Genomic analysis of a cypovirus isolated from the eucalyptus brown looper, *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae)

André Ballerini Horta[a],[1], Daniel Mendes Pereira Ardisson-Araujo[b],[1], Leonardo Assis da Silva[c],
Fernando Lucas de Melo[c], Fabricio da Silva Morgado[c], Manoel Victor Franco Lemos[d],
Zulene Antonio Ribeiro[e], Arlindo Leal Boiça Junior[e], Carlos Frederico Wilcken[a],
Bergmann Morais Ribeiro[c],*

[a] *Plant Protection Department, FCA/UNESP—São Paulo State University, Campus of Botucatu, 18610-307, Botucatu, SP, Brazil*
[b] *Biochemistry and Molecular Biology Department, Federal University of Santa Maria, Santa Maria, RS, Brazil*
[c] *Cell Biology Department, UnB—University of Brasília, 70910-900, Brasília, DF, Brazil*
[d] *Applied Biology Department, FCAV/UNESP—São Paulo State University, Campus of Jaboticabal, 14884-900, Jaboticabal, SP, Brazil*
[e] *Plant Protection Department, FCAV/UNESP—São Paulo State University, Campus of Jaboticabal, 14884-900, Jaboticabal, SP, Brazil*

ARTICLE INFO

ABSTRACT

The eucalyptus brown looper, *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae), is the main lepidopteran defoliator of eucalyptus plantations in Brazil. Outbreaks of this insect pest are common in Brazil and can affect the productivity of planted forests severely. *T. arnobia* caterpillars from a laboratory colony with viral infection symptoms were analyzed by electron microscopy that revealed polyhedral occlusion bodies (OBs) with several icosahedral virus particles embedded. Analysis of its genetic material showed ten segments of dsRNA, which confirmed this virus as a possible member of the genus *Cypovirus*. Phylogenetic analysis of the whole genome sequence revealed its close relationship with other isolates of *Cypovirus 14* species and according to these results we proposed the name Thyrinteina arnobia cypovirus 14 (TharCPV-14) for this new virus isolate. Further research will be necessary in order to analyze the potential of this virus as a biopesticide.

1. Introduction

Wood production in countries located in tropical and subtropical regions is based on forest plantations. Brazil has a prominent position as a global leader in forest productivity. According to the Brazilian Industry of Trees (Portuguese acronym IBÁ, 'Indústria Brasileira de Árvores') in 2016, the export revenue from the Brazilian planted tree industry reached US$ 8.9 billion; mainly due to *Eucalyptus*, the most planted hardwood species worldwide (Rockwood et al., 2008). The eucalyptus plantations occupy approximately 73% of 7.84 million hectares of planted forests in Brazil. The growing areas of non-native forests cultivated as monocultures have created conditions to the establishment and reproduction of many species of insect-pests at high population densities, which can severely reduce tree development and economic yields (Hurley et al., 2016).

One of the most important insect-pests of eucalyptus forests in Brazil is the larval stage of *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae), the eucalyptus brown looper (Oliveira et al., 2005; Ribeiro et al., 2016). The control of this insect is carried out mainly by biopesticides and releases of natural enemies, such as parasitoids and predatory bugs (Barbosa et al., 2016; Zanuncio et al., 2014). However, severe outbreaks in Brazil have led to the prospection of alternative methods for its effective control (Jesus et al., 2015), including new biological agents.

Cypoviruses, also known as cytoplasmic polyhedrosis viruses (CPVs), are insect viruses with a genome segmented consisting of 10 to 12 dsRNA molecules in a non-enveloped and icosahedral capsid that belong to the family *Reoviridae* (Attoui et al., 2012). Remarkably, during virus infection CPVs produce in the cell cytoplasm occlusion bodies (OBs) that protect virions from environmental adversities. The main infection route is by ingestion of OBs present on contaminated food followed by the dissolution in the alkaline pH of the caterpillar

* Corresponding author.
E-mail addresses: andre.b.horta@fcav.unesp.br (A.B. Horta), daniel.araujo@ufsm.br (D.M.P. Ardisson-Araujo), leocbq@unb.br (L.A. da Silva), flmelo@unb.br (F.L. de Melo), fabsmorga@gmail.com (F. da Silva Morgado), mvictor@fcav.unesp.br (M.V. Franco Lemos), zribeiro@fcav.unesp.br (Z.A. Ribeiro), aboicajr@fcav.unesp.br (A.L. Boiça), cwilcken@fca.unesp.br (C.F. Wilcken), bergmann@unb.br (B.M. Ribeiro).
[1] Equal contribution to this work.

midgut. The dissolution releases virions from OBs to infect the midgut epithelium cells (Attoui et al., 2012). In lethal infections, disease symptoms appear two or three days after the initial ingestion by the larva and include reduction of insect feeding and movement, alterations of tegument coloration, pasty excrements, and climbing up behavior to the plant top before death. After death, the OBs are released in the environment, contaminating the plant leaves, and favoring the horizontal transmission among susceptible hosts (Belloncik, 1989). Cypoviruses are considered less pathogenic than baculoviruses (another OB-producing insect virus with large double-stranded DNA genome) and have been shown to induce sublethal effects on some infected hosts such as lower weight of the infected inset, shorter adult lifespan, and development (for a review see Rothman and Myers, 1996). In this work, we characterized the first virus isolated from *T. arnobia,* which was shown to be an isolate belonging to the species *Cypovirus 14* and we tentatively named Thyrinteina arnobia cypovirus 14 (TharCPV-14). Moreover, we performed ultrastructural characterization, sequencing, phylogenetic analysis, and genome diversity description.

2. Materials and methods

2.1. Samples origin

The samples of *T. arnobia* dead caterpillars with symptoms of virus infection were collected from an insect colony at the Laboratory of Plant Resistance to Insects at São Paulo State University (USP) (Jaboticabal, Brazil). This population was obtained from a commercial eucalyptus plantation, being maintained healthy for several generations through insect feeding with leaves of *E. camaldulensis* under controlled conditions of temperature ($25\,°C \pm 2$), humidity (70%) and photoperiod (12 h). Caterpillars with symptoms of virus infection were collected before death and small drops of its regurgitation liquid were analyzed under a light microscope. One leaf of *E. camaldulensis* treated with these drops was offered to 5 apparently healthy 3rd instar *T. arnobia* caterpillars and mortality observed every 24 h.

2.2. Purification and ultrastructural analysis of OBs

Ten dead caterpillars of *T. arnobia* between the 5th to 6th instar were macerated in phosphate buffered saline (PBS: 137.0 mM NaCl, 2.7 mM KCl, 10.0 mM Na2HPO4, 2.0 mM KH2PO4, pH 7.4) and the homogenate was filtered once through cheesecloth and centrifuged once at $12,000 \times g$ for 10 min at 4 °C. The pellet was washed with PBS and centrifuged three times at the same conditions. The pellet was resuspended with distilled RNase-free water and placed onto a linear sucrose gradient (20, 40, 50, 60 and 80%) with distilled RNase-free water, and centrifuged at $140,000 \times g$ for 75 min at 4 °C. A main white

band containing OBs was collected, diluted 10 times in PBS and centrifuged again at $12,000 \times g$ for 10 min at 4 °C. The pellet was collected, resuspended in 1 mL of distilled RNase-free water, counted in a hemocytometer, as previously described (O'Reilly et al., 1992) and maintained at $-20\,°C$. For scanning electron microscopy (SEM), 100 μl (10%) of the OBs suspensions were incubated with 300 μl of acetone at 25 °C for 1 h. The suspensions were loaded onto two metallic stubs, dried overnight at 37 °C, coated with gold in a Sputter Coater (Balzers) for 3 min, and observed in a Scanning Electron Microscope Jeol JSM 840 A at 10 kV. For transmission electron microscopy (TEM), pellets of purified OBs were fixed in Karnovsky fixative (2.5% glutaraldehyde, 2% paraformaldehyde, in 0.1 M, cacodylate buffer, pH 7.2) for 2 h, post-fixed in 1% osmium tetroxide in the same buffer for 1 h and then stained *en bloc* with 0.5% aqueous uranyl acetate, dehydrated in acetone, and embedded in Spurr's low viscosity embedding medium. The ultrathin sections (60–80 nm) were obtained with a Leica ultramicrotome equipped with a diamond knife, contrasted with 2% uranyl acetate and observed in a ZEISS TEM 109 at 80 kV.

2.3. SDS-PAGE analysis

10 μl of purified OBs (10^6) were mixed with the same volume of loading buffer (0.25 M Tris-Cl, pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, and 0.02% bromophenol blue), heated for 5 min at 100 °C and subjected to electrophoresis in 12% SDS-PAGE gels using the Mini Protean Tetra Cell apparatus (BioRad) following the manufacturer's instructions. The gel was then photographed using an ImageQuant™ LAS 4000 (GE).

2.4. RNA isolation, genome sequencing, and analysis

RNA extraction was carried out using 200 μl (10^8/mL) of the purified OBs suspension using the TRIZOL® reagent (Invitrogen) according to the manufacturer's instructions. The RNA quality was checked by electrophoresis in an agarose gel (1%), stained with 0.01% ethidium bromide, and visualized under UV light and photographed in an AlphaImager® Mini (Alpha Innotech). The viral RNA genome segments were used to build cDNA libraries and sequenced using 2×100 bp read length on the HiSeq™ 2000 platform (Illumina, San Diego, CA, USA) at Macrogen Inc (Seoul, Republic of Korea). The resulting reads were trimmed and the genome *de novo* assembly was carried out using CLC Genome Workbench 6.5.2 (CLC bio, Denmark). The read mapping and genome annotation was performed using Geneious 7.1.8 (Kearse et al., 2012) and selecting the following parameters for Open Reading Frames (ORFs): AUG start codons (methionine), minimal overlapping of adjacent ORFs and at least 150 bp long. ORFs were annotated using BLASTx search against the NCBI non-redundant protein database. The

Table 1
Properties of the dsRNA segments of TharCPV-14. Segments with the respective GenBank accession numbers, segment sizes in nucleotides, including ORFs, protein's sizes in amino acids, putative function, nucleotide identity with other cypovirus 14 species.

Segment number	Accession number	Segment size (nt)	ORF posistion (nt)	Protein size (aa)	Putative Function	Nucleotide identity (%)		
						HearCPV/ TharCPV	LydiCPV/ TharCPV	HearCPV/ LydiCPV
S1	MF161423	4466	446–4363	1305	RdRp	84,5	83,6	97,5
S2	MF161424	4075	59–3970	1303	major capsid protein	84,3	84,3	97,7
S3	MF161425	3953	34–3834	1266	minor capsid protein	81,8	81,7	98,6
S4	MF161426	3358	46–3270	1074	mRNA 5′ capping synthesis	83,2	83,1	98,4
S5	MF161432	3119	58–3009	982	Unknown	65,2	65,0	97,9
S6	MF161427	1772	19–1671	550	RNA guanylyltransferase	84,0	83,9	99,3
S7	MF161428	1412	8–1279	423	NTPase activity	80,5	79,8	94,1
S8	MF161429	1332	43–1182	379	Unknown	81,3	81,2	99,0
S9	MF161430	1269	71–1024	317	Unknown	79,9	81,0	95,8
S10	MF161431	978	55–804	249	Occlusion body protein (POLH)	88,0	87,9	98,5

viral derived sequences were submitted to GenBank (Table 1).

2.5. Phylogenetic analysis

The phylogenetic analysis was conducted using all nucleotide sequences of the ten segments and compared to other viral sequences deposited in GenBank. Only those hits with e-values less than 10–20 were used. Redundant sequences (nucleotide identity higher than 90%) were omitted from our analysis. The MAFFT alignment method (Katoh et al., 2002) was carried out with the RNA-dependent RNA polymerase (RdRp) gene of several cypoviruses and other reoviruses publicly available strains. A maximum likelihood tree was inferred using the Fast-tree method (Stamatakis et al., 2008) implemented in Geneious R9 and the branch support was estimated by Shimodaira-Hasegawa-like test (Anisimova et al., 2011).

3. Results

3.1. Virus characterization

For almost twenty consecutive generations *T. arnobia* caterpillars were kept healthy at rearing conditions in a laboratory colony. Unexpectedly, a few caterpillars with symptoms of virus infection were identified and less than 15 days after this first observation, hundreds of caterpillars at fourth and fifth instars died stuck on leaves (Fig. 1A). The disease decimated completely the laboratorial insect population and extracts from these dead insects were analyzed by light and electron microscopy. Light microscopy revealed the presence of OBs that were purified and subjected to SEM and TEM. OBs with a polyhedral shape and non-regular size were observed (Fig. 1B) that formed a proteinaceous matrix surrounding several icosahedral virions (Fig. 1C). To confirm the protein nature of the OB structure, we subjected the OBs to

SDS-PAGE and found a strong band between 30–40 kDa, characteristic of the cypovirus polyhedrin with other protein bands above and below (Fig. 1D). Furthermore, we conducted a nucleic acid extraction from the OBs that showed an electrophoretic profile of ten putative segments of dsRNA (Fig. 1E), which indicated that the pathogen was possibly a cypovirus. Its pathogenicity was confirmed by the death of 3rd instar caterpillars of *T. arnobia* between 72 and 96 h after feeding with a leaf treated with vomit drops from dying caterpillars.

3.2. Genome sequencing of T. arnobia isolated cypovirus

In total, 10 genomic dsRNA segments were identified from the Illumina platform sequencing. The GenBank accession numbers, characteristics of each segment, and the putative annotated protein are shown in Table 1. We numbered the segments according to their size from the largest (S1) to the smallest (S10). The conserved terminal sequences were found in both the 5′ and 3′ noncoding regions (5′-AGAA... CAGCU -3′) of most segments, which are identical to those found in *Cypovirus 14* species (Attoui et al., 2012). All segments presented homologs in GenBank, mostly from the species *Cypovirus 14*. The largest segment of TharCPV-14 (S1) contains a long ORF that codes for the viral RNA polymerase (RdRp) and is closely related to the RdRps of both Lymantria dispar cypovirus 14 (LydiCPV-14) and Heliothis armigera cypovirus 14 (HearCPV-14) (Attoui et al., 2012). The segment 2 (S2) encodes the major core protein (VP1) and presents identity with the segment 2 of HearCPV-14 (GenBank accession number: ABD57841.1), segment 1 from Bombyx mori cypovirus 1 (BmCPV-1) (32% nt identity), and the segment 1 of Thaumetopoea pityocampa cypovirus 5 (25% nt identity). The segment 3 (S3) encodes a hypothetical protein with a predicted thiol peptidases domain (PPPDE superfamily) (Iyer et al., 2004) and shows identity with the minor capsid protein encoded by segment 1 of Antheraea mylitta cypovirus 4 (28% nt identity) and
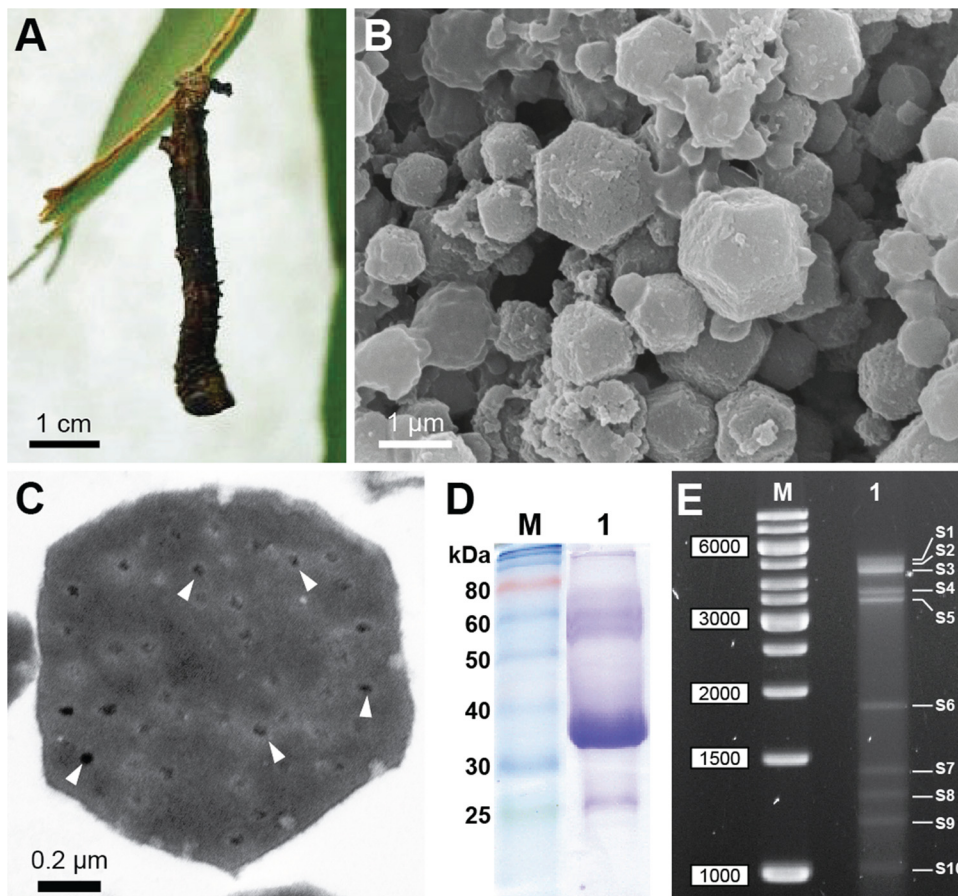

Fig. 1. Structural and ultrastructural characterization of the novel cypovirus isolated from dead *Thyrinteina arnobia* larvae showing symptoms of virus infection. A) Dead *T. arnobia* caterpillar stuck on the leaf with viral infection symptoms. (B) Scanning electron micrography of purified polyhedral occlusion bodies (OBs) from dead infected larval extract. (C) Transmission electron micrography of OBs in transversal sections showing the icosahedral virus particles (indicated with white arrow heads) occluded in the polyhedrin protein matrix. (D) SDS-PAGE (12%) of the solubilized TharCPV-14 OB, showing prominent band near 30 kDa, the expected size of polyhedrin. (E) Agarose gel electrophoretic patterns of putative dsRNA from TharCPV-14 identifying the segments numbered from S1 to S10.

the minor core protein (VP2) encoded by segment 2 of Orgyia pseudotsugata cypovirus 5 (26% nt identity). Segments 4, 6 and 7 encode proteins related to BmCPV-1 proteins involved in mRNA 5′ capping synthesis, RNA guanylyltransferase and protein with NTPase activity, respectively. Segments 5, 8 and 9 encode proteins with unknown function (hypothetical proteins). Interestingly, S8 is only present in *Cypovirus 14*, and seemed to be an autapomorphy for this species complex. Using the SMART domain prediction, we found a domain in S8 related to the eukaryotic initiation factor 6 (eIF6), related to protein translation. The segment (S10) encodes the major OB protein of TharCPV-14. The sequence of 249 amino acids with around 28.6 kDa has 98% amino acid identity with the same protein encoded by the segment 10 of Heliothis assulta cypovirus 14 (HeasCPV-14) and 97% amino acid identity with the polyhedrin encoded by the segment 10 of LydiCPV-14. Interestingly, we found an overlapping ORF (669 nt and 222 aa) in an opposite direction from the *polh* gene. This overlapping ORF was previously described in the genome of LydiCPV-14 (AF389461). There is no evidence of its translation and no homologs in other organisms were found.

3.3. Phylogenetic analysis and genome diversity

The species demarcation criteria are not strictly specified based on the complete genome sequence for all segments of CPVs. According to the ICTV, the criteria for the genus includes dsRNA electrophoretic migration, high degree of nucleotide or amino acid sequence conservation (estimated > 80% for the former), and cross-hybridization of genome segments. Therefore, we estimate the virus phylogeny based on the RdRp gene (Fig. 2). The isolated virus belongs to the Cypovirus 14 species, but with a long branch length suggesting high genetic heterogeneity. Moreover, we performed individual phylogenetic analyses for all the other segments of TharCPV-14 and, as expected, TharCPV-14 was found to be related to isolates from the species *Cypovirus 14* in all
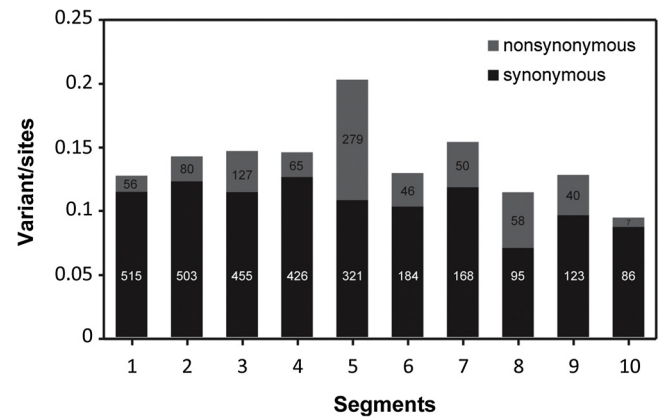

Fig. 3. Nucleotide diversity for the coding regions of TharCPV-14 segments against two other isolates from the species Cypovirus 14. We set TharCPV-14 segments as reference and count the total number of variant sites (NVS) corrected by the segment size. Nonsynonymous substitutions are shown in grey and synonymous substitutions in black. The absolute NVSs are also shown inside the bars.

phylogenies (Fig. S1). LydiCPV-14 and HearCPV-14 also belong to the species *Cypovirus 14*. In Table 1, we present the global identity for each segment comparing the ThaCPV-14 to its closely related isolates. We performed a nucleotide diversity analysis in relation to these completely sequenced *Cypovirus 14* isolates considering only the coding region inside the segments (Fig. 3). We found that TharCPV-14 presents several polymorphisms in comparison to the other isolates of *Cypovirus 14*. For this analysis, we counted the number of single nucleotide variants (SNVs) observed for each individual segment of TharCPV-14 in relation to the other isolates (LydiCPV-14 and HearCPV-14). We found that the amount of nucleotide variations increased with the size of the virus segment, with exceptions for the S1, S5, and S10 segments. The S1 and S10 presented slightly less variation than it was expected for their sizes and most of the SNVs did not change the protein sequence (data not shown). The most conserved segment among the isolates was the S10 (*polh* gene) since single non-synonymous mutation has the potential to drastically change OB formation and impairs the ability to occlude virions as observed for baculoviruses (Coulibaly et al., 2007; Ardisson-Araújo et al., 2018). On the other hand, the S5 segment presented more SNVs than that expected by its size. Most of the SNVs were non-synonymous as observed in Fig. 3. Interestingly, the S5 segment presents the smallest identity based on MAFFT alignment considering the S5 from the three *Cypovirus 14* isolates with 65% of global nucleotide identity. The nucleotide identity between the S5 of LydiCPV-14 and HearCPV-14 alone was 97.6%, depicting a small genetic distance between these two isolates.

4. Discussion

Sixteen species of the genus *Cypovirus* (1–16) are currently recognized by the ICTV. In this work, we found a new isolate of the species *Cypovirus 14* infecting a laboratory colony of the eucalyptus brown looper, *Thyrinteina arnobia*. We performed structural and ultrastructural characterization of this new virus and obtained its complete genome sequence. TharCPV-14 presented polyhedral-shaped OBs with embedded icosahedral virions. This new virus isolate contains a dsRNA genome with 10 segments and a highly diverse sequence compared to the other *Cypovirus 14* isolates, relating itself as closer to the hypothetical ancestor of LydiCPV-14 and HearCPV-14.

TharCPV-14, HearCPV-14, and LydiCPV-14 were isolated from different insect host species, but they belong to the same ICTV recognized species *Cypovirus 14* according to the species demarcation criteria. However, when we compared the nucleotide identity among these three isolates, we found that TharCPV-14 was the most divergent isolate, with
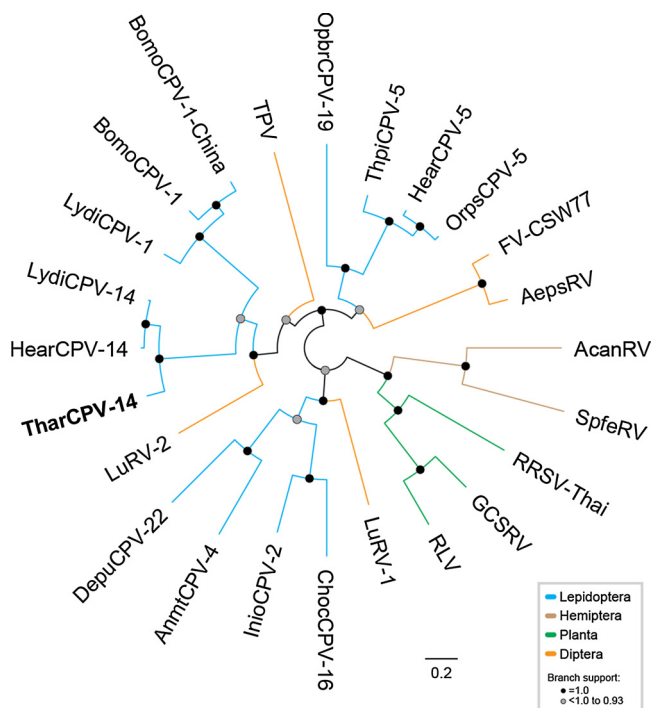

Fig. 2. Phylogenetic analysis of Thyrinteina arnobia *cypovirus 14* (TharCPV-14) with respect to other dsRNA viruses. The phylogeny was inferred based on the MAFFT alignment of several RdRp genes and the FastTree method with a Shimoidara-Hasengawa-like test for the branch support implemented in Geneious R7. The tree was midpoint rooted and presented here as a polar cladogram for clarity.

more SNVs when compared to the other isolates. A phylogenetic analysis based on the amino acid sequences of several reovirus RdRp proteins showed that TharCPV-14 nested together in a branch containing the other isolates of *Cypovirus 14* in a position closer to the hypothetical ancestor of this species. On the hand, viruses with different evolutionary origins were found infecting simultaneously the same insect host (Graham et al., 2007; Zhou et al., 2014). Interestingly, the *in silico* analysis showed that each of the segments of the TharCPV-14 genome contained a single ORF and the conserved terminal sequences at their 5′ and 3′ ends (5′ - AGAA…CAGCU -3′) as shown for the other two known cypoviruses from the *Cypovirus 14 species*. Each species of cypovirus has a specific sequence at their 5′ and 3′ ends (see Supplementary Table S1). Such conserved motifs are detected in many viruses containing segmented genomes and it has been suggested that they play an important role in transcription, replication, RNA packaging, and virus maturation (Anzola et al., 1987; Attoui et al., 2012; Xu et al., 1989). In CPVs, low nucleotide sequence identity (20–23%) was reported among different species. Nevertheless, considering the same species, the isolates showed higher nucleotide sequence identities (80–98%) (Attoui et al., 2012; Li et al., 2006). All the segments presented homologs in GenBank, mostly from the *Cypovirus 14* species. The segment 8 sequence is only present in *Cypovirus 14* isolates and seemed to be an autapomorphy for this species complex. We found a domain in the S8 segment related to the eukaryotic initiation factor 6 (eIF6), associated with protein translation (Manfrini et al., 2017). The impact and the function of each segment is not clear for most of the described CPVs so far. A reverse genetics system based for Bombyx mori cypovirus (BmCPV) has been recently developed (Guo et al., 2018). The system consists of 10 recombinant plasmids expressing the full-length cDNA of viral genome segments from S1 to S10 under the control of a T7 promoter for *in vitro* transcription. Both viral particles and OBs with embedded virions were generated from co-transfected cells and injected silkworms.

The reason for the sudden emergence of a cypovirus in the insect laboratory colony is unclear. In fact, the virus was likely present in the indigenous insect population used to establish the lab colony and much probably, it was not causing a clear symptom of disease. Similarly to *Helicoverpa zea single nucleopolyhedrovirus* (Ardisson-Araújo et al., 2014a) and *Bombyx mori nucleopolyhedrovirus* (Ardisson-Araújo et al., 2014b) which were probably introduced in Brazil by infected insects presenting either a sublethal or a latent infection. This characteristic seems to be crucial for the persistence of baculoviruses in the environment (Kukan, 1999). The high conspecific density itself may intensify the probability of a pathogen to find its host and replicate. Some gregarious living insects may evolve to counteract this improved risk by developing strategies such as density-dependent prophylaxis (Silva et al., 2016). Several studies indicate that cypoviruses can develop chronic diseases with sublethal effects (Mertens, 2004; Mertens et al., 1999) leading also to vertical transmission in some species (Graham et al., 2006). However, cypoviruses do seem to be able to interact with the host and change dramatically the genomic DNA methylation patterns in the midgut and fat body of the infected host (Hou et al., 2017).

5. Conclusion

A new pathogenic virus to *T. arnobia* was found and purified from dead insects from a lab colony. Its genome was shown to contain 10 segments and its non-enveloped viral particles were found occluded in large proteinaceous OBs. All genomic RNA segments have coding regions with high amino acid identity to proteins found in the other two isolates belonging to the Cypovirus 14 species. The constructed phylogenetic tree based on the RdRp gene confirms the genetic proximity of this virus to the HearCPV-14 and LydiCPV-14 isolates and we named this new virus Thyrinteina arnobia cypovirus 14 (TharCPV-14). Future studies will evaluate the TharCPV-14 potential as a viral biopesticide for the control of *T. arnobia* caterpillars in eucalyptus plantations.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2018.05.026.

References

Anisimova, M., Gil, M., Dufayard, J.F., Dessimoz, C., Gascuel, O., 2011. Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. Syst. Biol. 60, 685–699. http://dx.doi.org/10.1093/sysbio/syr041.

Anzola, J.V., Xu, Z.K., Asamizu, T., Nuss, D.L., 1987. Segment-specific inverted repeats found adjacent to conserved terminal sequences in wound tumor virus genome and defective interfering RNAs. Proc. Natl. Acad. Sci. U. S. A. 84, 8301–8305.

Ardisson-Araújo, D.M., Sosa-Gómez, D.R., Melo, F.L., Nair Báo, S., Ribeiro, B.M., 2014a. Characterization of *Helicoverpa zea single nucleopolyhedrovirus* isolated in Brazil during the first old world bollworm (Noctuidae: Helicoverpa armigera) nationwide outbreak. Virus Rev. Res. 20, 1–4. http://dx.doi.org/10.17525/vrrjournal.v20i1.254.

Ardisson-Araújo, D.M.P., Melo, F.L., de Souza Andrade, M., Brancalhão, R.M.C., Báo, S.N., Ribeiro, B.M., 2014b. Complete genome sequence of the first non-Asian isolate of Bombyx mori nucleopolyhedrovirus. Virus Genes 49, 477–484. http://dx.doi.org/10.1007/s11262-014-1112-6.

Ardisson-Araújo, D.M., da Silva, A.M.R., Melo, F.L., dos Santos, E.R., Sosa-Gómez, D.R., Ribeiro, B.M., 2018. A novel betabaculovirus isolated from the monocot pest *Mocis latipes* (Lepidoptera: Noctuidae) and the evolution of multiple-copy genes. Viruses 10 (3), 134. http://dx.doi.org/10.3390/v10030134.

Attoui, H., Mertens, P.P.C., Becnel, J., Belaganahalli, S., Bergoin, M., Brussaard, C.P., Chappell, J.D., Ciarlet, M., del Vas, M., Dermody, T.S., Dormitzer, P.R., Duncan, R., Fcang, Q., Graham, R., Guglielmi, K.M., Harding, R.M., Hillman, B., Makkay, A., Marzachì, C., Matthijnssens, J., Milne, R.G., Mohd Jaafar, F., Mori, H., Noordeloos, A.A., Omura, T., Patton, J.T., Rao, S., Maan, M., Stoltz, D., Suzuki, N., Upadhyaya, N.M., Wei, C., Zhou, H., 2012. Reoviridae. In: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier-Academic Press, San Diego pp. 540–537.

Barbosa, R.H., Zanuncio, J.C., Pereira, F.F., Kassab, S.O., Rossoni, C., 2016. Foraging activity of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) at various densities on pupae of the eucalyptus defoliator *Thyrinteina arnobia* (Lepidoptera: Geometridae). Florida Entomol. 99, 686–690. http://dx.doi.org/10.1653/024.099.0417.

Belloncik, S., 1989. Cytoplasmic polyhedrosis viruses - Reoviridae. Adv. Virus Res. 37, 173–209. http://dx.doi.org/10.1016/S0065-3527(08)60835-0.

Coulibaly, F., Chiu, E., Ikeda, K., Gutmann, S., Haebel, P.W., Schulze-Briese, C., Mori, H., Metcalf, P., 2007. The molecular organization of cypovirus polyhedra. Nature 446, 97–101. http://dx.doi.org/10.1038/nature05628.

Graham, R.I., Rao, S., Possee, R.D., Sait, S.M., Mertens, P.P.C., Hails, R.S., 2006. Detection and characterisation of three novel species of reovirus (Reoviridae), isolated from geographically separate populations of the winter moth *Operophtera brumata* (Lepidoptera: Geometridae) on Orkney. J. Invertebr. Pathol. 91, 79–87. http://dx.doi.org/10.1016/j.jip.2005.11.003.

Graham, R.I., Rao, S., Sait, S.M., Mertens, P.P.C., Hails, R.S., Possee, R.D., 2007. Characterization and partial sequence analysis of two novel cypoviruses isolated from the winter moth *Operophtera brumata* (Lepidoptera: Geometridae). Virus Genes 35, 463–471. http://dx.doi.org/10.1007/s11262-007-0113-0.

Guo, R., Cao, G., Xue, R., Kumar, D., Chen, F., Liu, W., Jiang, Y., Lu, Y., Zhu, L., Liang, Z., Kuang, S., 2018. Exogenous gene can be expressed by a recombinant *Bombyx mori* cypovirus. Appl. Microbiol. Biotechnol. 102, 1367–1379. http://dx.doi.org/10.1007/s00253-017-8667-9.

Hou, C., Annan, E., Wu, P., Shang, Q., Chen, T., Jie, W., Jiang, X., Guo, X., 2017. DNA methylation in silkworm genome may provide insights into epigenetic regulation of response to *Bombyx mori* cypovirus infection. Sci. Rep. 7http://dx.doi.org/10.1038/s41598-017-16357-7. 16013.0.

Hurley, B.P., Garnas, J., Wingfield, M.J., Branco, M., Richardson, D.M., Slippers, B., 2016. Increasing numbers and intercontinental spread of invasive insects on eucalypts. Biol.

Invasions 18, 921–933. http://dx.doi.org/10.1007/s10530-016-1081-x.

Iyer, L.M., Koonin, E.V., Aravind, L., 2004. Novel predicted peptidases with a potential role in the ubiquitin signaling pathway. Cell Cycle 3, 1440–1450. http://dx.doi.org/10.4161/cc.3.11.1206.

Jesus, F.G., Nogueira, L., Boiça Junior, A.L., Ribeiro, Z.A., Araújo, M.S., Zanuncio, J.C., 2015. Resistance of *Eucalyptus* spp. genotypes to eucalyptus brown looper *Thyrinteina arnobia* (Lepidoptera: Geometridae). Aust. J. Crop Sci. 9, 1016–1021.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast fourier transform. Nucleic Acids Res. 30, 3059–3066.

Kearse, M., Moir, R., Wilcon, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. http://dx.doi.org/10.1093/bioinformatics/bts199.

Kukan, B., 1999. Vertical transmission of nucleopolyhedrovirus in insects. J. Invertebr. Pathol. 74, 103–111. http://dx.doi.org/10.1006/JIPA.1999.4873.

Li, Y., Tan, L., Li, Y., Chen, W., Zhang, J., Hu, Y., 2006. Identification and genome characterization of *Heliothis armigera* cypovirus types 5 and 14 and *Heliothis assulta* cypovirus type 14. J. Gen. Virol. 87, 387–394. http://dx.doi.org/10.1099/vir.0.81435-0.

Manfrini, N., Ricciardi, S., Miluzio, A., Fedeli, M., Scagliola, A., Gallo, S., Brina, D., Adler, T., Busch, D.H., Gailus-Durner, V., Fuchs, H., Hrabě de Angelis, M., Biffo, S., 2017. High levels of eukaryotic Initiation Factor 6 (eIF6) are required for immune system homeostasis and for steering the glycolytic flux of TCR-stimulated CD4 + + T cells in both mice and humans. Dev. Comp. Immunol. 77, 69–76. http://dx.doi.org/10.1016/J.DCI.2017.07.022.

Mertens, P., 2004. The dsRNA viruses. Virus Res. 101, 3–13. http://dx.doi.org/10.1016/j.virusres.2003.12.002.

Mertens, P.P.C., Pedley, S., Crook, N.E., Rubinstein, R., Payne, C.C., 1999. A comparison of six cypovirus isolates by cross-hybridisation of their dsRNA genome segments. Arch. Virol. 144, 561–576. http://dx.doi.org/10.1007/s007050050525.

O'Reilly, D.R., Miller, L.K., Luckow, V.A., 1992. Baculovirus Expression Vector: A Laboratory Manual. WH Freeman and Company, New York. http://dx.doi.org/10.1016/0092-8674(93)90288-2.

Oliveira, H.N., de, Zanuncio, J.C., Pedruzzi, E.P., Espindula, M.C., 2005. Rearing of *Thyrinteina arnobia* (Lepidoptera: Geometridae) on guava and eucalyptus in laboratory. Braz. Arch. Biol. Technol. 48, 801–806. http://dx.doi.org/10.1590/S1516-89132005000600016.

Ribeiro, G.T., Zanuncio, J.C., de S Tavares, W., de S Ramalho, F., Serrão, J.E., 2016. Constancy, distribution, and frequency of Lepidoptera defoliators of *Eucalyptus grandis* and *Eucalyptus urophylla* (Myrtaceae) in four Brazilian regions. Neotrop. Entomol. 45, 629–636. http://dx.doi.org/10.1007/s13744-016-0410-1.

Rockwood, D.L., Rudie, A.W., Ralph, S.A., Zhu, J.Y., Winandy, J.E., 2008. Energy product options for *Eucalyptus* species grown as short rotation woody crops. Int. J. Mol. Sci. 9, 1361–1378. http://dx.doi.org/10.3390/ijms9081361.

Rothman, L.D., Myers, J.H., 1996. Debilitating effects of viral diseases on host Lepidoptera. J. Invertebr. Pathol. 67, 1–10. http://dx.doi.org/10.1006/jipa.1996.0001.

Silva, F.W.S., Serrão, J.E., Elliot, S.L., 2016. Density-dependent prophylaxis in primary anti-parasite barriers in the velvetbean caterpillar. Ecol. Entomol. 41, 451–458. http://dx.doi.org/10.1111/een.12315.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol. 57, 758–771. http://dx.doi.org/10.1080/10635150802429642.

Xu, Z., Anzola, J.V., Nalin, C.M., Nuss, D.L., 1989. The 3′-terminal sequence of a wound tumor virus transcript can influence conformational and functional properties associated with the 5′-terminus. Virology 170, 511–522. http://dx.doi.org/10.1016/0042-6822(89)90443-1.

Zanuncio, J.C., Tavares, W.S., Fernandes, B.V., Wilcken, C.F., Zanuncio, T.V., 2014. Production and use of Heteroptera predators for the biological control of *Eucalyptus* pests in Brazil. Ekoloji 23, 98–104. http://dx.doi.org/10.5053/ekoloji.2014.9112.

Zhou, Y., Qin, T., Xiao, Y., Qin, F., Lei, C., Sun, X., 2014. Genomic and biological characterization of a new Cypovirus isolated from *Dendrolimus punctatus*. PLoS One 9http://dx.doi.org/10.1371/jpornal.pone.0113201. e113201.