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GENÔMICA COMPARATIVA DE UM BACULOVÍRUS ISOLADO DA LAGARTA PRAGA *Rachiplusia nu* (GUENÉE) (LEPIDOPTERA: NOCTUIDAE)

Santa Maria, RS
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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciências Biológicas: Bioquímica Toxicológica.**

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Santa Maria, RS
2019

DEDICATÓRIA

Às minhas mães Simone e Nilsa

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RESUMO

GENÔMICA COMPARATIVA DE UM BACULOVÍRUS ISOLADO DA LAGARTA PRAGA *Rachiplusia nu* (GUENÉE) (LEPIDOPTERA: NOCTUIDAE)

AUTORA: Luana Beló Trentin
ORIENTADOR: Daniel Mendes Pereira Ardisson-Araújo

Descrevemos um novo baculovírus isolado da praga polífaga *Rachiplusia nu*. O vírus apresentou corpos de oclusão em forma de pirâmide (OBs) com um único nucleocapsídeos por envelope e uma dose de mortalidade de $6{,}9 \times 10^3$OBs / ml a larvas de terceiro instar de *R. nu*. O genoma do vírus possui 128.587 pb de comprimento, com um teor de G + C de 37,9% e 134 ORFs preditas. O vírus é um alphabaculovirus intimamente relacionado com Trichoplusia ni single nucleopolyhedrovirus, Chrysodeixis chalcites nucleopolyhedrovirus e Chrysodeixis includens nucleopolyhedrovirus. Surpreendentemente, encontramos co-evolução entre os vírus relacionados e seus hospedeiros em nível de espécie. Além disso, foram encontrados genes auxiliares com homólogos em outros baculovírus, por exemplo uma *CPD fotoliase*. O gene pareceu ser o resultado de um único evento de transferência horizontal de lepidópteros para alphabaculovírus, seguido por uma transferência de alfa para betabaculovírus. A proteína prevista parece ser uma enzima ativa que garante a proteção do DNA contra a luz solar.

Palavras-chave: Genômica. Baculovírus. *Alphabaculovirus. Rachiplusia nu nucleopolyhedrovirus*. Fotoliase.

ABSTRACT

GENÔMICA COMPARATIVA DE UM BACULOVÍRUS ISOLADO DA LAGARTA PRAGA *Rachiplusia nu* (GUENÉE) (LEPIDOPTERA: NOCTUIDAE)

AUTHOR: LUANA BELÓ TRENTIN
ADVISOR: DANIEL MENDES PEREIRA ARDISSON-ARAÚJO

We described a novel baculovirus isolated from the polyphagous insect pest *Rachiplusia nu*. The virus presented pyramidal-shaped occlusion bodies (OBs) with singly-embed nucleocapsids and a dose mortality response of 6.9×10^3OBs/ml to third-instar larvae of *R. nu*. The virus genome is 128,587 bp long with a G + C content of 37.9% and 134 predicted ORFs. The virus is an alphabaculovirus closely related to Trichoplusia ni single nucleopolyhedrovirus, Chrysodeixis chalcites nucleopolyhedrovirus, and Chrysodeixis includens single nucleopolyhedrovirus and may constitute a new species. Surprisingly, we found co-evolution among the related viruses and their hosts at species level. Besides, auxiliary genes with homologs in other baculoviruses were found, *e.g.* a *CPD-photolyase*. The gene seemed to be result of a single event of horizontal transfer from lepidopterans to alphabaculovirus, followed by a transference from alpha to betabaculovirus. The predicted protein appears to be an active enzyme that ensures likely DNA protection from sunlight.

Keywords: Genomic. Baculovirus. *Alphabaculovirus. Rachiplusia nu nucleopolyhedrovirus*. Photolyase.

LISTA DE ILUSTRAÇÕES

INTRODUÇÃO

ARTIGO 1

LISTA DE ABREVIATURAS E SIGLAS

ºC	graus *Celsius*
6-4PP	pirimidina-pirimidona fotoprodutos
8-HDF	8-hidroxi-7,8-didemethyl-5-deazaflavina
x *g*	velocidade de sedimentação gravitacional
AcMNPV	*Autographa californica multiple nucleopolyhedrovirus*
BV	do inglês, "budded vírus" (vírus brotado)
cDNA	DNA sintetizado a partir de um RNA mensageiro
ChchNPV	*Chrysodeixis chalcites nucleopolyhedrovirus*
ChinSNPV	*Chrysodeixis includens single*
CPD	dímeros de pirimidina
DNA	Ácido desoxirribonucléico
dNTP	2'-deoxinucleotídeos-5'-trifosfatos
EDTA	ácido etileneodiamina tetraacético
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
FAD	dinucleótido de flavina e adenina
g	grama
GV	*Granulovirus*
h	horas
h p. i.	horas pós-infecção
kDa	kiloDalton
kpb	kilo-pares de bases
lef-8	fator de expressão tardio 8
lef-9	fator de expressão tardio 9
lef-12	fator de expressão tardio 12
min	minutos
ml	mililitros
mM	milimolar
MNPV	*multiple nucleopolyhedrovirus*
MTHF	5,10 metentiltetrahidrofolato
NaCl	cloreto de sódio
ng	nanogramas
NPV	*Nucleopolyhedrovirus*
OB	do inglês, "occlusion body"
ODV	do inglês "occluded vírus

ORF	fase de leitura aberta (do inglês, “open reading frame”)
PBS	do inglês, “Phosphate buffered saline”
pb	pares de base
pH	concentração de íon hidroxônio livre
PIB	do inglês, “polyhedral inclusion bodies”
PIF	do inglês, “*per os* infectivity fator”
RanuNPV	Rachiplusia nu nucleopolyhedrovirus
RNA	ácido ribonucleico
rpm	rotação por minuto
RT-PCR	do inglês, “Reverse transcription polymerase chain”
SDS	do inglês, sodium dodecyl sulfate
SNPV	*Single Nucleopolyhedrovirus*
SfMNPV	*Spodoptera frugiperda multiple nucleopolyhedrovirus*
ssDNA	desoxirribonucleato de fita-simples
ssRNA	ribonucleato de fita-simples
Taq	*Termus aquaticus*
Tris	2-amino-2-hidroximetil-propano-1,3-diol
TnSNPV	*Trichoplusia ni single nucleopolyhedrovirus*
µg	microgramas
µg/ml	microgramas/mililitro
µl	microlitro
µm	micrômetro
µM	micromolar
UV	radiação ultravioleta

SUMÁRIO

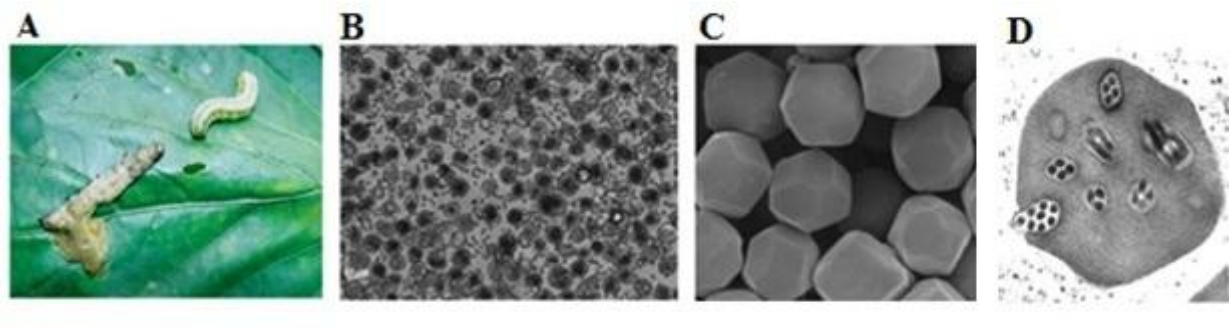
1 APRESENTAÇÃO

Esta dissertação aborda a montagem, descrição e caracterização do genoma de um novo baculovírus que está estruturada da seguinte forma: o item **INTRODUÇÃO**, contém uma breve revisão da literatura sobre os temas a serem abordados nesta dissertação; seguido dos **OBJETIVOS** do trabalho. Os **RESULTADOS** estão apresentados em forma de artigo com o título: "The complete genome of Rachiplusia nu nucleopolyhedrovirus (RanuNPV) and the identification of a baculoviral *CPD-photolyase* homolog". Este artigo contém as seções: **INTRODUCTION**; **MATERIAL AND METHODS; RESULTS AND DISCUSSION** e **CONCLUSION**. O item **CONCLUSÃO**, encontra-se no final desta dissertação. As **REFERÊNCIAS BIBLIOGRÁFICAS** correspondem somente às citações que aparecem no item **INTRODUÇÃO** da dissertação. As demais referências bibliográficas estão presentes no corpo do artigo publicado.

2 INTRODUÇÃO

A descoberta dos baculovírus está vinculada a prática chinesa milenar de produzir seda a partir de casulos de *Bombyx mori*, comumente conhecido como bicho-da-seda (ROHRMANN, 2013). Este vírus está entre os vários patógenos que afligem a sericultura no Brasil e no mundo, causando a doença conhecida desde 1.800 como poliedrose viral (Figura 1A) (ARDISSON- ARAÚJO, 2014). O termo poliedrose foi cunhado com base na presença de corpos de oclusão altamente refratáveis em microscopia de luz com o formato de poliedro (Figura 1B) presentes no extrato de lagartas mortas. No entanto, embora a presença de partículas infecciosas dentro dos corpos de oclusão poliédricos (Figura 1C) tenha sido sugerida anteriormente, foi somente no final de 1940 que a presença de vírions em forma de bastão foi demonstrada de forma convincente por microscopia eletrônica de transmissão (Figura 1D) (BERGOLD, 1947).

Figura 1- Morte característica de lagartas causada por infecção com baculovírus.

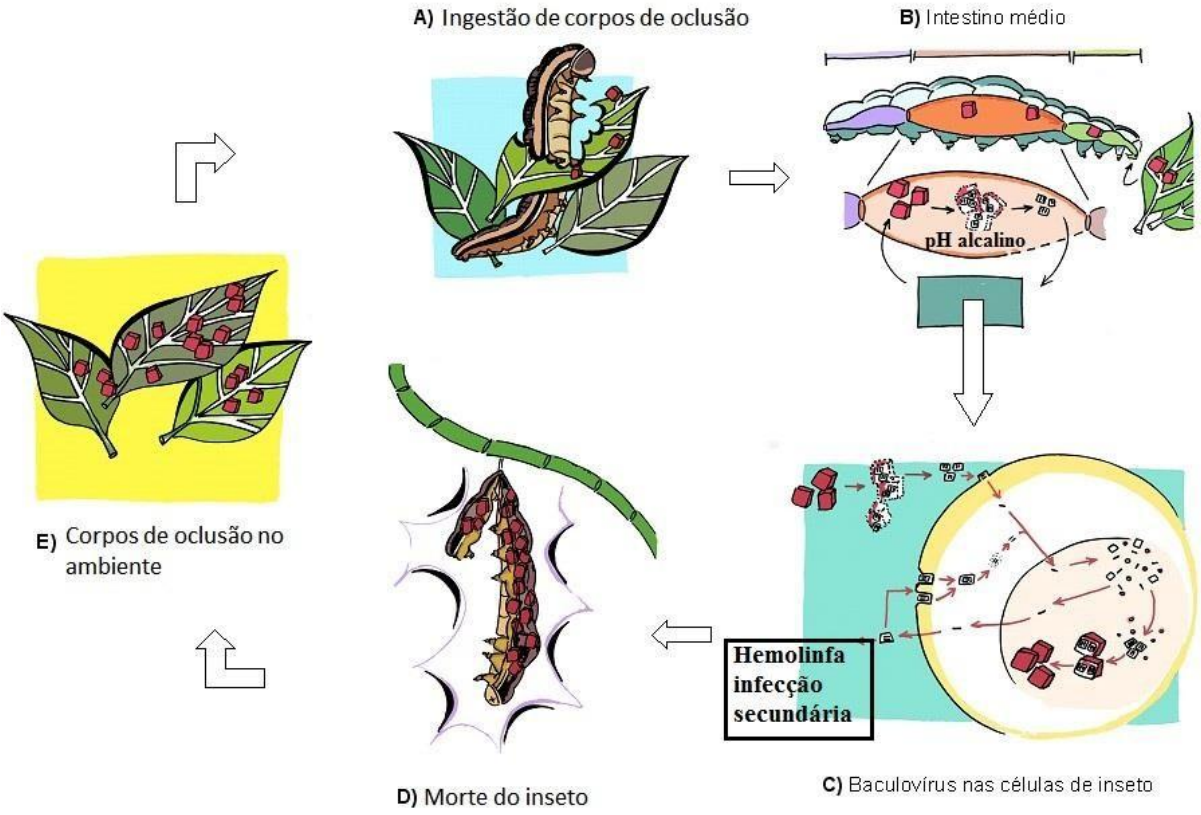


Fonte: Modificado de acervo pessoal e REBOLLEDO, Dulce *et al*., 2015.
(A) A poliedrose causa liquefação do corpo da larva. Na figura, larva de *Spodoptera exigua* morta por baculovírus em estado de liquefação e uma larva sadia (fonte: DAVID NANCE/USDA AGRICULTURAL RESEARCH SERVICE/BUGWOOD.ORG). (B) Fotografia de corpos de oclusão liberados no meio vistos por microscopia óptica e dentro de células hospedeiras (Fonte: Modificado de acervo pessoal). (C) Microscópia eletrônica mostrando a estrutura poliédrica dos vírus (D) Microscópia de transmissão mostrando a presença de vírions dentro dos corpos de oclusão.

O nome baculovírus foi proposto por Mauro Martignoni, sendo um nome derivado do latim *baculo* que significa bastão, devido ao formato do nucleocapsídeo viral. O nucleocapsídeo contém uma cópia do genoma de DNA dupla-fita circular com tamanho variando entre 80 e 180 kpb circundado por uma bicamada lipoprotéica, denominada envelope (ROHRMANN, 2013). Os baculovírus pertencem a família *Baculoviridae*, composta por quatro gêneros: *Alphabaculovirus* e *Betabaculovirus* infectivos para lepidópteros (estágio larval de borboletas e mariposas); *Gammabaculovirus* infectivos para himenópteros (estágio larval de vespas com comportamento semelhante ao de lagartas) e *Deltabaculovirus* infectivos para dípteros (estágio larval de mosquitos) (JEHLE *et al*, 2006b).

Em termos gerais, a infecção começa com o inseto ingerindo o vírus na forma de corpo de oclusão (OB, do inglês ‘occlusion body’) (Figura 2- A) que estabelece a infecção no intestino médio da larva (Figura 2-B). O vírus replica inicialmente neste órgão e posteriormente espalha a doença ao longo do corpo do hospedeiro (Figura 2-C). A doença culmina com a morte da larva, produção de mais OBs (Figura 2-D) e liquefação do cadáver como mecanismo de espalhamento ambiental (Figura 2- E). Os corpos de oclusão podem ser pulverizados numa plantação para controle de insetos praga. Atualmente, os baculovírus são utilizados no controle biológico de insetos praga por ser considerados seguros, seletivos e restritos a insetos (MOSCARDI, 1999).

Figura 2- Visão geral da infecção por baculovírus.



Fonte: Modificado de BARROS, 2012.
(A) O vírus é ingerido na forma de corpo de oclusão de alimento contaminado naturalmente ou por pulverização de lavoura. (B) O vírus atinge o intestino da lagarta. (C) replica e se espalha ao longo do corpo do inseto. (D) A replicação culmina com a morte do inseto que vira um saco de corpos de oclusão. (E) O inseto liquefaz e libera novas partículas infectivas no ambiente, fechando o ciclo.

O processo infeccioso por baculovírus produz dois tipos de fenótipos virais. Estes fenótipos são responsáveis por diferentes estágios da infecção pelo vírus. O primeiro fenótipo é o vírion derivado de oclusão (ODV, do inglês ‘occlusion-derived virion’) que é responsável

pela infecção primária oral e o segundo fenótipo é o vírion extracelular ou brotado (BV, do inglês, 'budded virion'), responsável pela disseminação da infecção ao longo do corpo do inseto hospedeiro e o estabelecimento da infecção secundária (CLEM & PASSARELI, 2013). Importante destacar que os ODVs estão oclusos num corpo cristalino proteico chamado OB, a mesma estrutura que levou a descoberta e ao nome da patologia causada por baculovírus (*i.e.* poliedrose). A principal função dos OBs é estabilizar os vírions no ambiente. OBs são constituídos principalmente por uma proteína única produzida em grande quantidade pela célula infectada que forma uma matriz cristalina ao redor dos vírions. Esta proteína pode ser a poliedrina ou granulina variando conforme o gênero do baculovírus. Os OBs possuem a função de garantir a viabilidade das partículas virais em função das adversidades ambientais (luz UV e umidade) até que sejam ingeridos por um hospedeiro. Assim, estes iniciam o ciclo de infecção quando as larvas consomem folhas, ramos, frutos, caules ou água contaminados (SLACK & ARIF, 2007) (Figura 3A.1), sendo em seguida dissolvidos em condições alcalinas (pH 10-11) (Figura 3A.2) (TERRA & FERREIRA, 1994) do intestino médio dos insetos. A dissolução da matriz leva a liberação dos ODVs (Figura 3A.3) e o estabelecimento da infecção primária do vírus em células epiteliais do intestino médio do hospedeiro. Para isto, os ODVs atravessam a membrana peritrófica (recobre todo o lúmen do intestino e serve de proteção inata contra injúrias dos alimentos e patógenos) (Figura 3A.4).

Os ODVs se fundem às membranas das microvilosidades (Figura 3B.1) (HORTON & BURAND,1993; HAAS-STAPLETON *et al.*, 2004). Este processo é mediado principalmente por proteínas do complexo PIFs (fatores de infecção "per os"), que estão presentes no envelope do ODV (PENG *et al*., 2012). A fusão leva a liberação dos nucleocapsídeos no citoplasma da célula (Figura 3B.2) que migram deste compartimento para a membrana do núcleo via filamentos de actina, passando pelo poro nuclear (Figura 3B-3) (GOLEY *et al*., 2006; OHKAWA *et al*., 2005). Os vírions possuem uma dimensão de 30-60 nm de diâmetro (JEHLE *et al*., 2006b) e atravessam o poro nuclear de dimensão entre 38-78 nm (ALBER et al., 2007). Os nucleocapsídeos são desmontados e o genoma do DNA viral é liberado no núcleo (Figura 3B.4). Os promotores dos genes virais são expostos e dentro de 30 minutos após a entrada no vírus na célula, dá-se início a fase precoce da infecção (CHISHOLM & HENNER, 1988).

Genes virais precoces são transcritos pela RNA polimerase II do hospedeiro (Figura 3B.5). Os mRNAs são enviados para o citosol onde ocorre tradução e produção de proteínas funcionais (Figura 3B.6, 7). As proteínas da fase precoce, juntamente com proteínas associadas ao vírion (que foram expostas durante a desmontagem do nucleocapsídeo no

núcleo) são responsáveis por subverter a maquinaria da célula hospedeira e preparar o ambiente para a fase tardia ou fase de replicação do genoma viral e montagem de novos vírions (Figura 3B. 8) (ROHRMANN, 2013; SLACK & ARIF, 2007). A fase tardia da infecção ocorre em média 12 h pós-infecção (p.i.), iniciando-se com a hipertrofia nuclear com a expansão do estroma virogênico (região de montagem viral) que preenche a maior parte do núcleo (SLACK & ARIF, 2007).

Assim, a fase tardia é marcada pela produção exacerbada de vírions na forma de BV que se estende de 12-20 h p.i.. Os nucleocapsídeos montados no núcleo migram para fora do estroma virogênico, através da zona do anel da membrana nuclear até o citosol (Figura 3B.9), onde adquirem um envelope da membrana plasmática para produzir BV (nucleocapsídeo mais envelope) (Figura 3B.10) (AU; WU; PANTE, 2013; SLACK & ARIF, 2007). O envelope que constitui o BV possui uma região denominada de peplômero que é formada pela proteína de fusão de envelope GP64 ou proteína F (Figura 3B.11) (VOLKMAN *et al*., 1984), a depender do tipo viral. Em seguida, esse vírion rapidamente brota do intestino médio em direção a hemolinfa do inseto (análoga ao sangue) para infectar outros tecidos (Figura 3B.12,13).

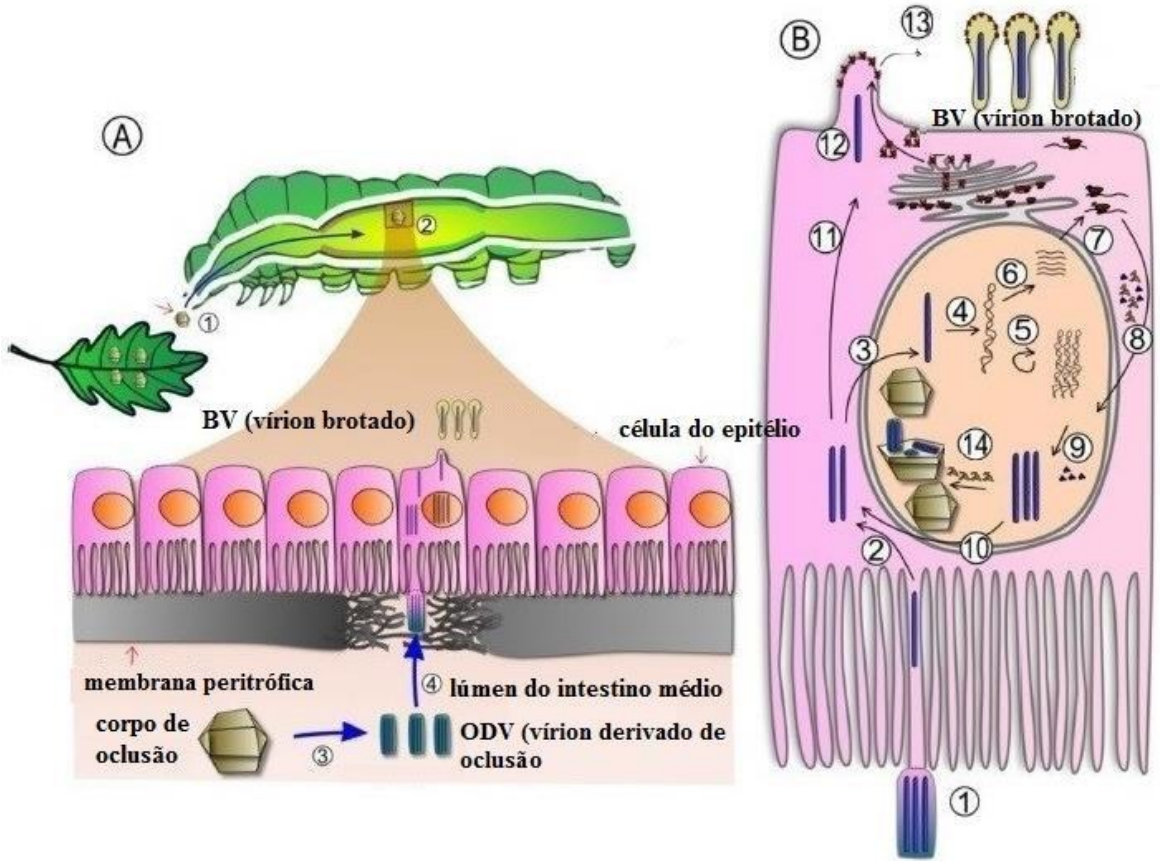
A proteína GP64 é encontrada nos *Alphabaculovirus* do grupo I (WHITFORD *et al*., 1989; ROHRMANN, 2013) e a proteína de fusão ou proteína F é encontrada tanto em *Alphabaculovirus* quanto em *Betabaculovirus* e *Deltabaculovirus*. Estas duas classes de proteínas são responsáveis pela capacidade que os BVs têm de infectar novas células. A infecção de células sadias por BV se dá por endocitose adsortiva induzida pela proteína de envelope. O BV é endocitado pela célula e, mediante diminuição do pH da vesícula, ocorre fusão da membrana do envelope com a membrana do endossomo, mediada pela proteína de envelope. Em seguida, os nucleocapsídeos são liberados e transportados para o núcleo via filamentos de actina, para que assim se inicie uma nova replicação viral. Esta nova célula infectada passará pela etapa precoce e tardia, de modo a produzir mais BVs. Os BVs produzidos *de novo* brotam das células infectadas (Figura 3B-13) e continuam com o espalhamento da infecção entre os vários tecidos internos do hospedeiro, incluindo traqueoblastos, hemócitos e corpos gordurosos (BLISSARD & ROHRMANN, 1990; SLACK & ARIF, 2007; HORTON & BURAND, 1993).

As células produtoras de BV agora iniciam uma terceira fase da infecção. A fase muito tardia inicia-se 20 h p.i., onde o estroma virogênico recua e a infecção viral prioriza agora a produção de ODVs e não mais BVs. Assim, o retículo endoplasmático, a membrana nuclear externa, membrana nuclear interna e o complexo de poros nucleares funcionam como uma rede contínua de membranas, utilizadas para fornecer proteínas ao envelope do ODV

(BRAUNAGEL *et al*., 2007; SLACK & ARIF, 2007). A membrana nuclear interna fornece a bicamada lipídica para os ODVs. O processo de oclusão dos OBs começa a ocorrer 24 h p.i. e em torno de 48 h p.i. esses OBs são liberados dos núcleos de células infectadas, sendo disseminados para o ambiente externo por liberação lítica (Figura 3B.14, 15).

Como abordado anteriormente, os OBs possibilitam que os baculovírus permaneçam num estado adormecido e viável no meio ambiente (BERGOLD, 2012), uma vez que os vírions dos baculovírus possuem um envelope constituído de uma bicamada lipídica, que torna o vírus suscetível à dessecação e à perda de viabilidade fora do hospedeiro (COX, 1989). Entretanto, os OBs estão susceptíveis a irradiação por luz UV. A radiação UV causa um efeito drástico sobre o material genético das partículas virais (RAUTH, 1965) mediante a produção de fotoprodutos gerados por UV como dímeros de pirimidina (FRIEDBERG *et al*., 2005). As partículas virais que possuem seu genoma constituído por DNA são mais suscetíveis à formação de fotoprodutos (MURPHY & GORDON, 1981). Nesse sentido, formam-se dois tipos de lesões de DNA: dímeros de pirimidina ciclobutano (CPDs) e o fotoproduto 6-4 pirimidina-pirimidona (6-4 PPs) (SANCAR, 2004).

Figura 3- Ciclo viral no inseto hospedeiro.



Fonte: Modificado de: HAASE; SCIOCCO-CAP; ROMANOWSKI, 2015.

Ciclo infeccioso do baculovírus. **A.1-** Esquema transversal de uma larva de inseto. Um corpo de oclusão de baculovírus (OB) sendo ingerido por meio de alimentos contaminados. **A.2-** Quando os OBs atingem o intestino médio alcalino, a matriz proteica é dissolvida, **A.3-** liberando ODVs. **A.4-** A membrana peritrófica é degradada por metaloproteases presentes na matriz cristalina junto às partículas virais. **B.1-** Os ODVs entram na célula por fusão com microvílosidades das células epiteliais do intestino médio do hospedeiro, **B.2-** liberando os nucleocapsídeos para o citoplasma. **B. 3-** Em seguida vão para o núcleo, **B.4-** sendo desmontados e liberado o genoma. **B.5** Então os genes iniciais são transcritos **B.6,7** e traduzidos. **B. 8** Algumas das proteínas translocam para o núcleo, participando da transcrição / replicação do genoma. Estas proteínas também participam da montagem dos nucleocapsídeos no núcleo. **B.9-** Nos primeiros estágios da infecção viral, o nucleocapsídeo é transportado para o citoplasma, **B. 10-** onde adquirem um envelope na membrana plasmática. **B.11-** Os BVs possuem uma região pleolômerica formada pelas proteínas de fusão (EFPs) GP64 e F. **B.12,13-** O fenótipo BV brota das células infectadas. **B.14,15-** Na fase muito tardia da infecção, os nucleocapsídeos são envolvidos pela membrana nuclear interna e são ocluídos nos corpos de oclusão (OBs).

Lesões de DNA do tipo CPDs ocorrem por meio de duas ligações covalentes entre os carbonos 5 e 6, enquanto que as lesões de DNA do tipo 6-4 PPs ocorrem entre os carbonos 6 e 4, ocorrendo por meio de uma ligação covalente entre as bases pirimidinas adjacentes (MOURET *et al*., 2006; DUNN *et al*., 2006). As lesões induzidas por UV no DNA podem inibir a progressão da RNA polimerase, durante o processo de transcrição ou inibir a DNA polimerase no processo de replicação o que pode levar ao surgimento de mutações e atenuação da infecção viral (WEBER, 2005; YOU *et al*., 2001; CHOI & PFEIFER, 2005). Assim, existe uma via específica de reparo de DNA induzida por UV, denominada de fotorreativação que reverte os dímeros de pirimidina para a sua forma monomérica, sendo esta catalisada por uma enzima denominada de fotoliase, que utiliza a luz visível como fonte de energia para catalisar esse processo (SANCAR 1994, 2000). Surpreendentemente, alguns baculovírus foram identificados codificando em seu genoma genes homólogos a fotoliase/criptocromo. Fotoliases (*phr*) e criptocromos (*cry*) pertencem a mesma família de flavoproteínas muito parecidos estruturalmente, devido a presença de um domínio central conservado de cerca de 500 aminoácidos (HUANG et al., 2006; TOKUTOMI; MATSUOKA; ZIKIHARA , 2008). A função dos criptocromos (*cry*) e das fotoliases (*phr*) é distinta, uma vez que as fotoliases utilizam a luz visível para reparar o dano do DNA induzido pelo ultravioleta e os criptocromos utilizam a luz visível para o controle circadiano (SANCAR, 2004; CASHMORE, 2003). Neste sentido uma das características estruturais mais marcantes que separam funcionalmente os criptocromos das fotoliases é a presença de extensões C-

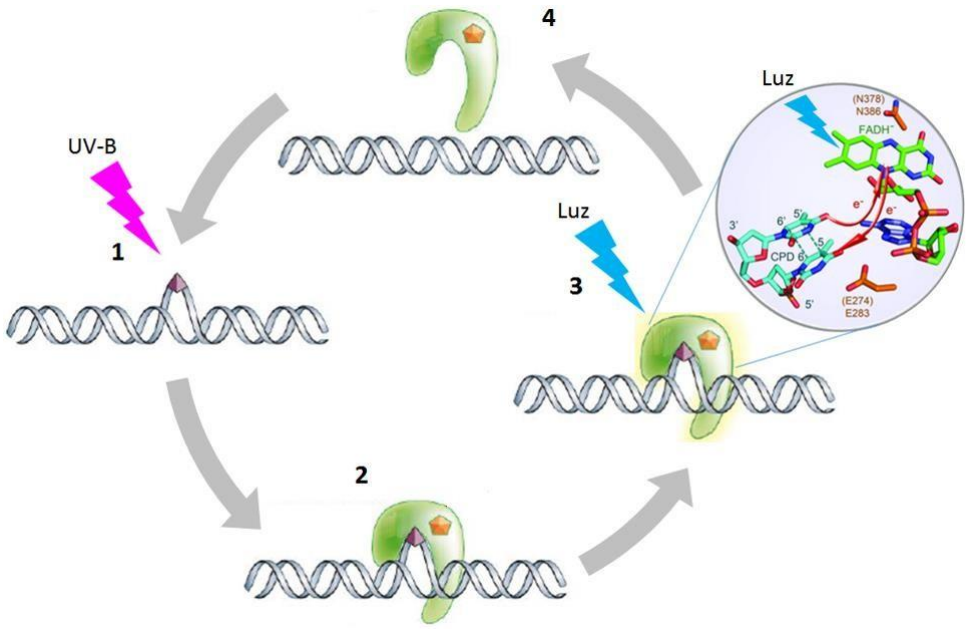
terminais diferentes (CHAVES et al., 2011).

As fotoliases podem ser divididas conforme a especificidade de seu substrato em CPD- fotoliase que repara lesões do tipo CPD e (6-4)-fotoliase que repara lesões do tipo (6-4) PPs no DNA (YASUI *et al*., 1994). As CPD-fotoliase estão presentes no genoma de baculovírus e podem ser ainda divididas em duas classes baseadas na identidade das sequências e filogenia (KANAI *et al*., 1997). As CPD-fotoliases de classe I estão presentes no genoma de eubactérias, fungos e arqueas halófilas (MCCREADY & MARCELLO, 2003). Enquanto que as CPD-fotoliases de classe II estão presentes no genoma de eucariotos, algumas eubactérias e agentes patogênicos como vírus e microsporídios parasitas de insetos (O'CONNOR *et al*., 1996; BENNETT *et al*., 2003; SRINIVASAN *et al*., 2001; SLAMOVITS & KEELING, 2004). Homólogos do gene da *CPD-fotoliase* de classe II são encontrados em vários baculovírus que, além de possuir a atividade fotorreativa, podem estar vinculados à atividade de controle do ciclo circadiano do hospedeiro (CHAVES *et al*., 2011), uma vez que já existem evidências de que os produtos de *phr1* e *phr2* de ChchNPV são capazes de interagir com a proteína CLOCK. No entanto, PHR2 se mostrou ser mais efetiva na ligação com a proteína, além de afetar a osciliação de fibroblastos embrionários de camundongos imortalizados, sugerindo que PHR2 pode regular o ciclo circadiano molecular (BIERNAT *et al*., 2012).

As CPD-fotoliases podem carregar dois cromóforos, sendo um destes o cofator catalítico o dinucleotídeo flavina adenina (FAD) que sempre está presente e o outro ou 5,10 metentiltetrahidrofolato (MTHF) ou 8-hidroxi-7,8-didemethyl-5-deazaflavin (8-HDF) que variam conforme a classe de CPD-fotoliase (DEISENHOFER, 2000; KLAR et al., 2006). Os cromóforos de antena MTH e 8-HDF não são essenciais para a função da enzima, mas podem aumentar a eficiência de absorção e ampliar o espectro de atividade da fotoliase (PAYNE & SANCAR, 1990; ESSEN & KLAR et al., 2006). Assim a incidência de luz UV no DNA pode levar a formação de lesões do tipo CPD no DNA, por meio de ligações covalentes entre as pirimidinas adjacentes ou vizinhas, sendo essas formadas principalmente pela incidência de luz UV-B (280-315 nm) (Figura 4-1). A CPD-fotoliase se liga ao dímero de pirimidina, formando um complexo estável (Figura 4-2) (BERG & SANCAR, 1998). Em seguida, o cromóforo FADH- (forma aniônica reduzida) absorve a luz em um comprimento de onda na ordem de 300-500, que é assim convertido para a forma excitada * FADH-. Assim, o * FADH- transfere um elétron para a lesão CPD de DNA levando a sua forma nativa, monómeros de pirimidina. O elétron é transferido de volta para restaurar a forma funcional de FADH-. (Figura 4-3), onde o DNA reparado é liberado da CPD-fotoliase e as duas cadeias de

DNA voltam a sua conformação nativa (Figura 4-4) (ESSEN, 2006; SANCAR, 2004; WEBER, 2005). A presença de um gene homólogo a CPD-fotoliase de classe II funcional em baculovírus parece ser uma estratégia adaptativa na luta contra os danos causados pela luz UV ao genoma viral.

Figura 4- O mecanismo de fotorreparo proposto para a CPD- fotoliase de classe II.



Fonte: Modificado de FRIEDBERG, 2005; ZHANG, WANG, ZHONG, 2017).

(1) O DNA exposto ao UV-B sofre lesões do tipo pirimidina *i.e* (CPDs). **(2)** Os dímeros de pirimidina CPD são reconhecidos e ligados pela CPD-fotoliase. **(3)** Após a absorção de luz em comprimentos de onda > 300 nm pelo cofator que assimila a energia da luz (FADH-), ocorre a transferência de um elétron para o dímero de pirimidina e os restaura para a conformação nativa. Por sua vez o FADH- é regenerado pelo elétron recebido a partir da conversão do dímero de pirimidina a monómeros de pirimidina. **(4)** O DNA é restaurado e ocorre a liberação da fotoliase.

A presença de um gene homólogo a CPD-fotoliase de classe II funcional em baculovírus parece ser uma estratégia adaptativa na luta contra os danos causados pela luz UV ao genoma viral. Atualmente, o controle de populações de insetos-praga, segundo Van Lenteren (2008), vem sendo realizado preferencialmente com a utilização de inseticidas químicos. Contudo, existe uma predisposição para se diminuir os investimentos e consumo dos mesmos, visto que os insetos podem desenvolver resistência aos princípios ativos dos produtos, além da intensa preocupação social relacionadas à conservação ambiental (FINKLER, 2013). Entretanto, um dos principais gargalos para o uso de baculovírus como

controlador eficiente de pragas da agricultura é sua fragilidade a luz UV, quando o vírus é administrado em lavouras.

O principal programa de controle biológico baseado em baculovírus aconteceu no Brasil para controle da lagarta da soja *Anticarsia gemmatalis*, com mais de dois milhões de hectares aplicados com formulados do vírus. Entretanto, com o uso incessante de outras medidas químicas para controle de fungos fitopatogênicos, houve uma mudança no cenário de importância de pragas e a *A. gemmatalis* deixou de ser a principal praga, sendo substituída pela lagarta *Chrysodeixis includens*. Atualmente, *C. includens* é a principal praga da soja junto a *A. gemmatalis* e outras espécies dentro do gênero *Spodoptera*. Esta lagarta pertence a subfamília Plusiinae, conhecida como lagartas falsa-medideira, uma vez que se desloca semelhante ao ato de medir com a palma da mão (SOSA-GÓMEZ, 2010). Junto a *C. includens*, outra falsa-medideira tem causado surtos ocasionais na cultura de soja e em várias outras culturas no Brasil, a lagarta da espécie *Rachiplusia nu* (Guenée, 1852) (Lepidoptera: Noctuidae, Plusiinae). *R. nu* é uma espécie polífaga amplamente distribuída na América do Sul (YOUNG & YEARIAN, 1983), ocorrendo na Argentina, Bolívia, Brasil, Chile, Paraguai, Peru e Uruguai. Os prejuízos causados por *Rachiplusia nu*, além da soja, estendem-se a várias culturas de alto valor econômico agregado como girassol (*Helianthus annus*); alfafa (*Medicago sativa*); algodão (*Gossypium hirsutum*); feijão (*Phaselous vulgaris*); linho (*Linum usitatissimum*) e tabaco (*Nicotiana tabacum*) (BARRIONUEVO et al., 2012).

O controle da espécie *R. nu* tem sido realizado preferencialmente por meio da utilização de inseticidas químicos, porém esta estratégia de controle tem se mostrado falha, uma vez que a espécie apresenta tolerância a vários químicos comumente utilizados (MASCARENHAS & BOETHEL, 2000). Neste sentido, existe uma necessidade de estudos que caracterizem novas espécies de baculovírus para o controle desta praga, devido às perdas econômicas que esta praga pode causar em várias culturas. Assim, na década de 1990 pesquisadores brasileiros, argentinos e uruguaios realizaram um projeto cooperativo com o objetivo de avaliar isolados de baculovírus que poderiam ser usados para controle de *R. nu* (MOSCARDI, F. & SOSA-GOMEZ, 1992). Na Argentina, já existe um estudo que caracterizou o genoma parcial de um baculovírus isolado de *R. nu*, denominado de Rachiplusia nu multiple nucleopolyhedrovirus (RanuMNPV), sendo este uma variante do vírus da espécie *Autographa californica multiple nucleopolyhedrovirus* (AcMNPV), mas com um espectro de hospedeiro diferente (RODRÍGUEZ, V.A *et al*., 2012).

As limitações encontradas relacionadas ao controle da praga *R. nu* tornam importantes os estudos que visam a caracterização de inseticidas alternativos como é o caso dos

biopesticida a base de baculovírus (THIEM, 2009). A Embrapa-Soja dispõe de uma coleção com quase 100 extratos de lagartas que apresentam características de infecção por baculovírus. Estas larvas foram isoladas em todo território brasileiro, mas principalmente no sul do país, e depositadas na coleção da Embrapa. Em colaboração, foi-nos enviado um extrato de indivíduos da espécie *R. nu* obtidos do campo e mortos com sintoma de infecção por baculovírus para caracterização. A base para o início de quaisquer estudos moleculares mais detalhados de novas espécies virais com potencial uso bioinseticida se inicia com sequenciamento do genoma completo. Assim, com o avanço das técnicas de sequenciamento de alto desempenho, novos genomas de baculovírus surgem de forma crescente permitindo um entendimento mais profícuo da história evolutiva da família viral. Além disso, é importante salientar que os dados gerados com sequenciamento influenciam diretamente no uso de baculovírus como agentes de controle biológicos bem como em seu melhoramento como vetor de expressão heteróloga. Nesta dissertação, foi sequenciado e caraterizado o genoma completo de um baculovírus isolado da espécie *R. nu*, aqui chamado Rachiplusia nu nucleopolyhedrovirus (RanuNPV). Descobriu-se que o vírus é uma nova espécie dentro do gênero *Alphabaculovirus*, completamente diferente daquele isolado na Argentina. Além disso, esse novo vírus contém em seu genoma um homólogo de uma CPD-fotoliase da classe II em seu genoma.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Caracterizar o genoma de um baculovírus isolado da espécie praga da soja *Rachiplusia nu* afim de entender a resistência a intempéries ambientais, interação vírus-hospedeiro e seu contexto evolutivo, bem como contribuir com o desenvolvimento de agentes efetivos de controle biológico de pragas.

3.2 OBJETIVOS ESPECÍFICOS

- Sequenciar por pirosequenciamento 454 (Roche) o genoma completo do baculovírus isolado da lagarta R. nu, aqui denominado Rachiplusia nu nucleopolyhedrovirus (RanuNPV).
- Montar o genoma completo de RanuNPV usando o método ‘de novo assembly’ com um algoritmo próprio implementado no programa Geneious 9.0.5.
- Descrever, caracterizar in silico, identificar e anotar todas as ORFs encontradas no genoma de RanuNPV por BLASTX e HMMER.
- Estabelecer as relações filogenéticas da nova espécie de baculovírus com as demais espécies já descritas.
- Analisar peculiaridades do genoma de RanuNPV como a presença de ORFs únicas.
- Aplicar o critério de demarcação de novas espécies a fim de descobrir se RanuNPV é uma nova espécie dentro da família Baculoviridae.
- Analisar in silico o gene homólogo de CPD-fotoliase da classe II a fim de estabelecer as relações filogenéticas do gene com espécies proximamente relacionadas, identificar aminoácidos conservados, avaliar a conservação em nível estrutural da proteína (secundária e terciária) por modelagem por homologia.

4 RESULTADOS

A metodologia, os resultados e a discussão inseridos nesta dissertação apresentam-se sob a forma de manuscrito científico. O manuscrito foi publicado na revista Virology. TRENTIN, Luana Beló *et al*. The complete genome of Rachiplusia nu nucleopolyhedrovirus (RanuNPV) and the identification of a baculoviral CPD-photolyase homolog. **Virology**, v. 534, p. 64-71, 2019.

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The complete genome of Rachiplusia nu nucleopolyhedrovirus (RanuNPV) and the identification of a baculoviral *CPD-photolyase* homolog

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ABSTRACT

We described a novel baculovirus isolated from the polyphagous insect pest *Rachiplusia nu*. The virus presented pyramidal-shaped occlusion bodies (OBs) with singly-embed nucleocapsids and a dose mortality response of 6.9×10^3 OBs/ml to third-instar larvae of *R. nu*. The virus genome is 128,587 bp long with a G + C content of 37.9% and 134 predicted ORFs. The virus is an alphabaculovirus closely related to Trichoplusia ni single nucleopolyhedrovirus, Chrysodeixis chalcites nucleopolyhedrovirus, and Chrysodeixis includens single nucleopolyhedrovirus and may constitute a new species. Surprisingly, we found co-evolution among the related viruses and their hosts at species level. Besides, auxiliary genes with homologs in other baculoviruses were found, *e.g.* a *CPD-photolyase*. The gene seemed to be result of a single event of horizontal transfer from lepidopterans to alphabaculovirus, followed by a transference from alpha to betabaculovirus. The predicted protein appears to be an active enzyme that ensures likely DNA protection from sunlight.

1. Introduction

Baculoviruses belong to a large family of rod-shaped insect-infecting enveloped viruses inside family *Baculoviridae*. The viruses harbor a supercoiled double-stranded DNA genome with size ranging from 80 to 180 kbp (Rohrmann, 2013). The family contains four genera that coevolved with their insect host at order level: members of *Alphabaculovirus* and *Betabaculovirus* are infectious to larvae of Lepidoptera (butterflies and moths), members of *Gammabaculovirus* are infectious to larvae of Hymenoptera (wasps with caterpillar-like behavior), and members of *Deltabaculovirus* are infectious to larvae of Diptera (mosquitoes). The virus infection process produces two viral phenotypes: (i) the occlusion-derived virus (ODV) and (ii) the budded virus (BV). These two morphologically different but genetically identical virions reflect their respective roles in insect-to-insect and cell-to-cell transmission, respectively. Moreover, as a hallmark of baculovirus, ODVs are occluded within a crystalline protein matrix, the occlusion body (OB). Based on OB morphology, baculoviruses are also classified into those that have a polyhedral-shaped OB called by nucleopolyhedrovirus (NPV) and those with a granular-shape OB called by granulovirus (GV) (Jehle et al., 2006b). This classification scheme is no longer used in baculovirus taxonomy, even though those two terms continue to be used in vernacular names of the viruses.

OB is a convergent adaptation in baculovirus and other insect viruses, including entomopoxviruses and cypovirus (Mitsuhashi et al., 2007; Axford et al., 2014). The OB protects the virion from environmental dissection and degradation and enables it to persist in a dormant form for a long period (Bergold, 2012). Nevertheless, the crystals are not able to protect baculovirus virions against ultraviolet (UV) radiation (Jeyarani et al., 2013), especially from the UV-B (290–320 nm). UV-B radiation may cause a drastic effect on the genomic viral DNA by means of forming two types of pyrimidine dimers, the more abundant cyclobutane-pyrimidine dimers (CPDs) and the less abundant (6–4)-photoproducts (6–4 PPs) (Sancar, 2004). Lesions might hamper polymerase activities through the DNA template, breaking down both transcription and replication processes, which may cause permanent mutation and virus attenuation (Weber, 2005; You et al., 2001; Choi and Pfeifer, 2005). In contrast, baculoviruses present in their genome an interesting

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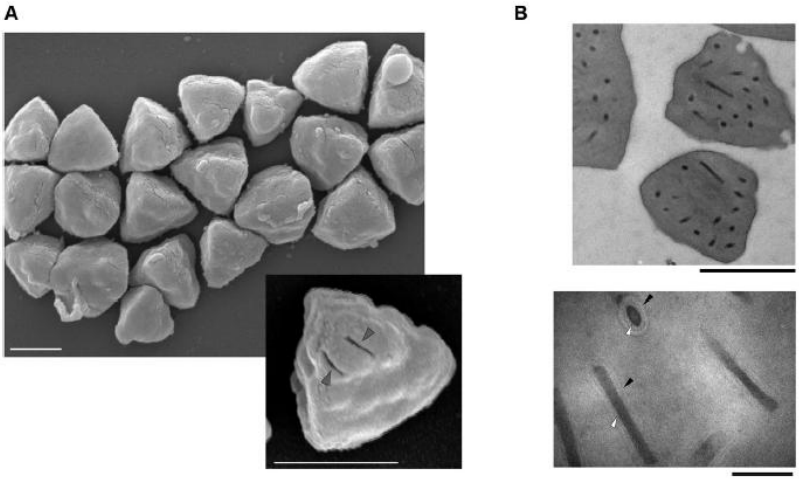

Fig. 1. Ultrastructure analyses of the RanuNPV occlusion bodies (OB). (A) Scanning electron micrography of OBs reveals pyramidal crystals with a predominant size of 1.2 µm. An inset reveals a reminiscent indentation of an immature OB that lost its ODV during preparation, which is pointed by the grey arrowhead. (B) Transmission electron micrography of sectioned OBs reveals several ODVs inside the proteinaceous polyhedrin occlusion. The ODVs present each a single rod-shaped nucleocapsid pointed by the white arrowhead and the envelopes by black. Scale bars = 1.0 µm.

Table 1
Dose-mortality response of third-instar larvae of *Rachiplusia nu* infected orally with RanuNPV-VPN54.

n[a]	Slope	LC_{50} (OB/ml)	Fiducial limits (95%)	X^2
270	1.018 ± 0.214	6,887.1	3,367.2–51,231.0	4.3271

[a] Number of insects tested.

strategy to overcome the UV impacts on DNA: several species genomes harbor CPD-Photolyase (CPD-Phr) homologs that repair CPD dimer at the damaged DNA (Gilbert et al., 2016). Large double-stranded DNA viruses exhibit genomic plasticity and may evolve by horizontal gene transfer (HGT). In this specific case of CPD-Phr, baculoviruses took advantage of an existing insect cell pathway and, through an unknown mechanism, incorporated it into their genome (Harrison et al., 2017).

The semilooper *Rachiplusia nu* (Guenée, 1852) (Lepidoptera: Noctuidae, Plusiinae) is a polyphagous leaf-feeder species widely distributed in South America (Young and Yearian, 1983). The main strategy for controlling the insect pest is based on chemical pesticides that may cause selection of resistant insects and pollute the environment. Therefore, biological control agents arise as an efficient approach to control insect pests in a green and safe fashion (Sun and Peng, 2007; Szewczyk et al., 2006), such as baculoviruses. Baculoviruses are specific to a narrow range of insects varying from one to dozens of hosts (Clem and Passarelli, 2013). This host specificity makes them widely used in the biological control of insect pests of forest and agricultural. For instance, in 1982 and 1983 a baculovirus-based biological control program was established using the Anticarsia gemmatalis multiple nucleopolyhedrovirus isolate 2D (AgMNPV-2D) to control a prevalent soybean (*Glycine max*) pest at that time, the velvetbean caterpillar *Anticarsia gemmatalis* (Moscardi, 1999; Sosa-Gómez, 2017). In this work, we characterized at biological and ultrastructural level a putative baculovirus found in larvae extracts of species *R. nu* with symptoms of baculovirus infection, the isolate VPN54. Moreover, we sequenced and described its complete genome. We found that Rachiplusia nu nucleopolyhedrovirus VPN54 (RanuNPV-VPN54) could be a representative of a novel species into genus *Alphabaculovirus* that nested close to the ancestor of three other previously characterized plusiinae-infecting virus species, including *Trichplusia ni single nucleopolyhedrvoirus*, *Chrysodeixis includens nucleopolyhedrovirus*, and *Chrysodeixis chalcites nucleopolyhedrovirus*. The related viruses presented strict genome architecture collinearity with no inversions. Moreover, we found several gene losses and acquisition along the genome, such as a homolog of the *late expression factor 12*, four *baculovirus repeat ORFs* (*bro-a, b, c,* and *d*), and a *bona fide* functional *CPD-photolyase class II* gene. We analyzed the evolution and structure of the CPD-Phr homolog in this work.

2. Materials and methods

2.1. Insects, virus purification, bioassay, and electron microscopy

RanuNPV was obtained from *R. nu* cadavers with characteristics of baculovirus infection death found in soybean crops in 1989. The place of collection was the city of Oliveiros (Santa Fe, Argentina). Cadavers were sent to EMBRAPA (Brazilian Agricultural Research Corporation) and kept frozen (catalog VPN54). The OBs in the extracts were used to infect field-collected caterpillars of species *R. nu* to confirm virus etiology and propagate the OBs. The insects were reared on soybean leaves and kept in an acclimatized room, at 26 ± 1 °C, 65 ± 10% RH, and a 12:12 (day:night) photoperiod. The cadavers were homogenized with the same volume of ddH2O (w/v), filtered on cotton gauze, and centrifuged at 5,000 × *g* for 10 min. The supernatant was discarded, the pellet suspended at the same volume of 0.5% SDS, and centrifuged at 5,000 × *g* for 10 min; the process was performed three more times. The pellet was suspended in 0.5 M NaCl, centrifuged at 5,000 × *g* for 10 min and suspended in 2 ml ddH2O. OBs were loaded onto a sucrose gradient (40–65%), centrifuged at 130,000 × *g* for 3 h. OBs were collected as a band and diluted five times with ddH2O. The suspension were collected by centrifugation at 7,000 × *g* for 10 min, diluted in ddH_2O (10^6 OBs/ml ddH_2O), and store at 4 °C (O'Reilly et al., 1992). For dose mortality response, eggs of *R. nu* caterpillars were obtained from soybean fields in Londrina (Paraná, Brazil) and hatched in laboratory. Third-instar caterpillars were fed on artificial diet (Greene et al., 1976). For bioassay, the diet was contaminated with six doses of RanuNPV-VPN54 OBs (3.12×10^2, 6.25×10^2, 1.25×10^3, 2.5×10^3, 5.0×10^3, and 1×10^4; an untreated group was established as control) and given in triplicate to 28–33 subjects of third instar larvae of *R. nu*. The insects were allowed to eat the contaminated food for 24 h. The infected insects were transferred to fresh diet and kept until death. Mortality was recorded by the number of dead insects for 12 days. The results were analyzed by Probit in PoloPlus version 1.0. Moreover, one hundred µl of the OB-containing suspension (10^5 OBs/ml of ddH2O) were used for Scanning electron microscopy and Transmission electron microscopy according to previously published protocols (Ardisson-Araújo et al., 2014).

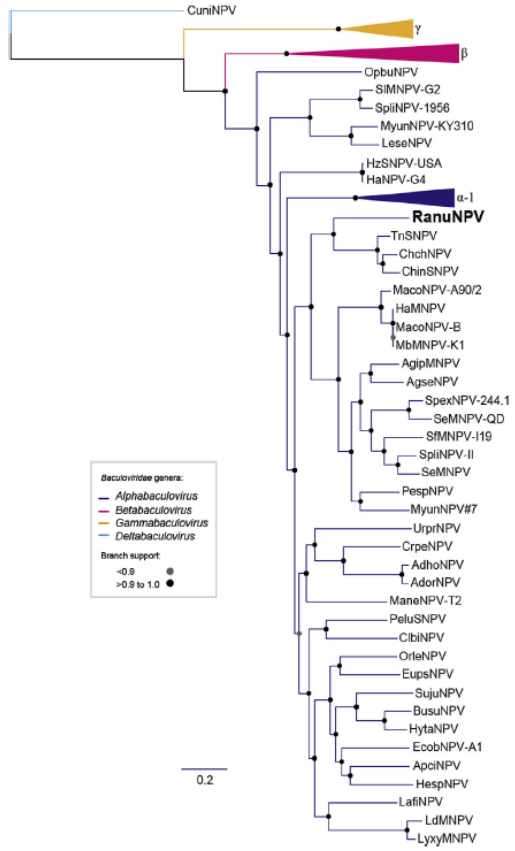

Fig. 2. RanuNPV is an alphabaculovirus. Maximum likelihood inference based on the concatenated nucleotide sequences of the 38 core genes from several selected baculovirus genomes (Table S1) using the FastTree method. The branch support was determined by a SH-like method (black and grey closed circles). Some branches were collapsed for clarity: alphabaculovirus group I, betabaculovirus (pink), gammabaculovirus (orange), and deltabaculovirus (CuniNPV, light blue). CuniNPV was used to root the tree. The genus *Alphabaculovirus* contained RanuNPV (boldface) that clustered together with TnNPV, ChchNPV, and ChinSNPV.

2.2. Viral genomic DNA extraction and amplification

For DNA purification, one hundred µl of the OB-containing suspension (10^6 OBs/ml ddH_2O) were heated for 20 min at 95 °C, placed on ice for 5 min, and treated with RQase RNase-Free DNase (Promega, Madison, WI, United States). The suspension was washed three times with SDS 0.5% and once with NaCl 0.5 M by centrifugation (7000 × *g* for 10 min), using equal volumes for each suspension. The resulting pellet was diluted in ddH2O and dissolved in alkaline solution for further DNA extraction (O'Reilly et al., 1992). The DNA pellet was dissolved in 10 µl of sterile ddH2O at 50 °C for 1 h and directly subjected to a rolling circle amplification (RCA) reaction using the phi29 DNA polymerase and a randon 3′-thiophosphate protected hexamer primer according to the manufacturer's protocols (New England Biolabs, Ipswich, MA, United States). The reaction was subjected to electrophoresis on a 0.8% agarose gel (w/v) (Sambrook and Russel, 2001), visualized, and photographed in AlphaImager® Mini (Alpha Innotech, San Leandro, CA, United States) (data not shown).

2.3. Sequencing and assembly of the viral genome and genome annotation

The sequencing of the viral DNA was performed with the 454 Genome Sequencer (GS) Titanium at the Macrogen (Seoul, South Korea). The reads were trimmed to remove regions of low quality sequencing. The *de novo* assembly method was used with no reference genome. Only one single contig was obtained by *de novo* assembly using an algorithm implemented in the Geneious 9.0 (Kearse et al., 2012). The open reading frames (ORFs) that started with a methionine codon (ATG) and encoded polypeptides of at least 50 amino acids were identified with Geneious 9.0 and annotated using BLAST-X (Altschul et al., 1997). The genomic DNA sequence was submitted to the GenBank with the accession number MK419956.

2.4. Analyses of gene content and genome comparison

The RanuNPV ORFs were compared with other baculoviruses by the BLASTX. We collected the identity of each gene with closely related baculoviruses, including Trichoplusia ni single nucleopolyhedrovirus (TnSNPV), Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV), and Chrysodeixis includens nucleopolyhedrovirus (ChinNPV) and also to Autographa californica multiple nucleopolyhedrovirus (AcMNPV), a virus in the type species baculovirus *Autographa californica nucleopolyhedrovirus*. The unique ORFs were submitted to the HMMER and the HHpred to search for conserved domains (Finn et al., 2011; Alva et al., 2016). We used the progressive Mauve algorithm implemented in the software Geneious R10 for genomic comparison and analysis. We re-annotated the genomes of TnSNPV, ChchNPV, and ChinSNPV according to the same criteria used for the RanuNPV and constructed a Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) to represent the number of ORFs shared among RanuNPV and the closest relatives.

2.5. Phylogeny of baculovirus and species demarcation criterion

Phylogenetic analysis was performed by the MAFFT method (Katoh et al., 2002) with the alignment of the baculovirus core genes from 97 publicly available baculovirus genomes (Table S1). A maximum likelihood tree was inferred using the Fast-tree method (Stamatakis et al., 2008) and a Shimodaira-Hasegawa-like test for branch support (Anisimova et al., 2011). To verify whether this virus corresponds to a new species, the nucleotide distances were estimated with the Kimura-2 parameter replacement model from partial sequences obtained from three conserved baculovirus genes, including *lef-8*, *lef-9*, and *polyhedrin* (Jehle et al., 2006a).

2.6. Analysis of congruence and virus co-evolution at species level

The virus phylogeny was inferred using the PhyML method (Guindon et al., 2010) based on the concatenated alignment of the 38 baculovirus core genes from nineteen species with the substitution model GTR + G (1.13) + I (0.20). For the host phylogeny, the inference was performed based on the mitochondrial *cytochrome oxidase I* (*coi*) gene with several noctuids from where the virus was isolated, using the PhyML method (Guindon et al., 2010) under the substitution model GTR + G (0.4) + I (0.4). A bombycoid host (*Bombyx mori*) and its virus isolate (Bombyx mori nucleopolyhedrovirus, BmNPV) was used as external group. The trees were presented as cladograms and the topologies were compared. For the branch statistical support, we performed Bootstrap analysis with 100 replicates.

2.7. In silico characterization of the RanuNPV phr reveals how this gene envolved in alphabaculovirus

A homolog of the insect photolyase protein (Phr) was identified in the RanuNPV genome (RanuNPV-ORF-68). The protein was

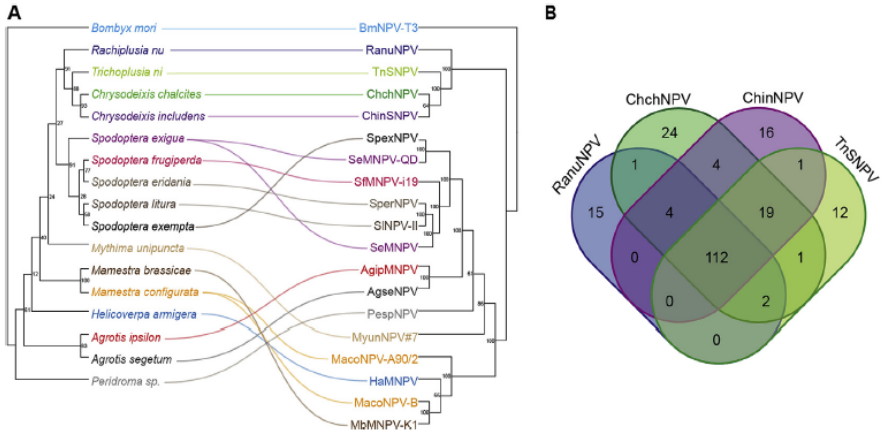

Fig. 3. Topology congruence plusiinaean hosts/alphabaculoviruses and RanuNPV gene content analyses. (A) Baculoviral tree topology of RanuNPV and other alphabaculoviruses against their respective hosts. We used the *cytochrome oxidase I* (*coi*) of mtDNA to infer lepidopteran phylogeny, especially for members of Noctuidae. Surprisingly, RanuNPV and its closest relatives, *i.e.* TnNPV, ChinSNPV, and ChchNpV presented congruence with host tree topology. (B) Venn diagram showing the number of genes shared among RanuNPV and its plusiinaean-infecting viruses.

characterized *in silico* in order to understand the conservation of the structure and putative functionality. Phylogenetic analyses based on the RanuNPV *phr* was performed using sequences retrieved from the BLASTX, with e-value less than 10^{-5}. The sequences were aligned by the MAFFT method (Katoh et al., 2002) and the alignment used for phylogenetic inference with the PhyML method (Guindon et al., 2010) under the substitution models LG + G (0.68). The model was predicted by the MEGA7 software (Kumar et al., 2016). To understand the evolution and acquisition of the *photolyase* gene, the genome context was evaluated in relation to the genome from closely related species. In addition, the conservation of secondary and tertiary structure were evaluated based on the homology model. The templates for three dimensional (3D) structure prediction of Phr were searched in Expasy SWISS-MODEL Server 59 (Schwede et al., 2003) using the predicted amino acid sequence as reference. The photolyase structure model was obtained from the crystallized functional enzyme model 5o8d. The model was validated by SAVES v. 5.0 (http://servicesn.mbi.ucla.edu/SAVES/) to obtain the Ramachandran plot. As a control, the same method was applied for ChchNPV phr1 and phr2.

3. Results and discussion

3.1. Ultrastructure of RanuNPV occlusion bodies (OBs) and bioassay

We evaluated the RanuNPV purified OBs by transmission and scanning electron microscopy (TEM and SEM). By SEM, the OBs were found to be pyramidal (Fig. 1A). We found that the mean size of the pyramidal OBs was 1.4 ± 0.2 µm. The biggest size was 2.1 µm in length, whereas the smallest was 0.8 µm. We calculated that based on three representative fields by considering the pyramid as a planar figure, and measuring the highest length of 150 polyhedra. Immature OBs revealed rod-shaped indentations on the surfaces of OBs, which likely corresponded to ODV that were lost during isolation (inset's grey arrowhead, Fig. 1A). RanuNPV OB sections revealed several embedded ODVs with singly-enveloped nucleocapsids (Fig. 1B), in agreement to other plusiinaean-infecting alphabaculovirus. We analyzed several works where the authors had described OB shapes of alphabaculoviruses at ultrastructural levels (list of references and virus isolates in Table S3) and found no virus with that morphology found for RanuNPV OBs. The usual OB morphology of alphabaculovirus is polyhedral. The major protein responsible for OB formation is polyhedrin and RanuNPV polyhedrin presents one punctual mutation (A197N) in comparison to other closely related polyhedral OB-forming viruses (data not shown), *i.e.* ChchNPV and ChinNPV (Xu et al., 2010; Alexandre et al., 2010; Arneodo et al., 2018). An OB produced by the recombinant AcMNPV containing the SeMNPV polyhedrin had an altered morphology, being pyramidal with less virions occluded in comparison with the parental AcMNPV (Hu et al., 1999). Maybe, not only the polyhedrin sequence but also the cell machenary or other viral factor may alter OB shape and nucleocapside envelopement in OB-forming viruses (Silva et al., 2019).

To confirm the infection etiology found in *R. nu* subjects, we carried out a dose-mortality response in *R. nu* insects collected from soybean fields. We confirmed that the virus was lethal to third-instar subjects of *R. nu* with a LD50 of 6.9×10^3 OBs/ml. The infected caterpillars presented yellowish and liquefied tegument with melanotic pigments, as typical for other baculovirus infections (Rohrmann, 2013). The lethal dose obtained here is similar to that observed for several isolates of ChinNPV infecting third-instar *C. includens* larvae, which ranged from 2.5×10^3 to 9.3×10^3 OBs/ml (Alexandre et al., 2010) (see Table 1).

3.2. Properties of the RanuNPV genome sequence

We sequenced the genome of RanuNPV by the 454 Genome Sequencer (GS) FLX ™ Titanium method (Macrogen Inc., Korea). No virus isolated from *R. nu* had been sequenced completely and described so far. We assembled the reads from sequencing of RanuNPV DNA into a circular genome contig of 128,587 bp long. 8,305 reads with a mean size of 776.1 ± 210.7 nt were obtained and mapped, allowing for a coverage of 50.2 ± 12.4 X. The size of the genome and the G + C nucleotide distribution (37.9%) were within the range of genome sizes and nucleotide distributions that have been reported for other alphabaculoviruses (Table S1). Moreover, a total of 134 ORFs were annotated (Table S2).

3.3. Relationship of RanuNPV to other baculoviruses

RanuNPV belongs to genus *Alphabaculovirus* as a basal species of the clade formed by the plusiinaean-infecting alphabaculoviruses of species *Trichoplusia ni single nucleopolyhedrovirus*, *Chrysodeixis chalcites nucleopolyhedrovirus*, and *Chrysodeixis includens single nucleopolyhedrovirus* (Fig. 2). The phylogeny was based on the concatenated nucleotide sequence alignment of the 38 baculovirus core genes from several

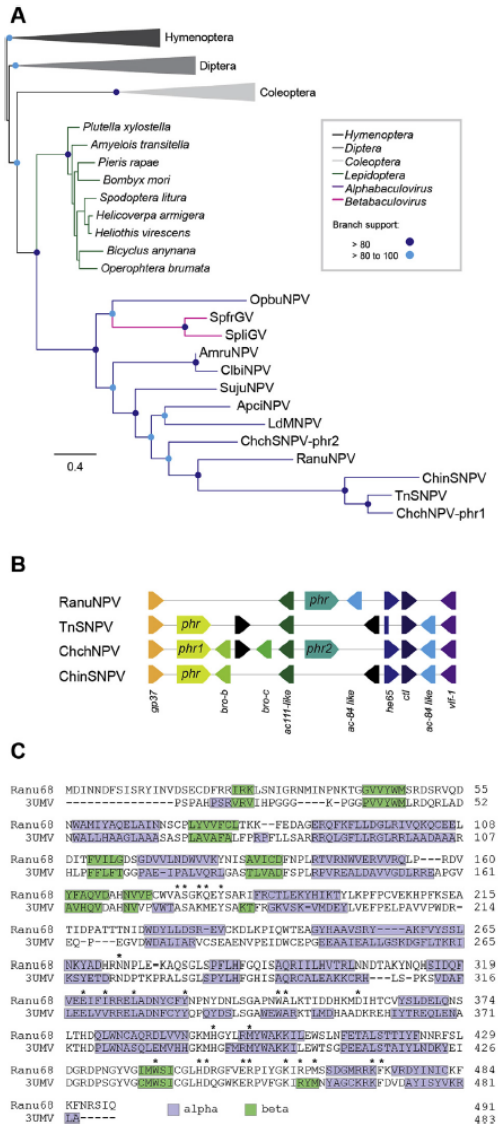

Fig. 4. Photolyase evolution in RanuNPV-related alphabaculovirus and alignment with a homolog Phr crystal. (A) Phylogeny of Phr homologs based on the predicted amino acid sequences by the PhyML method under LG + G model. We hypothesized that a single event of horizontal transfer of the *phr* gene from lepidopterans to alphabaculovirus (blue branches) and then to betabaculovirus (pink braches). (B) The genomic contexts of the photolyase in the RanuNPV and related species, including TnSNPV, ChchNPV, and ChinNPV. The arrowhead depicts gene direction in the genome. Arrowheads with similar colors depict orthology, and the black ones independent acquisitions. (C) Individual alignments of the RanuNPV *phr* gene with the crystal structure of the 3UMV protein identifying the secondary structures. Conserved β-sheets and α-helices are boxed in green and purple, respectively.

baculovirus genomes publicly available. The pairwise nucleotide identity of the RanuNPV core genes with all completely sequenced alphabaculovirus genome is shown in Table S1. The mean identity of RanuNPV and the closely related species were about 59.4%, which depicts a very divergent lineage inside the genus. Indeed, the branch length separating RanuNPV from the other virus species is in a range similar to that observed for other recognized baculovirus species, pointing RanuNPV as a representative of a putative novel virus species.

3.4. RanuNPV may represent a novel species inside Alphabaculovirus

There are only 45 species recognized by the International Committee on Taxonomy of Virus (ICTV) within genus *Alphabaculovirus*. From those, only four had been described in Brazil, *Anticarsia gemmatalis multiple nucleopolyhedrovirus*, *Chrysodeixes includes nucleopolyhedrovirus*, *Lonomia obliqua nucleopolyhedrovirus*, and *Perigonia lusca nucleopolyhedrovirus*. ChinNPV is the unique isolate described in Brazil from *Chrysodeixes includens*, one of the most important soybean pests (Craveiro et al., 2015). A specific demarcation criterion was established for baculoviruses (Jehle et al., 2006a), which is based on the comparative analyses of the conserved nucleotide region from three genes, *polyhedrin*, *lef-8*, and *lef-9* by using the Kimura-2-parameters model (K2P).

A virus is considered to be a member of a novel species when the number of substitutions is higher than 0.05 per site. We suggest that RanuNPV is a member of a new species, which we are tentatively naming as *Rachiplusia nu nucleopolyhedrovirus* (Table S2). In two previous works, a *R. nu*-infecting virus was isolated and described (Rodríguez et al., 2012; Young and Yearian, 1983). In Young and Yearian (1983), a nucleopolyhedrovirus (NPV) was isolated from larvae collected from soybean in Argentina in 1980. The virus was lethal to larvae of *R. ou* and presented OBs similar to that observed for RanuNPV in the present work, regarding shape and number of nucleocapsids per envelope. In Rodríguez et al., 2012, the virus was found to have OBs with multiply-enveloped ODVs and was called Rachiplusia nu multiple nucleopolyhedrovirus (RanuMNPV). The partial sequences of the genes *p74*, *polyhedrin*, *v-cathepsin*, *v-chitinase*, *lef-8*, and *lef-9* genes revealed that this virus is a closely related variant of AcMNPV found in a different host range. *Autographa californica*, *R. ou*, and *R. nu* belongs to subfamily Plusiinae.

3.5. Coevolution analysis at species level

We evaluated whether there would be congruence between the baculoviral tree topology of RanuNPV, TnSNPV, ChchNPV, and ChinSNPV against their respective hosts, including *R. nu*, *T. ni*, *C. chalcites*, and *C. includens*. RanuNPV and its related viruses were isolated from hosts that belong to family Noctuidae and subfamily Plusiinae. Surprisingly, we found strict congruence between the trees, as observed in Fig. 3A. We constructed the trees based on the mtDNA *cytochrome oxidase subunit i* (*COI*) for hosts, which is widely used to infer phylogenies of lepidopterans (Taft and Cognato, 2017; Kirichenko et al., 2018) and concatenated core genes for baculoviruses. We selected the non-plusiinaean bombycoid host *B. mori* and the alphabaculovirus isolated from this species as external groups to the trees. Baculoviruses evolved with their host at insect order level generating alpha, beta, gamma, and deltabaculovirus (Herniou et al., 2004). Coevolution, a reciprocal evolution in interacting species driven by natural selection, is a powerful determinant for the biology and genetics of infection, pointing to the historical association between pathogens and their hosts (Woolhouse et al., 2002). The selection pressure exerted on baculoviruses makes them subject to coevolution (Herniou et al., 2004). Nevertheless, different phylogeny-trait correlation tests show unequivocal significant associations between the taxonomy of insect hosts at superfamily, family, and subfamily and the BV species phylogeny (Thèzè et al., 2018). In contrast, virus and host coevolution at species level is not clear.

3.6. RanuNPV ORF content

The RanuNPV genome contains all the 38 core genes identified to date in every baculovirus genome, including the recently described gene *ac110* (*pif-7*, the RanuNPV-ORF-93) (Javed et al., 2017). The genome also presents the 26 ORFs identified by Garavaglia et al. (2012) as present in genomes of alpha- and betabaculoviruses (Table S2). We also found four copies of the *baculovirus repeated ORF* (*bro*), and homologs of auxiliary genes such as the *inhibitor of apoptosis 2* (*iap-2*, the RanuNPV-ORF-61) and *iap-3* (RanuNPV-ORF-39), *chitinase* (RanuNPV-ORF-65), *cathepsin* (RanuNPV-ORF-64), and *he65* (RanuNPV-ORF-70). Among the hypothetical ORFs annotated, nine were found to be unique (RanuNPV-ORF-5, RanuNPV-ORF-6, RanuNPV-ORF-9, RanuNPV-ORF-20, RanuNPV-ORF-33, RanuNPV-ORF-40, RanuNPV-ORF-112, RanuNPV-ORF-115, and RanuNPV-ORF-125), *i.e.* not found in any other baculovirus genomes so far (Table S3). Only one hypothetical gene (RanuNPV-ORF-5) did not show any hit with any other gene from Genbank and no domains were found using either the HHpred or Smart. We also performed an ORF content comparison among RanuNPV and its closest relatives (*i.e.* TnNPV, ChchNPV, and ChinSNPV) and we plotted the result in a Venn Diagram (Fig. 3B). A total of 211 different genes were found considering the four species genomes. For this comparison, we reannotated the four genomes under the same criterion and found 32 new genes not annotated before, including three in the TnNPV, 16 in the ChchNPV, and 13 in the ChinSNPV. Only 112 genes were shared among the four species. Fifteen ORFs were found only in the RanuNPV genome: nine unique in baculovirus, three *baculovirus repeat ORFs* (*bro-a*, *bro-c*, and *bro-d*) with no ortholog in the related species, one *late expression factor 12* (*lef-12*, the RanuNPV-ORF-34), and two other hypothetical proteins (RanuNPV-ORF-63, RanuNPV-ORF-128). The RanuNPV-ORF-63 did not present hit using BLASTX; however, we did found hit with the alphabaculovirus Agrotis segetum nucleopolyedrovirus (nt identity of 25.7%, e-value 2.2E-11) by using the HMMER algorithm. The RanuNPV-ORF-128 was found to be related to Spodoptera frugiperda multiple nucleopolyhedrovirus (nt identity of 38%, e-value 3.00E-12). Two ORFs were shared solely by RanuNPV and both TnSNPV and ChchNPV (RanuNPV-ORF-31 and RanuNPV-ORF-35 [ac43-like]) and one single ORF was shared between RanuNPV and ChchNPV, a *CPD-phr* homolog (RanuNPV-ORF-68).

3.7. The evolution of photolyase genes in plusiinaean-infecting alphabaculovirus

The RanuNPV genome presents a homolog of a *CPD-phr* gene (RanuNPV-ORF68). As a virus related to the most recent common ancestor of ChchNPV, ChinNPV, and TnSNPV, the ortholog of a *CPD-phr* found in the genome of RanuNPV, allowed for a wide comprehension of *CPD-phr* in *Baculoviridae*. To investigate the evolutionary history of this gene, we performed a BLASTX search in the NCBI non-redundant database to find homologs. The retrieved sequences were aligned by the MAFFT and used to infer the phylogeny by the PhyML. We found that the *CPD-phr* gene is present in several baculoviruses, including members of *Alphabaculovirus* and *Betabaculovirus* (Fig. 4A). The baculovirus clade nested as a monophyletic group sharing a unique ancestor with lepidopterans (Biernat et al., 2012). This finding was previously observed (Harrison el al., 2017; van Oers et al., 2004; Willis et al., 2005). Some alphabaculoviruses that infect also members of subfamily Plusiinae do not contain *phr* genes, including AcMNPV, Thysanoplusia orichalcea nucleopolyhedrovirus (ThorNPV), and Rachiplusia ou multiple nucleopolyhedrovirus (RoMNPV) (Van Oers and Vlak, 2007). The gene topology in Fig. 4A depicts a single event of HGT from insects to baculovirus, specifically to alphabaculoviruses. This ability to acquire genes from insects is a remarkable feature in baculoviruses, which include genes related to nucleotide metabolism (Ardisson-Araújo et al., 2016), nucleases (Ardisson-Araújo et al., 2018), innate immune response (Ardisson-Araújo et al., 2015), and apoptosis control (Harrison et al., 2016). In the specific case of *phr*, the gene was acquired by alphabaculovirus and transferred to betabaculovirus. Viruses of only two *Spodoptera*-infecting betabaculovirus species harbor a *phr* homolog. ChchNPV is the unique virus harboring two copies of the *phr*, called by *phr1* and *phr2*. van Oers et al. (2005) described the gene as a product of duplication rather than independent horizontal gene transfer. In a wide perspective, the enzymes are divided into two classes based on the differences in the amino acid sequences of the organisms that harbor the gene (Kanai et al., 1997). Interestingly, the RanuNPV added a small piece to this puzzle that clarifies the evolution of *phr* in baculovirus. We took advantage of the genome *loci* for the related species to understand the gene evolution (Fig. 4B). RanuNPV is a virus closely related to the ancestor of the plusiinae-infecting alphabaculoviruses and presents a *phr2* homolog but not a *phr1*. Two main hypothetical pictures might be draw from this evolutionary scenario. In the first, *phr2* is present in the most recent common ancestor (m.r.c.a.) of RanuNPV-related species. In the primary speciation event, the RanuNPV lineage maintained the *phr2* and the lineage that originate the m.r.c.a. of TnSNPV, ChchNPV, and ChinNPV underwent a duplication event of *phr2* that led to *phr1* followed by two independent losses, one by the TnSNPV lineage and the other one by the ChinNPV lineage. In the second scenario, the most recent common ancestor (m.r.c.a) of TnSNPV, ChchNPV, and ChinNPV acquired the *phr1*. After, the ChchNPV lineage underwent an independent duplication that generated *phr2* and transferred the gene to the RanuNPV lineage. Besides being less parsimonious than the second picture, the first hypothesis is reinforced by two important facts. First, the *phr1* homologs seemed to have lost the CPD-repairing activity whereas the *phr2* maintained this activity (Van Oers et al., 2008). Second, the hypothetical independent loss of *phr2* undergone by TnSNPV lineage is reinforced by the presence of a reminiscent non-coding fragment of *he65* (Fig. 4B).

3.8. Ranu68 is a bona fide functional CPD-Photolyase

Since most of the *phrs* described so far have the same basic architecture, we took advantage of a previously established crystal structure to solve by homology modeling the predicted RanuNPV *phr*. Therefore, in order to determine whether the predicted amino acid sequence of RanuNPV-ORF68 potentially encodes a functional Phr, we performed an alignment against homologs with solved crystal structures and built a 3D model (Fig. S1A). The identity between the viral sequences and its homolog was 44% (PDB ID: 5o8d). The model was checked by the SAVES v5.0 and we found that 91.3% of the amino acid residues were in most favored regions and only three residues were in disallowed regions, which represents 0.7% of the residues (Fig. S1A). Essentially, the RanuNPV Phr model represents a globular protein that is composed by two defined domains, an N-terminal α/β-domain (residues 1–200) and a C-terminal α-helical domain (residues 227–486) (Fig. S1A). A long inter-domain loop (residues 201–226) links the two major domains. Most of the secondary structures observed for the Phr model is predicted for the Ranu068, as also observed for the both fuctional ChchNPV CPD-Phr2 (Fig. S1B) and Phr1 (Fig. S1C). Importantly, for ChchNPV CPD-Phr2 92.1% of the amino acid residues were in most favored regions and only four residues were in disallowed regions, which represents 0.9% of the residues. In contrast, phr1 presented 86.8% of the amino acid residues were in most favored regions and four residues were in disallowed regions, which represents 0.9% of the residues. In a previous work characterizing the activity of baculoviral Phrs, Van Oers et al. (2008) identified several directly conserved amino acids among the active baculoviral enzymes and other characterized enzymes. Interestingly, *phr1* of ChchNPV does not encode a functional CPD-disrupting enzyme, besides sharing a similar ancestor to *phr2*, the CPD-disrupting functional enzyme. Twenty-six amino acid residues were conserved among ChchNPV Phr2 and other functionally tested Phr homologs and not conserved in the non-functional counterpart Phr1 (Fig. 4C). Of those, Ranu068 conserved 25 residues with only one

mutation at position 325. Conversely, all the analyzed homologs, including Ranu068 presented a hydrophobic amino acid residue at that changed position (valine or isoleucine). Therefore, based on the tertiary, secondary and, primary conservation of the Phr, we believe that Ranu068 is likely a *bona fide* active CPD-Phr. Active CPD-photolyases can revert CPD lesion by means of a catalytic cofactor, the FAD that assimilates light energy and reverts the dimer (Deisenhofer, 2000; Eker et al., 2009). Photoreactivation is a pathway used to revert the pyrimidine dimers to their monomeric form (Sancar, 2004). An enzyme activity remains to be carried out in order to elucidate the functional activity of Ranu068.

4. Conclusion

In this work, we have characterized at the ultrastructural and genomic level a baculovirus isolated from the plusiinaean soybean pest *R. nu*. The virus presented the peculiar feature of having a pyramidal-shaped OB with several singly-enveloped ODVs within and a lethal dose of 6.9 OBs/ml to the third-instar field-derived caterpillars of species *R. nu*. The virus could represent a novel species in genus *Alphabaculovirus* and be correlated with members that infect other insects in subfamily Plusiinae. It has 134 ORFs and nine were shown to be unique in baculovirus genomes. In addition, we identified a hypothetical *phr* gene with a predicted role for the repair of CPD lesions in DNA. In particular, it was found that the *phr* gene underwent a single event of horizontal transfer from lepidopteran to alphabaculovirus and then to betabaculovirus. The RanuNPV genome provides a key baculovirus genome to clarify the evolution of the *phr* in alphabaculovirus, since it is related to the m.r.c.an of the plusiinaean-isolated viruses. Overall, alphabaculovirus genome sequencing is of importance to the field as few genomes are publicly accessible. RanuNPV is a widely distributed polyphagous pest in South America that causes great damage to several crops of economic importance. Certainly, both discovery and description of novel baculoviruses may lead to the development of greener and safer pesticides in order to counteract and effectively control crop damage-causing insect populations. Moreover, that allows us to understand the evolution of baculovirus in a wider perspective.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virol.2019.05.019.

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5 CONCLUSÃO

A presente dissertação dedicou-se a descrever em nível ultraestrutural e genômico um novo baculovírus isolado do inseto-praga da soja *R. nu*. O novo vírus Rachiplusia nu nucleopolyhedrovirus constitui uma nova espécie dentro do gênero *Alphabaculovírus* que se correlaciona com membros dos vírus infectivos da subfamília Plusiinae. Neste contexto, o vírus apresentou 134 ORFs, sendo que nove destas são únicas na família *Baculoviridae*. O vírus apresentou a característica peculiar de corpos de oclusão OBs em forma de pirâmide, contendo vários ODVs por envelope e uma dose letal de 6,9 OBs / ml para a lagarta de terceiro instar da espécie *R. nu*. Além disso, identificou-se um gene hipotético *phr* previsto para exercer o reparo de lesões de CPD no DNA. Em particular, verificou-se que o gene *phr* sofreu um único evento de transferência horizontal de lepidópteros para alphabaculovírus e depois para betabaculovírus. O genoma de RanuNPV pode fornecer um esclarecimento para a evolução do gene *phr* nos alphabaculovirus, uma vez que está relacionado com o m.r.c.a dos vírus isolados de plusiinae. Além disso, RanuNPV podem levar o desenvolvimento de biopesticidas mais seguros para o controle efetivo das populações de insetos causadores de danos às culturas agrícolas, uma vez que a lagarta praga *R. nu* é uma espécie polífaga amplamente distribuída na América do Sul, causando grandes danos a várias culturas de importância econômica.

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APÊNDICE A – FIGURA SUPLEMENTAR 1

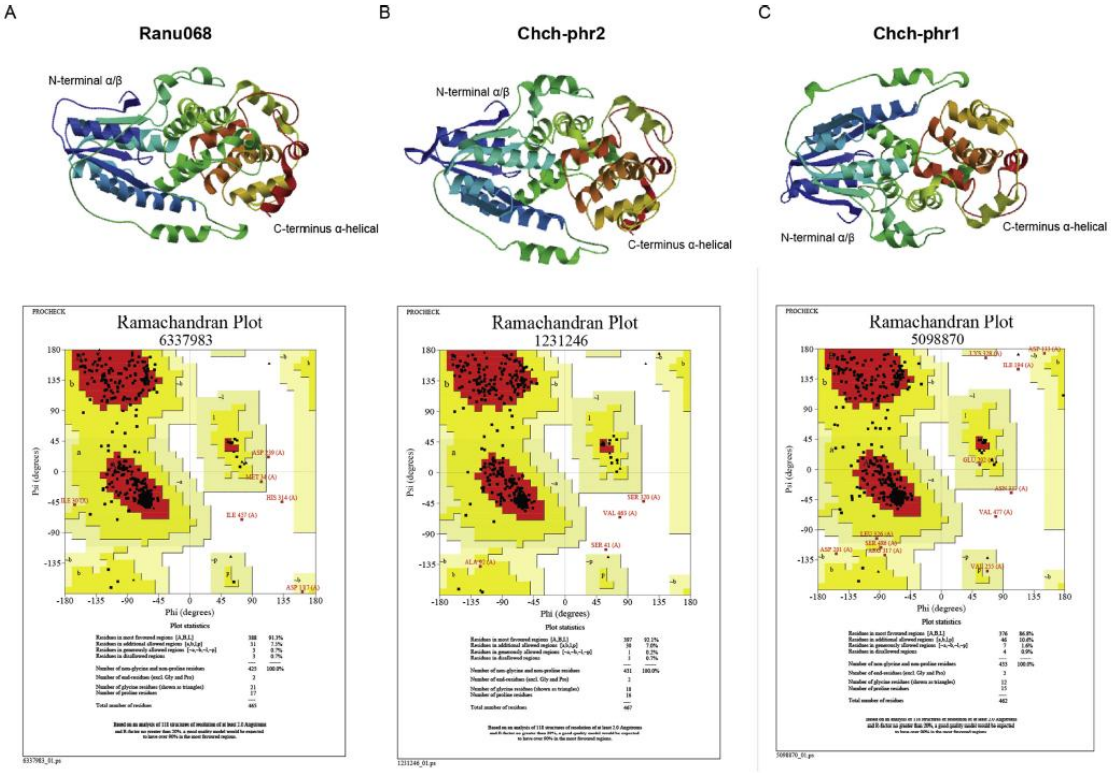

Figure S1. Predicted structure of the RanuNPV CPD-photolyase based on homology modeling. Homology modeling and Ramachandran plot for the proposed protein model of (A) Ranu068, (B) ChchNPV-Phr2, and (C) ChchNPV-Phr2.

APÊNDICE B – TABELA SUPLEMENTAR 1

Table S1. Genomes used in this paper for reconstruction of the baculovirus phylogeny in both the Fig. 2. The isolates placed into the genera *Alphabaculovirus* (dark blue), *Betabaculovirus* (pink), *Gammabaculovirus* (orange), and *Deltabaculovirus* (light blue) are presented together with the acronym used in the main text, the host family where the virus was isolated from, the G+C content, the Genbank accession number, the genome size, and the global identity in relation to RanuNPV (using a pairwise alignment of the concatenated nucleotide sequence of the 38 baculovirus core genes).

	Baculovirus	Acronym	Host family	G + C content (%)	Accession number	Genome Size (bp)	Id (%)
1	Adoxophyes honmai nucleopolyhedrovirus	AdhoNPV	Tortricidae	35.6	AP006270	113220	51.8
2	Adoxophyes orana nucleopolyhedrovirus	AdorNPV	Tortricidae	35	EU591746	111724	51.8
3	Agrotis ipsilon multiple nucleopolyhedrovirus strain Illinois	AgipMNPV	Noctuidae	48.6	EU839994	155122	54.8
4	Agrotis segetum nucleopolyhedrovirus	AgseNPV	Noctuidae	45.7	DQ123841	147544	55.2
5	Antheraea pernyi nucleopolyhedrovirus isolate L2	AnpeNPV-L2	Saturniidae	53.5	EF207986	126246	45.0
6	Anticarsia gemmatalis multiple nucleopolyhedrovirus	AgMNPV	Noctuidae	44.5	DQ813662	132239	46.5
7	Apocheima cinerarium nucleopolyhedrovirus	ApciNPV	Geometridae	33.4	FJ914221	123876	48.6
8	Autographa californica multiple nucleopolyhedrovirus clone C6	AcMNPV-C6	Noctuidae	40.7	L22858	133894	48.3
9	Bombyx mandarina nucleopolyhedrovirus S2	BomaNPV-S2	Bombycidae	40.4	JQ071499	129646	48.3
10	Bombyx mori nucleopolyhedrovirus strain T3	BmNPV-T3	Bombycidae	40.4	L33180	128413	48.2
11	Buzura suppressaria nucleopolyhedrovirus	BusuNPV	Geometridae	36.8	KF611977	120420	52.2
12	Catopsilia pomona nucleopolyhedrovirus	CapoNPV	Pieridae	39.7	KU565883	128058	47.0
13	Choristoneura fumiferana defective multiple nucleopolyhedrovirus	CfDEFMNPV	Tortricidae	45.8	AY327402	131160	46.4
14	Choristoneura fumiferana multiple nucleopolyhedrovirus	CfMNPV	Tortricidae	50.1	AF512031	129593	45.9
15	Choristoneura murinana nucleopolyhedrovirus	ChmuNPV	Tortricidae	50	KF894742	124688	45.6
16	Choristoneura occidentalis nucleopolyhedrovirus	ChocNPV	Tortricidae	50.1	KC961303	128446	45.8

17	Choristoneura rosaceana nucleopolyhedrovirus	ChroNPV	Tortricidae	48.6	KC961304	129052	45.8
18	Chrysodeixis chalcites nucleopolyhedrovirus	ChchNPV	Noctuidae	39	AY864330	149622	59.2
19	Chrysodeixis includens single nucleopolyhedrovirus isolate IF	ChinSNPV-IF	Noctuidae	39.2	KU669293	139181	59.2
20	Clanis bilineata nucleopolyhedrovirus	ClbiNPV	Sphingidae	37.7	DQ504428	135454	52.8
21	Condylorrhiza vestigialis multiple nucleopolyhedrovirus	CoveMNPV	Crambidae	42.9	KJ631623	125767	46.7
22	Cryptophlebia peltastica ucleopolyhedrovirus	CrpeNPV	Tortricidae	37.2	MH394321	115728	52.7
23	Cyclophragma undans nucleopolyhedrovirus	CyunNPV	Lasiocampidae	45.1	KT957089	140418	46.8
24	Dasychra pudibunda nucleopolyhedrovirus	DapuNPV	Lymantriidae	54.4	KP747440	136761	45.2
25	Dendrolimus kikuchii nucleopolyhedrovirus	DekiNPV	Lasiocampidae	48	JX193905	141454	46.4
26	Ecotropis obliqua nucleopolyhedrovirus strain A1	EcobNPV-A1	Geometridae	37.6	DQ837165	131204	52.2
27	Epiphyas postvittana nucleopolyhedrovirus	EppoNPV	Tortricidae	40.7	AY043265	118584	46.9
28	Euproctis pseudoconspersa nucleopolyhedrovirus	EupsNPV	Lymantriidae	40.3	FJ227128	141291	51.8
29	Helicoverpa armigera multiple nucleopolyhedrovirus	HaMNPV	Noctuidae	40.1	EU730893	154196	55.3
30	Helicoverpa armigera nucleopolyhedrovirus G4	HaNPV-G4	Noctuidae	39	AF271059	130759	52.0
31	Helicoverpa zea single nucleopolyhedrovirus USA	HzSNPV-USA	Noctuidae	39.1	AF334030	130869	52.4
32	Hemileuca sp. nucleopolyhedrovirus	HespNPV	Saturniidae	38.1	KF158713	140633	51.4
33	Hyphantria cunea nucleopolyhedrovirus	HycuNPV	Arctiidae	45.5	AP009046	132959	46.3
34	Hyposidra talaca nucleopolyhedrovirus	HytaNPV	Geometridae	39.6	MH261376	139089	52.1
35	Lambdina fiscellaria nucleopolyhedrovirus	LafiNPV	Geometridae	43.7	KP752043	157977	49.2
36	Leucania separata nuclear polyhedrovirus strain AH1	LeseNPV-AH1	Noctuidae	48.6	AY394490	168041	48.4
37	Lonomia obliqua multiple nucleopolyhedrovirus	LoobMNPV	Saturniidae	35.7	KP763670	120022	48.3
38	Lymantria dispar multiple nucleopolyhedrovirus	LdMNPV	Lymantriidae	57.5	AF081810	161046	49.2
39	Lymantria xylina multiple nucleopolyhedrovirus	LyxyMNPV	Lymantriidae	53.5	GQ202541	156344	50.1
40	Malacosoma neustria nucleopolyhedrovirus isolate T2	ManeNPV-T2	Lasiocampidae	38.2	KY968317	130202	52.9
41	Mamestra brassicae multiple nucleopolyhedrovirus strain K1	MbMNPV-K1	Noctuidae	40.1	JQ798165	152710	55.3
42	Mamestra configurata nucleopolyhedrovirus B	MacoNPV-B	Noctuidae	40	AY126275	158482	55.3

43	Mamestra configurata nucleopolyhedrovirus-A strain 90/2	MacoNPV-A 90/2	Noctuidae	41.7	U59461	155060	54.9
44	Maruca vitrata multiple nucleopolyhedrovirus	MaviMNPV	Crambidae	38.6	EF125867	111953	48.4
45	Mythimna unipuncta nucleopolyhedrovirus strain #7	MyunNPV#7	Noctuidae	48.6	MF375894	148482	49.1
46	Mythimna unipuncta nucleopolyhedrovirus strain KY310	MyunNPV-KY310	Noctuidae	43.9	MH124167	156647	46.4
47	Operophtera brumata nucleopolyhedrovirus	OpbuNPV	Geometridae	38.9	MF614691	119054	48.0
48	Orgyia leucostigma nucleopolyhedrovirus isolate CFS-77	OrleNPV	Lymantriidae	39.9	EU309041	156179	52.1
49	Orgyia pseudotsugata multiple nucleopolyhedrovirus	OpMNPV	Lymantriidae	55.1	U75930	131995	45.1
50	Oxyplax ochracea nucleopolyhedrovirus	OxocNPV	Limacodidae	31.2	MF143631	113971	49.3
51	Peridroma sp. nucleopolyhedrovirus	PespNPV	Noctuidae	53.2	KM009991	151109	54.4
52	Perigonia lusca single nucleopolyhedrovirus	PeluSNPV	Sphingidae	39.6	KM596836	132831	52.2
53	Philosamia cynthia ricini nucleopolyhedrovirus	PhcyNPV	Saturniidae	53.7	JX404026	125376	44.9
54	Plutella xylostella multiple nucleopolyhedrovirus isolate CL3	PlxyMNPV	Plutellidae	40.7	DQ457003	134417	48.4
55	**Rachiplusia nu nucleopolyhedrovirus**	**RanuNPV**	**Noctuidae**	**37.9**	**MK419956**	**128587**	**100**
56	Rachiplusia ou multiple nucleopolyhedrovirus	RoMNPV	Noctuidae	39.1	AY145471	131526	48.4
57	Spilosoma obliqua nucleopolyhedrosis virus isolate IIPR	SpobNPV-IIPR	Erebidae	45.5	KY550224	136141	46.3
58	Spodoptera exempta nucleopolyhedrovirus strain 244.1	SpexNPV-244.1	Noctuidae	41.2	MH717816	129528	52.2
59	Spodoptera exigua multiple nucleopolyhedrovirus	SeMNPV-US1	Noctuidae	43.8	AF169823	135611	55.1
60	Spodoptera exigua multiple nucleopolyhedrovirus isolate QD	SeMNPV-QD	Noctuidae	37.4	MH370144	128525	55.3
61	Spodoptera frugiperda multiple nucleopolyhedrovirus isolate 19	SfMNPV-I19	Noctuidae	40.3	EU258200	132565	55.5
62	Spodoptera litoralis nucleopolyhedrovirus isolate AN1956	SpliNPV-1956	Noctuidae	44.7	JX454574	137998	48.4
63	Spodoptera litura multiple nucleopolyhedrovirus G2	SlMNPV-G2	Noctuidae	42.8	AF325155	139342	48.7
64	Spodoptera litura nucleopolyhedrovirus II	SliNPV-II	Noctuidae	45	EU780426	148634	54.9
65	Sucra jujuba nucleopolyhedrovirus	SujuNPV	Geometridae	38.7	KJ676450	135952	51.9
66	Thysanoplusia orichalcea nucleopolyhedrovirus	ThorNPV	Noctuidae	39.5	JX467702	132978	48.3
67	Trichoplusia ni single nucleopolyhedrovirus	TnSNPV	Noctuidae	39	DQ017380	134394	59.7
68	Urbanus proteus nucleopolyhedrovirus	UrprNPV	Hesperiidae	34.7	KR011717	105555	51.0

69	Adoxophyes orana granulovirus	AdorGV	Tortricidae	34.5	AF547984	99657	40.0
70	Agrotis segetum granulovirus-L1	AgseGV-L1	Noctuidae	37.3	KC994902	131442	39.4
71	Artogeia rapae granulovirus isolate Wuhan	ArraGV-Wuhan	Pieridae	33.2	GQ884143	108592	40.3
72	Choristoneura fumiferana granulovirus	ChfuGV	Tortricidae	50.1	DQ333351	104710	39.9
73	Clostera anastomosis granulovirus	CaLGV	Notodontidae	46.7	KC179784	101818	38.3
74	Clostera anastomosis granulovirus isolate ClanGV-B	ClanGV-B	Notodontidae	37.8	KR091910	107409	39.2
75	Clostera anachoreta granulovirus	ClanGV	Notodontidae	44.4	HQ116624	101487	38.3
76	Cnaphalocrocis medinalis granulovirus	CnmeGV	Crambidae	35.2	KP658210	112060	38.3
77	Cryptophlebia leucotreta granulovirus isolate CV3	CrleGV	Tortricidae	32.4	AY229987	110907	39.9
78	Cydia pomonella granulovirus	CpGV	Tortricidae	45.3	U53466	123500	38.6
79	Diatraea saccharalis granulovirus	DisaGV	Crambidae	34.9	KP296186	98392	39.8
80	Epinotia aporema granulovirus	EpapGV	Tortricidae	41.5	JN408834	119082	39.4
81	Erinnyis ello granulovirus	ErelGV	Sphingidae	38.7	KJ406702	102759	39.3
82	Helicoverpa armigera granulovirus	HaGV	Noctuidae	40.8	EU255577	169794	39.7
83	Mocis latipes granulovírus	MolaGV	Noctuidae	38.3	KR011718	134272	39.9
84	Mythimna unipuncta granulovírus	MyunGV#8	Noctuidae	49.9	KX855660	144673	38.9
85	Phthorimaea operculella granulovirus	PhopGV	Gelechiidae	35.7	AF499596	119217	39.3
86	Pieris rapae granulovirus isolate E3	PiraGV-E3	Pieridae	33.2	GU111736	108476	40.3
87	Plodia interpunctella granulovirus	PiGV	Pyralidae	44.2	KX151395	112536	39.2
88	Plutella xylostella granulovirus	PlxyGV	Plutellidae	40.7	AF270937	100999	39.1
89	Pseudaletia unipuncta granulovirus strain Hawaiin	PsunGV-Hawaiin	Noctuidae	39.8	EU678671	176677	39.8
90	Spodoptera frugiperda granulovirus isolate VG008	SpfrGV-VG008	Noctuidae	46.2	KM371112	140913	39.3
91	Spodoptera litura granulovirus isolate K1	SpliGV	Noctuidae	38.8	DQ288858	124121	38.8
92	Trichoplusia ni granulovirus	TnGV	Noctuidae	39.8	KU752557	175360	39.8
93	Xestia c-nigrum granulovirus	XcGV	Noctuidae	40.7	AF162221	178733	39.6
94	Neodiprion abietis nucleopolyhedrovirus	NeabNPV	Diprionidae	33.4	DQ317692	84264	35.5

95	Neodiprion lecontei nucleopolyhedrovirus	NeleNPV	Diprionidae	33.4	AY349019	81755	35.6
96	Neodiprion sertifer nucleopolyhedrovirus	NeseNPV	Diprionidae	33.8	AY430810	86462	35.9
97	Culex nigripalpus nucleopolyhedrovirus	CuniNPV	Culicidae	50.9	AF403738	108252	29.3

APÊNDICE C – TABELA SUPLEMENTAR 2

Table S2. Characteristics of the Rachiplusia nu nucleopolyhedrovirus (RanuNPV) genome: number, position, nucleotide and amino acid size of each ORF and homology search. Predicted ORFs are compared with homolog genes in four related genomes: Trichoplusia ni nucleopolyhedrovirus (TnNPV), Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV), Chrysodeixis includens single nucleopolyhedrovirus isolate IF (ChinSNPV-IF), and Autographa californica multiple nucleopolyhedrovirus clone C6 (AcMNPV-C6).

					TnNPV		ChchNPV		AcMNPV-C6		ChinNPV-IF	
ORF	**Name**	**Position**	**Size (nt)**	**Size (aa)**	**ORF**	**Max ID (%)**	**ORF**	**Max ID (%)**	**ORF**	**Max ID (%)**	**ORF**	**Max ID (%)**
1	*polh*	1 > 741	741	246	1	99	1	99	8	92	1	94
2	*pp78/83*	738 < 1,955	1,218	405	2	39	2	49	9	18	2	39
3	*pk-1*	1,979 > 2,806	828	275	3	56	3	55	10	40	3	54
4	*hoar*	2,988 < 4,898	1,911	636	4	27	4	28	-	-	4	19
5	*RanuNPV-ORF5*	4,903 < 5,163	261	86	-	-	-	-	-	-	-	-
6	*RanuNPV-ORF6*	5,684 > 6,886	1,203	400	-	-	-	-	-	-	-	-
7	*odv-e56 (pif-5)*	7,062 > 8,15	1,089	362	8	72	7	75	148	52	9	74
8	*me-53*	8,418 < 9,416	999	332	9	61	8	60	139	27	10	54
9	*RanuNPV-ORF9*	9,415 > 9,798	384	127	-	-	-	-	-	-	-	-
10	*exon0*	9,922 > 10,761	840	279	10	49	10	46	141	28	11	37
11	*p49*	10,78 > 12,201	1,422	473	11	78	11	78	142	50	75	77
12	*odv-e18*	12,223 > 12,471	249	82	12	90	12	88	143	60	7	86
13	*odv-ec27*	12,533 > 13,372	840	279	13	65	13	64	144	46	14	62
14	*chtBD2*	13,391 > 13,672	282	93	14	66	14	67	145	42	15	68
15	*ep23*	13,748 < 14,368	621	206	15	57	15	60	146	31	16	58
16	*ie-1*	14,541 > 16,913	2,373	790	16	40	16	40	147	19	17	24
17	*p74(pif-0)*	17,012 > 19,006	1,995	664	17	71	17	70	138	54	18	70
18	*p10*	19,059 < 19,328	270	89	18	64	18	65	137	28	19	58
19	*p26a*	19,386 < 20,087	702	233	19	57	19	60	136	27	20	51
20	*RanuNPV-ORF20*	20,327 < 20,488	162	53	-	-	-	-	-	-	-	-
21	*ac29-like*	20,467 > 20,679	213	70	20	41	20	46	29	34	21	29

22	*lef-6*	20,768	<	21,559	792	263	21	58	21	57	28	17	22	30
23	*dbp*	21,535	<	22,551	1,017	338	22	27	22	26	25	19	23	26
24	*ac26-like*	22,64	>	23,014	375	124	23	38	23	44	26	22	24	33
25	*ac34-like*	23,748	<	24,332	585	194	24	51	25	49	34	24	26	41
26	*ubiquitin*	24,48	>	24,749	270	89	25	91	26	88	35	66	27	89
27	*RanuNPV-ORF27*	24,712	>	24,921	210	69	25b	40	27	42	-	-	28	38
28	*39k*	25,003	<	25,98	978	325	26	37	28	38	36	27	29	34
29	*lef-11*	26,021	<	26,479	459	152	27	63	29	62	37	24	30	76
30	*nudix*	26,338	<	27,126	789	262	28	57	30	57	38	46	31	55
31	*RanuNPV-ORF31*	27,236	<	27,802	567	188	-	-	-	-	-	-	-	-
32	*p47*	27,948	<	29,177	1,230	409	31	64	33	64	40	51	34	64
33	*RanuNPV-ORF33*	29,21	<	29,362	153	50	-	-	-	-	-	-	-	-
34	*lef-12*	29,309	>	30,148	840	279	-	-	-	-	41	31	-	-
35	*ac43-like*	30,087	>	30,338	252	83	32	43	35	53	43	25	-	-
36	*bro-a*	30,359	<	31,501	1,143	380	-	-	-	-	-	-	-	-
37	*lef-8*	31,705	<	34,335	2,631	876	33	68	37	68	50	60	37	68
38	*bjdp*	34,359	>	35,426	1,068	355	34	46	38	47	51	5	38	46
39	*iap-3*	35,466	<	36,194	729	242	35	32	39	31			39	30
40	*RanuNPV-ORF40*	36,345	>	36,578	234	77	-	-	-	-	-	-	-	-
41	*ac52-like*	36,588	<	37,142	555	184	37	35	40	34	52	14	41	36
42	*ac53-like*	37,203	>	37,622	420	139	38	63	41	63	53	40	42	66
43	*RanuNPV-ORF43*	37,663	<	38,469	807	268	39	54	42	53	-	-	43	42
44	*RanuNPV-ORF44*	38,856	<	39,083	228	75	40	56	43	55	-	-	44	55
45	*lef-10*	39,043	>	39,267	225	74	41	67	44	62	53-a	47	45	68
46	*vp-1054*	39,131	>	40,138	1,008	335	42	64	45	65	54	39	46	64
47	*ac55-like*	40,257	>	40,478	222	73	43	46	46	51	55	29	47	56
48	*ac56-like*	40,396	>	40,749	354	117	44	47	47	50	56	19	76	9
49	*ac57-like*	41,024	>	41,569	546	181	45	57	48	57	57	34	49	49
50	*ChaB-like*	41,594	<	42,154	561	186	46	64	49	67	50/59	8	50	38
51	*ChaB-like*	42,213	<	42,509	297	98	47	57	50	54	60	34	51	52

52	*bro-b*	42,581	<	43,066	486	161	-	-	55	47	-	-	33	14
53	*fp-25k*	43,258	<	44,007	750	249	48	87	51	88	61	49	52	65
54	*lef-9*	44,078	>	45,571	1,494	498	49	77	52	77	62	60	53	76
55	*ac76-like*	45,988	>	46,245	258	85	52	91	56	92	76	43	55	91
56	*ac75-like*	46,257	>	46,643	387	128	53	65	57	66	75	16	56	65
57	*dna-pol*	46,719	<	49,967	3,249	1,082	54	62	58	62	65	41	57	59
58	*desmoplakin*	49,969	>	52,197	2,229	742	55	47	59	45	66	21	58	45
59	*lef-3*	52,328	<	53,479	1,152	383	56	32	60	31	67	18	59	25
60	*ac68-like (pif-6)*	54,045	>	54,449	405	134	57	72	61	70	68	25	60	58
61	*iap-2*	54,491	>	55,342	852	283	58	51	62	50	71	28	61	53
62	*p26a*	55,406	>	56,122	717	238	59	66	63	67	136	20	62	63
63	*RanuNPV-ORF63*	56,162	<	56,848	687	228	-	-	-	-	-	-	-	-
64	*v-cath*	56,908	<	57,957	1,050	349	60	71	64	71	127	54	63	72
65	*chi*	58,037	>	59,746	1,710	569	63	75	65	78	126	67	64	76
66	*gp-37*	59,809	>	60,642	834	277	64	82	67	81	64	51	67	78
67	*ac111-like*	60,727	<	60,927	201	66	66	23	71	49	111	4	70	30
68	*phr-2*	61,338	>	62,813	1,476	491	67	44	72	56	-	-	68	42
69	*ac84-like*	62,94	<	63,458	519	172	62	26	75	26	84	41	73	26
70	*he65*	63,707	>	64,411	705	234	-	-	73	42	105	26	71	43
71	*ctl*	64,486	>	64,635	150	49	-	-	74	84	3	53	72	12
72	*vlf-1*	64,632	<	65,783	1,152	383	70	90	76	90	77	69	74	82
73	*ac78-like*	65,796	<	66,155	360	119	71	40	77	44	78	36	75	41
74	*gp41*	66,176	<	67,075	900	299	72	74	78	75	80	48	76	73
75	*ac81-like*	67,146	<	67,793	648	215	74	78	79	78	81	53	77	65
76	*ac82-like*	67,75	<	68,553	804	267	75	69	80	54	82	29	78	48
77	*vp91*	68,423	>	70,834	2,412	803	76	49	81	47	83	41	79	49
78	*vp39*	70,928	<	71,962	1,035	344	77	42	82	42	89	38	80	42
79	*lef-4*	71,961	>	73,412	1,452	483	78	61	83	61	90	42	81	61
80	*p33*	73,452	<	74,231	780	259	79	68	84	66	92	47	82	66
81	*p18*	74,224	>	74,706	483	160	80	78	85	78	93	54	83	78

82	*odv-e25*	74,703	>	75,362	660	219	81	83	86	81	94	44	84	82
83	*dna-helicase*	75,501	<	79,289	3,789	1,262	82	56	87	51	95	35	85	56
84	*pif-4*	79,255	>	79,773	519	172	83	63	88	64	96	53	86	59
85	*38k*	79,995	<	80,93	936	311	86	61	91	60	98	44	89	59
86	*lef-5*	80,823	>	81,713	891	296	87	68	92	68	99	48	90	69
87	*p6.9*	81,71	<	82,009	300	99	89	23	93	78	100	50	91	72
88	*p40*	82,066	<	83,25	1,185	394	89	63	94	59	101	39	92	63
89	*p12*	83,278	<	83,529	252	83	90	66	95	66	102	32	93	72
90	*p48*	83,621	<	84,763	1,143	380	91	77	96	77	103	50	94	75
91	*vp80*	84,827	>	86,584	1,758	585	92	64	97	59	104	30	95	48
92	*pif-7*	86,587	>	86,772	186	61	93	61	98	61	110	29	-	-
93	*odv-ec43*	86,756	>	87,829	1,074	357	94	75	99	75	109	44	96	75
94	*ac108-like*	87,845	>	88,153	309	102	95	60	100	59	108	35	97	59
95	*odv-e66*	88,206	<	90,203	1,998	665	96	72	101	75	46	44	98	71
96	*p13*	90,263	<	91,108	846	281	97	55	102	55	-	-	99	56
97	*ac106/107-like*	91,632	<	92,354	723	240	102	72	107	72	106	63	104	75
98	*ac114-like*	92,392	<	93,996	1,605	534	103	49	108	50	114	29	105	49
99	*pif-3*	94,024	<	94,638	615	204	105	38	110	38	115	44	107	37
100	*bro-c*	94,721	<	95,065	345	114	136	20	55	24	2	17	33	24
101	*ac30-like*	95,363	>	95,944	582	193	107	66	112	68	30	16	109	69
102	*sod*	95,994	>	96,449	456	151	109	79	115	79	31	70	110	78
103	*dUTPase*	96,53	<	97,024	495	164	-	-	119	37	-	-	114	22
104	*pep*	97,641	>	98,6	960	319	113	83	121	83	131	30	115	75
105	*rr2*	98,652	<	99,608	957	318	114	64	122	64	-	-	116	63
106	*ac132-like*	99,744	>	100,169	426	141	115	40	123	38	132	18	117	36
107	*RanuNPV-ORF107*	100,176	>	101,33	1,155	384	116	66	124	67	-	-	118	67
108	*ac18-like*	101,364	<	102,599	1,236	411	117	43	125	41	18	23	119	43
109	*RanuNPV-ORF109*	102,601	>	102,93	330	109	118	53	126	50	-	-	120	45
110	*alk-exo*	103,048	>	104,256	1,209	402	119	45	127	45	133	38	121	43
111	*RanuNPV-ORF111*	104,286	<	105,041	756	251	120	55	128	57	-	-	122	54

112	*RanuNPV-ORF112*	105,227	<	105,385	159	52	-	-	-	-	-	-	-
113	*fgf*	105,513	>	106,382	870	289	122	37	130	43	32	33	123	34
114	*pif-1*	106,425	<	107,987	1,563	520	123	52	131	52	119	48	124	54
115	*RanuNPV-ORF115*	108,051	<	108,629	579	192	-	-	-	-	-	-	-	-
116	*gp-16*	108,693	<	108,98	288	95	125	57	133	58	130	37	126	54
117	*p24*	108,996	<	109,733	738	245	126	59	134	63	129	38	127	57
118	*RanuNPV-ORF118*	109,829	>	110,293	465	154	-	-	135	40	-	-	-	-
119	*lef-2*	110,268	>	110,888	621	206	128	57	136	57	6	41	129	55
120	*38.7 kDa*	111,122	<	112,213	1,092	363	129	41	137	43	13	47	130	39
121	*lef-1*	112,233	<	112,886	654	217	130	60	138	59	14	46	131	57
122	RanuNPV-ORF122	112,948	>	113,385	438	145	131	64	139	66	-	-	132	51
123	*ptp*	113,513	<	113,908	396	131	132	56	140	56	1	12	133	50
124	*egt*	114,182	>	115,765	1,584	527	133	77	141	75	15	46	134	75
125	RanuNPV-ORF125	115,738	<	115,902	165	54	-	-	-	-	-	-	-	-
126	RanuNPV-ORF126	115,989	>	116,288	300	99	135	62	142	62	-	-	135	55
127	RanuNPV-ORF127	116,927	<	119,554	2,628	875	136	53	143	55	-	-	136	47
128	RanuNPV-ORF128	119,728	>	120,147	420	139	-	-	-	-	-	-	87	40
129	*pkip*	120,163	>	120,675	513	170	139	44	146	44	24	24	137	43
130	*arif-1*	120,72	<	121,727	1,008	335	140	38	147	47	20/21	28	138	39
131	*pif-2*	121,782	>	122,876	1,095	364	141	67	148	66	22	61	139	67
132	*bro-d*	122,943	>	123,635	693	230	108	37	-	-	-	-	-	-
133	*fp*	123,721	<	125,721	2,001	666	143	61	150	63	23	18	140	60
134	*rr1*	125,972	<	128,338	2,367	788	144	57	151	55	-	-	141	54

APÊNDICE D – TABELA SUPLEMENTAR 3

Table S3. OB morphology in several some alphabaculovirus related to RanuNPV.

Baculovirus	Reference	OB
Operophtera brumata nucleopolyhedrovirus	Harrison et al., 2017.	Polyhedral
Mythimna unipuncta nucleopolyhedrovirus strain KY310	Harrison et al., 2019	Polyhedral
Helicoverpa zea single nucleopolyhedrovirus	Ardisson-Araújo et al., 2015	Polyhedral
Rachiplusia nu nucleopolyhedrovirus	**In this work**	**Pyramidal**
Chrysodeixis chalcites nucleopolyhedrovirus	Xu et al., 2010	Polyhedral
Chrysodeixis includens single nucleopolyhedrovirus	Alexandre et al., 2010;	Polyhedral
Spodoptera exigua multiple nucleopolyhedrovirus isolate QD	Chen et al., 2019.	Polyhedral
Mythimna unipuncta nucleopolyhedrovirus strain #7	Harrison et al., 2018.	Polyhedral
Urbanus proteus nucleopolyhedrovirus	Santos et al., 2018	Polyhedral
Cryptophlebia peltastica nucleopolyhedrovirus	Marsberg et al., 2018	Polyhedral
Malacosoma neustria nucleopolyhedrovirus	Demir et al., 2013	Polyhedral
Perigonia lusca single nucleopolyhedrovirus	Ardisson-Araújo et al, 2016	Polyhedral
Euproctis pseudoconspersa nucleopolyhedrovirus	Tang et al., 2009	Polyhedral
Ectropis obliqua nucleopolyhedrovirus	Ma et al., 2006	Polyhedral
Lambdina fiscellaria nucleopolyhedrovirus	Whittome-Waygood et al., 2009	Polyhedral

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