

A highly effective nucleopolyhedrovirus against *Malacosoma* spp. (Lepidoptera: Lasiocampidae) from Turkey: isolation, characterization, phylogeny, and virulence

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Abstract: A nucleopolyhedrovirus (NPV) was isolated from diseased larvae of *Malacosoma franconicum* (Denis & Schiffermüller, 1775), collected from the northeastern part of Turkey. Light and electron microscopic observations showed that this virus is a multiple nucleopolyhedrovirus (MNPV). The dimensions of the polyhedral inclusion bodies varied from 1.0 to 2.1 µm, and the sizes of the virions were also measured to be approximately 194.5 nm. Restriction analysis of the new isolate and ManeNPV-T2 (previously isolated from *M. neustria* larvae) showed that the new virus is closely related to ManeNPV-T2. Furthermore, sequence and phylogenetic analysis of *polh* and *lef-8* also revealed a high degree of homology to *M. neustria* NPV. Our results indicate that the new virus is another isolate of ManeNPV that infects *M. franconicum* larvae naturally. Therefore, it was named ManeNPV-T3. The virulence of ManeNPV-T3, which was tested against the third instar of *M. franconicum* and *M. neustria* larvae under laboratory conditions, indicated that 0.6×10^5 occlusion bodies per larvae caused 100% mortality within 10 days. The same dose also showed viral infection in *Malacosoma castrensis*, *Plodia interpunctella*, *Thaumetopoea pityocampa*, and *Galleria mellonella* larvae causing 65%, 60%, 35%, and 25% mortalities, respectively. The findings indicate that ManeNPV-T3 is a promising microbial agent for the biological control of *Malacosoma* species.

Key words: *Malacosoma* spp., nucleopolyhedrovirus, virus isolation, biological control

1. Introduction

Malacosoma species are very common in Turkey and cause extensive economic losses in a number of plants (Doğanlar, 1975). One of these species, *Malacosoma franconicum* (Denis & Schiffermüller, 1775) (Lepidoptera: Lasiocampidae), is a pest native to Turkey (Stene, 1914). The presence of *M. franconicum* has been reported in Turkey until now, and we also detected it in Gümüşhane, Turkey. With the great importation of indoor plants to Turkey, it is possible that this insect was introduced in this way.

Malacosoma spp. is particularly injurious to apple, cherry, and other fruit trees but can also be destructive to other deciduous trees (Leathers and Gupta, 1993). Furthermore, its caterpillars induce mare reproductive loss syndrome, which causes early fetal losses and late-term abortion in horses (Webb et al., 2004). Control of this pest is difficult, largely because of its congregation behavior. Larvae spend the day inside their protective tents where they are largely shielded from exposure to insecticides. At night and in rainy weather, they emerge to forage, at which time a widely disseminated control agent would be necessary.

Another species, *Malacosoma neustria* (Linnaeus, 1758) (Lepidoptera: Lasiocampidae), known as the European tent caterpillar, is an important defoliator of various fruit trees and ornamental trees, particularly in eastern and central Turkey (Özbek and Çalmasur, 2005; Özbek and Çoruh, 2010). The caterpillars first feed on the buds and then on the leaves of the trees. Within a few years, they become so widespread that they leave the trees completely bare. Since 1970, outbreaks have been observed at about 3- to 7-year intervals in the province of Erzurum (Özbek and Çoruh, 2010).

Nucleopolyhedroviruses (NPVs), members of the family Baculoviridae, are one of the most promising biological and biotechnological control agents of insects to date (Demir et al., 2008). They are enveloped viruses that have double-stranded, circular DNA genomes ranging in size from 80 to 180 kbp (Theilmann et al., 2005). These viruses have been used as biopesticides to control the population of insect pests in agriculture, forestry, and pastures (Moscardi, 1999; Lacey et al., 2001; Szweczyk et al., 2006). The susceptibility of *M. americanum* and *M. neustria* to nucleopolyhedroviruses has been noted on

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numerous occasions. The susceptibility of *M. americanum* to NPV has been reported by several authors (Nordin, 1974; Progar et al., 2010). A *M. americanum* NPV was isolated from this insect in Kentucky and it killed less than 5% of the population sample (Nordin, 1974). Moreover, some morphological properties of this NPV were studied by Ackermann and Smirnof (1983), but more detailed information about this *M. americanum* NPV, such as restriction endonuclease enzyme profile, genome size, phylogenetic analysis, and host spectrum, was lacking. Furthermore, distinctive isolates of NPV have been studied from populations of *M. neustria* and have been investigated in terms of their virulence against pests (Biliotti, 1955; Günther, 1958; Magnoler, 1985; Kikhno and Stokovskaya, 1997; Jankevica et al., 1998; Demir et al., 2009a, 2009b, 2013).

In this study, in order to determine the feasibility of using a nucleopolyhedrovirus as a biological control agent of *Malacosoma* spp., a new nucleopolyhedrovirus isolated from *Malacosoma franconicum* in Turkey was characterized based on its morphological and molecular features, as well as partial *polyhedrin* (*polh*) and *late expression factor-8* (*lef-8*) gene analysis, using electron microscopy, restriction endonuclease, and phylogenetic analysis. In addition, its virulence on some lepidopteran larvae including *M. franconicum* and *M. neustria* species was determined.

2. Materials and methods

2.1. Virus and insects

Dead and diseased *Malacosoma franconicum* larvae with nucleopolyhedrosis virus symptoms (geotropism, diarrhea, and liquefied body) were collected from various host plants in the Eastern Black Sea Region of Turkey. These larvae were checked under a light microscope to determine whether or not they included occlusion bodies (OBs). The ones that had OBs were stored at -20°C .

Infection experiments were conducted using third instar larvae of insects from different species of Lepidoptera: *M. franconicum* (Fam.: Lasiocampidae), *M. neustria* (lackey moth, Fam.: Lasiocampidae), *M. castrensis* (ground lackey, Fam.: Lasiocampidae), *Plodia interpunctella* (Indian meal moth, Fam.: Pyralidae), *Thaumetopoea pityocampa* (pine processionary, Fam.: Thaumetopoeidae), and *Galleria mellonella* (honeycomb moth, Fam.: Pyralidae). *G. mellonella* and *P. interpunctella* were raised from eggs in the laboratory and fed an artificial diet until the larval stage. The other species were collected from the fields as larvae in the appropriate seasons.

2.2. Determination, isolation and propagation of the virus
Dead larvae with symptoms of baculovirus infection were dissected in Ringer's solution, and wet smears were

examined under a phase-contrast microscope (Nikon Eclipse E600) for virus observation at 400 \times magnification. The fat body, the tracheal body, and the midgut of each insect were thoroughly examined to observe viral infections in the insect bodies and were photographed using a digital camera (Nikon Coolpix 5000). The larvae that had died due to virus were stored at -20°C .

After defrosting, the larvae were ground in sterile water and filtered through cheesecloth to remove the larval debris. The OBs were purified according to the procedure described by Muñoz et al. (1997). Amplification of the virus was performed in healthy *M. franconicum* larvae in the laboratory. The larvae were placed in infection dishes, fed with 10^6 OBs applied on the leaf surface, and maintained at 25°C to develop infection. The OBs were purified from larvae by homogenization and density gradient centrifugation using microcentrifuge tubes as described by Ishii et al. (2003). The concentration of OBs was determined using a Thoma hemocytometer under a phase-contrast microscope and the OBs were stored at -20°C .

2.3. Electron microscopy

A suspension of purified polyhedra was placed on a round coverslip and allowed to air dry. The coverslip was glued onto a 1.27-cm aluminum stub and sputter-coated with gold for 3 min and examined in a JSM 6400 scanning electron microscope.

For transmission electron microscopy, the pellets of purified OBs were fixed in a modified Karnovsky fixative (2% glutaraldehyde, 2% paraformaldehyde in a 0.05 M pH 7.2 cacodylate buffer + 0.001 M CaCl_2) for 2 h, postfixed in 1% OsO_4 in the same buffer for 1 h, and embedded in resin. The resulting blocks were sectioned in a Leica Ultracut UCT ultramicrotome, stained with 3% aqueous uranyl acetate Reynold's lead citrate and examined on a Zeis EM 900 transmission electron microscope at 80 kV. The sizes of the viral OBs and the sizes of the nucleocapsids were measured directly from the amplified photographs using a precision ruler and dividing the value by the magnification of the photograph.

2.4. DNA extraction and restriction enzyme analysis

Purified polyhedra were dissolved in 0.1 M sodium carbonate (final pH \sim 11) by incubating for 30 min at 37°C . Large debris was removed by centrifuging for 5 min at 1000 rpm and the supernatant was centrifuged for 30 min at 14,000 rpm in order to pellet the occluded virions. The DNA was isolated according to Reed et al. (2003) and dialyzed for 24 h at 4°C against a 0.1X TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 7.5). The quantity and quality of the isolated DNA were determined spectrophotometrically and by electrophoresis in 0.7% agarose gel. For the restriction enzyme analyses, 5 μg of

DNA was digested with *KpnI*, *PstI*, and *BamHI* restriction enzymes (Promega) at 37 °C for 3.5 h. The digested fragments were separated in 0.7% agarose gel in a TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 15 mA for 18 h. *HindIII*-digested λ DNA was used as the molecular size marker. The gel was stained with ethidium bromide and photographed on a UV transilluminator.

2.5. PCR amplification and sequence analysis of conserved *polh* and *lef-8* genes

PCR was used to amplify *polh* and *lef-8* genes from viral DNA. The degenerate primer set (F: 5'- GTA AAA CGA CGG CCA GTT YIK IGG ICC IGG IAA RAA - 3' and R: 5'- AAC AGC TAT GAC CAT GTC IGG IGC RAA YTC YTT - 3') for the *polh* gene previously described by de Moraes and Maruniak (1997) was used for the amplification. The primer pair (F: 5'- GTA AAA CGA CGG CCA GTT YTT YCA YGG NGA RAT GAC -3' and R: 5'- AAC AGC TAT GAC CAT GGN AYR TAN GGR TCY TCN GC -3') described by Herniou et al. (2004) was used for amplification of the *lef-8* gene.

Each 25 μ L of PCR reaction mixture contained 30–50 ng of viral DNA; 400 nM of each primer; 0.2 mM each of dATP, dCTP, dGTP, and dTTP; 0.5 U of *Taq* DNA polymerase (Promega); 1.5 mM MgCl₂; and 2.5 μ L of 10X reaction buffer (Promega). Reactions were carried out in a Bio-Rad thermocycler using the following parameters. One 5-min cycle at 95 °C (initial denaturation step) was firstly followed by 10 cycles of 60 s at 94 °C, 45 s at 45 °C, and 60 s at 72 °C, and then followed by 25 cycles of 45 s at 94 °C, 30 s at 50 °C, and 60 s 72 °C. Finally, the amplification was completed with a final extension step of 5 min at 72 °C. The PCR products were cloned into pGEM-T easy vector (Promega). The nucleotide sequences of the PCR products were determined by automated sequencing (Macrogen).

2.6. Phylogenetic analysis

To show the position of the NPV isolate of this study relative to the other NPVs in GenBank, we performed a phylogenetic analysis using the partial *polh* and *lef-8* DNA sequences. Nucleotide sequences were aligned in Clustal X. Maximum parsimony analysis was performed using Mega 5. The robustness of the phylogenetic tree was tested by bootstrap analysis of 500 replications. The *polh* and *lef-8* sequences used for this analysis were retrieved from GenBank (Table).

2.7. Pathogenicity experiment

The biological activity of the new NPV isolate against third instar larvae of 6 different hosts (*M. franconicum*, *M. neustria*, *M. castrensis*, *P. interpunctella*, *T. pityocampa*, and *G. mellonella*) was determined. An OB suspension was prepared as 1×10^6 ODs/mL. Experiments were performed with 15 larvae per pest and were replicated 3 times for each pest. Larvae that had been starved for 6 h prior to the virus application were fed with natural foliage

contaminated with an OB suspension and incubated at 24 °C with 16 h light/8 h dark (Lucarotti and Morin, 1997). After 24 h, all larvae were fed on fresh foliage and held in the same conditions for 10 days. As a control, the same number of larvae were prepared separately for each pest and were fed on foliage treated with water. The experiments were repeated 3 times. Mortality was assessed daily for 10 days; dead larvae were removed and checked for NPV infection under a phase-contrast microscope. All mortalities were evaluated using Abbott's formula (Abbott, 1925). To determine differences among densities, the data were subjected to analysis of variance and subsequently to Duncan's multiple comparison tests. All analyses were performed using SPSS 21.0.

3. Results

3.1. Microscopy

During light microscopic studies of the dead larvae with typical baculovirus infection symptoms, it was determined that some larvae had been infected with a nucleopolyhedrovirus. In particular, it was shown that there were a lot of polyhedra in some cells around the tracheal tissue (Figure 1). The electron micrograph studies also revealed typical baculovirus OBs. The scanning electron micrograph showed that the OBs were irregular in shape and ranged in size from 1.0 to 2.1 μ m in diameter with a mean diameter of 1.46 μ m (Figure 2A). The transmission electron micrograph revealed that OBs were occupied by several virions with multiple nucleocapsids packaged within a single viral envelope (Figure 2B). The length of a rod-shaped nucleocapsid was approximately 194.5 nm with a width of approximately 40 nm.

3.2. Restriction endonuclease analysis of virus DNA

Restriction enzyme analysis of the new NPV genome purified from viral inclusion bodies yielded 9 *KpnI*, 8 *PstI*, and 7 *BamHI* visible fragments on agarose gel. All restriction endonuclease reactions resulted in different fragment profiles from the literature. The sizes of all restriction endonuclease fragments were observed clearly on 0.7% agarose gels (Figure 3).

3.3. Phylogenetic analysis of the *polh* and *lef-8* genes

The purpose of the phylogenetic analysis was to show the taxonomic position of the new NPV relative to the other NPVs according to partial sequences of the *polh* and *lef-8* genes. Sequences obtained for *polh* were nearly 500 nucleotides and those for *lef-8* were nearly 700 nucleotides. These sequences were compared with the other *polh* and *lef-8* sequences in the literature using the BLAST program. The phylogenetic analysis of the new NPV indicated its closeness to the ManeNPV from group II (Figure 4).

3.4. Pathogenicity and host range of virus

The infectivity of the new NPV on 6 different insect pests was determined using bioassays. For each pest, 15 larvae

Table. Sequences of baculoviruses used for phylogenetic analysis.

Virus	Abbreviation	GenBank accession no.	
		<i>polh</i>	<i>lef-8</i>
<i>Autographa californica</i> MNPV	AcMNPV	L22858	L22858
<i>Hyphantria cunea</i> NPV	HycuNPV	AF300872	AY706560
<i>Bombyx mori</i> NPV	BmNPV	U75359	L33180
<i>Anagrapha falcifera</i> MNPV	AnfaNPV	U64896	JN674780
<i>Antheraea pernyi</i> MNPV	AnpeNPV	AB062454	DQ486030
<i>Anticarsia gemmatilis</i> MNPV	AgMNPV	Y17753	NC_008520
<i>Epiphyas postvittana</i> MNPV	EppoNPV	AF061578	Ay043265
<i>Choristoneura fumiferana</i> MNPV	CfMNPV	U40833	AF081810
<i>Leucoma salicis</i> MNPV	LesaNVP	AY729808	
<i>Orgyia pseudotsugata</i> MNPV	OpMNPV	M14885	NC001875
<i>Orgyia pseudotsugata</i> SNPV	OpSNPV	M32433	
<i>Spodoptera littoralis</i> MNPV	SpliNPV	D01017	Y10669
<i>Heliocoverpa armigera</i> SNPV	HaSNPV	AF271059	
<i>Spodoptera exigua</i> MNPV	SeMNPV	AF169823	AF169823
<i>Xestia c-nigrum</i> GV	XcGV	AF169823	AF162221
<i>Malacosoma</i> sp. NPV	MaspNPV	AY519240	AY519241
<i>Mamestra brassicae</i> NPV	MabrNPV		JQ798165
<i>Malacosoma neustria</i> NPV isolate 1	ManeNPV-1	AJ277555	AY706569
<i>Malacosoma neustria</i> NPV isolate 2	ManeNPV-2	AY127899	AY519244
<i>Malacosoma neustria</i> NPV isolate 3	ManeNPV-3	AY519243	
<i>Malacosoma neustria</i> NPV isolate 4	ManeNPV-4	AY706708	
<i>Malacosoma neustria</i> NPV isolate 5	ManeNPV-5	X55658	
<i>Malacosoma americanum</i> NPV isolate 1	MaamNPV-1	AY589504	AY706565
<i>Malacosoma americanum</i> NPV isolate 2	MaamNPV-2	AY706704	
<i>Malacosoma californicum</i> NPV	MacaNPV	AY519237	AY519238
<i>Malacosoma disstria</i> NPV	MadiNPV	U61732	
<i>Malacosoma neustria</i> NPV Turkish isolate 2	ManeNPV-T2	Demir et al., 2013	
<i>Malacosoma neustria</i> NPV Turkish isolate 3	ManeNPV-T3	This study	This study
<i>Mamestra configurata</i> NPV isolate 1	MacoNPV-1		U59461
<i>Mamestra configurata</i> NPV isolate 2	MacoNPV-2		AY126275

were infected and the test was performed 3 times (totally 135 larvae in 3 replicates). The mortality levels varied for all pests within 10 days. While the 0.6×10^5 OBs/larva dose had 100% insecticidal activity on *M. franconicum* and *M. neustria*, the same dose caused 65%, 60%, 35%, and 25% mortality for *M. castrensis*, *P. interpunctella*, *T. pityocampa*, and *G. mellonella* larvae, respectively ($F = 1390.122$; $df = 5.17$; $P < 0.05$) (Figure 5). No mortality was observed in any of the control groups in host-range testing.

4. Discussion

To date, genetically engineered baculoviruses have introduced a promising research line to overcome the

slow action of baculoviruses as biocontrol agents. On the other hand, searching for new natural baculovirus isolates with better insecticidal characteristics is still a developing subject of work (so that they can be safer and there can be no risk from releasing a genetically engineered product into nature).

A nucleopolyhedrovirus was isolated from diseased *Malacosoma franconicum* larvae collected from fields in Turkey. Because baculoviruses are named according to the insect host species from which they were first isolated, the new NPV isolate was first thought to be a *M. franconicum* NPV Turkish isolate. However, phylogenetic analysis of its *polh* and *lef-8* genes conserved among lepidopteran

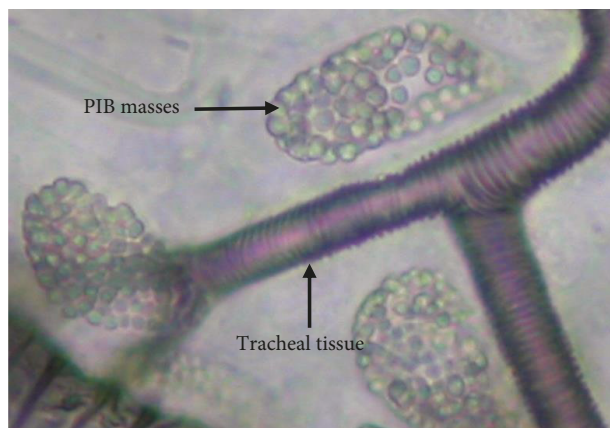


Figure 1. The light micrograph of a nucleopolyhedrovirus from *M. franconicum*. The PIBs are seen as a bright crystal mass in the infected cell nucleus around the tracheal tissue (15×40).

baculoviruses showed that it is closely related to *M. neustria* NPV-T2 characterized by Demir et al. (2013) from Turkey, and that it was a ManeNPV infection in a field population of *M. franconicum*. Since it is the third isolate of *M. neustria* in Turkey, it was named ManeNPV-T3.

Figure 1 shows a great number of polyhedral inclusion bodies (PIBs) as a bright crystal mass in an infected cell nucleus around the tracheal tissue of larvae that had died due to viral infection. The OBs of the NPVs are most easily seen under a light microscope due to their larger size and their light refractory polyhedral structure. Scanning

electron microscope results showed that ManeNPV-T3 has PIB sizes between 1.0 and 2.1 μm in diameter (Figure 2A). While these dimensions are greater than the PIB sizes of ManeNPV-T2 (Demir et al., 2013), the dimensions of OBs were within the usual size range of polyhedra. In a previous study, Ackermann and Smirnov (1983) investigated the morphological characteristics of 23 baculoviruses that also include *M. americanum*, and reported that the mean dimensions of MaamNPV polyhedra varied between 1.1 and 1.7 μm , with an average of 1.44 μm . All of these findings demonstrated that the size of the polyhedra in the new isolate is compatible with baculoviruses. Transmission electron microscopic observations confirmed that ManeNPV-T3 was a multiple-nucleocapsid nucleopolyhedrovirus, as common as earlier investigated viruses isolated from several *Malacosoma* species such as *M. neustria*, *M. disstria*, and *M. apicola* (Keddie and Erlandson 1995; Demir et al., 2013). Figure 2B shows that nucleocapsids, multiple virions, virogenic stroma, and developing polyhedra were visible in the hypertrophied nuclei of an infected cell. In a cross-section of some polyhedra, it was evident that multiple virions contain more than one nucleocapsid per virion, and the virions contained enveloped, rod-shaped nucleocapsids. Nucleocapsid sizes were identified to be 194.5 nm in length and 40 nm in width. These sizes are smaller than those of ManeNPV, which have nucleocapsid lengths and widths of 250 and 50 nm, respectively. The results obtained in this study and described by Demir et al. (2013) seemed to be closely correlated.

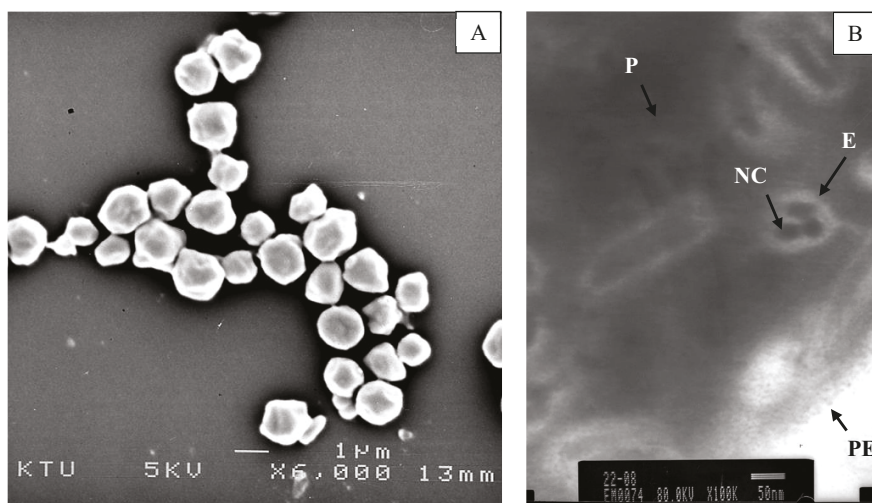


Figure 2. Electron micrographs of purified polyhedral occlusion bodies. A: Scanning electron micrograph showing purified polyhedra. B: Transmission electron micrograph of a section of a purified polyhedron inclusion body, with details of a polyhedron showing multiple nucleocapsids surrounded by a single membrane. The polyhedron envelope (PE), the polyhedrin matrix (P), the virion envelope (E), and the rod-like nucleocapsid (NC) are indicated.

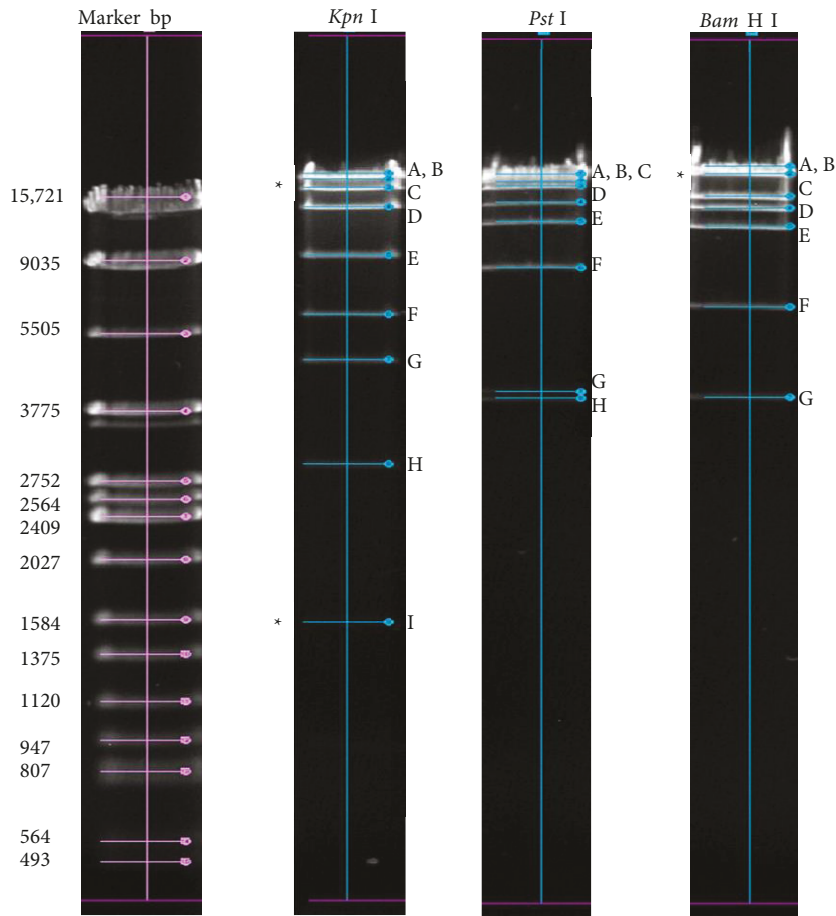


Figure 3. Restriction fragmentation patterns of viral genomic DNA using the restriction endonucleases *KpnI*, *PstI*, and *BamHI*. Restriction fragments were separated on a 1% agarose gel at 20 V. λ *HindIII* digest was used as the molecular size marker. The letters to the right of both lines indicate the fragments generated by each restriction enzyme. The asterisk indicates different fragments of ManeNPV-T3 compared to ManeNPV-T2.

Restriction endonuclease (RE) analysis is an important technique for comparing different geographical isolates of the same virus (Murillo et al., 2001). In the present study, ManeNPV-T3 DNA was digested with *KpnI*, *PstI*, and *BamHI* enzymes. In a previous study, the restriction endonuclease analysis of a ManeNPV-T2 isolate was also performed with *BamHI*, *KpnI*, *PstI*, and *EcoRI* enzymes. *PstI* restriction analysis of both viral DNA shared the same bands and profiles. However, the *KpnI* and *BamHI* profiles of ManeNPV-T3 had more bands than the ManeNPV-T2 isolate with the same enzymes (Demir et al., 2013). Evaluation of these restriction profile differences showed that ManeNPV-T3 is different from the other ManeNPV isolates and is a new Turkish isolate of ManeNPV.

Sequence analysis of ManeNPV-T3 *polh* and *lef-8* genes was performed. Alignment of the *polh* sequence in the nucleotide BLAST program from NCBI showed

that the isolate of this study is very similar to *M. neustria* NPV-T2. They have 95% similarity according to their partial *polh* gene sequences. Phylogenetic analysis was performed using partial *polh* and *lef-8* gene sequences. The resulting trees linked ManeNPV-T3 together with other *M. neustria* NPVs from GenBank according to neighbor joining analysis. In the *polh* tree, ManeNPV-T3 clustered first with ManeNPV-2. These 2 NPVs later clustered with ManeNPV-T2. In the *lef-8* tree, ManeNPV-T3 again localized together with ManeNPV-T2, which supports the *polh* phylogeny. In these trees, while 2 *M. americanum* NPVs were localized together, *M. californicum* NPV and *Malacosoma* spp. NPVs were clustered together. However, *M. distria* NPV was clustered in a position quite far from the other *Malacosoma* NPVs. Moreover, the tree also reveals that the *Malacosoma* NPVs are closer to the Group II NPVs than the Group I NPVs.

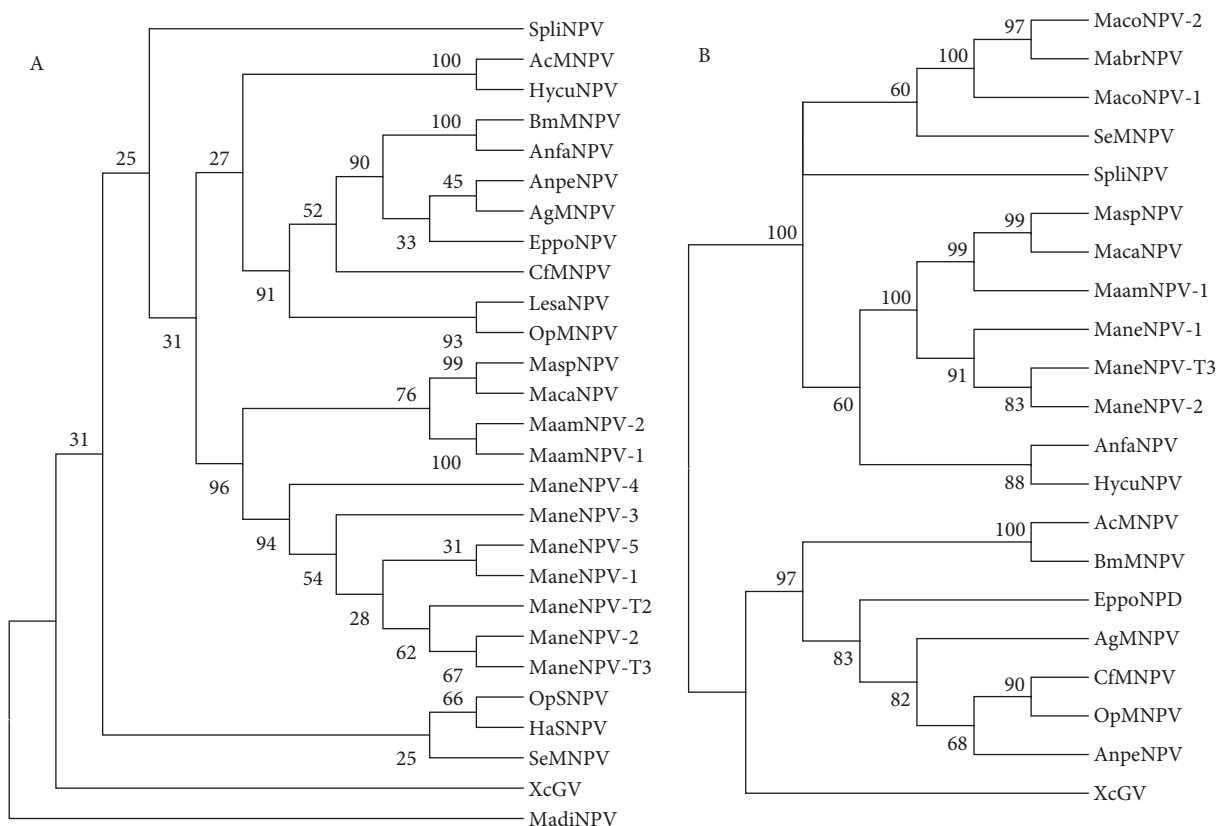


Figure 4. Neighbor joining trees based on partial *polyhedrin* (A) and *late expression factor-8* (B) sequences. The numbers indicate the bootstrap scores.

The biological activity of ManeNPV-T3 was tested on *M. franconicum*, *M. neustria*, *M. castrensis*, *P. interpunctella*, *T. pityocampa*, and *G. mellonella* larvae. For all insects, 15 larvae (replicated 3 times) were infected with 1×10^6 OBs/mL doses. The isolate had different insecticidal activity and pathogenesis on different lepidopteran hosts. The mortality rate varied from 25% to 100% among the insects used in bioassays. Yearian and Young (1976) applied *M. americanum* NPV to fourth instar larvae and found 61.4% mortality at 10^6 PIBs/mL viral concentrations within 14 days. By contrast, Progar et al. (2010) found fourth instar larvae of *M. americanum* to be highly resistant to the virus at similar concentrations. In our study, the 10^6 OBs/mL dose of ManeNPV-T3 was enough to provide 100% mortality in *M. franconicum* and *M. neustria* within 10 days. Although the new virus was isolated from *M. franconicum* and is a *M. neustria* NPV, it also has significant insecticidal activity on both *M. franconicum* and *M. neustria* larvae. The insecticidal activity of ManeNPV-T2 on *M. neustria* was determined to be 100% with 10^6 PIBs/mL concentration within 10 days, despite being isolated from *M. neustria* larvae (Demir et al., 2013). This shows that the new isolate is as effective as ManeNPV-T2 on *M. neustria*. The biological activity of our study is much greater than that

of the study conducted by Jankevica and Zarins (1999), and indicates the virulence of a *M. neustria* NPV Latvian isolate within 16 days. A comparison of results showed *M. franconicum* and *M. neustria* larvae to be highly susceptible

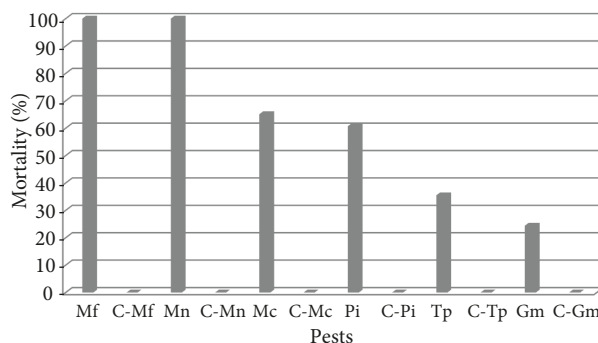


Figure 5. The mortality percentages of 0.6×10^5 OBs/larva on each third instar pest larva within 10 days of infection. Values were corrected according to Abbott's formula (Abbott, 1925). Ma: *Malacosoma franconicum*, Mn: *Malacosoma neustria*, Mc: *Malacosoma castrensis*, Pi: *Plodia interpunctella*, Tp: *Thaumetopoea pityocampa*, Gm: *Galleria mellonella*, and C: control.

to ManeNPV-T3. These data are the strongest evidence to support the idea that *Malacosoma* spp. can be difficult to control because of their congregational behavior inside a tent that protects them against their predators and insecticides.

Although the NPV sample was obtained from *Malacosoma franconicum*, the results from BLAST and the phylogenetic analysis found it to be closer to *Malacosoma neustria* NPVs. Because of that similarity, we thought that it is ManeNPV and the *M. franconicum* larvae take the virus in naturally through contaminated foods during feeding. While the virus belonged to *M. neustria*, it affected *M. franconicum* larvae, and produced progeny virus in that host with a properly productive baculovirus infection cycle. In addition, findings from the virulence study indicate that ManeNPV-T3 appears to be a promising biocontrol agent for use against *Malacosoma* spp. including *M. franconicum*

and *M. neustria*, and further studies should include its field efficacy and the investigation of the predisposition of that isolate for mass production.

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