

First report of *Chrysodeixis includens* nucleopolyhedrovirus (ChinNPV) infecting *Chrysodeixis includens* (Lepidoptera: Noctuidae) in Argentina

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Chrysodeixis (= *Pseudoplusia*) *includens* Walker (Lepidoptera: Noctuidae) is a polyphagous Neotropical pest, especially on soybean and cotton. Control failures due to insecticide resistance are a major concern (Owen et al. 2013), partially overcome by new-generation insecticides, transgenic crops, and natural enemies (Ramos et al. 2017). Also, a highly virulent baculovirus, *Chrysodeixis includens* nucleopolyhedrovirus (ChinNPV), has been proposed as a promising bioinsecticide candidate for the specific control of *C. includens* (Alexandre et al. 2010).

In 2017, baculovirus infection symptoms (sluggish movement, body color change, and tissue liquefaction) were recorded in laboratory-reared *C. includens* larvae in Tucumán, Argentina. The colony originated from specimens collected on soybean and bean in the northwest of the country. Light microscopy of larval homogenates revealed presumed occlusion bodies (proteinaceous structures that protect the virions contained within them from environmental factors). Based on this observation, additional analyses were conducted to elucidate the disease etiology.

Symptomatic larvae were ground in water + 0.5% sodium dodecyl sulfate (SDS) and centrifuged at low speed. The supernatant was filtered, centrifuged at 7,000 rpm for 20 min, and the occlusion bodies were re-suspended in water. Some occlusion bodies were dehydrated, metallized, and observed in a scanning electron microscope Quanta 250 (FEI Co., Eindhoven, The Netherlands). Occlusion bodies were variable in shape and size, with a quite irregular appearance, compared to other baculovirus species. Their diam ranged from about 0.7 µm to about 1.6 µm, and adopted distinct polyhedral morphologies (Fig. 1A-C). For molecular characterization, occlusion bodies were dissolved with sodium carbonate. The sample was adjusted to 10 mM Tris-HCl, 10 mM EDTA, 0.25% SDS, and 500 µg per ml proteinase K, and incubated overnight at 37 °C. Viral DNA was extracted with chloroform/isoamyl alcohol (24:1) (12,000 rpm, 10 min, 3 times) and precipitated at 12,000 rpm for 15 min with isopropanol + 0.2 M NaCl. The taxonomically informative polyhedrin, lef-8 and lef-9 baculovirus genes were partially amplified by PCR with primers prPH1/prPH2, prL8-2/prL8-1.b, and prL9-1/prL9-2, respectively, as mentioned elsewhere (Jehle et al. 2006). Purified products were directly sequenced (both strands) in an ABI PRISM 3500 XL genetic analyzer (Applied Biosystems, Foster City, California, USA) at Instituto de Biotecnología-INTA (Hurlingham, Argentina). Each gene was amplified independently and sequenced twice. BLAST searches for NCBI databases identified the virus under study as a new variant of

ChinNPV, hereafter ChinNPV-Tuc. Sequences exhibited ≥ 99% nucleotide identity to those from ChinNPV isolates infecting *C. includens* in other Latin American countries (Rowley et al. 2011; Craveiro et al. 2016). Polyhedrin, lef-8 and lef-9 partial sequences of ChinNPV-Tuc were deposited at GenBank under accession numbers MG865665, MG865666, and MG865667, respectively. They were aligned with ClustalW, concatenated (1,463 nt in total) and compared to all known ChinNPV isolates by maximum-likelihood phylogenetic analysis (1,000 bootstrap replicates) using MEGA7 (Kumar et al. 2016). *Chrysodeixis chalcites* nucleopolyhedrovirus served as the outgroup. The phylogeny resulted in 2 major ChinNPV clades. One clade grouped ChinNPV-Tuc from Argentina with 3 additional Brazilian isolates (ChinNPV-IE, -IF and -IG), whereas the other included isolates from Guatemala (ChinNPV-IA), Colombia (ChinNPV-458), and Brazil (ChinNPV-IB, -IC and -ID) (Fig. 1D).

The persistent agricultural damage caused by *C. includens* has shifted attention toward alternative control measures. Differentially virulent ChinNPV isolates have been characterized (Alexandre et al. 2010; Craveiro et al. 2016), supporting the search for novel, genetically diverse variants that may be suitable for controlling *C. includens*. This is the first description of a ChinNPV isolate from Argentina. The pathogenicity of ChinNPV-Tuc and its potential use for pest management should be assessed.

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Summary

Typical baculovirus infection symptoms were observed in *Chrysodeixis includens* Walker (Lepidoptera: Noctuidae) larvae from a laboratory rearing in Tucumán, Argentina. Scanning electron microscopy revealed the occurrence of polyhedral occlusion bodies displaying a heterogeneous morphology. Amplification, sequencing, and phylogenetic analysis of conserved polyhedrin, lef-8 and lef-9 genes identified the pathogen, for the first time in Argentina, as a variant of *Chrysodeixis includens* nucleopolyhedrovirus (ChinNPV). The biocontrol potential of this new isolate is worthy of future research.

Key Words: biological control; Plusiinae; baculovirus; characterization

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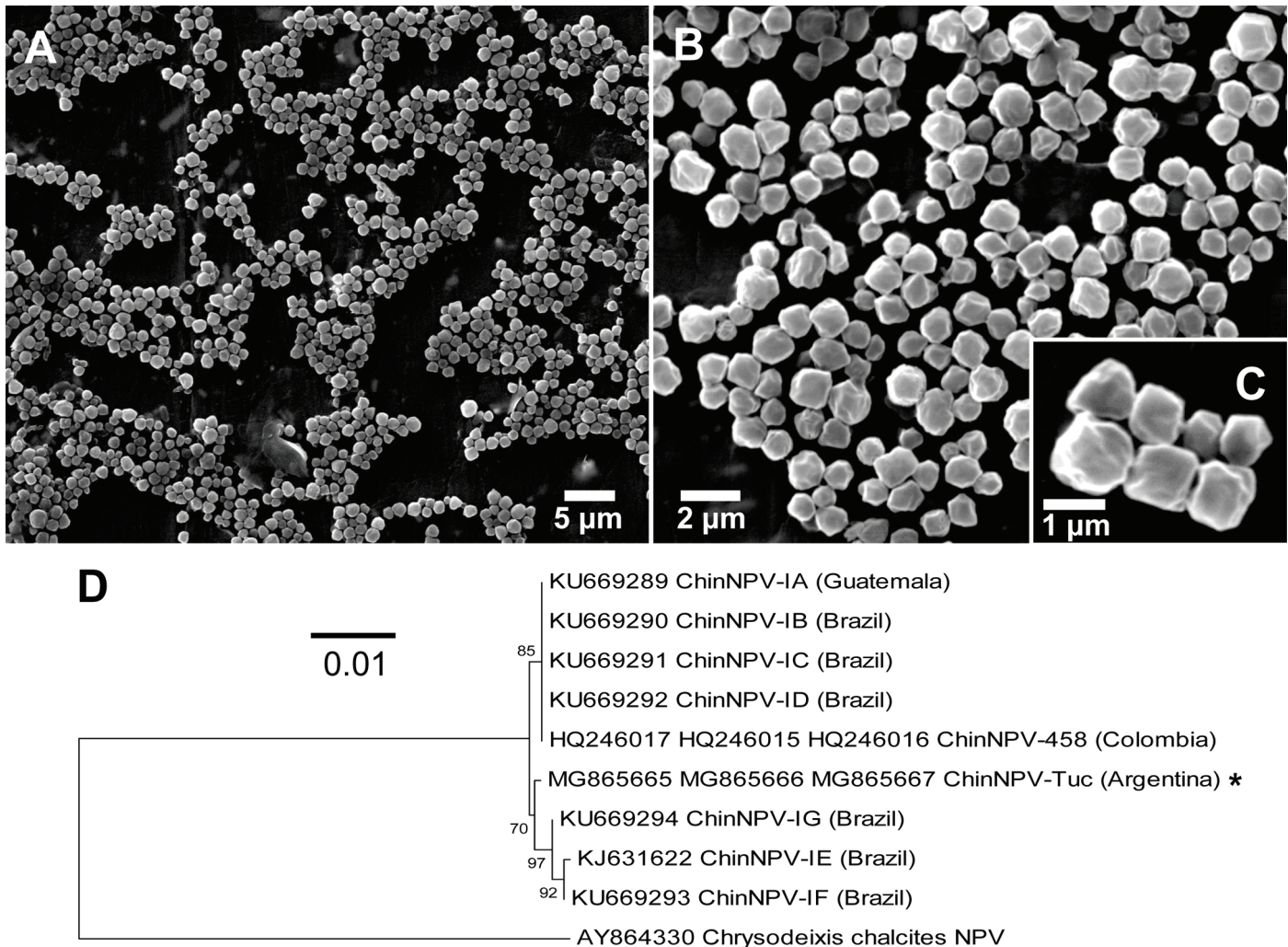


Fig. 1. *Chrysodeixis includens* nucleopolyhedrovirus, Tucumán isolate (ChinNPV-Tuc). A-C: scanning electron micrographs of occlusion bodies at different magnifications. D: Maximum-likelihood phylogeny of concatenated polyhedrin, lef-8 and lef-9 ChinNPV partial sequences. ChinNPV-Tuc is highlighted by an asterisk (*). Bootstrap support (1,000 repetitions) is indicated at the nodes. GenBank accessions, isolate name, and geographic origin are given. Scale bar represents substitutions per site.

Sumario

Se observaron síntomas típicos de infección por baculovirus en larvas de *Chrysodeixis includens* Walker (Lepidoptera: Noctuidae) criadas en laboratorio en Tucumán, Argentina. Mediante microscopía electrónica de barrido, se observaron cuerpos de oclusión poliédricos de morfología heterogénea. La amplificación, secuenciación y análisis filogenético de tres genes conservados (poliedrina, lef-8 y lef-9) permitieron identificar al patógeno, por primera vez en Argentina, como una variante de *Chrysodeixis includens* nucleopolyhedrovirus (ChinNPV). Se prevé investigar el potencial biocontrolador de este nuevo aislamiento.

Palabras Clave: control biológico; Plusiinae; baculovirus, caracterización

References Cited

Alexandre TM, Ribeiro ZMA, Craveiro SR, Cunha F, Fonseca ICB, Moscardi F, Castro MEB. 2010. Evaluation of seven viral isolates as potential biocontrol

- agents against *Pseudoplusia includens* (Lepidoptera: Noctuidae) caterpillars. *Journal of Invertebrate Pathology* 105: 98–104.
- Craveiro SR, Santos LAVM, Togawa RC, Inglis PW, Grynberg P, Ribeiro ZMA, Ribeiro BM, Castro MEB. 2016. Complete genome sequences of six *Chrysodeixis includens* nucleopolyhedrovirus isolates from Brazil and Guatemala. *Genome Announcements* 4: e01192–16.
- Jehle JA, Lange M, Wang H, Hu Z, Wang Y, Hauschild R. 2006. Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. *Virology* 346: 180–193.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetic Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.
- Owen LN, Catchot AL, Musser FR, Gore J, Cook DC, Jackson R. 2013. Susceptibility of *Chrysodeixis includens* (Lepidoptera: Noctuidae) to reduced risk-insecticides. *Florida Entomologist* 96: 554–559.
- Ramos RS, Sedyama CS, Queiroz EA, Costa TL, Martins JC, Araújo TA, Picanço MC. 2017. Toxicity of insecticides to *Chrysodeixis includens* and their direct and indirect effects on the predator *Blaptostethus palllescens*. *Journal of Applied Entomology* 141: 677–689.
- Rowley DL, Popham HJR, Harrison RL. 2011. Genetic variation and virulence of nucleopolyhedroviruses isolated worldwide from the Heliiothine pests *Helicoverpa armigera*, *Helicoverpa zea*, and *Heliothis virescens*. *Journal of Invertebrate Pathology* 107: 112–126.