB	H	H	BP	1	BP1	0.76028	1.25627	1.01126	2.65872	1.05540
FEB	H	H	BP	1	BP1	0.78092	1.22612	1.05259	4.05905	1.14897
FEB	H	H	BP	1	BP1	0.93026	1.10565	1.16402	2.24251	1.39994
FEB	H	H	BP	1	BP1	0.95416	1.20392	0.99822	3.31675	1.14920
FEB	H	H	BP	1	BP1	1.06014	1.21981	0.96403	3.92704	1.39389
FEB	U	U	BP	2	BP2	0.77611	0.73801	0.33652	4.50375	1.17110
FEB	U	U	BP	2	BP2	0.90836	0.97417	0.59170	4.20709	1.23665
FEB	U	U	BP	2	BP2	0.96147	0.89965	0.71688	2.69329	1.11045
FEB	U	U	BP	2	BP2	0.97089	0.90928	1.00416	3.72209	1.25183
FEB	U	U	BP	2	BP2	1.13867	1.08658	0.88982	4.16470	1.23829
FEB	U	U	BP	3	BP3	0.91174	1.17937	1.55670	3.00213	1.34081
FEB	U	U	BP	3	BP3	0.95301	1.06870	1.22893	4.04815	1.58224
FEB	U	U	BP	3	BP3	1.01468	1.19193	1.42675	3.30598	1.48822
FEB	U	U	BP	3	BP3	1.12005	0.77627	1.50121	3.24259	1.27729
FEB	U	U	BP	3	BP3	1.32552	1.50106	1.52947	2.54451	1.77481
FEB	H	H	PA	1	PA1	0.69974	0.67245	0.96946	3.13858	0.69615
FEB	H	H	PA	1	PA1	0.89655	0.71890	0.24830	2.16123	0.65533
FEB	H	H	PA	1	PA1	0.96277	0.81484	1.00835	3.16287	0.63907
FEB	H	H	PA	1	PA1	1.03705	0.68380	1.00425	2.94774	0.15975

FEB	H	H	PA	1	PA1	1.14693	1.00413	1.06463	2.37282	0.83289
FEB	U	SU	PA	2	PA2	0.82417	1.21281	1.50307	3.77168	1.37775
FEB	U	SU	PA	2	PA2	0.94122	0.52800	0.84997	1.88611	1.07685
FEB	U	SU	PA	2	PA2	1.08991	1.44593	1.64496	4.05538	1.60738
FEB	U	SU	PA	2	PA2	1.13557	0.53498	0.86608	2.30362	1.03911
FEB	U	SU	PA	2	PA2	1.18294	1.91891	1.92203	4.25835	1.80484
FEB	U	SU	PA	3	PA3	0.94980	0.91465	0.85386	2.19873	0.78099
FEB	U	SU	PA	3	PA3	1.05728	0.90186	0.82027	0.67346	NaN
FEB	U	SU	PA	3	PA3	1.06825	0.88541	1.02837	2.44390	0.86088
FEB	U	SU	PA	3	PA3	1.13985	1.73046	1.49921	2.87407	0.97242
FEB	U	SU	PA	3	PA3	1.23820	0.52761	1.06986	2.59854	0.33361
JAN	H	H	BP	1	BP1	0.37127	NaN	1.28160	3.55489	1.44594
JAN	H	H	BP	1	BP1	0.79236	0.61127	0.11911	2.64655	1.08757
JAN	H	H	BP	1	BP1	1.01856	1.10064	0.37454	1.98306	1.11360
JAN	H	H	BP	1	BP1	1.51512	2.44836	2.54207	4.37752	2.34298
JAN	H	H	BP	1	BP1	1.55136	2.42430	2.38124	4.29870	2.04590
JAN	U	U	BP	2	BP2	0.71417	0.71919	0.59688	3.74611	0.83746
JAN	U	U	BP	2	BP2	0.80112	0.62632	0.46447	2.55849	0.86880
JAN	U	U	BP	2	BP2	0.97809	0.89465	0.09376	1.90109	0.59685
JAN	U	U	BP	2	BP2	0.99047	0.84732	0.28709	2.08675	1.04941
JAN	U	U	BP	2	BP2	1.23933	1.00088	0.81547	3.27345	1.22975
JAN	U	U	BP	3	BP3	0.93298	1.06957	0.67682	1.79251	0.71114
JAN	U	U	BP	3	BP3	1.10896	1.12940	1.13665	2.18712	1.33554
JAN	U	U	BP	3	BP3	1.28247	1.06699	1.39808	1.68420	1.17887
JAN	U	U	BP	3	BP3	1.53135	1.25788	NaN	2.62480	1.17820
JAN	U	U	BP	3	BP3	NaN	NaN	NaN	NaN	1.86391
JAN	H	H	PA	1	PA1	0.12789	NaN	1.37761	3.09950	2.07374
JAN	H	H	PA	1	PA1	0.66631	NaN	1.37910	2.73021	1.71248
JAN	H	H	PA	1	PA1	0.74127	NaN	2.17882	NaN	2.24292
JAN	H	H	PA	1	PA1	1.37855	0.83050	0.76219	2.58040	1.07201
JAN	U	SU	PA	2	PA2	0.77554	1.09550	NaN	4.63275	1.63251
JAN	U	SU	PA	2	PA2	0.95132	0.42822	0.73009	1.96430	0.61312
JAN	U	SU	PA	2	PA2	0.96355	0.78150	0.63044	1.99150	1.04367
JAN	U	SU	PA	2	PA2	1.32419	0.30406	0.74546	3.11081	1.53931
JAN	U	SU	PA	3	PA3	0.74546	0.58250	0.69443	4.33833	1.10272
JAN	U	SU	PA	3	PA3	0.90377	0.82394	0.89278	1.72744	1.32499
JAN	U	SU	PA	3	PA3	0.91116	0.62447	0.47443	3.59957	0.90087
JAN	U	SU	PA	3	PA3	1.20689	0.96052	0.92592	2.93313	1.56133
JAN	U	SU	PA	3	PA3	1.21183	0.43547	0.92755	3.08372	1.30555
MAR	H	H	BP	1	BP1	1.00237	1.18329	0.75738	2.21579	1.12978
MAR	H	H	BP	1	BP1	1.11375	1.40617	1.14949	2.45166	1.60400
MAR	H	H	BP	1	BP1	1.22787	1.18323	1.40423	2.71378	1.68929
MAR	H	H	BP	1	BP1	1.27892	1.47250	1.11996	3.65666	1.74396
MAR	H	H	BP	1	BP1	1.40087	1.38446	1.32351	2.95647	1.57923
MAR	U	U	BP	2	BP2	0.71132	0.95508	0.79277	3.66182	0.78778
MAR	U	U	BP	2	BP2	1.16379	1.55710	1.16630	4.53431	1.20592
MAR	U	U	BP	2	BP2	1.16816	1.43266	0.63443	2.09140	1.03616
MAR	U	U	BP	2	BP2	NaN	NaN	0.86603	2.06460	0.35814
MAR	U	U	BP	3	BP3	0.37412	0.33498	0.51874	2.45968	0.92161
MAR	U	U	BP	3	BP3	0.59895	0.79401	1.07109	3.78976	0.90351
MAR	U	U	BP	3	BP3	1.06476	1.12739	1.17176	3.22656	1.21752

MAR	U	U	BP	3	BP3	1.26362	1.13792	1.12021	3.53408	1.51012
MAR	H	H	PA	1	PA1	0.28170	NaN	NaN	2.84397	0.04623
MAR	H	H	PA	1	PA1	0.83792	1.30258	0.84565	2.27927	1.26173
MAR	H	H	PA	1	PA1	0.92956	1.32466	0.47103	2.48985	1.29336
MAR	H	H	PA	1	PA1	0.97290	1.30035	0.51909	2.29260	1.40017
MAR	H	H	PA	1	PA1	1.04688	1.21635	0.69896	3.51554	1.49378
MAR	U	SU	PA	2	PA2	0.80296	0.93231	0.88155	1.38305	1.17709
MAR	U	SU	PA	2	PA2	1.19940	1.57735	0.77582	1.77445	1.13982
MAR	U	SU	PA	2	PA2	1.42161	1.32443	1.00711	1.51384	1.50673
MAR	U	SU	PA	2	PA2	1.49562	1.75613	1.18692	1.43628	1.76001
MAR	U	SU	PA	2	PA2	1.56496	1.67173	1.09681	2.11148	1.81762
MAR	U	SU	PA	3	PA3	0.45121	0.60011	0.47875	1.86376	1.04327
MAR	U	SU	PA	3	PA3	0.57028	NaN	NaN	2.92598	1.58267
MAR	U	SU	PA	3	PA3	1.07956	1.14165	0.78750	2.35862	1.38272
MAR	U	SU	PA	3	PA3	1.09680	1.23807	0.80069	3.43498	1.66468
MAR	U	SU	PA	3	PA3	1.15657	1.33334	0.67823	2.29559	1.52276

Table S3(b): Lowest daily temperature and highest daily humidity for each colony. In addition to the information on the previous tab, there is also information on Syndrome days (NO = without syndrome symptoms, YES = with syndrome symptoms).

Month	Health in March	Syndrome days	Locality	Sisters	Colony	Day	Temperature morning	Humidity noon
DEC	H	NO	BP	1	BP1	12/01/18	23.09	93.98
DEC	H	NO	BP	1	BP1	12/02/18	18.27	92.51
DEC	H	NO	BP	1	BP1	12/03/18	13.59	92.24
DEC	H	NO	BP	1	BP1	12/04/18	13.31	84.24
DEC	H	NO	BP	1	BP1	12/05/18	15.92	85.18
DEC	H	NO	BP	1	BP1	12/06/18	15.99	82.13
DEC	H	NO	BP	1	BP1	12/07/18	17.03	79.95
DEC	H	NO	BP	1	BP1	12/08/18	11.42	74.54
DEC	H	NO	BP	1	BP1	12/09/18	14.7	71.63
DEC	H	NO	BP	1	BP1	12/10/18	20.56	70.54
DEC	H	NO	BP	1	BP1	12/11/18	19.32	70.36
DEC	H	NO	BP	1	BP1	12/12/18	26.57	69.08
DEC	H	NO	BP	1	BP1	12/13/18	23.4	72.89
DEC	H	NO	BP	1	BP1	12/14/18	25.38	61.4
DEC	H	NO	BP	1	BP1	12/15/18	24.34	79.95
DEC	H	NO	BP	1	BP1	12/16/18	25.11	75.6
DEC	H	NO	BP	1	BP1	12/17/18	26.38	53.68
DEC	H	NO	BP	1	BP1	12/18/18	25.4	84.71
DEC	H	NO	BP	1	BP1	12/19/18	24.29	78.09
DEC	H	NO	BP	1	BP1	12/20/18	24.85	71.29
DEC	H	NO	BP	1	BP1	12/21/18	23.69	88.29
DEC	H	NO	BP	1	BP1	12/22/18	23.09	70.34
DEC	H	NO	BP	1	BP1	12/23/18	22.73	72.29
DEC	H	NO	BP	1	BP1	12/24/18	21.8	77.69
DEC	H	NO	BP	1	BP1	12/25/18	21.25	67.45
DEC	H	NO	BP	1	BP1	12/26/18	22.75	60.4
DEC	H	NO	BP	1	BP1	12/27/18	22.01	58.1
DEC	H	NO	BP	1	BP1	12/28/18	24.85	55.17
DEC	H	NO	BP	1	BP1	12/29/18	22.2	54.2
DEC	H	NO	BP	1	BP1	12/30/18	24.58	52.7
DEC	H	NO	BP	1	BP1	12/31/18	28.39	71.3
JAN	H	NO	BP	1	BP1	01/01/19	26.28	54.94
JAN	H	NO	BP	1	BP1	01/02/19	28.59	53.39
JAN	H	NO	BP	1	BP1	01/03/19	30.95	70.51
JAN	H	NO	BP	1	BP1	01/04/19	23.47	86.47
JAN	H	NO	BP	1	BP1	01/05/19	22.8	86.57
JAN	H	NO	BP	1	BP1	01/06/19	23.47	72.87
JAN	H	NO	BP	1	BP1	01/07/19	27.88	71.15
JAN	H	NO	BP	1	BP1	01/08/19	25.48	72
JAN	H	NO	BP	1	BP1	01/09/19	26.13	80.39
JAN	H	NO	BP	1	BP1	01/10/19	24.75	81.1
JAN	H	NO	BP	1	BP1	01/11/19	24.53	86.55
JAN	H	NO	BP	1	BP1	01/12/19	23.91	89.9
JAN	H	NO	BP	1	BP1	01/13/19	24.92	77.49
JAN	H	NO	BP	1	BP1	01/14/19	25.09	81.18
JAN	H	NO	BP	1	BP1	01/15/19	26.79	66.39
JAN	H	NO	BP	1	BP1	01/16/19	25.5	72.47
JAN	H	NO	BP	1	BP1	01/17/19	27.36	84.21
JAN	H	NO	BP	1	BP1	01/18/19	22.66	87.92

JAN	H	NO	BP	1	BP1	01/19/19	25.67	90.29
JAN	H	NO	BP	1	BP1	01/20/19	22.51	88.06
JAN	H	NO	BP	1	BP1	01/21/19	23.76	90.94
JAN	H	NO	BP	1	BP1	01/22/19	22.06	74.14
JAN	H	NO	BP	1	BP1	01/23/19	24	67.39
JAN	H	NO	BP	1	BP1	01/24/19	26.4	68.36
JAN	H	NO	BP	1	BP1	01/25/19	23.69	67.14
JAN	H	NO	BP	1	BP1	01/26/19	25.07	66.49
JAN	H	NO	BP	1	BP1	01/27/19	25.94	64.19
JAN	H	NO	BP	1	BP1	01/28/19	25.62	55.48
JAN	H	NO	BP	1	BP1	01/29/19	27.11	57.7
JAN	H	NO	BP	1	BP1	01/30/19	27.55	53.01
JAN	H	NO	BP	1	BP1	01/31/19	27.33	56.19
FEB	H	NO	BP	1	BP1	02/01/19	24.97	64.75
FEB	H	NO	BP	1	BP1	02/02/19	25.48	75.77
FEB	H	NO	BP	1	BP1	02/03/19	23.52	86.07
FEB	H	NO	BP	1	BP1	02/04/19	19.87	85.19
FEB	H	NO	BP	1	BP1	02/05/19	20.37	79.39
FEB	H	NO	BP	1	BP1	02/06/19	18.08	77.51
FEB	H	NO	BP	1	BP1	02/07/19	20.1	74.68
FEB	H	NO	BP	1	BP1	02/08/19	22.01	67.61
FEB	H	NO	BP	1	BP1	02/09/19	23.45	61.55
FEB	H	NO	BP	1	BP1	02/10/19	26.89	75.38
FEB	H	NO	BP	1	BP1	02/11/19	25.19	75.64
FEB	H	NO	BP	1	BP1	02/12/19	24.15	84.37
FEB	H	NO	BP	1	BP1	02/13/19	18.65	85.79
FEB	H	NO	BP	1	BP1	02/14/19	20.08	71.54
FEB	H	NO	BP	1	BP1	02/15/19	24.22	88.33
FEB	H	NO	BP	1	BP1	02/16/19	22.61	88.68
FEB	H	NO	BP	1	BP1	02/17/19	22.15	79.83
FEB	H	NO	BP	1	BP1	02/18/19	23.26	78.94
FEB	H	NO	BP	1	BP1	02/19/19	22.47	80.61
FEB	H	NO	BP	1	BP1	02/20/19	23.71	72.2
FEB	H	NO	BP	1	BP1	02/21/19	21.68	72.51
FEB	H	NO	BP	1	BP1	02/22/19	21.13	69.72
FEB	H	NO	BP	1	BP1	02/23/19	24.27	63.44
FEB	H	NO	BP	1	BP1	02/24/19	27.36	54.4
FEB	H	NO	BP	1	BP1	02/25/19	24.48	85.73
FEB	H	NO	BP	1	BP1	02/26/19	21.27	86.35
FEB	H	NO	BP	1	BP1	02/27/19	19.79	86.6
FEB	H	NO	BP	1	BP1	02/28/19	19.37	84.08
MAR	H	NO	BP	1	BP1	03/01/19	18.32	82.11
MAR	H	NO	BP	1	BP1	03/02/19	20.98	66.29
MAR	H	NO	BP	1	BP1	03/03/19	21.75	76.9
MAR	H	NO	BP	1	BP1	03/04/19	22.08	68.22
MAR	H	NO	BP	1	BP1	03/05/19	26.82	60.11
MAR	H	NO	BP	1	BP1	03/06/19	23.62	79.93
MAR	H	NO	BP	1	BP1	03/07/19	25.36	75.67
MAR	H	NO	BP	1	BP1	03/08/19	25.14	86.5
MAR	H	NO	BP	1	BP1	03/09/19	24.39	90.21
MAR	H	NO	BP	1	BP1	03/10/19	21.2	89.53
MAR	H	NO	BP	1	BP1	03/11/19	20.29	86.07
MAR	H	NO	BP	1	BP1	03/12/19	21.06	89.58
MAR	H	NO	BP	1	BP1	03/13/19	22.61	91.13

MAR	H	NO	BP	1	BP1	03/14/19	21.99	86.2
MAR	H	NO	BP	1	BP1	03/15/19	19.51	83.28
MAR	H	NO	BP	1	BP1	03/16/19	21.77	85.28
MAR	H	NO	BP	1	BP1	03/17/19	24.05	88.64
MAR	H	NO	BP	1	BP1	03/18/19	24.51	90.02
MAR	H	NO	BP	1	BP1	03/19/19	22.82	89.96
MAR	H	NO	BP	1	BP1	03/20/19	21.32	90.58
MAR	H	NO	BP	1	BP1	03/21/19	19.75	89.58
MAR	H	NO	BP	1	BP1	03/22/19	16.25	89.72
MAR	H	NO	BP	1	BP1	03/23/19	18.08	87.71
MAR	H	NO	BP	1	BP1	03/24/19	18.27	84.39
MAR	H	NO	BP	1	BP1	03/25/19	20.22	84.57
MAR	H	NO	BP	1	BP1	03/26/19	16.53	82.58
MAR	H	NO	BP	1	BP1	03/27/19	18.89	80.03
MAR	H	NO	BP	1	BP1	03/28/19	19.91	83.41
MAR	H	NO	BP	1	BP1	03/29/19	19.44	83.41
MAR	H	NO	BP	1	BP1	03/30/19	22.73	73.98
MAR	H	NO	BP	1	BP1	03/31/19	23.76	81.74
APR	H	NO	BP	1	BP1	04/01/19	23.14	85.57
APR	H	NO	BP	1	BP1	04/02/19	22.13	81.2
APR	H	NO	BP	1	BP1	04/03/19	24.36	73.4
APR	H	NO	BP	1	BP1	04/04/19	24.51	77.87
APR	H	NO	BP	1	BP1	04/05/19	24.82	83.98
APR	H	NO	BP	1	BP1	04/06/19	21.25	87.99
APR	H	NO	BP	1	BP1	04/07/19	22.06	89.17
APR	H	NO	BP	1	BP1	04/08/19	21.41	87.78
APR	H	NO	BP	1	BP1	04/09/19	17.13	87.42
APR	H	NO	BP	1	BP1	04/10/19	16.94	86.89
APR	H	NO	BP	1	BP1	04/11/19	20.32	83.4
APR	H	NO	BP	1	BP1	04/12/19	21.77	87.51
APR	H	NO	BP	1	BP1	04/13/19	20.98	88.58
APR	H	NO	BP	1	BP1	04/14/19	20.58	82.95
APR	H	NO	BP	1	BP1	04/15/19	22.18	85.69
APR	H	NO	BP	1	BP1	04/16/19	23.11	89.76
APR	H	NO	BP	1	BP1	04/17/19	20.96	89.97
APR	H	NO	BP	1	BP1	04/18/19	19.1	85.81
APR	H	NO	BP	1	BP1	04/19/19	18.68	88.05
APR	H	NO	BP	1	BP1	04/20/19	20.67	88.34
APR	H	NO	BP	1	BP1	04/21/19	22.32	90.14
APR	H	NO	BP	1	BP1	04/22/19	19.46	88.94
APR	H	NO	BP	1	BP1	04/23/19	20.08	83.65
APR	H	NO	BP	1	BP1	04/24/19	21.15	81.12
APR	H	NO	BP	1	BP1	04/25/19	22.75	89.3
APR	H	NO	BP	1	BP1	04/26/19	23.04	92.34
APR	H	NO	BP	1	BP1	04/27/19	23.3	92.02
APR	H	NO	BP	1	BP1	04/28/19	22.32	92.18
APR	H	NO	BP	1	BP1	04/29/19	18.7	91.76
APR	H	NO	BP	1	BP1	04/30/19	16.06	89.8
MAY	H	NO	BP	1	BP1	05/01/19	20.41	89.57
MAY	H	NO	BP	1	BP1	05/02/19	17.89	87.32
MAY	H	NO	BP	1	BP1	05/03/19	20.91	75.16
MAY	H	NO	BP	1	BP1	05/04/19	24.1	91.96
MAY	H	NO	BP	1	BP1	05/05/19	24.27	89.2
MAY	H	NO	BP	1	BP1	05/06/19	23.3	91.27

MAY	H	NO	BP	1	BP1	05/07/19	20.37	91.23
MAY	H	NO	BP	1	BP1	05/08/19	16.39	87.56
MAY	H	NO	BP	1	BP1	05/09/19	20.37	92.31
MAY	H	NO	BP	1	BP1	05/10/19	22.32	89.1
MAY	H	NO	BP	1	BP1	05/11/19	21.08	90.24
MAY	H	NO	BP	1	BP1	05/12/19	18.03	92.17
MAY	H	NO	BP	1	BP1	05/13/19	19.46	91.51
MAY	H	NO	BP	1	BP1	05/14/19	18.46	91.77
MAY	H	NO	BP	1	BP1	05/15/19	19.08	98.24
MAY	H	NO	BP	1	BP1	05/16/19	20.96	75
MAY	H	NO	BP	1	BP1	05/17/19	20.89	61.56
MAY	H	NO	BP	1	BP1	05/18/19	21.39	69.02
MAY	H	NO	BP	1	BP1	05/19/19	22.27	71.12
MAY	H	NO	BP	1	BP1	05/20/19	22.94	74.21
MAY	H	NO	BP	1	BP1	05/21/19	23.81	75.2
MAY	H	NO	BP	1	BP1	05/22/19	22.44	79.89
DEC	U	NO	BP	2	BP2	12/01/18	24.17	94.02
DEC	U	NO	BP	2	BP2	12/02/18	18.18	91.25
DEC	U	NO	BP	2	BP2	12/03/18	14.79	93.68
DEC	U	NO	BP	2	BP2	12/04/18	14.22	91.84
DEC	U	NO	BP	2	BP2	12/05/18	16.58	87.05
DEC	U	NO	BP	2	BP2	12/06/18	16.89	88.02
DEC	U	NO	BP	2	BP2	12/07/18	17.56	84.48
DEC	U	NO	BP	2	BP2	12/08/18	12.1	85.13
DEC	U	NO	BP	2	BP2	12/09/18	15.53	84.08
DEC	U	NO	BP	2	BP2	12/10/18	20.94	80.28
DEC	U	NO	BP	2	BP2	12/11/18	19.98	73.05
DEC	U	NO	BP	2	BP2	12/12/18	27.41	69.59
DEC	U	NO	BP	2	BP2	12/13/18	23.88	81.62
DEC	U	NO	BP	2	BP2	12/14/18	25.4	75.69
DEC	U	NO	BP	2	BP2	12/15/18	22.2	79.16
DEC	U	NO	BP	2	BP2	12/16/18	23.81	87.32
DEC	U	NO	BP	2	BP2	12/17/18	25.19	73.74
DEC	U	NO	BP	2	BP2	12/18/18	23.67	84.05
DEC	U	NO	BP	2	BP2	12/19/18	22.54	89.26
DEC	U	NO	BP	2	BP2	12/20/18	22.9	85.73
DEC	U	NO	BP	2	BP2	12/21/18	21.82	91.04
DEC	U	NO	BP	2	BP2	12/22/18	21.84	92.23
DEC	U	NO	BP	2	BP2	12/23/18	21.18	90.85
DEC	U	NO	BP	2	BP2	12/24/18	20.1	86.93
DEC	U	NO	BP	2	BP2	12/25/18	19.98	84.57
DEC	U	NO	BP	2	BP2	12/26/18	22.27	83.16
DEC	U	NO	BP	2	BP2	12/27/18	21.06	81.39
DEC	U	NO	BP	2	BP2	12/28/18	24.22	76.03
DEC	U	NO	BP	2	BP2	12/29/18	21.41	74.64
DEC	U	NO	BP	2	BP2	12/30/18	23.3	67.66
DEC	U	NO	BP	2	BP2	12/31/18	26.72	76.24
JAN	U	NO	BP	2	BP2	01/01/19	25.11	75.66
JAN	U	NO	BP	2	BP2	01/02/19	27.8	68.32
JAN	U	NO	BP	2	BP2	01/03/19	30.09	68.24
JAN	U	NO	BP	2	BP2	01/04/19	21.34	80.29
JAN	U	NO	BP	2	BP2	01/05/19	21.37	85.6
JAN	U	NO	BP	2	BP2	01/06/19	21.99	87.72
JAN	U	NO	BP	2	BP2	01/07/19	26.94	81.67

JAN	U	NO	BP	2	BP2	01/08/19	24.17	84.46
JAN	U	NO	BP	2	BP2	01/09/19	25.21	81.37
JAN	U	NO	BP	2	BP2	01/10/19	23.88	85.26
JAN	U	NO	BP	2	BP2	01/11/19	23.3	89.08
JAN	U	NO	BP	2	BP2	01/12/19	22.61	90.71
JAN	U	NO	BP	2	BP2	01/13/19	23.79	93.51
JAN	U	NO	BP	2	BP2	01/14/19	23.71	91.2
JAN	U	NO	BP	2	BP2	01/15/19	25.91	84.3
JAN	U	NO	BP	2	BP2	01/16/19	25.87	89.12
JAN	U	NO	BP	2	BP2	01/17/19	27.63	90.04
JAN	U	NO	BP	2	BP2	01/18/19	22.59	94.31
JAN	U	NO	BP	2	BP2	01/19/19	25.74	94.97
JAN	U	NO	BP	2	BP2	01/20/19	22.25	96.08
JAN	U	NO	BP	2	BP2	01/21/19	23.47	96.17
JAN	U	NO	BP	2	BP2	01/22/19	22.32	94.94
JAN	U	NO	BP	2	BP2	01/23/19	24.29	90.59
JAN	U	NO	BP	2	BP2	01/24/19	26.33	88.88
JAN	U	NO	BP	2	BP2	01/25/19	24.12	87.95
JAN	U	NO	BP	2	BP2	01/26/19	25.43	83.11
JAN	U	NO	BP	2	BP2	01/27/19	26.52	77.35
JAN	U	NO	BP	2	BP2	01/28/19	26.09	77.24
JAN	U	NO	BP	2	BP2	01/29/19	27.24	76.08
JAN	U	NO	BP	2	BP2	01/30/19	27.7	72.01
JAN	U	NO	BP	2	BP2	01/31/19	27.41	74.92
FEB	U	NO	BP	2	BP2	02/01/19	25.19	83.92
FEB	U	NO	BP	2	BP2	02/02/19	25.7	86.83
FEB	U	NO	BP	2	BP2	02/03/19	23.42	92.12
FEB	U	NO	BP	2	BP2	02/04/19	20.32	94.11
FEB	U	NO	BP	2	BP2	02/05/19	20.72	93.56
FEB	U	NO	BP	2	BP2	02/06/19	18.87	93.61
FEB	U	NO	BP	2	BP2	02/07/19	20.51	92.55
FEB	U	NO	BP	2	BP2	02/08/19	22.23	87.52
FEB	U	NO	BP	2	BP2	02/09/19	23.59	83.71
FEB	U	NO	BP	2	BP2	02/10/19	26.62	83.56
FEB	U	NO	BP	2	BP2	02/11/19	25.11	88.49
FEB	U	NO	BP	2	BP2	02/12/19	23.86	91.25
FEB	U	NO	BP	2	BP2	02/13/19	18.75	92.2
FEB	U	NO	BP	2	BP2	02/14/19	20.34	91.27
FEB	U	NO	BP	2	BP2	02/15/19	23.93	91.54
FEB	U	NO	BP	2	BP2	02/16/19	22.47	94.1
FEB	U	NO	BP	2	BP2	02/17/19	21.8	91.93
FEB	U	NO	BP	2	BP2	02/18/19	22.94	91.95
FEB	U	NO	BP	2	BP2	02/19/19	22.2	92.22
FEB	U	NO	BP	2	BP2	02/20/19	23.64	89.52
FEB	U	NO	BP	2	BP2	02/21/19	21.8	87.15
FEB	U	NO	BP	2	BP2	02/22/19	21.37	82.5
FEB	U	NO	BP	2	BP2	02/23/19	24.41	76.96
FEB	U	NO	BP	2	BP2	02/24/19	27.43	67.11
FEB	U	NO	BP	2	BP2	02/25/19	24.2	84.45
FEB	U	NO	BP	2	BP2	02/26/19	20.89	89.3
FEB	U	NO	BP	2	BP2	02/27/19	19.6	91.9
FEB	U	NO	BP	2	BP2	02/28/19	19.13	93.01
MAR	U	NO	BP	2	BP2	03/01/19	18.41	90.7
MAR	U	NO	BP	2	BP2	03/02/19	21.29	83.77

MAR	U	NO	BP	2	BP2	03/03/19	21.25	87.52
MAR	U	NO	BP	2	BP2	03/04/19	21.65	84.3
MAR	U	NO	BP	2	BP2	03/05/19	26.74	73.99
MAR	U	NO	BP	2	BP2	03/06/19	23.21	84.19
MAR	U	NO	BP	2	BP2	03/07/19	25.04	85.83
MAR	U	NO	BP	2	BP2	03/08/19	24.82	86.73
MAR	U	NO	BP	2	BP2	03/09/19	24.15	90.73
MAR	U	NO	BP	2	BP2	03/10/19	20.98	93
MAR	U	NO	BP	2	BP2	03/11/19	20.08	91.77
MAR	U	NO	BP	2	BP2	03/12/19	20.89	92.66
MAR	U	NO	BP	2	BP2	03/13/19	22.35	92.71
MAR	U	NO	BP	2	BP2	03/14/19	21.96	94.53
MAR	U	NO	BP	2	BP2	03/15/19	19.41	90.88
MAR	U	NO	BP	2	BP2	03/16/19	21.27	91.58
MAR	U	NO	BP	2	BP2	03/17/19	23.42	92.58
MAR	U	NO	BP	2	BP2	03/18/19	24	93.62
MAR	U	NO	BP	2	BP2	03/19/19	22.2	94.95
MAR	U	NO	BP	2	BP2	03/20/19	20.79	93.48
MAR	U	NO	BP	2	BP2	03/21/19	19.06	95.04
MAR	U	NO	BP	2	BP2	03/22/19	15.27	95.39
MAR	U	NO	BP	2	BP2	03/23/19	17.39	96.03
MAR	U	YES	BP	2	BP2	03/24/19	17.27	94.87
MAR	U	YES	BP	2	BP2	03/25/19	19.32	91.52
MAR	U	YES	BP	2	BP2	03/26/19	15.03	92.09
MAR	U	YES	BP	2	BP2	03/27/19	17.42	93.07
MAR	U	YES	BP	2	BP2	03/28/19	18.53	93.61
MAR	U	YES	BP	2	BP2	03/29/19	18.3	92.89
MAR	U	YES	BP	2	BP2	03/30/19	22.18	87.61
MAR	U	NO	BP	2	BP2	03/31/19	22.8	85.76
APR	U	NO	BP	2	BP2	04/01/19	22.32	90.07
APR	U	NO	BP	2	BP2	04/02/19	21.49	87.16
APR	U	NO	BP	2	BP2	04/03/19	23.91	86.26
APR	U	NO	BP	2	BP2	04/04/19	23.88	83.09
APR	U	NO	BP	2	BP2	04/05/19	23.69	85.45
APR	U	NO	BP	2	BP2	04/06/19	20.17	89.52
APR	U	NO	BP	2	BP2	04/07/19	20.98	91.1
APR	U	NO	BP	2	BP2	04/08/19	20.41	93.38
APR	U	NO	BP	2	BP2	04/09/19	19.79	87.41
APR	U	NO	BP	2	BP2	04/10/19	16.46	91.91
APR	U	NO	BP	2	BP2	04/11/19	19.98	89.54
APR	U	NO	BP	2	BP2	04/12/19	20.89	89.25
APR	U	NO	BP	2	BP2	04/13/19	20.17	92.01
APR	U	NO	BP	2	BP2	04/14/19	19.79	92.77
APR	U	NO	BP	2	BP2	04/15/19	21.7	90.37
APR	U	NO	BP	2	BP2	04/16/19	22.39	91.36
APR	U	NO	BP	2	BP2	04/17/19	20.41	93.84
APR	U	NO	BP	2	BP2	04/18/19	18.37	95.14
APR	U	NO	BP	2	BP2	04/19/19	18.18	93.94
APR	U	NO	BP	2	BP2	04/20/19	20.03	92.42
APR	U	NO	BP	2	BP2	04/21/19	21.63	93.36
APR	U	NO	BP	2	BP2	04/22/19	18.65	93.85
APR	U	NO	BP	2	BP2	04/23/19	19.41	95.42
APR	U	NO	BP	2	BP2	04/24/19	19.89	97.06
APR	U	NO	BP	2	BP2	04/25/19	21.94	94.36

APR	U	NO	BP	2	BP2	04/26/19	22.37	94.97
APR	U	NO	BP	2	BP2	04/27/19	22.35	95.94
APR	U	NO	BP	2	BP2	04/28/19	21.6	97.42
APR	U	NO	BP	2	BP2	04/29/19	17.82	98.06
APR	U	NO	BP	2	BP2	04/30/19	15.61	98.57
MAY	U	NO	BP	2	BP2	05/01/19	19.46	97.81
MAY	U	NO	BP	2	BP2	05/02/19	17.01	98.83
MAY	U	NO	BP	2	BP2	05/03/19	20.17	97.25
MAY	U	NO	BP	2	BP2	05/04/19	22.99	97.23
MAY	U	NO	BP	2	BP2	05/05/19	23.42	96.73
MAY	U	NO	BP	2	BP2	05/06/19	22.49	97.69
MAY	U	NO	BP	2	BP2	05/07/19	19.46	99.1
MAY	U	NO	BP	2	BP2	05/08/19	15.41	99.84
MAY	U	NO	BP	2	BP2	05/09/19	19.32	99.22
MAY	U	NO	BP	2	BP2	05/10/19	21.41	97.86
MAY	U	NO	BP	2	BP2	05/11/19	20.17	98.86
MAY	U	NO	BP	2	BP2	05/12/19	16.75	100
MAY	U	NO	BP	2	BP2	05/13/19	18.41	100
MAY	U	NO	BP	2	BP2	05/14/19	17.63	100
MAY	U	NO	BP	2	BP2	05/15/19	18.03	100
MAY	U	NO	BP	2	BP2	05/16/19	20.96	77.15
MAY	U	NO	BP	2	BP2	05/17/19	20.91	62.9
MAY	U	NO	BP	2	BP2	05/18/19	21.41	70.05
MAY	U	NO	BP	2	BP2	05/19/19	22.27	72.49
MAY	U	NO	BP	2	BP2	05/20/19	22.97	75.33
MAY	U	NO	BP	2	BP2	05/21/19	23.88	76.02
MAY	U	NO	BP	2	BP2	05/22/19	22.47	84.17
DEC	U	NO	BP	4	BP extra x	12/01/18	23.12	94.9
DEC	U	NO	BP	4	BP extra x	12/02/18	18.3	93.41
DEC	U	NO	BP	4	BP extra x	12/03/18	13.61	93.14
DEC	U	NO	BP	4	BP extra x	12/04/18	13.32	85.07
DEC	U	NO	BP	4	BP extra x	12/05/18	15.94	86.01
DEC	U	NO	BP	4	BP extra x	12/06/18	16.01	82.93
DEC	U	NO	BP	4	BP extra x	12/07/18	17.06	80.73
DEC	U	NO	BP	4	BP extra x	12/08/18	11.44	75.27
DEC	U	NO	BP	4	BP extra x	12/09/18	14.72	72.33
DEC	U	NO	BP	4	BP extra x	12/10/18	20.58	71.23
DEC	U	NO	BP	4	BP extra x	12/11/18	19.35	71.05
DEC	U	NO	BP	4	BP extra x	12/12/18	26.61	69.76
DEC	U	NO	BP	4	BP extra x	12/13/18	23.43	73.6
DEC	U	NO	BP	4	BP extra x	12/14/18	25.42	62
DEC	U	NO	BP	4	BP extra x	12/15/18	24.37	80.73
DEC	U	NO	BP	4	BP extra x	12/16/18	25.15	76.33
DEC	U	NO	BP	4	BP extra x	12/17/18	26.41	54.2
DEC	U	NO	BP	4	BP extra x	12/18/18	25.44	85.54
DEC	U	NO	BP	4	BP extra x	12/19/18	24.32	78.86
DEC	U	NO	BP	4	BP extra x	12/20/18	24.88	71.99
DEC	U	NO	BP	4	BP extra x	12/21/18	23.72	89.15
DEC	U	NO	BP	4	BP extra x	12/22/18	23.12	71.03
DEC	U	NO	BP	4	BP extra x	12/23/18	22.76	73
DEC	U	NO	BP	4	BP extra x	12/24/18	21.83	78.45
DEC	U	NO	BP	4	BP extra x	12/25/18	21.28	68.11
DEC	U	NO	BP	4	BP extra x	12/26/18	22.78	60.99
DEC	U	NO	BP	4	BP extra x	12/27/18	22.04	58.67

DEC	U	NO	BP	4	BP extra x	12/28/18	24.88	55.71
DEC	U	NO	BP	4	BP extra x	12/29/18	22.23	54.73
DEC	U	NO	BP	4	BP extra x	12/30/18	24.62	53.22
DEC	U	NO	BP	4	BP extra x	12/31/18	28.43	71.99
JAN	U	NO	BP	4	BP extra x	01/01/19	26.32	55.48
JAN	U	NO	BP	4	BP extra x	01/02/19	28.63	53.92
JAN	U	NO	BP	4	BP extra x	01/03/19	30.99	71.2
JAN	U	NO	BP	4	BP extra x	01/04/19	23.5	87.31
JAN	U	NO	BP	4	BP extra x	01/05/19	22.83	87.42
JAN	U	NO	BP	4	BP extra x	01/06/19	23.5	73.58
JAN	U	NO	BP	4	BP extra x	01/07/19	27.91	71.85
JAN	U	NO	BP	4	BP extra x	01/08/19	25.51	72.7
JAN	U	NO	BP	4	BP extra x	01/09/19	26.17	81.18
JAN	U	NO	BP	4	BP extra x	01/10/19	24.78	81.89
JAN	U	NO	BP	4	BP extra x	01/11/19	24.57	87.4
JAN	U	NO	BP	4	BP extra x	01/12/19	23.94	90.78
JAN	U	NO	BP	4	BP extra x	01/13/19	24.95	78.25
JAN	U	NO	BP	4	BP extra x	01/14/19	25.12	81.98
JAN	U	NO	BP	4	BP extra x	01/15/19	26.83	67.04
JAN	U	NO	BP	4	BP extra x	01/16/19	25.54	73.18
JAN	U	NO	BP	4	BP extra x	01/17/19	27.4	85.03
JAN	U	NO	BP	4	BP extra x	01/18/19	22.69	88.78
JAN	U	NO	BP	4	BP extra x	01/19/19	25.71	91.17
JAN	U	NO	BP	4	BP extra x	01/20/19	22.54	88.92
JAN	U	NO	BP	4	BP extra x	01/21/19	23.79	91.83
JAN	U	NO	BP	4	BP extra x	01/22/19	22.09	74.86
JAN	U	NO	BP	4	BP extra x	01/23/19	24.04	68.04
JAN	U	NO	BP	4	BP extra x	01/24/19	26.44	69.03
JAN	U	NO	BP	4	BP extra x	01/25/19	23.72	67.79
JAN	U	NO	BP	4	BP extra x	01/26/19	25.1	67.14
JAN	U	NO	BP	4	BP extra x	01/27/19	25.98	64.82
JAN	U	NO	BP	4	BP extra x	01/28/19	25.66	56.02
JAN	U	NO	BP	4	BP extra x	01/29/19	27.15	58.26
JAN	U	NO	BP	4	BP extra x	01/30/19	27.59	53.53
JAN	U	NO	BP	4	BP extra x	01/31/19	27.37	56.74
FEB	U	NO	BP	4	BP extra x	02/01/19	25	65.38
FEB	U	NO	BP	4	BP extra x	02/02/19	25.51	76.52
FEB	U	NO	BP	4	BP extra x	02/03/19	23.55	86.91
FEB	U	NO	BP	4	BP extra x	02/04/19	19.89	86.02
FEB	U	NO	BP	4	BP extra x	02/05/19	20.39	80.17
FEB	U	NO	BP	4	BP extra x	02/06/19	18.11	78.27
FEB	U	NO	BP	4	BP extra x	02/07/19	20.13	75.41
FEB	U	NO	BP	4	BP extra x	02/08/19	22.04	68.27
FEB	U	NO	BP	4	BP extra x	02/09/19	23.48	62.16
FEB	U	NO	BP	4	BP extra x	02/10/19	26.93	76.12
FEB	U	NO	BP	4	BP extra x	02/11/19	25.22	76.38
FEB	U	NO	BP	4	BP extra x	02/12/19	24.18	85.2
FEB	U	NO	BP	4	BP extra x	02/13/19	18.68	86.63
FEB	U	NO	BP	4	BP extra x	02/14/19	20.11	72.24
FEB	U	NO	BP	4	BP extra x	02/15/19	24.25	89.19
FEB	U	NO	BP	4	BP extra x	02/16/19	22.64	89.55
FEB	U	NO	BP	4	BP extra x	02/17/19	22.19	80.61
FEB	U	NO	BP	4	BP extra x	02/18/19	23.29	79.71
FEB	U	NO	BP	4	BP extra x	02/19/19	22.5	81.4

FEB	U	NO	BP	4	BP extra x	02/20/19	23.75	72.9
FEB	U	NO	BP	4	BP extra x	02/21/19	21.71	73.22
FEB	U	NO	BP	4	BP extra x	02/22/19	21.16	70.4
FEB	U	NO	BP	4	BP extra x	02/23/19	24.3	64.06
FEB	U	NO	BP	4	BP extra x	02/24/19	27.4	54.94
FEB	U	NO	BP	4	BP extra x	02/25/19	24.52	86.57
FEB	U	NO	BP	4	BP extra x	02/26/19	21.3	87.19
FEB	U	NO	BP	4	BP extra x	02/27/19	19.82	87.45
FEB	U	NO	BP	4	BP extra x	02/28/19	19.39	84.9
MAR	U	NO	BP	4	BP extra x	03/01/19	18.34	82.92
MAR	U	NO	BP	4	BP extra x	03/02/19	21.01	66.94
MAR	U	NO	BP	4	BP extra x	03/03/19	21.78	77.65
MAR	U	NO	BP	4	BP extra x	03/04/19	22.11	68.89
MAR	U	NO	BP	4	BP extra x	03/05/19	26.86	60.7
MAR	U	NO	BP	4	BP extra x	03/06/19	23.65	80.71
MAR	U	NO	BP	4	BP extra x	03/07/19	25.39	76.41
MAR	U	NO	BP	4	BP extra x	03/08/19	25.17	87.35
MAR	U	NO	BP	4	BP extra x	03/09/19	24.42	91.09
MAR	U	NO	BP	4	BP extra x	03/10/19	21.23	90.41
MAR	U	NO	BP	4	BP extra x	03/11/19	20.32	86.91
MAR	U	NO	BP	4	BP extra x	03/12/19	21.09	90.46
MAR	U	NO	BP	4	BP extra x	03/13/19	22.64	92.02
MAR	U	NO	BP	4	BP extra x	03/14/19	22.02	87.05
MAR	U	NO	BP	4	BP extra x	03/15/19	19.54	84.09
MAR	U	NO	BP	4	BP extra x	03/16/19	21.8	86.11
MAR	U	NO	BP	4	BP extra x	03/17/19	24.08	89.51
MAR	U	NO	BP	4	BP extra x	03/18/19	24.54	90.9
MAR	U	NO	BP	4	BP extra x	03/19/19	22.86	90.84
MAR	U	NO	BP	4	BP extra x	03/20/19	21.35	91.46
MAR	U	NO	BP	4	BP extra x	03/21/19	19.77	90.46
MAR	U	NO	BP	4	BP extra x	03/22/19	16.27	90.6
MAR	U	NO	BP	4	BP extra x	03/23/19	18.11	88.56
MAR	U	NO	BP	4	BP extra x	03/24/19	18.3	85.22
MAR	U	NO	BP	4	BP extra x	03/25/19	20.25	85.4
MAR	U	NO	BP	4	BP extra x	03/26/19	16.56	83.38
MAR	U	NO	BP	4	BP extra x	03/27/19	18.92	80.81
MAR	U	NO	BP	4	BP extra x	03/28/19	19.94	84.23
MAR	U	NO	BP	4	BP extra x	03/29/19	19.46	84.23
MAR	U	NO	BP	4	BP extra x	03/30/19	22.76	74.7
MAR	U	NO	BP	4	BP extra x	03/31/19	23.79	82.54
APR	U	NO	BP	4	BP extra x	04/01/19	23.17	86.41
APR	U	NO	BP	4	BP extra x	04/02/19	22.16	81.99
APR	U	NO	BP	4	BP extra x	04/03/19	24.4	74.12
APR	U	NO	BP	4	BP extra x	04/04/19	24.54	78.63
APR	U	NO	BP	4	BP extra x	04/05/19	24.86	84.8
APR	U	NO	BP	4	BP extra x	04/06/19	21.28	88.85
APR	U	NO	BP	4	BP extra x	04/07/19	22.09	90.04
APR	U	NO	BP	4	BP extra x	04/08/19	21.44	88.64
APR	U	NO	BP	4	BP extra x	04/09/19	17.15	88.27
APR	U	NO	BP	4	BP extra x	04/10/19	16.96	87.74
APR	U	NO	BP	4	BP extra x	04/11/19	20.35	84.21
APR	U	NO	BP	4	BP extra x	04/12/19	21.8	88.36
APR	U	NO	BP	4	BP extra x	04/13/19	21.01	89.44
APR	U	NO	BP	4	BP extra x	04/14/19	20.61	83.76

APR	U	NO	BP	4	BP extra x	04/15/19	22.21	86.53
APR	U	NO	BP	4	BP extra x	04/16/19	23.14	90.63
APR	U	NO	BP	4	BP extra x	04/17/19	20.99	90.85
APR	U	NO	BP	4	BP extra x	04/18/19	19.13	86.65
APR	U	NO	BP	4	BP extra x	04/19/19	18.7	88.92
APR	U	NO	BP	4	BP extra x	04/20/19	20.7	89.2
APR	U	NO	BP	4	BP extra x	04/21/19	22.35	91.02
APR	U	NO	BP	4	BP extra x	04/22/19	19.49	89.81
APR	U	NO	BP	4	BP extra x	04/23/19	20.11	84.47
APR	U	NO	BP	4	BP extra x	04/24/19	21.18	81.91
APR	U	NO	BP	4	BP extra x	04/25/19	22.78	90.17
APR	U	NO	BP	4	BP extra x	04/26/19	23.07	93.24
APR	U	NO	BP	4	BP extra x	04/27/19	23.34	92.92
APR	U	NO	BP	4	BP extra x	04/28/19	22.35	93.08
APR	U	NO	BP	4	BP extra x	04/29/19	18.73	92.66
APR	U	NO	BP	4	BP extra x	04/30/19	16.08	90.68
MAY	U	NO	BP	4	BP extra x	05/01/19	20.44	90.44
MAY	U	NO	BP	4	BP extra x	05/02/19	17.92	88.17
MAY	U	NO	BP	4	BP extra x	05/03/19	20.94	75.9
MAY	U	NO	BP	4	BP extra x	05/04/19	24.13	92.86
MAY	U	NO	BP	4	BP extra x	05/05/19	24.3	90.07
MAY	U	NO	BP	4	BP extra x	05/06/19	23.34	92.17
MAY	U	NO	BP	4	BP extra x	05/07/19	20.39	92.12
MAY	U	NO	BP	4	BP extra x	05/08/19	16.42	88.42
MAY	U	NO	BP	4	BP extra x	05/09/19	20.39	93.21
MAY	U	NO	BP	4	BP extra x	05/10/19	22.35	89.97
MAY	U	NO	BP	4	BP extra x	05/11/19	21.11	91.12
MAY	U	NO	BP	4	BP extra x	05/12/19	18.06	93.08
MAY	U	NO	BP	4	BP extra x	05/13/19	19.49	92.4
MAY	U	NO	BP	4	BP extra x	05/14/19	18.49	92.67
MAY	U	NO	BP	4	BP extra x	05/15/19	19.11	99.2
MAY	U	NO	BP	4	BP extra x	05/16/19	20.99	75.73
MAY	U	NO	BP	4	BP extra x	05/17/19	20.92	62.17
MAY	U	NO	BP	4	BP extra x	05/18/19	21.42	69.7
MAY	U	NO	BP	4	BP extra x	05/19/19	22.31	71.81
MAY	U	NO	BP	4	BP extra x	05/20/19	22.98	74.94
MAY	U	NO	BP	4	BP extra x	05/21/19	23.84	75.93
MAY	U	NO	BP	4	BP extra x	05/22/19	22.47	80.67
DEC	H	NO	PA	1	PA1	12/01/18	23.91	86.54
DEC	H	NO	PA	1	PA1	12/02/18	18.15	82.41
DEC	H	NO	PA	1	PA1	12/03/18	16.08	78.45
DEC	H	NO	PA	1	PA1	12/04/18	16.34	74.65
DEC	H	NO	PA	1	PA1	12/05/18	20.6	78.77
DEC	H	NO	PA	1	PA1	12/06/18	18.06	79.01
DEC	H	NO	PA	1	PA1	12/07/18	17.49	73.93
DEC	H	NO	PA	1	PA1	12/08/18	13.88	78.23
DEC	H	NO	PA	1	PA1	12/09/18	16.37	81.34
DEC	H	NO	PA	1	PA1	12/10/18	20.27	77.26
DEC	H	NO	PA	1	PA1	12/11/18	21.77	74.26
DEC	H	NO	PA	1	PA1	12/12/18	26.55	76.73
DEC	H	NO	PA	1	PA1	12/13/18	24.17	80.96
DEC	H	NO	PA	1	PA1	12/14/18	23.98	78.82
DEC	H	NO	PA	1	PA1	12/15/18	23.62	81.4
DEC	H	NO	PA	1	PA1	12/16/18	24.36	83.49

DEC	H	NO	PA	1	PA1	12/17/18	25.45	71.51
DEC	H	NO	PA	1	PA1	12/18/18	24.15	80.64
DEC	H	NO	PA	1	PA1	12/19/18	23.59	83.16
DEC	H	NO	PA	1	PA1	12/20/18	24.36	82.62
DEC	H	NO	PA	1	PA1	12/21/18	22.78	85.73
DEC	H	NO	PA	1	PA1	12/22/18	23.91	86.06
DEC	H	NO	PA	1	PA1	12/23/18	22.51	83.05
DEC	H	NO	PA	1	PA1	12/24/18	20.63	81.69
DEC	H	NO	PA	1	PA1	12/25/18	20.41	80.2
DEC	H	NO	PA	1	PA1	12/26/18	23.69	80.04
DEC	H	NO	PA	1	PA1	12/27/18	23.18	79.34
DEC	H	NO	PA	1	PA1	12/28/18	22.87	79.42
DEC	H	NO	PA	1	PA1	12/29/18	24.53	73.83
DEC	H	NO	PA	1	PA1	12/30/18	24.77	76
DEC	H	NO	PA	1	PA1	12/31/18	27.11	77.15
JAN	H	NO	PA	1	PA1	01/01/19	26.74	79.72
JAN	H	NO	PA	1	PA1	01/02/19	26.82	77.26
JAN	H	NO	PA	1	PA1	01/03/19	29.59	74.56
JAN	H	NO	PA	1	PA1	01/04/19	22.66	83.85
JAN	H	NO	PA	1	PA1	01/05/19	21.77	83.9
JAN	H	NO	PA	1	PA1	01/06/19	22.87	81.71
JAN	H	NO	PA	1	PA1	01/07/19	27.01	76.99
JAN	H	NO	PA	1	PA1	01/08/19	24.44	78.6
JAN	H	NO	PA	1	PA1	01/09/19	26.06	78.32
JAN	H	NO	PA	1	PA1	01/10/19	25.02	79.88
JAN	H	NO	PA	1	PA1	01/11/19	25.87	80.35
JAN	H	NO	PA	1	PA1	01/12/19	23.79	84.08
JAN	H	NO	PA	1	PA1	01/13/19	24.58	85.84
JAN	H	NO	PA	1	PA1	01/14/19	25.84	80.77
JAN	H	NO	PA	1	PA1	01/15/19	25.89	82.07
JAN	H	NO	PA	1	PA1	01/16/19	25.33	81.64
JAN	H	NO	PA	1	PA1	01/17/19	27.83	83.08
JAN	H	NO	PA	1	PA1	01/18/19	23.57	87.29
JAN	H	NO	PA	1	PA1	01/19/19	24.82	89.13
JAN	H	NO	PA	1	PA1	01/20/19	20.87	90.56
JAN	H	NO	PA	1	PA1	01/21/19	23.09	89.31
JAN	H	NO	PA	1	PA1	01/22/19	22.11	88.76
JAN	H	NO	PA	1	PA1	01/23/19	24.05	86.45
JAN	H	NO	PA	1	PA1	01/24/19	27.73	83.03
JAN	H	NO	PA	1	PA1	01/25/19	23.57	84.45
JAN	H	NO	PA	1	PA1	01/26/19	25.04	81.85
JAN	H	NO	PA	1	PA1	01/27/19	24.27	80.66
JAN	H	NO	PA	1	PA1	01/28/19	25.31	78.75
JAN	H	NO	PA	1	PA1	01/29/19	26.38	73.07
JAN	H	NO	PA	1	PA1	01/30/19	27.06	71.35
JAN	H	NO	PA	1	PA1	01/31/19	28.15	70.24
FEB	H	NO	PA	1	PA1	02/01/19	24.77	79.51
FEB	H	NO	PA	1	PA1	02/02/19	25.4	82.15
FEB	H	NO	PA	1	PA1	02/03/19	22.44	84.87
FEB	H	NO	PA	1	PA1	02/04/19	19.46	83.07
FEB	H	NO	PA	1	PA1	02/05/19	18.25	81.66
FEB	H	NO	PA	1	PA1	02/06/19	19.46	80.53
FEB	H	NO	PA	1	PA1	02/07/19	21.32	80.9
FEB	H	NO	PA	1	PA1	02/08/19	22.73	80.9

FEB	H	NO	PA	1	PA1	02/09/19	23.86	79.57
FEB	H	NO	PA	1	PA1	02/10/19	27.7	73.33
FEB	H	NO	PA	1	PA1	02/11/19	24.87	80.34
FEB	H	NO	PA	1	PA1	02/12/19	23.62	81.06
FEB	H	NO	PA	1	PA1	02/13/19	19.2	82.38
FEB	H	NO	PA	1	PA1	02/14/19	21.56	80.88
FEB	H	NO	PA	1	PA1	02/15/19	23.5	80.73
FEB	H	NO	PA	1	PA1	02/16/19	22.59	85.03
FEB	H	NO	PA	1	PA1	02/17/19	21.75	84.89
FEB	H	NO	PA	1	PA1	02/18/19	24.24	82.58
FEB	H	NO	PA	1	PA1	02/19/19	23.57	82.09
FEB	H	NO	PA	1	PA1	02/20/19	24.32	81.23
FEB	H	NO	PA	1	PA1	02/21/19	22.71	79.21
FEB	H	NO	PA	1	PA1	02/22/19	23.74	79.06
FEB	H	NO	PA	1	PA1	02/23/19	24.75	80.77
FEB	H	NO	PA	1	PA1	02/24/19	25.91	76.49
FEB	H	NO	PA	1	PA1	02/25/19	23.88	84.09
FEB	H	NO	PA	1	PA1	02/26/19	20.84	87.7
FEB	H	NO	PA	1	PA1	02/27/19	19.84	88.64
FEB	H	NO	PA	1	PA1	02/28/19	18.18	87.87
MAR	H	NO	PA	1	PA1	03/01/19	19.27	88.15
MAR	H	NO	PA	1	PA1	03/02/19	21.89	87.31
MAR	H	NO	PA	1	PA1	03/03/19	23.76	84.47
MAR	H	NO	PA	1	PA1	03/04/19	23.26	85.78
MAR	H	NO	PA	1	PA1	03/05/19	25.28	83
MAR	H	NO	PA	1	PA1	03/06/19	22.94	85.86
MAR	H	NO	PA	1	PA1	03/07/19	23.88	87.24
MAR	H	NO	PA	1	PA1	03/08/19	25.33	85.14
MAR	H	NO	PA	1	PA1	03/09/19	23.95	87.54
MAR	H	NO	PA	1	PA1	03/10/19	20.48	88.25
MAR	H	NO	PA	1	PA1	03/11/19	19.2	86.04
MAR	H	NO	PA	1	PA1	03/12/19	20.94	85.93
MAR	H	NO	PA	1	PA1	03/13/19	23.67	87.13
MAR	H	NO	PA	1	PA1	03/14/19	21.84	87.29
MAR	H	NO	PA	1	PA1	03/15/19	21.25	85.1
MAR	H	NO	PA	1	PA1	03/16/19	22.66	85.61
MAR	H	NO	PA	1	PA1	03/17/19	24.34	84.88
MAR	H	NO	PA	1	PA1	03/18/19	23.81	88.94
MAR	H	NO	PA	1	PA1	03/19/19	23.71	89.5
MAR	H	NO	PA	1	PA1	03/20/19	21.18	86.59
MAR	H	NO	PA	1	PA1	03/21/19	17.99	86.57
MAR	H	NO	PA	1	PA1	03/22/19	16.73	85.81
MAR	H	NO	PA	1	PA1	03/23/19	18.94	85.71
MAR	H	NO	PA	1	PA1	03/24/19	19.44	86.48
MAR	H	NO	PA	1	PA1	03/25/19	20.6	79.79
MAR	H	NO	PA	1	PA1	03/26/19	19.39	80.53
MAR	H	NO	PA	1	PA1	03/27/19	19.58	82.79
MAR	H	NO	PA	1	PA1	03/28/19	20.63	86.16
MAR	H	NO	PA	1	PA1	03/29/19	22.42	85.17
MAR	H	NO	PA	1	PA1	03/30/19	20.29	84.86
MAR	H	NO	PA	1	PA1	03/31/19	22.99	86.29
APR	H	NO	PA	1	PA1	04/01/19	24.41	87.29
APR	H	NO	PA	1	PA1	04/02/19	22.15	85.97
APR	H	NO	PA	1	PA1	04/03/19	24.82	84.19

APR	H	NO	PA	1	PA1	04/04/19	24.87	84.81
APR	H	NO	PA	1	PA1	04/05/19	24.92	86.04
APR	H	NO	PA	1	PA1	04/06/19	20.72	87.41
APR	H	NO	PA	1	PA1	04/07/19	22.18	89.82
APR	H	NO	PA	1	PA1	04/08/19	19.94	90.43
APR	H	NO	PA	1	PA1	04/09/19	16.32	89.14
APR	H	NO	PA	1	PA1	04/10/19	17.96	88.63
APR	H	NO	PA	1	PA1	04/11/19	20.08	86.98
APR	H	NO	PA	1	PA1	04/12/19	23.33	87.87
APR	H	NO	PA	1	PA1	04/13/19	21.82	89.58
APR	H	NO	PA	1	PA1	04/14/19	23.35	89.36
APR	H	NO	PA	1	PA1	04/15/19	21.96	87.52
APR	H	NO	PA	1	PA1	04/16/19	22.23	87.96
APR	H	NO	PA	1	PA1	04/17/19	18.53	90.78
APR	H	NO	PA	1	PA1	04/18/19	17.63	91.27
APR	H	NO	PA	1	PA1	04/19/19	18.37	91.03
APR	H	NO	PA	1	PA1	04/20/19	20.01	89.87
APR	H	NO	PA	1	PA1	04/21/19	22.59	90.16
APR	H	NO	PA	1	PA1	04/22/19	19.44	90.33
APR	H	NO	PA	1	PA1	04/23/19	20.44	90.48
APR	H	NO	PA	1	PA1	04/24/19	21.41	89.84
APR	H	NO	PA	1	PA1	04/25/19	24.03	89.29
APR	H	NO	PA	1	PA1	04/26/19	23.83	91.46
APR	H	NO	PA	1	PA1	04/27/19	23.83	92.16
APR	H	NO	PA	1	PA1	04/28/19	21.65	92.45
APR	H	NO	PA	1	PA1	04/29/19	18.13	91.54
APR	H	NO	PA	1	PA1	04/30/19	14.7	91.63
MAY	H	NO	PA	1	PA1	05/01/19	20.39	90.78
MAY	H	NO	PA	1	PA1	05/02/19	16.42	89.8
MAY	H	NO	PA	1	PA1	05/03/19	19.6	89.82
MAY	H	NO	PA	1	PA1	05/04/19	23.83	92.27
MAY	H	NO	PA	1	PA1	05/05/19	22.92	92.79
MAY	H	NO	PA	1	PA1	05/06/19	22.85	92.75
MAY	H	NO	PA	1	PA1	05/07/19	18.22	93.55
MAY	H	NO	PA	1	PA1	05/08/19	14.65	92.54
MAY	H	NO	PA	1	PA1	05/09/19	20.2	91.9
MAY	H	NO	PA	1	PA1	05/10/19	21.32	91.35
MAY	H	NO	PA	1	PA1	05/11/19	21.68	93.14
MAY	H	NO	PA	1	PA1	05/12/19	16.8	94.21
MAY	H	NO	PA	1	PA1	05/13/19	18.51	93.89
MAY	H	NO	PA	1	PA1	05/14/19	17.72	94.25
MAY	H	NO	PA	1	PA1	05/15/19	18.94	94.18
MAY	H	NO	PA	1	PA1	05/16/19	17.99	94.64
MAY	H	NO	PA	1	PA1	05/17/19	14.86	93.84
MAY	H	NO	PA	1	PA1	05/18/19	15.06	91.7
MAY	H	NO	PA	1	PA1	05/19/19	18.01	90.42
MAY	H	NO	PA	1	PA1	05/20/19	16.75	89.74
MAY	H	NO	PA	1	PA1	05/21/19	21.03	89.53
MAY	H	NO	PA	1	PA1	05/22/19	20.37	91.78
DEC	U	NO	PA	2	PA2	12/01/18	21.41	99.32
DEC	U	NO	PA	2	PA2	12/02/18	16.08	100
DEC	U	NO	PA	2	PA2	12/03/18	13.28	99.75
DEC	U	NO	PA	2	PA2	12/04/18	13.52	99.17
DEC	U	NO	PA	2	PA2	12/05/18	17.58	96.47

DEC	U	NO	PA	2	PA2	12/06/18	15.77	95.1
DEC	U	NO	PA	2	PA2	12/07/18	15.44	93.16
DEC	U	NO	PA	2	PA2	12/08/18	11.27	92.64
DEC	U	NO	PA	2	PA2	12/09/18	13.43	92.04
DEC	U	NO	PA	2	PA2	12/10/18	17.51	90
DEC	U	NO	PA	2	PA2	12/11/18	19.32	85.13
DEC	U	NO	PA	2	PA2	12/12/18	24.48	81.99
DEC	U	NO	PA	2	PA2	12/13/18	21.94	80.27
DEC	U	NO	PA	2	PA2	12/14/18	22.18	79.48
DEC	U	NO	PA	2	PA2	12/15/18	21.75	80.24
DEC	U	NO	PA	2	PA2	12/16/18	22.32	82.27
DEC	U	NO	PA	2	PA2	12/17/18	23.04	78.3
DEC	U	NO	PA	2	PA2	12/18/18	22.13	79.74
DEC	U	NO	PA	2	PA2	12/19/18	21.65	82.05
DEC	U	NO	PA	2	PA2	12/20/18	22.42	80.25
DEC	U	NO	PA	2	PA2	12/21/18	21.37	83.7
DEC	U	NO	PA	2	PA2	12/22/18	22.13	86.8
DEC	U	NO	PA	2	PA2	12/23/18	20.44	84.51
DEC	U	NO	PA	2	PA2	12/24/18	18.51	82.74
DEC	U	NO	PA	2	PA2	12/25/18	18.63	80.88
DEC	U	NO	PA	2	PA2	12/26/18	21.89	77.49
DEC	U	NO	PA	2	PA2	12/27/18	21.08	77.83
DEC	U	NO	PA	2	PA2	12/28/18	20.98	76.7
DEC	U	NO	PA	2	PA2	12/29/18	22.99	63.07
DEC	U	NO	PA	2	PA2	12/30/18	22.68	72.3
DEC	U	NO	PA	2	PA2	12/31/18	25.14	72.54
JAN	U	NO	PA	2	PA2	01/01/19	24.8	75.66
JAN	U	NO	PA	2	PA2	01/02/19	24.7	74.4
JAN	U	NO	PA	2	PA2	01/03/19	27.58	71.04
JAN	U	NO	PA	2	PA2	01/04/19	21.06	73.68
JAN	U	NO	PA	2	PA2	01/05/19	19.89	76.3
JAN	U	NO	PA	2	PA2	01/06/19	20.98	78.62
JAN	U	NO	PA	2	PA2	01/07/19	24.73	77.36
JAN	U	NO	PA	2	PA2	01/08/19	22.42	78.82
JAN	U	NO	PA	2	PA2	01/09/19	24.2	77.47
JAN	U	NO	PA	2	PA2	01/10/19	23.3	77.68
JAN	U	NO	PA	2	PA2	01/11/19	24.03	76.47
JAN	U	NO	PA	2	PA2	01/12/19	22.27	79.25
JAN	U	NO	PA	2	PA2	01/13/19	22.8	81.92
JAN	U	NO	PA	2	PA2	01/14/19	24.2	80.21
JAN	U	NO	PA	2	PA2	01/15/19	24.48	80.41
JAN	U	NO	PA	2	PA2	01/16/19	24.15	81.61
JAN	U	NO	PA	2	PA2	01/17/19	26.65	81.95
JAN	U	NO	PA	2	PA2	01/18/19	23.04	86.28
JAN	U	NO	PA	2	PA2	01/19/19	24.17	87.22
JAN	U	NO	PA	2	PA2	01/20/19	20.22	88.69
JAN	U	NO	PA	2	PA2	01/21/19	22.44	89.13
JAN	U	NO	PA	2	PA2	01/22/19	21.41	88.19
JAN	U	NO	PA	2	PA2	01/23/19	23.06	85.3
JAN	U	NO	PA	2	PA2	01/24/19	26.38	83.39
JAN	U	NO	PA	2	PA2	01/25/19	22.61	84.78
JAN	U	NO	PA	2	PA2	01/26/19	23.86	83.47
JAN	U	NO	PA	2	PA2	01/27/19	23.18	80.93
JAN	U	NO	PA	2	PA2	01/28/19	24.46	79.02

JAN	U	NO	PA	2	PA2	01/29/19	25.67	76
JAN	U	NO	PA	2	PA2	01/30/19	26.16	74.49
JAN	U	NO	PA	2	PA2	01/31/19	26.84	72.22
FEB	U	NO	PA	2	PA2	02/01/19	23.57	74.65
FEB	U	NO	PA	2	PA2	02/02/19	24.12	77.53
FEB	U	NO	PA	2	PA2	02/03/19	21.51	81.06
FEB	U	NO	PA	2	PA2	02/04/19	18.63	81.13
FEB	U	NO	PA	2	PA2	02/05/19	17.37	80.14
FEB	U	NO	PA	2	PA2	02/06/19	18.51	80.64
FEB	U	NO	PA	2	PA2	02/07/19	19.89	80.59
FEB	U	NO	PA	2	PA2	02/08/19	20.84	79.76
FEB	U	NO	PA	2	PA2	02/09/19	22.42	77.52
FEB	U	NO	PA	2	PA2	02/10/19	26.23	73.3
FEB	U	NO	PA	2	PA2	02/11/19	23.42	76.91
FEB	U	NO	PA	2	PA2	02/12/19	22.61	79.03
FEB	U	NO	PA	2	PA2	02/13/19	17.84	80.96
FEB	U	NO	PA	2	PA2	02/14/19	20.1	80.6
FEB	U	NO	PA	2	PA2	02/15/19	22.06	80.98
FEB	U	NO	PA	2	PA2	02/16/19	20.75	83.18
FEB	U	NO	PA	2	PA2	02/17/19	20.17	82.89
FEB	U	NO	PA	2	PA2	02/18/19	22.87	82.08
FEB	U	NO	PA	2	PA2	02/19/19	22.18	82.41
FEB	U	NO	PA	2	PA2	02/20/19	22.85	83.32
FEB	U	NO	PA	2	PA2	02/21/19	21.32	79.79
FEB	U	NO	PA	2	PA2	02/22/19	22.06	78.73
FEB	U	NO	PA	2	PA2	02/23/19	23.33	75.99
FEB	U	NO	PA	2	PA2	02/24/19	24.2	71.45
FEB	U	NO	PA	2	PA2	02/25/19	22.56	79.21
FEB	U	NO	PA	2	PA2	02/26/19	19.79	83.23
FEB	U	NO	PA	2	PA2	02/27/19	18.53	87
FEB	U	NO	PA	2	PA2	02/28/19	17.08	91.23
MAR	U	NO	PA	2	PA2	03/01/19	17.8	93.82
MAR	U	NO	PA	2	PA2	03/02/19	20.2	95.54
MAR	U	NO	PA	2	PA2	03/03/19	22.23	94.35
MAR	U	NO	PA	2	PA2	03/04/19	21.27	98.01
MAR	U	NO	PA	2	PA2	03/05/19	23.76	90.62
MAR	U	NO	PA	2	PA2	03/06/19	21.29	92.19
MAR	U	NO	PA	2	PA2	03/07/19	22.27	95.16
MAR	U	NO	PA	2	PA2	03/08/19	24.29	92.38
MAR	U	NO	PA	2	PA2	03/09/19	22.56	94.47
MAR	U	YES	PA	2	PA2	03/10/19	19.01	96.7
MAR	U	YES	PA	2	PA2	03/11/19	17.39	97.45
MAR	U	YES	PA	2	PA2	03/12/19	19.22	97.51
MAR	U	YES	PA	2	PA2	03/13/19	22.11	96.05
MAR	U	YES	PA	2	PA2	03/14/19	20.22	91.11
MAR	U	YES	PA	2	PA2	03/15/19	19.56	91.28
MAR	U	YES	PA	2	PA2	03/16/19	21.18	87.43
MAR	U	NO	PA	2	PA2	03/17/19	22.85	89.64
MAR	U	NO	PA	2	PA2	03/18/19	22.25	92.62
MAR	U	NO	PA	2	PA2	03/19/19	22.32	92.62
MAR	U	NO	PA	2	PA2	03/20/19	19.7	91.78
MAR	U	NO	PA	2	PA2	03/21/19	16.15	90
MAR	U	NO	PA	2	PA2	03/22/19	12.97	93.3
MAR	U	NO	PA	2	PA2	03/23/19	16.46	93.56

MAR	U	NO	PA	2	PA2	03/24/19	16.77	93.33
MAR	U	NO	PA	2	PA2	03/25/19	18.77	81.44
MAR	U	NO	PA	2	PA2	03/26/19	16.42	88.34
MAR	U	NO	PA	2	PA2	03/27/19	17.06	90.07
MAR	U	NO	PA	2	PA2	03/28/19	18.91	92.06
MAR	U	NO	PA	2	PA2	03/29/19	20.51	91.48
MAR	U	NO	PA	2	PA2	03/30/19	18.37	91.19
MAR	U	NO	PA	2	PA2	03/31/19	21.34	90.74
APR	U	NO	PA	2	PA2	04/01/19	22.66	90.2
APR	U	NO	PA	2	PA2	04/02/19	20.46	88.77
APR	U	NO	PA	2	PA2	04/03/19	23.21	87.16
APR	U	NO	PA	2	PA2	04/04/19	23.23	86.01
APR	U	NO	PA	2	PA2	04/05/19	23.52	84.66
APR	U	NO	PA	2	PA2	04/06/19	19.56	88.71
APR	U	NO	PA	2	PA2	04/07/19	20.77	91.09
APR	U	NO	PA	2	PA2	04/08/19	18.87	92.38
APR	U	NO	PA	2	PA2	04/09/19	15.01	93.29
APR	U	NO	PA	2	PA2	04/10/19	16.13	93.86
APR	U	NO	PA	2	PA2	04/11/19	18.56	93.94
APR	U	NO	PA	2	PA2	04/12/19	21.84	92.52
APR	U	NO	PA	2	PA2	04/13/19	20.2	94.26
APR	U	NO	PA	2	PA2	04/14/19	21.84	94.58
APR	U	NO	PA	2	PA2	04/15/19	20.56	93.87
APR	U	NO	PA	2	PA2	04/16/19	20.51	93.91
APR	U	NO	PA	2	PA2	04/17/19	16.61	95.66
APR	U	NO	PA	2	PA2	04/18/19	16.15	96.58
APR	U	NO	PA	2	PA2	04/19/19	16.61	97.33
APR	U	NO	PA	2	PA2	04/20/19	18.49	97.03
APR	U	NO	PA	2	PA2	04/21/19	20.87	96.52
APR	U	NO	PA	2	PA2	04/22/19	17.89	97.09
APR	U	NO	PA	2	PA2	04/23/19	18.68	97.38
APR	U	NO	PA	2	PA2	04/24/19	19.79	98.09
APR	U	NO	PA	2	PA2	04/25/19	22.32	96.91
APR	U	NO	PA	2	PA2	04/26/19	22.23	97.64
APR	U	NO	PA	2	PA2	04/27/19	22.3	98.06
APR	U	NO	PA	2	PA2	04/28/19	20.6	98.3
APR	U	NO	PA	2	PA2	04/29/19	16.87	99.19
APR	U	NO	PA	2	PA2	04/30/19	13.45	99.61
MAY	U	NO	PA	2	PA2	05/01/19	18.68	98.58
MAY	U	NO	PA	2	PA2	05/02/19	14.96	99
MAY	U	NO	PA	2	PA2	05/03/19	17.84	99.57
MAY	U	NO	PA	2	PA2	05/04/19	22.23	98.09
MAY	U	NO	PA	2	PA2	05/05/19	21.58	98.47
MAY	U	NO	PA	2	PA2	05/06/19	21.75	98
MAY	U	NO	PA	2	PA2	05/07/19	17.37	98.58
MAY	U	NO	PA	2	PA2	05/08/19	13.76	99.35
MAY	U	NO	PA	2	PA2	05/09/19	18.79	98.46
MAY	U	NO	PA	2	PA2	05/10/19	20.01	97.7
MAY	U	NO	PA	2	PA2	05/11/19	20.46	97.81
MAY	U	NO	PA	2	PA2	05/12/19	15.96	98.8
MAY	U	NO	PA	2	PA2	05/13/19	17.46	98.45
MAY	U	NO	PA	2	PA2	05/14/19	16.99	99.15
MAY	U	NO	PA	2	PA2	05/15/19	17.89	98.69
MAY	U	NO	PA	2	PA2	05/16/19	17.08	99.38

MAY	U	NO	PA	2	PA2	05/17/19	14.27	100
MAY	U	NO	PA	2	PA2	05/18/19	14.39	99.71
MAY	U	NO	PA	2	PA2	05/19/19	17.18	99.07
MAY	U	NO	PA	2	PA2	05/20/19	16.03	98.86
MAY	U	NO	PA	2	PA2	05/21/19	19.94	97.94
MAY	U	NO	PA	2	PA2	05/22/19	19.22	96.25
DEC	U	NO	PA	5	PA extra y	12/01/18	23.5	92.2
DEC	U	NO	PA	5	PA extra y	12/02/18	18.49	94.5
DEC	U	NO	PA	5	PA extra y	12/03/18	16.32	93.55
DEC	U	NO	PA	5	PA extra y	12/04/18	16.18	93.91
DEC	U	NO	PA	5	PA extra y	12/05/18	19.84	89.78
DEC	U	NO	PA	5	PA extra y	12/06/18	18.03	88.95
DEC	U	NO	PA	5	PA extra y	12/07/18	17.61	87.94
DEC	U	NO	PA	5	PA extra y	12/08/18	14.03	87.26
DEC	U	NO	PA	5	PA extra y	12/09/18	15.84	87.03
DEC	U	NO	PA	5	PA extra y	12/10/18	19.7	84.12
DEC	U	NO	PA	5	PA extra y	12/11/18	21.32	76.66
DEC	U	NO	PA	5	PA extra y	12/12/18	26.11	75.51
DEC	U	NO	PA	5	PA extra y	12/13/18	23.38	78.31
DEC	U	NO	PA	5	PA extra y	12/14/18	23.35	81.94
DEC	U	NO	PA	5	PA extra y	12/15/18	22.9	81.9
DEC	U	NO	PA	5	PA extra y	12/16/18	23.38	82.39
DEC	U	NO	PA	5	PA extra y	12/17/18	24.51	73.26
DEC	U	NO	PA	5	PA extra y	12/18/18	23.33	79.97
DEC	U	NO	PA	5	PA extra y	12/19/18	22.54	82.94
DEC	U	NO	PA	5	PA extra y	12/20/18	23.69	82.51
DEC	U	NO	PA	5	PA extra y	12/21/18	22.23	87.04
DEC	U	NO	PA	5	PA extra y	12/22/18	23.35	87.7
DEC	U	NO	PA	5	PA extra y	12/23/18	21.94	85
DEC	U	NO	PA	5	PA extra y	12/24/18	19.89	84.74
DEC	U	NO	PA	5	PA extra y	12/25/18	19.96	82.57
DEC	U	NO	PA	5	PA extra y	12/26/18	22.92	82.12
DEC	U	NO	PA	5	PA extra y	12/27/18	22.23	78.54
DEC	U	NO	PA	5	PA extra y	12/28/18	22.3	73.7
DEC	U	NO	PA	5	PA extra y	12/29/18	24.1	62.63
DEC	U	NO	PA	5	PA extra y	12/30/18	23.95	72.06
DEC	U	NO	PA	5	PA extra y	12/31/18	26.43	75.76
JAN	U	NO	PA	5	PA extra y	01/01/19	25.89	72.43
JAN	U	NO	PA	5	PA extra y	01/02/19	26.11	71.55
JAN	U	NO	PA	5	PA extra y	01/03/19	28.87	72.3
JAN	U	NO	PA	5	PA extra y	01/04/19	21.87	80.38
JAN	U	NO	PA	5	PA extra y	01/05/19	21.37	81.87
JAN	U	NO	PA	5	PA extra y	01/06/19	22.27	80.4
JAN	U	NO	PA	5	PA extra y	01/07/19	26.06	78.05
JAN	U	NO	PA	5	PA extra y	01/08/19	23.81	77.47
JAN	U	NO	PA	5	PA extra y	01/09/19	25.31	78.54
JAN	U	NO	PA	5	PA extra y	01/10/19	24.39	79.61
JAN	U	NO	PA	5	PA extra y	01/11/19	25.23	81.14
JAN	U	NO	PA	5	PA extra y	01/12/19	23.04	84.57
JAN	U	NO	PA	5	PA extra y	01/13/19	23.86	84.66
JAN	U	NO	PA	5	PA extra y	01/14/19	25.45	78.89
JAN	U	NO	PA	5	PA extra y	01/15/19	25.55	78.7
JAN	U	NO	PA	5	PA extra y	01/16/19	25.11	79.69
JAN	U	NO	PA	5	PA extra y	01/17/19	26.92	80.53

JAN	U	NO	PA	5	PA extra y 01/18/19	23.04	84.2
JAN	U	NO	PA	5	PA extra y 01/19/19	24.34	86.57
JAN	U	NO	PA	5	PA extra y 01/20/19	20.34	87.59
JAN	U	NO	PA	5	PA extra y 01/21/19	22.54	87.71
JAN	U	NO	PA	5	PA extra y 01/22/19	21.46	86.92
JAN	U	NO	PA	5	PA extra y 01/23/19	23.18	83.65
JAN	U	NO	PA	5	PA extra y 01/24/19	26.74	80.46
JAN	U	NO	PA	5	PA extra y 01/25/19	22.9	82.01
JAN	U	NO	PA	5	PA extra y 01/26/19	24.2	79.54
JAN	U	NO	PA	5	PA extra y 01/27/19	23.52	76.45
JAN	U	NO	PA	5	PA extra y 01/28/19	25.02	71.83
JAN	U	NO	PA	5	PA extra y 01/29/19	26.3	66.88
JAN	U	NO	PA	5	PA extra y 01/30/19	26.55	64.92
JAN	U	NO	PA	5	PA extra y 01/31/19	27.26	68.39
FEB	U	NO	PA	5	PA extra y 02/01/19	24.1	74.23
FEB	U	NO	PA	5	PA extra y 02/02/19	24.48	76.95
FEB	U	NO	PA	5	PA extra y 02/03/19	21.75	81.38
FEB	U	NO	PA	5	PA extra y 02/04/19	18.84	81.36
FEB	U	NO	PA	5	PA extra y 02/05/19	17.51	80.92
FEB	U	NO	PA	5	PA extra y 02/06/19	18.65	81.35
FEB	U	NO	PA	5	PA extra y 02/07/19	20.22	81.27
FEB	U	NO	PA	5	PA extra y 02/08/19	21.06	80.85
FEB	U	NO	PA	5	PA extra y 02/09/19	22.63	78.34
FEB	U	NO	PA	5	PA extra y 02/10/19	26.52	72.42
FEB	U	NO	PA	5	PA extra y 02/11/19	23.62	77.57
FEB	U	NO	PA	5	PA extra y 02/12/19	22.75	79.58
FEB	U	NO	PA	5	PA extra y 02/13/19	17.99	81.47
FEB	U	NO	PA	5	PA extra y 02/14/19	20.27	81.66
FEB	U	NO	PA	5	PA extra y 02/15/19	22.13	80.53
FEB	U	NO	PA	5	PA extra y 02/16/19	20.87	83.4
FEB	U	NO	PA	5	PA extra y 02/17/19	20.32	84.57
FEB	U	NO	PA	5	PA extra y 02/18/19	22.99	83.93
FEB	U	NO	PA	5	PA extra y 02/19/19	22.27	84.52
FEB	U	NO	PA	5	PA extra y 02/20/19	23.06	84.51
FEB	U	NO	PA	5	PA extra y 02/21/19	21.6	83.49
FEB	U	NO	PA	5	PA extra y 02/22/19	22.37	81.89
FEB	U	NO	PA	5	PA extra y 02/23/19	23.74	80.12
FEB	U	NO	PA	5	PA extra y 02/24/19	24.56	74.3
FEB	U	NO	PA	5	PA extra y 02/25/19	22.94	82.66
FEB	U	NO	PA	5	PA extra y 02/26/19	20.01	88.43
FEB	U	NO	PA	5	PA extra y 02/27/19	18.89	89.57
FEB	U	NO	PA	5	PA extra y 02/28/19	17.63	90.31
MAR	U	NO	PA	5	PA extra y 03/01/19	18.41	90.64
MAR	U	NO	PA	5	PA extra y 03/02/19	20.84	89.54
MAR	U	NO	PA	5	PA extra y 03/03/19	22.99	87.57
MAR	U	NO	PA	5	PA extra y 03/04/19	22.23	90.13
MAR	U	NO	PA	5	PA extra y 03/05/19	24.39	84.77
MAR	U	NO	PA	5	PA extra y 03/06/19	21.77	89.38
MAR	U	NO	PA	5	PA extra y 03/07/19	22.99	85.22
MAR	U	NO	PA	5	PA extra y 03/08/19	24.75	89.05
MAR	U	NO	PA	5	PA extra y 03/09/19	23.4	91.8
MAR	U	NO	PA	5	PA extra y 03/10/19	19.98	88.6
MAR	U	NO	PA	5	PA extra y 03/11/19	18.41	90.94
MAR	U	NO	PA	5	PA extra y 03/12/19	20.22	93.06

MAR	U	NO	PA	5	PA extra y 03/13/19	22.68	92.59
MAR	U	NO	PA	5	PA extra y 03/14/19	20.75	92.93
MAR	U	NO	PA	5	PA extra y 03/15/19	20.03	91.57
MAR	U	NO	PA	5	PA extra y 03/16/19	21.82	91.72
MAR	U	NO	PA	5	PA extra y 03/17/19	23.33	90.71
MAR	U	YES	PA	5	PA extra y 03/18/19	22.92	94.17
MAR	U	YES	PA	5	PA extra y 03/19/19	23.02	95.11
MAR	U	YES	PA	5	PA extra y 03/20/19	20.22	91.93
MAR	U	YES	PA	5	PA extra y 03/21/19	16.75	92.94
MAR	U	YES	PA	5	PA extra y 03/22/19	14	94.57
MAR	U	YES	PA	5	PA extra y 03/23/19	17.37	93.78
MAR	U	YES	PA	5	PA extra y 03/24/19	17.65	93.31
MAR	U	NO	PA	5	PA extra y 03/25/19	19.58	86.45
MAR	U	NO	PA	5	PA extra y 03/26/19	17.51	89
MAR	U	NO	PA	5	PA extra y 03/27/19	17.84	90.09
MAR	U	NO	PA	5	PA extra y 03/28/19	19.06	91.08
MAR	U	NO	PA	5	PA extra y 03/29/19	20.94	91.11
MAR	U	NO	PA	5	PA extra y 03/30/19	18.75	89.69
MAR	U	NO	PA	5	PA extra y 03/31/19	21.77	87.99
APR	U	NO	PA	5	PA extra y 04/01/19	22.94	87.78
APR	U	NO	PA	5	PA extra y 04/02/19	20.77	87.28
APR	U	NO	PA	5	PA extra y 04/03/19	23.59	82.02
APR	U	NO	PA	5	PA extra y 04/04/19	23.57	79.89
APR	U	NO	PA	5	PA extra y 04/05/19	23.67	82.48
APR	U	NO	PA	5	PA extra y 04/06/19	19.98	85.67
APR	U	NO	PA	5	PA extra y 04/07/19	21.03	87.93
APR	U	NO	PA	5	PA extra y 04/08/19	19.13	88.36
APR	U	NO	PA	5	PA extra y 04/09/19	15.29	89.47
APR	U	NO	PA	5	PA extra y 04/10/19	16.37	88.78
APR	U	NO	PA	5	PA extra y 04/11/19	18.94	88.43
APR	U	NO	PA	5	PA extra y 04/12/19	22.18	86.66
APR	U	NO	PA	5	PA extra y 04/13/19	20.56	87.85
APR	U	NO	PA	5	PA extra y 04/14/19	22.3	88.79
APR	U	NO	PA	5	PA extra y 04/15/19	20.72	87.93
APR	U	NO	PA	5	PA extra y 04/16/19	20.7	88.57
APR	U	NO	PA	5	PA extra y 04/17/19	16.75	91.17
APR	U	NO	PA	5	PA extra y 04/18/19	16.27	92.38
APR	U	NO	PA	5	PA extra y 04/19/19	16.94	93.19
APR	U	NO	PA	5	PA extra y 04/20/19	18.82	93
APR	U	NO	PA	5	PA extra y 04/21/19	21.1	92.41
APR	U	NO	PA	5	PA extra y 04/22/19	18.11	93.27
APR	U	NO	PA	5	PA extra y 04/23/19	18.79	93.22
APR	U	NO	PA	5	PA extra y 04/24/19	19.94	93.83
APR	U	NO	PA	5	PA extra y 04/25/19	22.49	93.18
APR	U	NO	PA	5	PA extra y 04/26/19	22.54	94.06
APR	U	NO	PA	5	PA extra y 04/27/19	22.44	94.54
APR	U	NO	PA	5	PA extra y 04/28/19	20.72	95.31
APR	U	NO	PA	5	PA extra y 04/29/19	16.99	95.88
APR	U	NO	PA	5	PA extra y 04/30/19	13.35	96.12
MAY	U	NO	PA	5	PA extra y 05/01/19	18.77	95.47
MAY	U	NO	PA	5	PA extra y 05/02/19	14.79	95.7
MAY	U	NO	PA	5	PA extra y 05/03/19	17.77	96.17
MAY	U	NO	PA	5	PA extra y 05/04/19	22.27	95.43
MAY	U	NO	PA	5	PA extra y 05/05/19	21.6	96.16

MAY	U	NO	PA	5	PA extra y 05/06/19	21.89	96.16
MAY	U	NO	PA	5	PA extra y 05/07/19	17.32	96.72
MAY	U	NO	PA	5	PA extra y 05/08/19	13.59	97.44
MAY	U	NO	PA	5	PA extra y 05/09/19	18.72	96.45
MAY	U	NO	PA	5	PA extra y 05/10/19	20.01	95.55
MAY	U	NO	PA	5	PA extra y 05/11/19	20.46	95.98
MAY	U	NO	PA	5	PA extra y 05/12/19	15.92	97.14
MAY	U	NO	PA	5	PA extra y 05/13/19	17.42	96.75
MAY	U	NO	PA	5	PA extra y 05/14/19	16.84	97.48
MAY	U	NO	PA	5	PA extra y 05/15/19	17.89	97.3
MAY	U	NO	PA	5	PA extra y 05/16/19	16.99	97.97
MAY	U	NO	PA	5	PA extra y 05/17/19	14.03	98.46
MAY	U	NO	PA	5	PA extra y 05/18/19	14.12	98.22
MAY	U	NO	PA	5	PA extra y 05/19/19	17.01	97.73
MAY	U	NO	PA	5	PA extra y 05/20/19	15.87	98.49
MAY	U	NO	PA	5	PA extra y 05/21/19	19.87	97.21
MAY	U	NO	PA	5	PA extra y 05/22/19	19.22	95.74

Table S3(c): whole bee weight.

Month	Health in march	Intensy	Locality	Sisters	Colony	Weight (g)
APR	H	H	BP	1	BP1	0.0695
APR	H	H	BP	1	BP1	0.0611
APR	H	H	BP	1	BP1	0.0679
APR	H	H	BP	1	BP1	0.0635
APR	H	H	BP	1	BP1	0.0751
DEC	H	H	BP	1	BP1	0.0594
DEC	H	H	BP	1	BP1	0.0642
DEC	H	H	BP	1	BP1	0.0642
DEC	H	H	BP	1	BP1	0.0648
DEC	H	H	BP	1	BP1	0.0493
FEB	H	H	BP	1	BP1	0.0716
FEB	H	H	BP	1	BP1	0.0679
FEB	H	H	BP	1	BP1	0.0664
FEB	H	H	BP	1	BP1	0.0731
FEB	H	H	BP	1	BP1	0.0789
JAN	H	H	BP	1	BP1	0.0694
JAN	H	H	BP	1	BP1	0.0744
JAN	H	H	BP	1	BP1	0.0573
JAN	H	H	BP	1	BP1	0.0743
JAN	H	H	BP	1	BP1	0.0716
MAY	H	H	BP	1	BP1	0.0689
MAY	H	H	BP	1	BP1	0.0588
MAY	H	H	BP	1	BP1	0.0695
MAY	H	H	BP	1	BP1	0.0708
MAY	H	H	BP	1	BP1	0.0674
MAR	H	H	BP	1	BP1	0.0619
MAR	H	H	BP	1	BP1	0.0633
MAR	H	H	BP	1	BP1	0.0650
MAR	H	H	BP	1	BP1	0.0649
MAR	H	H	BP	1	BP1	0.0613
APR	U	U	BP	2	BP2	0.0644
APR	U	U	BP	2	BP2	0.0663
APR	U	U	BP	2	BP2	0.0625
APR	U	U	BP	2	BP2	0.0547
APR	U	U	BP	2	BP2	0.0608
DEC	U	U	BP	2	BP2	0.0588
DEC	U	U	BP	2	BP2	0.0798
DEC	U	U	BP	2	BP2	0.0678
DEC	U	U	BP	2	BP2	0.0660
DEC	U	U	BP	2	BP2	0.0718
FEB	U	U	BP	2	BP2	0.0750
FEB	U	U	BP	2	BP2	0.0810
FEB	U	U	BP	2	BP2	0.0657
FEB	U	U	BP	2	BP2	0.0746
FEB	U	U	BP	2	BP2	0.0790
JAN	U	U	BP	2	BP2	0.0792
JAN	U	U	BP	2	BP2	0.0655
JAN	U	U	BP	2	BP2	0.0789
JAN	U	U	BP	2	BP2	0.0736

JAN	U	U	BP	2	BP2	0.0694
MAY	U	U	BP	2	BP2	0.0557
MAY	U	U	BP	2	BP2	0.0668
MAY	U	U	BP	2	BP2	0.0711
MAY	U	U	BP	2	BP2	0.0635
MAY	U	U	BP	2	BP2	0.0544
MAR	U	U	BP	2	BP2	0.0680
MAR	U	U	BP	2	BP2	0.0670
MAR	U	U	BP	2	BP2	0.0592
MAR	U	U	BP	2	BP2	0.0574
MAR	U	U	BP	2	BP2	0.0671
APR	U	U	BP	3	BP3	0.0647
APR	U	U	BP	3	BP3	0.0681
APR	U	U	BP	3	BP3	0.0624
APR	U	U	BP	3	BP3	0.0581
APR	U	U	BP	3	BP3	0.0567
DEC	U	U	BP	3	BP3	0.0683
DEC	U	U	BP	3	BP3	0.0681
DEC	U	U	BP	3	BP3	0.0694
DEC	U	U	BP	3	BP3	0.0655
DEC	U	U	BP	3	BP3	0.0707
FEB	U	U	BP	3	BP3	0.0715
FEB	U	U	BP	3	BP3	0.0776
FEB	U	U	BP	3	BP3	0.0776
FEB	U	U	BP	3	BP3	0.0706
FEB	U	U	BP	3	BP3	0.0726
JAN	U	U	BP	3	BP3	0.0956
JAN	U	U	BP	3	BP3	0.0691
JAN	U	U	BP	3	BP3	0.0849
JAN	U	U	BP	3	BP3	0.0811
JAN	U	U	BP	3	BP3	0.0717
MAY	U	U	BP	3	BP3	0.0554
MAY	U	U	BP	3	BP3	0.0551
MAY	U	U	BP	3	BP3	0.0665
MAY	U	U	BP	3	BP3	0.0684
MAY	U	U	BP	3	BP3	0.0629
MAR	U	U	BP	3	BP3	0.0588
MAR	U	U	BP	3	BP3	0.0560
MAR	U	U	BP	3	BP3	0.0567
MAR	U	U	BP	3	BP3	0.0597
MAR	U	U	BP	3	BP3	0.0593
JAN	H	H	PA	1	PA1	0.0669
JAN	U	SU	PA	2	PA2	0.0841
JAN	U	SU	PA	3	PA3	0.0701
APR	H	H	PA	1	PA1	0.0686
APR	H	H	PA	1	PA1	0.0688
APR	H	H	PA	1	PA1	0.0652
APR	H	H	PA	1	PA1	0.0558
APR	H	H	PA	1	PA1	0.0652
DEC	H	H	PA	1	PA1	0.0611
DEC	H	H	PA	1	PA1	0.0736

DEC	H	H	PA	1	PA1	0.0747
DEC	H	H	PA	1	PA1	0.0812
DEC	H	H	PA	1	PA1	0.0725
FEB	H	H	PA	1	PA1	0.0633
FEB	H	H	PA	1	PA1	0.0718
FEB	H	H	PA	1	PA1	0.0707
FEB	H	H	PA	1	PA1	0.0755
FEB	H	H	PA	1	PA1	0.0635
JAN	H	H	PA	1	PA1	0.0711
JAN	H	H	PA	1	PA1	0.0617
JAN	H	H	PA	1	PA1	0.0787
JAN	H	H	PA	1	PA1	0.0716
MAY	H	H	PA	1	PA1	0.0764
MAY	H	H	PA	1	PA1	0.0760
MAY	H	H	PA	1	PA1	0.0806
MAY	H	H	PA	1	PA1	0.0757
MAY	H	H	PA	1	PA1	0.0700
MAR	H	H	PA	1	PA1	0.0626
MAR	H	H	PA	1	PA1	0.0608
MAR	H	H	PA	1	PA1	0.0712
MAR	H	H	PA	1	PA1	0.0668
MAR	H	H	PA	1	PA1	0.0650
APR	U	SU	PA	2	PA2	0.0657
APR	U	SU	PA	2	PA2	0.0606
APR	U	SU	PA	2	PA2	0.0591
APR	U	SU	PA	2	PA2	0.0666
APR	U	SU	PA	2	PA2	0.0717
DEC	U	SU	PA	2	PA2	0.0720
DEC	U	SU	PA	2	PA2	0.0756
DEC	U	SU	PA	2	PA2	0.0654
DEC	U	SU	PA	2	PA2	0.0774
DEC	U	SU	PA	2	PA2	0.0801
FEB	U	SU	PA	2	PA2	0.0726
FEB	U	SU	PA	2	PA2	0.0744
FEB	U	SU	PA	2	PA2	0.0715
FEB	U	SU	PA	2	PA2	0.0692
FEB	U	SU	PA	2	PA2	0.0625
JAN	U	SU	PA	2	PA2	0.0764
JAN	U	SU	PA	2	PA2	0.0736
JAN	U	SU	PA	2	PA2	0.0802
JAN	U	SU	PA	2	PA2	0.0833
MAY	U	SU	PA	2	PA2	0.0806
MAY	U	SU	PA	2	PA2	0.0757
MAY	U	SU	PA	2	PA2	0.0721
MAY	U	SU	PA	2	PA2	0.0652
MAY	U	SU	PA	2	PA2	0.0695
MAR	U	SU	PA	2	PA2	0.0633
MAR	U	SU	PA	2	PA2	0.0622
MAR	U	SU	PA	2	PA2	0.0615
MAR	U	SU	PA	2	PA2	0.0697
MAR	U	SU	PA	2	PA2	0.0753

APR	U	SU	PA	3	PA3	0.0577
APR	U	SU	PA	3	PA3	0.0688
APR	U	SU	PA	3	PA3	0.0728
APR	U	SU	PA	3	PA3	0.0862
APR	U	SU	PA	3	PA3	0.0713
DEC	U	SU	PA	3	PA3	0.0645
DEC	U	SU	PA	3	PA3	0.0655
DEC	U	SU	PA	3	PA3	0.0601
DEC	U	SU	PA	3	PA3	0.0785
DEC	U	SU	PA	3	PA3	0.0633
FEB	U	SU	PA	3	PA3	0.0674
FEB	U	SU	PA	3	PA3	0.0874
FEB	U	SU	PA	3	PA3	0.0633
FEB	U	SU	PA	3	PA3	0.0715
FEB	U	SU	PA	3	PA3	0.0695
JAN	U	SU	PA	3	PA3	0.0657
JAN	U	SU	PA	3	PA3	0.0672
JAN	U	SU	PA	3	PA3	0.0655
JAN	U	SU	PA	3	PA3	0.0717
MAY	U	SU	PA	3	PA3	0.0676
MAY	U	SU	PA	3	PA3	0.0667
MAY	U	SU	PA	3	PA3	0.0698
MAY	U	SU	PA	3	PA3	0.0780
MAY	U	SU	PA	3	PA3	0.0655
MAR	U	SU	PA	3	PA3	0.0684
MAR	U	SU	PA	3	PA3	0.0628
MAR	U	SU	PA	3	PA3	0.0458
MAR	U	SU	PA	3	PA3	0.0635
MAR	U	SU	PA	3	PA3	0.0659

Table S3(d): glucose oxidase (GOX) activity from a five-head pool, represented by the Relative Fluorescence Unit per min/ μ L and /g

Month	Health in March	Intensity	Locality	Sisters	Colony	RFU/min/uL	RFU/min/g
APR	H	H	BP	1	BP1	3.28	3.89
APR	U	U	BP	2	BP2	2.76	3.58
APR	U	U	BP	3	BP3	2.85	3.68
APR	H	H	PA	1	PA1	3.86	4.77
APR	U	SU	PA	2	PA2	3.43	4.24
APR	U	SU	PA	3	PA3	3.38	3.79
FEB	H	H	BP	1	BP1	3.46	3.86
FEB	U	U	BP	2	BP2	3.52	3.75
FEB	U	U	BP	3	BP3	3.80	4.11
FEB	H	H	PA	1	PA1	3.08	3.57
FEB	U	SU	PA	2	PA2	4.06	4.64
FEB	U	SU	PA	3	PA3	3.49	3.89
JAN	H	H	BP	1	BP1	3.27	3.77
JAN	U	U	BP	2	BP2	3.36	3.67
JAN	U	U	BP	3	BP3	4.64	4.61
JAN	H	H	PA	1	PA1	2.44	2.79
JAN	U	SU	PA	2	PA2	2.93	2.94
JAN	U	SU	PA	3	PA3	3.41	4.01
MAR	H	H	BP	1	BP1	3.12	3.95
MAR	U	U	BP	2	BP2	2.80	3.52
MAR	U	U	BP	3	BP3	3.28	4.51
MAR	H	H	PA	1	PA1	3.18	3.90
MAR	U	SU	PA	2	PA2	8.33	10.04
MAR	U	SU	PA	3	PA3	3.15	4.11

Table S3(e): taxonomic characterization (at Family and/or genus level) of stored pollen of different colonies.

Month	Health in March	Locality	Colony	Eucalyptus	Myrtaceae	Pleroma cf. granulosa	Asteraceae	Solanum	Parapiptadenia rigida	Moraceae	Allophylus edulis	Salix humboldtiana	Sebastiania commersoniana
				Myrtaceae	Myrtaceae	Melastomataceae	Asteraceae	Solanaceae	Fabaceae	Moraceae	Sapindaceae	Salicaceae	Euphorbioideae
FEB	H	BP	BP b	1	26	0	0	0	0	0	0	0	0
FEB	H	BP	BP1	1	1	0	0	0	0	0	0	0	0
FEB	H	PA	PA3	26	8	0	0	0	0	0	0	0	0
JAN	H	BP	BP b	175	204	0	0	7	0	0	0	0	0
JAN	H	PA	PA3	1	522	0	0	1	1	0	1	0	0
MAR	U	BP	BP e	55	13	0	0	0	0	0	0	0	0
MAR	U	BP	BP f	148	1	0	0	0	0	0	0	0	0
MAR	H	BP	BP b	11	8	0	0	0	0	0	0	0	0
MAR	H	BP	BP1	53	103	0	0	0	0	0	0	0	0
MAR	U	BP	BP c	177	22	0	0	0	0	0	0	0	0
MAR	U	BP	BP d	10	9	0	0	0	0	0	0	0	0
MAR	H	PA	PA1	4	5	0	0	1	0	0	0	0	0
MAR	U	PA	PA2	1	56	0	0	0	0	0	0	0	0
MAR	U	SL	SL	1	98	0	1	1	1	0	1	0	0
NOV	H	BP	BP b	4	540	0	0	56	0	0	0	0	0
NOV	H	BP	BP1	1	500	0	0	65	0	0	0	0	0
NOV	U	BP	BP2	1	508	0	1	5	0	0	0	0	0
NOV	U	PA	PA g	365	259	0	1	25	15	0	0	0	0
NOV	H	PA	PA1	119	456	0	1	0	21	0	0	0	0
NOV	U	PA	PA2	431	48	0	1	2	59	0	0	0	0
OCT	H	BP	BP a	94	533	0	0	0	1	0	0	0	0
OCT	H	BP	BP b	11	592	0	0	0	1	0	0	0	0
OCT	H	BP	BP1	248	335	1	0	1	1	0	0	0	0
OCT	U	BP	BP2	4	530	0	0	1	0	0	0	0	0
OCT	U	PA	PA g	560	36	0	1	2	1	0	0	0	0
OCT	H	PA	PA1	205	350	2	1	3	1	0	0	0	0
OCT	U	PA	PA2	154	377	4	1	4	1	0	0	0	0
SET	H	BP	BP b	109	124	37	1	98	11	46	3	16	0
SET	H	BP	BP1	8	111	271	0	8	0	0	0	0	2
SET	U	PA	PA g	109	175	7	2	10	0	0	0	0	0
SET	H	PA	PA1	146	260	1	0	1	0	0	0	0	0

Table S4: Detection limits of high performance liquid chromatography coupled with sequential mass spectrometry

Parameter	Unit	Quantification limit
3 - Hydroxy carbofuran	mg/kg	0.01
Abamectin	mg/kg	0.01
Acephate	mg/kg	0.01
Acetamiprid	mg/kg	0.01
Acetochlor	mg/kg	0.01
Acrinathrin	mg/kg	0.01
Aldicarb (SUM)	mg/kg	0.01
Aldicarb sulfoxide	mg/kg	0.01
Aldicarb sulphone	mg/kg	0.01
Ametryn	mg/kg	0.01
Amicarbazone	mg/kg	0.01
Aminocarb	mg/kg	0.01
Amitraz	mg/kg	0.01
Anilazine	mg/kg	0.01
Atrazine	mg/kg	0.1
Azaconazole	mg/kg	0.01
Azimsulfuron	mg/kg	0.02
Azinphos ethyl	mg/kg	0.01
Azinphos methyl	mg/kg	0.01
Azocyclotin	mg/kg	0.01
Benalaxyl	mg/kg	0.01
Benfuracarb	mg/kg	0.01
Bitertanol	mg/kg	0.01
Boscalid	mg/kg	0.01
Bromacyl	mg/kg	0.01
Bupirimate	mg/kg	0.01
Buprofezin	mg/kg	0.01
Cadusafos	mg/kg	0.01
Carbaryl	mg/kg	0.01
Carbendazin (Benomyl, thiophanate methyl)	mg/kg	0.01
Carbofuran	mg/kg	0.01
Carbophenothion	mg/kg	0.01
Carbosulfan	mg/kg	0.01
Carboxin	mg/kg	0.01
Carfentrazone ethyl	mg/kg	0.01
Carpropamid	mg/kg	0.01
Chlorimuron ethyl	mg/kg	0.01
Chlorpyrifos (dursban/lorsba)	mg/kg	0.01
Chlorthiophos	mg/kg	0.01
Clothianidin	mg/kg	0.01
Coumaphos	mg/kg	0.01
Cyanazine	mg/kg	0.01
Cyanofenphos	mg/kg	0.01
Cyazofamid	mg/kg	0.01
Cyproconazole	mg/kg	0.01
Dichlofluanid	mg/kg	0.01
Dichlorvos	mg/kg	0.01
Diclofop methyl	mg/kg	0.01

Dicrotophos	mg/kg	0.01
Difenoconazole 1, 2	mg/kg	0.01
Diflubenzuron	mg/kg	0.01
Dimethanamid	mg/kg	0.01
Dimethoate	mg/kg	0.01
Dimethomorph (SUM)	mg/kg	0.01
Diniconazole	mg/kg	0.01
Disulfoton sulfone	mg/kg	0.01
Disulfoton sulfoxide	mg/kg	0.01
Diuron	mg/kg	0.01
Dodemorph	mg/kg	0.01
EPN	mg/kg	0.01
Epoxiconazole	mg/kg	0.01
Ethiofencarb	mg/kg	0.01
Ethiofencarb-sulfone	mg/kg	0.01
Ethion (nialate)	mg/kg	0.01
Ethiprole	mg/kg	0.01
Ethoprophos	mg/kg	0.01
Ethoxysulfuron	mg/kg	0.01
Etoxazole	mg/kg	0.01
Etrimfos	mg/kg	0.01
Famoxadone	mg/kg	0.01
Fenamidone	mg/kg	0.01
Fenamiphos sulfone	mg/kg	0.01
Fenamiphos sulfoxide	mg/kg	0.01
Fenarimol	mg/kg	0.01
Fenbuconazole	mg/kg	0.01
Fenhexamid	mg/kg	0.01
Fenoxycarb	mg/kg	0.01
Fenpropimorph	mg/kg	0.01
Fenpyroximate	mg/kg	0.01
Fenthion	mg/kg	0.01
Fenthion sulfone	mg/kg	0.01
Fenthion sulfoxide	mg/kg	0.01
Fipronil	mg/kg	0.03
Flazasulforon	mg/kg	0.01
Flufenacet	mg/kg	0.01
Flufenoxuron	mg/kg	0.01
Fluopicolide	mg/kg	0.1
Fluquinconazole	mg/kg	0.01
Flusilazol	mg/kg	0.01
Flutolanil	mg/kg	0.01
Fomezafen	mg/kg	0.01
Fonofos (SUM)	mg/kg	0.01
Fostiazate	mg/kg	0.01
Furathiocarb	mg/kg	0.01
Hexaconazole	mg/kg	0.01
Hexazinone	mg/kg	0.01
Hexythiazox	mg/kg	0.01
Imazalil	mg/kg	0.01
Imazapic	mg/kg	0.01

Imazapyr	mg/kg	0.01
Imazaquim	mg/kg	0.01
Imibenconazole	mg/kg	0.01
Imidacloprid	mg/kg	0.01
Indoxacarb	mg/kg	0.01
Iprovalicarb	mg/kg	0.01
Isofenphos	mg/kg	0.01
Isoxaflutole	mg/kg	0.01
Linuron	mg/kg	0.01
Lufenuron	mg/kg	0.01
Malaoxon	mg/kg	0.01
Metalaxyl (SUM)	mg/kg	0.01
Metconazole	mg/kg	0.01
Methamidophos	mg/kg	0.01
Methidathion	mg/kg	0.01
Methiocarb	mg/kg	0.01
Methiocarb sulfone	mg/kg	0.01
Methiocarb sulfoxide	mg/kg	0.01
Methobromuron	mg/kg	0.01
Methomyl	mg/kg	0.01
Metribuzin	mg/kg	0.01
Metsulfuron methyl	mg/kg	0.01
Mevinphos (SUM)	mg/kg	0.01
Monocrotophos	mg/kg	0.01
Monuron	mg/kg	0.01
Myclobutanil	mg/kg	0.03
Napropamide	mg/kg	0.01
Novaluron	mg/kg	0.01
Omethoate	mg/kg	0.01
Oxadixyl	mg/kg	0.01
Oxamyl	mg/kg	0.01
Oxasulfuron	mg/kg	0.01
Oxycarboxin	mg/kg	0.01
Paclobutrazol	mg/kg	0.01
Paraoxon ethyl	mg/kg	0.01
Paraoxon methyl	mg/kg	0.01
Penconazole	mg/kg	0.01
Pencycuron	mg/kg	0.01
Pendimethalin	mg/kg	0.01
Phorate	mg/kg	0.01
Phosalone	mg/kg	0.01
Phosmet	mg/kg	0.01
Phosphamidon (SUM)	mg/kg	0.01
Picoxystrobin	mg/kg	0.01
Pirimicarb	mg/kg	0.01
Pirimiphos ethyl	mg/kg	0.01
Pirimiphos methyl	mg/kg	0.01
Prochloraz	mg/kg	0.01
Profenophos	mg/kg	0.01
Promecarb	mg/kg	0.01
Prometon	mg/kg	0.01

Prometryn	mg/kg	0.01
Propamocarb	mg/kg	0.01
Propargite	mg/kg	0.01
Propiconazole I, II	mg/kg	0.1
Propoxur	mg/kg	0.01
Pymetrozine	mg/kg	0.01
Pyraclostrobin	mg/kg	0.01
Pyrazophos	mg/kg	0.01
Pyrethrins	mg/kg	0.01
Pyridaben	mg/kg	0.01
Pyridaphenthion	mg/kg	0.01
Pyrifenox	mg/kg	0.01
Pyrimethanil	mg/kg	0.01
Pyriproxyfen	mg/kg	0.01
Quinoxifen	mg/kg	0.01
Quinalofpop-p-ethyl	mg/kg	0.01
Rotenone	mg/kg	0.01
Simazine	mg/kg	0.01
Spinosad	mg/kg	0.01
Spiroxamine	mg/kg	0.01
Sulfentrazone	mg/kg	0.01
Sulfometuron-methyl	mg/kg	0.01
Sulfotep	mg/kg	0.01
Sulprofos	mg/kg	0.01
Tebuconazole	mg/kg	0.01
Tebufenozide	mg/kg	0.01
Tetraconazole	mg/kg	0.01
Thiabendazole	mg/kg	0.01
Thiacloprid	mg/kg	0.01
Thiamethoxam	mg/kg	0.01
Thiobencarb	mg/kg	0.01
Thiodicarb	mg/kg	0.01
Thionazin	mg/kg	0.01
Thiophanate methyl	mg/kg	0.01
Tolyfluanid	mg/kg	0.01
Triadimefon	mg/kg	0.01
Triadimenol	mg/kg	0.01
Triazophos	mg/kg	0.01
Trichlorfon	mg/kg	0.01
Tricyclazole	mg/kg	0.01
Tridemorph	mg/kg	0.01
Trifloxystrobin	mg/kg	0.01
Triflumizole	mg/kg	0.01
Triflumuron	mg/kg	0.01
Triticonazole	mg/kg	0.01
Vamidathion	mg/kg	0.01
Penoxulam	µg/kg	0.01

CAPÍTULO 5

Recomendações para o manejo da mandaçaia no RS

Este capítulo apresenta um folder de divulgação científica direcionado, principalmente, aos meliponicultores.

Recomendações para o manejo da abelha mandaçaia no RS

por Lílian Caesar e Karen Luisa Haag

A mandaçaia (*Melipona quadrifasciata*) é uma abelha sem ferrão amplamente distribuída no Brasil e, desde os aborígenes, cultivada para a obtenção de mel. No nosso Estado, Rio Grande do Sul (RS), ocorre uma subespécie desta abelha (*M. q. quadrifasciata*) que é evolutivamente divergente da mandaçaia do norte do Brasil (*M. q. anthidioides*, encontrada a partir do Estado de São Paulo). Isso significa que, embora muito parecidas, estas duas subespécies possuem adaptações próprias ao seu habitat (clima, flores utilizadas, etc) e também uma composição genética diferente.

No RS há vários anos ou talvez décadas, não se encontram mais ninhos da mandaçaia na natureza, com poucas exceções. Toda colônia de mandaçaia cultivada hoje no RS foi então obtida de outra colônia por divisão, um processo que vem acontecendo por muitas gerações. Portanto, pode-se considerar que a mandaçaia no RS é uma abelha domesticada, assim como a *Apis mellifera*, que foi introduzida da Europa e depois africanizou-se. A diferença entre a *A. mellifera* e a mandaçaia, no que diz respeito à domesticação, é que a abelha africanizada ainda tem a capacidade de enxamear e produzir ninhos silvestres, apesar da domesticação. Trata-se de uma abelha muito bem adaptada ao nosso ambiente, que forma colônias gigantescas comparativamente as nossas abelhas nativas e com extrema capacidade competitiva quanto à utilização de recursos.

Anualmente no RS os meliponicultores observam que uma grande quantidade de colônias de mandaçaia adoecem sempre no final do verão (normalmente em março). A doença pode ser tão severa que toda a colônia entra em colapso, lembrando bastante uma doença séria da *A. mellifera* chamada de “Desordem do Colapso das Colméias” (CCD), frequente nos Estados Unidos desde 2006. Até hoje não existe uma causa única que explique a CCD, mas alguns fatores importantes são a varroa, os vírus e os agroquímicos. No caso da mandaçaia, diferentes possibilidades foram levantadas pelos meliponicultores, como a toxicidade de alguma(s) planta(s) e os agroquímicos.

Por isso, nosso grupo de pesquisas do Laboratório de Genômica Evolutiva do Departamento de Genética da UFRGS, em colaboração com vários outros pesquisadores desta e de outras instituições, tem direcionado esforços no sentido de investigar as causas envolvidas na mortalidade anual das mandaçaia no nosso Estado.

Resumidamente, as conclusões dos nossos trabalhos – alguns deles já publicados em revistas científicas de alto nível – são as seguintes:

1. Há uma contínua **perda de peso** das forrageiras **entre janeiro e março**. A perda de peso das forrageiras está correlacionada ao tamanho da colônia em abelhas sociais de uma forma geral, então isso nos permite concluir que abelhas individuais, bem como a colmeia, enfraquecem neste período.

2. No período que antecede a síndrome também há uma **mudança nos hábitos alimentares** das mandaçaias, pois o pólen predominantemente armazenado nas colônias a partir de fevereiro deixa de ser de eucaliptos e passa a ser do maricá. É possível que a mandaçaias esteja sendo excluída pelo seus competidores - incluindo as *A. mellifera* - das áreas contendo as flores do eucalipto, e passa a coletar pólen de maricá que entra em floração em fevereiro.

3. Outra observação importante é que as abelhas doentes - e também as abelhas saudáveis, isto é, aquelas que não apresentam sintomas da doença - **podem abrigar os mais variados vírus**. Alguns desses vírus ocorrem também na *A. mellifera*, indicando que o contato direto - ou indireto, através das flores que ambas visitam - entre as duas espécies de abelhas permite a transmissão dos vírus uma à outra. **Em alguns meliponários** do RS as mandaçaias de colônias doentes apresentam **sintomas muito semelhantes aos sintomas neurológicos causados por infecções virais** em outras abelhas - raramente estes sintomas são também observados em outras espécies de abelha sem ferrão na mesma época. No entanto esses sintomas observados nas mandaçaias não são generalizados, variando enormemente entre colônias de distintos meliponários. O que todos os meliponários que observam a síndrome têm em comum é uma **mortalidade alta e incomum em março da espécie *M. quadrifasciata***.

4. É possível que a maior vulnerabilidade das mandaçaias em relação às outras abelhas nativas e a *A. mellifera* tenha relação com o **afrouxamento da seleção relacionado a algumas práticas de manejo**, as quais não necessariamente favorecem as abelhas no seu ambiente natural. Por exemplo, se por várias gerações as abelhas foram acostumadas a receber alimento regularmente, com o passar de muitas gerações poderá haver uma piora no desempenho destas abelhas em procurar alimento por conta própria.

5. Outro aspecto a ser levado em consideração é o **uso de agroquímicos** nas áreas de forrageio das abelhas. Mesmo que elas não estejam sujeitas a doses letais desses agentes químicos, podem haver efeitos subletais que irão se manifestar com o passar do tempo. Esses efeitos podem ser tardios e também cumulativos (vão se tornando cada vez mais graves). Um estudo recente feito na Europa mostrou que a presença de doses muito baixas de um pesticida em larvas e pupas das abelhas leva a um baixo desenvolvimento dos órgãos do sistema nervoso necessários para que a abelha quando adulta desempenhe suas atividades normais. (...)

(continuação) O forrageio é um comportamento que requer inteligência e reflexo. O mau funcionamento do sistema nervoso diminui o desempenho da forrageira, e conseqüentemente a nutrição da colônia.

6. Acompanhando três pares de colônias de mandaçaias em dois locais diferentes, nós observamos **efeitos tardios do mal funcionamento de genes relacionados com a detoxificação de compostos, bem como com a imunidade e a saúde geral das abelhas**. Isto sugere que não apenas podem estar ocorrendo efeitos subletais devido a exposição das mandaçaias a agroquímicos, como também uma perda na capacidade de defender-se de seus inimigos naturais, como vírus e outros parasitas.

Dadas as conclusões listadas acima, nossos estudos nos levam a sugerir as seguintes **medidas profiláticas no manejo das mandaçaias no RS**, que podem ser ampliadas ao manejo de outras abelhas sem ferrão, com o intuito de fortalecer as colônias e diminuir a incidência da síndrome:

1. Cultivar as abelhas em **condições similares ao ambiente natural**, procurando não fornecer alimento desnecessariamente e não alterando o microclima natural das colônias, como a temperatura. Desta forma, com o passar das gerações, diminuirão os possíveis efeitos danosos de algumas práticas de manejo.

2. Não introduzir abelhas de outros locais indiscriminadamente. Muito embora a mistura de abelhas originárias de locais distintos possa contribuir para aumentar a variabilidade genética, há o grande risco de serem introduzidos patógenos contra os quais as mandaçaias de populações locais não apresentam defesa.

3. Prover **quantidade de pasto apícola proporcional à quantidade de abelhas cultivadas** com o intuito de diminuir a competição por alimento. Esta recomendação é ainda mais importante se as mandaçaias estiverem sendo mantidas na proximidade de colônias de *A. mellifera*.

4. Evitar o uso de agroquímicos nas áreas de forrageio das mandaçaias.

5. As recomendações 3 e 4 são particularmente importantes nos meses anteriores à síndrome (**a partir de janeiro**), levando em consideração os efeitos tardios observados nos nossos estudos, que podem culminar com a síndrome.

Mais informações em: <http://www.ufrgs.br/genomicaevolutiva/manejo-de-abelhas>

SUGESTÕES PARA LEITURA

Título em português: Relato sobre a microbiota de *Melipona quadrifasciata* afetada por doença recorrente

Título original: Report on the microbiota of *Melipona quadrifasciata* affected by a recurrent disease

Link oficial: <https://www.sciencedirect.com/science/article/abs/pii/S002220111630218X?via%3Dihub>

Destaques: Neste trabalho mostramos que as principais bactérias associadas ao intestino de mandaçaia são parecidas àquelas que estão também no de *Apis*, desempenhando funções importantes para a saúde da abelha. Também mostramos que não estão presentes bactérias patogênicas já conhecidas por infectar outras abelhas, nem mesmo nas colônias afetadas pela síndrome. Não foram também detectados, nas mesmas colônias doentes e saudáveis avaliadas, patógenos como *Nosema* ou *Crithidia* (um tripanossomatídeo).

Título em português: O viroma de uma abelha sem ferrão em risco de extinção que sofre de uma mortalidade anual no sul do Brasil

Título original: The virome of an endangered stingless bee suffering from annual mortality in southern Brazil

Link oficial: <https://www.microbiologyresearch.org/content/journal/jgv/10.1099/jgv.0.001273#tab7>

Destaques: Neste trabalho mostramos que mandaçaias afetadas pela síndrome carregam muito mais vírus que as saudáveis. Dentre eles vírus, sete novos vírus foram descritos e dois deles são parecidos com vírus patogênicos de abelhas melíferas (que causam tremores e paralisia nelas). Estes vírus não são a causa principal da síndrome, mas alguns deles estão mais associados a abelhas doentes em um meliponário, onde os sintomas da síndrome foram mais intensos, e podem estar contribuindo para o colapso de colônias neste local.

Título em português: Definindo a saúde dos polinizadores: uma abordagem holística baseada em fatores ecológicos, genéticos e fisiológicos

Título original: Defining pollinator health: A Holistic Approach Based on Ecological, Genetic, and Physiological Factors

Link oficial: <https://www.annualreviews.org/doi/abs/10.1146/annurev-animal-020518-115045>

Destaques: Nesta revisão os autores discutem que a saúde dos polinizadores não pode ser reduzida à ausência de doença ou colônias cheias de indivíduos, mas sim um estado que permite que os indivíduos vivam mais e/ou se reproduzam mais, mesmo na presença de patógenos pelo ambiente. Também falam sobre o uso de biomarcadores padronizados para avaliar a saúde nos diferentes níveis de complexidade das abelhas sociais: a nível individual (ex: tamanho corporal, microbiota intestinal, fisiologia), da colônia (ex: imunidade social, tamanho da colônia) e da população (ex: diversidade genética). Reforçam que estas medidas de saúde usando biomarcadores podem guiar decisões sobre o manejo de colônias, sobre a conservação de abelhas selvagens e políticas ambientais.

Título em português: As práticas meliponícolas e a distância geográfica, não o uso da terra, direcionam o fluxo gênico nas abelhas tropicais

Título original: Beekeeping practices and geographic distance, not land use, drive gene flow across tropical bees

Link oficial: <https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.13852>

Destaques: Neste trabalho os autores mostram que o fluxo de genes (quando uma população recebe genes de outra) ocorre pela diminuição do isolamento geográfico devido ao manejo e troca de colônias, o que não é observado nas colônias selvagens. Isto pode levar à mistura entre espécies, resultando na substituição completa das características da espécie original. Também mostram que o que mantém o alto fluxo de genes nas abelhas sem ferrão são ambientes com paisagens heterogêneas, como florestas nativas conservadas. Por isso, os autores sugerem que para a preservação das abelhas haja regulação das práticas de meliponicultura para manter o fluxo gênico natural, como também melhorar os habitats amigáveis aos polinizadores.

Título em português: Cria pútrida européia em abelhas sem ferrão (Apidae: Meliponini) no Brasil: doença antiga, ameaça renovada

Título original: European Foulbrood in stingless bees (Apidae: Meliponini) in Brazil: old disease, renewed threat

Link oficial: <https://www.sciencedirect.com/science/article/abs/pii/S002220112030063X>

Destaques: Neste estudo foi detectado pela primeira vez a bactéria patogênica *Melissococcus plutonius* e os sintomas da cria pútrida européia em espécies de *Melipona*, o que estava levando à morte da prole e em alguns casos ao colapso de colônias. Essa bactéria infectou as colônias através do pólen de *Apis* contaminado e usado para alimentar as abelhas sem ferrão. Para evitar a transmissão de patógenos, os autores recomendam que meliponicultores evitem a introdução de rainhas, enxames ou colônias em meliponários sem considerar critérios sanitários; evitem o uso de utensílios contaminados, desinfetando apropriadamente, e que limitem o uso de suplementos à base de pólen para sustentar colônias de *Melipona*.

Título em português: Particionamento de recursos florais entre abelhas nativas do gênero *Melipona* e a abelha africanizada introduzida na floresta tropical atlântica brasileira

Título original: Floral resource partitioning between native *Melipona* bees and the introduced Africanized honey bee in the Brazilian Atlantic rain forest

Link oficial:

https://www.apidologie.org/articles/apido/abs/1997/05/Apidologie_0044-8435_1997_28_6_ART0002/Apidologie_0044-8435_1997_28_6_ART0002.html

Destaques: Neste estudo os autores mostram que *Melipona* e *Apis mellifera* na Mata Atlântica competem por recursos das mesmas flores, principalmente do grupo Myrtaceae (como o Eucalipto). O pico de coleta de pólen por *Melipona* foi justamente no período em que a *Apis* competiu menos por esses recursos. Isso por que enquanto *Melipona* usa poucas plantas como recurso, principalmente Myrtaceae e Melastomataceae, a *Apis mellifera* usa mais plantas, como Myrtaceae, Asteraceae, Euphorbiaceae e Arecaceae para pólen e Cunoniaceae, Rubiaceae e Myrtaceae para néctar.

Título em português: A exposição a inseticidas durante o desenvolvimento da prole ou no início da idade adulta reduz o crescimento cerebral e prejudica a aprendizagem de abelhas adultas.

Título original: Insecticide exposure during brood or early-adult development reduces brain growth and impairs adult learning in bumblebees

Link oficial: <https://royalsocietypublishing.org/doi/10.1098/rspb.2019.2442>

Destaques: Neste trabalho os autores mostram que houve uma redução no desenvolvimento de partes do cérebro de abelhas que foram expostas a agroquímicos (neonicotinóides) durante qualquer um dos estágios do desenvolvimento. Essa malformação não foi revertida mesmo quando a abelha não teve mais contato com o agroquímico. Estas abelhas também apresentaram uma resposta menor ao oferecimento de açúcar e dificuldade na aprendizagem.

Título em português: Abordagens ecológicas e evolutivas para o manejo de doenças de abelhas

Título original: Ecological and evolutionary approaches to managing honeybee disease

Link oficial: <https://www.nature.com/articles/s41559-017-0246-z>

Destaques: Neste trabalho os autores mostram que apiários muito populosos aumentam a chance de espalhar parasitas entre colônias, assim como colônias muito populosas podem ter dificuldade de desempenhar a imunidade social contra parasitas. Também mostram como a translocação de colônias leva novos patógenos a outros apiários e contribui para seleção de parasitas mais virulentos. Para evitar isso, os autores sugerem conservar a diversidade genotípica das colônias; disponibilizar maior diversidade alimentar e diminuir a dependência das colônias por açúcares processados; promover “estoque de sobreviventes”, ou seja, manter colônias naturalmente resistentes e permitir que colônias com baixa resistência a parasitas morram naturalmente ao invés de forçar medicações.

PERGUNTAS FREQUENTES

A síndrome é causada por alguma planta tóxica (ex: lanceta ou mata-campo)?

Análises do tipo de pólen coletado por mandaçaia de dois meliponários do RS, onde a síndrome ocorreu, não detectaram a presença de pólen de lanceta ou mata-campo, então estas plantas não são os agentes causadores da síndrome. Com esta análise percebemos que houve uma troca do consumo de pólen de eucalipto para pólen de maricá próximo ao período da síndrome. O maricá é uma planta nativa da região e não é tóxica para insetos. Esta troca pode ser resultado da competição por recursos com outros polinizadores e por isso, ao começar a florada de maricá, a mandaçaia passa a usá-lo como recurso.

Não seria então causada pelo besouro vaquinha que está nas plantas que a mandaçaia visita?

Não. Ao longo de nossas análises o besouro vaquinha (*Astylus variegatus*) não foi observado parasitando colônias de mandaçaia, nem mesmo mandaçaia que estavam forrageando. Este besouro também não está associado a plantas que a mandaçaia costuma visitar, como o eucalipto.

A síndrome é causada por agrotóxicos?

Análises de resíduos de colônias afetadas pela síndrome não detectaram agrotóxicos. No entanto, em um experimento recente que fizemos, observamos um mal funcionamento de um gene relacionado à detoxificação de compostos químicos nas colônias que adoeceram no período da síndrome. Este resultado sugere que embora as abelhas não morram imediatamente por doses altas de agroquímicos, as doses sub-letais podem estar contribuindo para a síndrome.

Esta síndrome é uma forma de controle populacional antes do inverno?

De fato a abelha mandaçaia não costuma fazer diapausa, que é uma forma de diminuir a atividade da colônia durante o inverno quando há menos recursos. No entanto, a síndrome já foi observada em colônias grandes, mas também em colônias jovens/pequenas, o que vai contra esta ideia. Também, os sintomas observados em mandaçaia de alguns meliponários (tremores e paralisia) sugerem algum problema neurológico causado por um parasita ou composto químico tóxico.

A síndrome afeta apenas mandaçaia?

A mandaçaia é a principal abelha afetada por esta síndrome. Em alguns meliponários, embora não em todos os anos, já foram observados sintomas similares em outras abelhas sem ferrão, como a *Melipona bicolor*, *M. seminigra*, *M. scutellaris* e *Scaptotrigona postica*. Amostras destas espécies já foram coletadas para futuras análises.

A síndrome ocorre também em outros estados além do RS?

Sim. Além do Rio Grande do Sul, a síndrome também ocorre em meliponários de Santa Catarina. Há também relatos de mortalidade de mandaçaia em março no Paraná, Minas Gerais, Rio de Janeiro e Bahia, embora não possamos garantir que seja exatamente a mesma síndrome.

Fechar a colônia antes do período da síndrome evita a alta mortalidade?

Até o momento não realizamos experimentos controlados testando os efeitos de fechar as colônias no início de março para prevenir a síndrome. Por isso, não podemos recomendar esta prática. O que se sabe são relatos de meliponicultores que ao fechar as colônias notaram que elas não adoeceram. Esta prática pode estar evitando o contato das abelhas com algum agente que contribua para a ocorrência da síndrome e também estimulando cuidados internos na colônia. Nossa estratégia é entender a síndrome para então pensar em estratégias de prevenção.

Carvão ativado é uma solução para a síndrome?

Para os efeitos do carvão ativado em colônias de mandaçaia também não realizamos experimentos controlados e, portanto, não recomendamos a aplicação como um remédio. O que se sabe no momento são relatos de meliponicultores que observam que algumas colônias que receberam uma solução de carvão ativado não adoeceram. Reforçamos que nossa estratégia é entender a síndrome para então pensar em estratégias de prevenção, assim também não precisaremos medicar as colônias.

A síndrome é pilhagem?

Não. A pilhagem é o furto entre colônias, principalmente nas colônias menores ou mais fracas, o que pode levar a brigas entre indivíduos e decapitação. Nada disso foi observado ao longo de nossos estudos sobre a síndrome, pelo contrário, as colônias somente são invadidas por parasitas (como outras abelhas ou forídeos) quando já morreram muitas operárias.

A síndrome é causada por forídeos?

Não, embora forídeos possam se aproveitar do enfraquecimento das colônias para invadi-las, contribuindo para o colapso final da colônia.

CAPÍTULO 6

Discussão e conclusão

6. Discussão e conclusão

Doenças de abelhas e o colapso de suas colônias tem desafiado pesquisadores na busca dos fatores causais. Embora a CCD explique apenas uma parte do colapso de colônias nos EUA, ela ficou conhecida pelo grande esforço feito por parte de diversos laboratórios para encontrar a causa da desordem (Williams et al. 2010). Os estudos foram primeiramente conduzidos com o intuito de buscar um agente causador único, mas o que foi encontrado até o momento é que múltiplos fatores podem estar envolvidos no colapso, como patógenos, desnutrição e agroquímicos (Cox-Foster et al. 2007; vanEngelsdorp et al. 2009; VanEngelsdorp et al. 2010). Efeitos subletais destes diversos fatores, agindo ou não de forma sinérgica, passaram a explicar grande parte do colapso de colônias pelo mundo (Bryden et al. 2013; Goulson et al. 2015). O que aumenta ainda mais o desafio do estudo do colapso de colônias é também a complexidade inerente às abelhas sociais, pois as respostas de como estes fatores agem podem estar no nível do indivíduo, da colônia ou mesmo da população (López-Uribe et al. 2020).

O estudo sobre a mortalidade anual de mandaiaias no RS se mostrou também bastante desafiador. Ao longo de nossas observações e amostragens percebemos que a síndrome, apesar de sazonal, ocorre em meliponários com distintos contextos ambientais e mostram uma variedade de sintomas com diferentes intensidades. Também não são todas as colônias de mandaiaias que são afetadas. A dificuldade de observarmos um fator em comum em todos locais e colônias afetadas indicava a possibilidade da ação de múltiplos fatores para o desfecho da síndrome. Investigando aspectos chave da saúde das mandaiaias, em nível de indivíduo e de colônia, identificamos alguns fatores relacionados à ocorrência da síndrome.

Primeiramente investigamos o papel do microbioma bacteriano das abelhas na ocorrência da síndrome (Díaz et al. 2017; Capítulo 2). Nosso estudo foi o primeiro a caracterizar a microbiota de uma abelha nativa do Brasil, a qual se mostrou bastante diversa e composta principalmente por bactérias do grupo Firmicutes (como diferentes tipos de *Lactobacillus*), assim como em *A. mellifera*. Entretanto, diferentemente da abelha melífera, que possui alta conservação nos grupos de bactérias associadas ao intestino (Martinson et al. 2011), *M. quadrifasciata* apresenta grande variação na composição da microbiota entre

abelhas de diferentes colônias (Díaz et al. 2017). É possível que tais padrões tenham relação com o processo de domesticação que a *A. mellifera* já sofre há mais tempo e mais intensamente (Zheng et al. 2018). Concluímos com esta investigação que a síndrome anual não é causada por alguma bactéria patogênica e também não parece estar relacionada à disbiose. Ou seja, não há diferença na composição geral da microbiota comparando abelhas doentes e saudáveis, apesar dos resultados sugerirem que a composição da microbiota nos diferentes *status* de saúde é dependente do ano. No entanto, não podemos descartar que a falta de algum padrão claro esteja relacionado a grande variação na microbiota. Um estudo acompanhando flutuações na composição da microbiota temporalmente poderia complementar os achados deste trabalho e faz parte das perspectivas desta tese. Já temos o DNA abdominal de 120 amostras de mandaçaia (ANEXO I), coletadas entre 2018-2019 para o estudo do Capítulo 4 (5 operárias/colônia/localidade/mês, utilizadas para pesagem e quantificação da atividade de GOX), a serem submetidas à amplificação de fragmentos de 16S (bactérias) e ITS (fungos) por PCR, sequenciamento e análises comparativas.

A partir da observação dos sintomas de mandaçaia afetadas pela síndrome, direcionamos as investigações para a possibilidade de uma infecção viral (Caesar et al. 2019; Capítulo 3). Tremores, paralisia e incapacidade de voar são alguns dos sintomas que podem ser observados em operárias de colônias afetadas. Tais sintomas assemelham-se aos observados em infecções por dicistrovírus em *Apis* spp. (McMenamin e Genersch 2015), mas nenhum destes vírus (ex: ABPV, IAPV, KBV) foi detectado nas mandaçaia. Porém, novos vírus da família *Dicistroviridae* (MqV1a e MqV1b) foram descritos, assim como da família *Circoviridae* (MqC, MqC1 e MqC2) e *Densoviridae* (MqD). Nenhum deles foi consistentemente relacionado à ocorrência da síndrome, mas alguns destes novos vírus foram mais detectados em um meliponário onde os sintomas observados durante a síndrome foram mais intensos (Caesar et al. 2019). Uma investigação envolvendo a quantificação da densidade da infecção viral poderia contribuir para entender a sua relação com os sintomas observados e faz parte das perspectivas desta tese. Amostras de colônias do meliponário onde observam-se sintomas mais intensos nas forrageiras afetadas foram coletadas durante a síndrome de 2019 e armazenadas a -80 °C (ANEXO II) para futura extração de DNA ou RNA, síntese de cDNA e quantificação absoluta dos vírus por qPCR.

Outro achado interessante desta etapa do estudo foi a detecção de vírus da manduca também em *A. mellifera*. Embora não possamos confirmar a direção da transmissão dos vírus, este dado chama atenção para a ocorrência de troca de patógenos entre abelhas exóticas e nativas.

A ausência de algum patógeno consistentemente associado às abelhas de colônias que sofreram mortalidade nos levou então a uma investigação integrativa sobre o *status* de saúde das colônias no período que engloba a síndrome. Avaliamos diversas características das manducaias tanto em nível de indivíduo quanto de colônia (Caesar et al. *em preparação*; Capítulo 4). Planejamos este estudo de forma a discriminar a contribuição genética da colônia bem como do ambiente para o desfecho da síndrome. As características em nível de colônia foram as que mais explicaram o *status* de saúde, como a perda do controle da umidade e temperatura, principalmente em colônias afetadas pela síndrome. Tais traços, no entanto, são consequência de diversos processos biológicos que ocorrem em nível individual. Nós verificamos que forrageiras das colônias que adoeceram tiveram uma menor expressão de genes relacionados, na abelha modelo *A. mellifera*, à capacidade de detoxificação de agroquímicos (*p450*) e resposta imune (*Vg* e *PO*). Assim como observado em outros eventos de colapso de colônias, sugerimos com este estudo que o efeito subletal de estressores como agroquímicos, competição por recursos e exposição a patógenos possam estar na raiz desta síndrome.

Todos estes achados foram usados como base para a elaboração de um manual de recomendações aos meliponicultores (Capítulo 5), os quais trouxeram aos pesquisadores o problema da síndrome anual das manducaias. A meliponicultura é uma atividade que tem tomado proporções cada vez maiores no Brasil, sendo que até 2015 estimava-se que haviam mais de 5.000 meliponicultores (Jaffé et al. 2015). A criação e manejo de abelhas sem ferrão pode ajudar comunidades como uma forma de renda adicional através da venda de produtos das colônias, e também incentiva a proteção de habitats naturais como fontes de alimento e locais de nidificação para as abelhas, assim como contribui na prestação de serviços de polinização da flora selvagem e de cultivares agrícolas (Slaa et al. 2006; Jaffé et al. 2015). A legislação atual, em nível nacional, impõe algumas regras aos meliponicultores, como o registro daqueles que possuem mais de 50 colônias, a

comercialização de colônias e produtos derivados apenas mediante autorização e restringe a translocação de colônias entre regiões (CONAMA, 2004). No entanto, além destas normas não serem suficientes para a preservação das espécies nativas, elas também não são estritamente seguidas, principalmente pela burocracia envolvida, como relatam os meliponicultores (Jaffé et al. 2015).

A translocação de colônias, por exemplo, é uma prática comum e um dos fatores que mais influencia nos padrões de diversidade genética entre espécies de abelhas sem ferrão das Américas (Jaffé et al. 2016). Alguns meliponicultores costumam comprar colônias de outras regiões do Brasil com o objetivo de fortalecer as colônias. No entanto, esta prática pode levar à homogeneização do perfil genético das populações e perda de adaptações locais importantes para a sobrevivência da espécie, como uma maior resistência a baixas temperaturas ou a agroquímicos (Jaffé et al. 2016). Nós observamos que a síndrome anual das mandaaias tem um componente genético, ou seja, há colônias mais suscetíveis aos efeitos subletais de estressores ambientais e também mais resistentes. A homogeneização do perfil genético das populações pode levar à perda do perfil genético destas colônias que respondem melhor aos fatores que contribuem para a ocorrência da síndrome. Por outro lado, certos genótipos também estão sendo grandemente expandidos através da comercialização de colméias obtidas por divisão, uma prática comum para obtenção de novas colônias (Jaffé et al. 2015). A mistura de genótipos através da translocação de colônias, combinada com a sua expansão pela divisão, altera completamente a estrutura genética das populações de abelhas sem ferrão. Esta prática pode ter afetado mais intensamente a mandaia, que é uma das espécies mais manejada historicamente (Jaffé et al. 2015). Isto, associado ao fato de que não há mais ninhos silvestres, com indivíduos capazes de fornecer genes às colônias manejadas, sugere que a questão genética é um fator importante para a ocorrência da síndrome.

Outro impacto resultante da translocação de colônias é a introdução de patógenos. Estudos realizados em diferentes estados do Brasil detectaram recentemente a presença de microsporídios, bactérias e vírus potencialmente patogênicos em abelhas sem ferrão (de Souza et al. 2019; Guimarães-Cestaro et al. 2020; Teixeira et al. 2020). Embora nem todas as colônias nas quais estes patógenos foram detectados apresentavam sintomas típicos de

cada infecção, em outros contextos a infecção por eles pode levar a diferentes desfechos. Sabe-se, por exemplo, que o DWV é mais virulento no inverno, levando ao colapso de inúmeras colônias de *A. mellifera* na Europa (Natsopoulou et al. 2017). Desta forma, caso este vírus seja trazido para o sul do Brasil, onde o inverno é mais rigoroso, ele poderia ser mais virulento e causar sintomas observáveis e danosos a diversas colônias. Além disso, há evidências de troca de patógenos entre abelhas sem ferrão e *A. mellifera*, inclusive em nosso estudo, sugerindo que ambas possam ser afetadas com a introdução de novos patógenos (Caesar et al. 2019; de Souza et al. 2019; Guimarães-Cestaro et al. 2020).

Outra prática bastante usada na meliponicultura é a suplementação alimentar de colônias, principalmente em períodos de maior escassez de recursos. Nós constatamos que a perda de peso das abelhas, possivelmente relacionada a competição por recursos, é um fator implicado na ocorrência da síndrome das mandaias. Porém, ao mesmo tempo que fornecer alimento às abelhas pode fortalecer as colônias para o período da síndrome, a suplementação alimentar também irá mascarar colônias geneticamente suscetíveis à mortalidade anual. Além disso, a dieta natural das abelhas é bastante equilibrada, enquanto o néctar fornece a principal fonte de carboidratos, o pólen fornece proteínas, lipídios e outros micronutrientes (Vaudo et al. 2015). Em *A. mellifera* e *B. terrestris* já foi mostrado que a atividade de forrageio é altamente regulada de acordo com as necessidades das colônias (Paoli et al. 2014; Stabler et al. 2015). Portanto, o uso de xarope e pólen apícola como suplementação alimentar deve ser evitado, e se for feito, é de extrema importância que o meliponicultor tome medidas sanitárias adequadas. Um estudo recente, que diagnosticou pela primeira vez a bactéria *M. plutonius* e os sintomas da doença conhecida como cria pútrida européia em *Melipona* spp., mostrou a presença do patógeno no pólen apícola oferecido às colônias que se infectaram (Teixeira et al. 2020).

A melhor forma de garantir a qualidade nutricional das colônias é promover pasto apícola diverso nos arredores do meliponário, proporcional à quantidade de colônias manejadas, como através da preservação de matas nativas (Vaudo et al. 2015). Com isso também será possível amenizar os efeitos da competição por recursos. Um estudo conduzido em região de Mata Atlântica mostrou que *M. quadrifasciata* e *A. mellifera* competem pelos recursos das mesmas flores, e o pico de coleta da abelha sem ferrão

ocorreu quando a abelha melífera não estava competindo pelo mesmo recurso (Wilms e Wiechers 1997). Com uma menor sobreposição dessas duas espécies também haverá uma diminuição no risco da troca de patógenos.

Outro fator de risco às mandaçaias, e mesmo a outras abelhas sem ferrão, é o uso de agroquímicos. Nossos resultados indicam diferenças entre colônias na capacidade de detoxificação destes compostos, o que por sua vez influencia no desfecho da síndrome. Por mais que os agroquímicos sejam ministrados de forma correta e nenhuma letalidade seja observada no período de aplicação, efeitos subletais podem afetar a saúde das colônias, e apenas tardiamente serem observados os efeitos. Em *B. terrestris* expostas à neonicotinóides durante qualquer um dos estágios do desenvolvimento há uma redução no desenvolvimento de partes do cérebro das abelhas que, apenas semanas depois, quando adultas, apresentaram uma resposta menor ao oferecimento de açúcar e dificuldade na aprendizagem (Smith et al. 2020). Portanto, é importante evitar o uso de agroquímicos nas proximidades do meliponário ou áreas de forrageio.

Por fim, concluímos que a síndrome anual das mandaçaias é resultado da combinação de efeitos subletais de múltiplos fatores, como a genética da colônia, competição por recursos, exposição a patógenos e agroquímicos. Nós acreditamos que há formas de amenizar os efeitos da mortalidade anual das mandaçaias, e que preservando a espécie *M. quadrifasciata* estaremos também contribuindo para a manutenção de outras abelhas sem ferrão que possam estar sofrendo pela ação dos mesmos fatores.

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ANEXO I

ID amostra	Status de saúde	Colônia	Amostra da colônia	Mês	DNA ng/ul	260/280
41	saudável	PA1	1	Jan	63,8	2,13
42	saudável	PA1	2	Jan	65	2,19
43	saudável	PA1	3	Jan	120,2	2,13
44	saudável	PA1	4	Jan	75,3	2,1
45	saudável	PA1	5	Jan	106,7	2,17
51	doente	PA2	1	Jan	71,5	2,15
52	doente	PA2	2	Jan	107,3	2,18
53	doente	PA2	3	Jan	128,4	2,15
54	doente	PA2	4	Jan	116,1	2,15
55	doente	PA2	5	Jan	139,1	2,12
56	doente	PA3	1	Jan	178,4	2,19
57	doente	PA3	2	Jan	199,7	2,21
58	doente	PA3	3	Jan	261,2	2,18
59	doente	PA3	4	Jan	278,7	2,13
60	doente	PA3	5	Jan	274,4	2,17
61	doente	BP3	1	Jan	245,5	2,16
62	doente	BP3	2	Jan	213,1	2,16
63	doente	BP3	3	Jan	267,2	2,14
64	doente	BP3	4	Jan	303,4	2,16
65	doente	BP3	5	Jan	226,7	2,21
66	doente	BP2	1	Jan	126,3	2,08
67	doente	BP2	2	Jan	118,1	2,12
68	doente	BP2	3	Jan	146,7	2,19
69	doente	BP2	4	Jan	84,3	2,19
70	doente	BP2	5	Jan	114,4	2,06
76	saudável	BP1	1	Jan	74,7	2,17
77	saudável	BP1	2	Jan	115	2,17
78	saudável	BP1	3	Jan	111,3	2,13
79	saudável	BP1	4	Jan	106,2	2,17
80	saudável	BP1	5	Jan	66,2	2,2
81	saudável	PA1	1	Fev	76,6	2,09
82	saudável	PA1	2	Fev	36,6	1,94
83	saudável	PA1	3	Fev	61,5	2,1
84	saudável	PA1	4	Fev	119,4	2,04
85	saudável	PA1	5	Fev	37,3	1,83
91	doente	PA2	1	Fev	59	2,15
92	doente	PA2	2	Fev	78,2	2,17
93	doente	PA2	3	Fev	74,1	2,28
94	doente	PA2	4	Fev	64,6	2,29
95	doente	PA2	5	Fev	70,3	2,24
96	doente	PA3	1	Fev	98,6	2,24
97	doente	PA3	2	Fev	158,5	2,17
98	doente	PA3	3	Fev	179,4	2,2
99	doente	PA3	4	Fev	152,9	2,21
100	doente	PA3	5	Fev	191	2,18
101	saudável	BP1	1	Fev	156,5	2,18
102	saudável	BP1	2	Fev	113,5	2,19
103	saudável	BP1	3	Fev	115,8	2,15
104	saudável	BP1	4	Fev	123,4	1,92
105	saudável	BP1	5	Fev	179,8	2,19
111	doente	BP2	1	Fev	106,3	2,19
112	doente	BP2	2	Fev	108,7	2,13
113	doente	BP2	3	Fev	61,8	1,98
114	doente	BP2	4	Fev	155,3	2,14
115	doente	BP2	5	Fev	111,8	2,14

116	saudável	BP1	1	Fev	102,6	2,12
117	saudável	BP1	2	Fev	71,5	2,18
118	saudável	BP1	3	Fev	111	2,15
119	saudável	BP1	4	Fev	77,9	2,04
120	saudável	BP1	5	Fev	58,3	1,97
121	saudável	PA1	1	Mar	100	2,25
122	saudável	PA1	2	Mar	100,8	2,24
123	saudável	PA1	3	Mar	132,2	2,25
124	saudável	PA1	4	Mar	160,6	2,23
125	saudável	PA1	5	Mar	152,8	2,14
131	doente	PA2	1	Mar	275,5	2,22
132	doente	PA2	2	Mar	153,3	2,25
133	doente	PA2	3	Mar	129,7	2,22
134	doente	PA2	4	Mar	141,3	2,25
135	doente	PA2	5	Mar	339,7	2,21
136	doente	PA3	1	Mar	148,8	2,13
137	doente	PA3	2	Mar	151,7	2,31
138	doente	PA3	3	Mar	150,8	2,27
139	doente	PA3	4	Mar	87,4	2,22
140	doente	PA3	5	Mar	113,6	2,25
141	doente	BP3	1	Abr	149,6	2,09
142	doente	BP3	2	Abr	118,8	2,25
143	doente	BP3	3	Abr	125,8	2,23
144	doente	BP3	4	Abr	149	2,3
145	doente	BP3	5	Abr	106,1	2,26
146	doente	BP2	1	Abr	115,5	2,24
147	doente	BP2	2	Abr	141,7	2,25
148	doente	BP2	3	Abr	102,6	2,3
149	doente	BP2	4	Abr	84,6	2,33
150	doente	BP2	5	Abr	73,7	2,36
156	saudável	BP1	1	Abr	90,9	2,36
157	saudável	BP1	2	Abr	146,9	2,27
158	saudável	BP1	3	Abr	118,2	2,32
159	saudável	BP1	4	Abr	48,6	2,29
160	saudável	BP1	5	Abr	138,6	2,34
161	saudável	PA1	1	Abr	54,4	2,12
162	saudável	PA1	2	Abr	42,6	2,33
163	saudável	PA1	3	Abr	59,5	2,16
164	saudável	PA1	4	Abr	100,3	2,27
165	saudável	PA1	5	Abr	40,6	2,29
171	doente	PA2	1	Abr	38,5	2,17
172	doente	PA2	2	Abr	80	2,38
173	doente	PA2	3	Abr	60,6	2,21
174	doente	PA2	4	Abr	35,3	2,21
175	doente	PA2	5	Abr	59,8	2,24
176	doente	PA3	1	Abr	60,6	2,14
177	doente	PA3	2	Abr	54,7	2,17
178	doente	PA3	3	Abr	64,6	2,24
179	doente	PA3	4	Abr	60,3	2,16
180	doente	PA3	5	Abr	78,2	2,36
181	saudável	BP1	1	Mar	176,7	2,26
182	saudável	BP1	2	Mar	149	2,31
183	saudável	BP1	3	Mar	127,6	2,23
184	saudável	BP1	4	Mar	137,6	2,28
185	saudável	BP1	5	Mar	135,3	2,28
191	doente	BP2	1	Mar	79,2	2,2
192	doente	BP2	2	Mar	80,7	2,3

193	doente	BP2	3	Mar	99,1	2,28
194	doente	BP2	4	Mar	165,3	2,28
195	doente	BP2	5	Mar	105,5	2,38
196	doente	BP3	1	Mar	133,7	2,29
197	doente	BP3	2	Mar	89,8	2,29
198	doente	BP3	3	Mar	82,8	2,23
199	doente	BP3	4	Mar	140,8	2,3
200	doente	BP3	5	Mar	62,1	2,33

ANEXO II

Colônia	Espécie	Status de saúde	Operárias	Larvas	Pupas	Ovos	Rainhas	Ácaros	Formigas	Fezes	Mel	Pólen
BLA	<i>M. quadrifasciata</i>	Doentes	5		10			x		x	x	
BLB	<i>M. quadrifasciata</i>	Doentes	5		10			x	x	x	x	x
BLC	<i>M. quadrifasciata</i>	Doentes - com carvão ativado	5		10			x			x	x
BLD	<i>M. bicolor</i>	Doentes	5		5				x		x	x
BLE	<i>M. scutellaris</i>	Doentes	5		6			x	x		x	x
BLF	<i>M. seminigra</i>	Saudáveis	5							x	x	x
BLG	<i>M. seminigra</i>	Doentes	5		10			x		x	x	x
BLH	<i>M. scutellaris</i>	Doentes - com carvão ativado	5		4					x	x	x
BLI	<i>S. postica</i>	Doentes	5		5						x	x
BLJ	<i>M. quadrifasciata</i>	Saudáveis – no cativeiro	5		10			x			x	
BLK	<i>M. quadrifasciata</i>	Saudáveis – no cativeiro	5	10				x			x	
BLL	<i>M. scutellaris</i>	Saudáveis – no cativeiro	5									
BLM	<i>M. seminigra</i>	Saudáveis – no cativeiro	5					x			x	
BLN	<i>A. melifera</i>	Saudáveis	5									x
BLO	<i>A. melifera</i>	Saudáveis	5									x
BLP	<i>A. melifera</i>	Saudáveis	5									x
BLQ	<i>M. quadrifasciata</i>	Doentes	5	10	10	10	1				x	x
BLR	<i>M. quadrifasciata</i>	Doentes	5	10	10	10	1				x	x