A novel putative virus of *Gremmeniella abietina* type B (Ascomycota: Helotiaceae) has a composite genome with endornavirus affinities

Tero T. Tuomivirta,¹ Juha Kaitera² and Jarkko Hantula¹

¹Finnish Forest Research Institute, Vantaa Research Unit, Jokiniemenkuja 1, PO Box 18, FI-01301 Vantaa, Finland
²Finnish Forest Research Institute, Muhos Research Unit, Kirkkosaarentie 7, FI-91500 Muhos, Finland

Ascospore and mycelial isolates of *Gremmeniella abietina* type B were found to contain three different dsRNA molecules with approximate lengths of 11, 5 and 3 kb. The 11 kb dsRNA encoded the genome of a putative virus and is named *Gremmeniella abietina* type B RNA virus XL (GaBRV-XL). GaBRV-XL probably exists in an unencapsulated state. We identified two distinct dsRNAs (10 374 and 10 375 bp) of GaBRV-XL, both of which coded for the same putative polyprotein (3249 amino acids) and contained four regions similar to putative viral methyltransferases, DExH box helicases, viral RNA helicase 1 and RNA-dependent RNA polymerases. While a cysteine-rich region with several CxCC motifs in GaBRV-XL was similar to that of putative endornaviruses, cluster analyses of conserved regions revealed GaBRV-XL to be distinct from a broad range of viral taxa but most closely related to *Discula destructiva* virus 3. Collectively, these findings suggest that GaBRV-XL represents a novel virus group related to endornaviruses.

INTRODUCTION

Recently recognized by the International Committee on Taxonomy of Viruses (ICTVdB Management, 2006), endornaviruses are usually cryptic and non-enveloped plant and fungal dsRNA viruses that spread efficiently through mitotic and meiotic cells but not through grafts (Fukuhara et al., 1995; Moriyama et al., 1999; Wakarchuk & Hamilton, 1990; Pfeiffer, 1998). Almost all of the 14 000-18 000 nucleotides comprising endornavirus genomes code for a putative polyprotein and exhibit a characteristic nick near the 5′ end of the coding strand. Similar putative viruses have been found in the Stramenopila (*Phytophthora*; Hacker et al., 2005) and in the basidiomycete *Helicobasidium mompa* (Fukuhara et al., 2005; Osaki et al., 2006), both of which are pathogens of plants.

*Gremmeniella abietina* var. *abietina* is the causal agent of scleroderris canker on coniferous trees. In northern Europe, this ascomycete is commonly recognized as a species complex (e.g. Uotila et al., 2000) with two types (A and B) that mainly infect Scots pine (*Pinus sylvestris*). Fungal viruses of the *G. abietina* species complex have been studied thoroughly in type A and are known to include putative members of the virus families Naraviridae, Totiviridae and Partitiviridae, some of which can co-infect a single fungal isolate (Tuomivirta et al., 2002; Tuomivirta & Hantula, 2005).

The goals of this study include a survey of dsRNA molecules in *G. abietina* type B, molecular characterization of the most common dsRNA type and its comparison to representatives of similar viral taxa.

METHODS

*G. abietina* type B strains. *G. abietina* type B strains (see Supplementary Table S1, available in JGV Online) were identified by the random amplified microsatellite (RAMS) technique of Hantula & Müller (1997). Branches from *P. sylvestris* and *Pinus contorta* <2 m in height (<20 years old) with symptoms of scleroderris canker were collected in artificially or naturally regenerated stands between June 1994 and June 1995 in northern Finland. Disease symptoms included death of lateral branches over several internodes, cankers, yellow–green woody tissues, pycnidia and apothecia. Fungi were isolated either from pycnidia or from infected wood adjacent to apothecia.

Nucleic acid isolation and electrophoresis. *G. abietina* type B isolates were grown at 20 °C on modified orange serum agar covered with a cellophane membrane (Müller et al., 1994). dsRNA isolation
was based on the protocol of Morris & Dodds (1979) as modified by Tuomivirta et al. (2002) and Tuomivirta & Hantula (2003). dsRNA molecules bind specifically to CF-11 cellulose (Whatman) in the presence of 15% ethanol and, in order to test for dsRNA encapsidation, the protocol was also conducted with non-phenol or chloroform extractions in osmotic stabilizer buffer (0.6 M NaCl in 0.1 M phosphate buffer, pH 6.0; Phillips, 1993). dsRNA recovery was quantified by electrophoresis according to Tuomivirta et al. (2002).

Buoyant densities were determined according to Tuomivirta & Hantula (2005) and each of 10 CsCl gradient fractions was tested for the presence of dsRNA through agarose gel electrophoresis. cDNA synthesis and sequencing followed Tuomivirta & Hantula (2005) except that dsRNA from isolates AU58 (Müller & Uotila, 1997) and E46 (Table 1) were ligated using T4 RNA ligase (Fermentas) to 5'-phosphorylated and 3'-inactivated adapters T4III (5'-GCATTCCGACCCGGGGTT-3') and T4III (5'-AGAGACCCAGTCTGAGCTCCA-GT-3'), respectively. Also, eight (AU58) and 10 (E46) specific primers (available on request) were designed for cloning or direct sequencing.

Sequences were compiled, aligned and analysed using Vector NTI Advance 10 (Invitrogen). Complete nucleic acid and putative amino acid sequences were screened using the protein BLAST (Altschul et al., 1997) search engine including CD-Search (Marchler-Bauer & Bryant, 2004) of the National Center for Biotechnology Information (NCBI) and the InterProScan (Zdobnov & Apweiler, 2001) protein signature search engine of the European Bioinformatics Institute (EBI). Alignment algorithms were from CLUSTAL W (Thompson et al., 1994). Phylogenetic analyses of resulting alignments were conducted according to the neighbour-joining method as applied in MEGA 3.1 (Kumar et al., 2004), Bayesian inference under a mixed amino acid model (ngen = 10,000, samplefreq = 10, burnin = 250) in MrBayes 3.12 (Huelsenbeck et al., 2001; Ronquist & Huelsenbeck, 2003) and fast maximum-likelihood using PhyML (Guindon et al., 2005). We used ProtTest (Abascal et al., 2005) to identify and apply the best-fit models of sequence evolution in the PhyML analysis. Finally, we used TreeView 1.6.6 (Page, 1996) to inspect the resultant dendrograms and their branch support values.

### Table 1. Viral amino acid sequences used in this study

Details of other sequences used in the construction of Supplementary Fig. S4 are given in Supplementary Table S2.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Abbreviation (strain)</th>
<th>Region of similarity to GaBRV-XL genome</th>
<th>Accession number (protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gremmeniella abietina type B RNA virus XL1</td>
<td>GaBRV-XL1</td>
<td>–</td>
<td>ABD73305</td>
</tr>
<tr>
<td>Gremmeniella abietina type B RNA virus XL2</td>
<td>GaBRV-XL2</td>
<td>–</td>
<td>ABD73306</td>
</tr>
<tr>
<td>Chinese wheat mosaic virus</td>
<td>CWMV-(Changdong)</td>
<td>Viral methyltransferase</td>
<td>CAC39427</td>
</tr>
<tr>
<td>Chinese wheat mosaic virus</td>
<td>CWMV-(Yantai)</td>
<td>Viral methyltransferase</td>
<td>NP_059514</td>
</tr>
<tr>
<td>Fragaria chiloensis latent virus</td>
<td>FCILV</td>
<td>Viral methyltransferase</td>
<td>YP_164801</td>
</tr>
<tr>
<td>Oat golden stripe virus</td>
<td>OGSV-(UK)</td>
<td>Viral methyltransferase</td>
<td>CAB57883</td>
</tr>
<tr>
<td>Pepper mild mottle virus</td>
<td>PMoMoV-(Ia)</td>
<td>Viral methyltransferase</td>
<td>CAC59956</td>
</tr>
<tr>
<td>Pepper mild mottle virus</td>
<td>PMoMoV-(Kr)</td>
<td>Viral methyltransferase</td>
<td>BAD90599</td>
</tr>
<tr>
<td>Soil-borne cereal mosaic virus</td>
<td>SBCMV-(France)</td>
<td>Viral methyltransferase</td>
<td>NP_059451</td>
</tr>
<tr>
<td>Soil-borne cereal mosaic virus</td>
<td>SBCMV-(Germany)</td>
<td>Viral methyltransferase</td>
<td>AAFF333</td>
</tr>
<tr>
<td>Soil-borne wheat mosaic virus</td>
<td>SBWMV-(UK)</td>
<td>Viral methyltransferase</td>
<td>CAC40982</td>
</tr>
<tr>
<td>Soil-borne wheat mosaic virus</td>
<td>SBWMV-(US)</td>
<td>Viral methyltransferase</td>
<td>NP_049335</td>
</tr>
<tr>
<td>Bovine viral diarrhea virus</td>
<td>BDV-(T20)</td>
<td>DExH box helicase</td>
<td>BAD4938</td>
</tr>
<tr>
<td>Classical swine fever virus</td>
<td>CSFV-(CS)</td>
<td>DExH box helicase</td>
<td>AAC9302</td>
</tr>
<tr>
<td>Classical swine fever virus</td>
<td>CSFV-(Estrup)</td>
<td>DExH box helicase</td>
<td>AAG59829</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>HCV-(BEBE1)</td>
<td>DExH box helicase</td>
<td>BAA0891</td>
</tr>
<tr>
<td>Hepatitis G virus</td>
<td>GB virus B-(FL3)</td>
<td>DExH box helicase</td>
<td>CAD21957</td>
</tr>
<tr>
<td>Shallot yellow stripe virus</td>
<td>SYSV</td>
<td>DExH box helicase</td>
<td>YP_331418</td>
</tr>
<tr>
<td>Sorghum mosaic virus</td>
<td>SrMV</td>
<td>DExH box helicase</td>
<td>AAB70862</td>
</tr>
<tr>
<td>Cherry green ring mottle virus</td>
<td>CGRMV</td>
<td>Viral RNA helicase</td>
<td>CAC18740</td>
</tr>
<tr>
<td>Cherry necrotic rusty mottle virus</td>
<td>CNRMV</td>
<td>Viral RNA helicase</td>
<td>AAFF8211</td>
</tr>
<tr>
<td>Phaseolus vulgaris endornavirus</td>
<td>PVuV</td>
<td>Viral RNA helicase</td>
<td>BAD8806</td>
</tr>
<tr>
<td>Discila destructiva virus 3</td>
<td>DDV-3</td>
<td>RNA_dep_RNApol2</td>
<td>AAKS403</td>
</tr>
<tr>
<td>Grapevine rootstock stem lesion-associated virus</td>
<td>GRSLaV</td>
<td>RNA_dep_RNApol2</td>
<td>NP_835244</td>
</tr>
<tr>
<td>Helicobasidium monop (endornavirus 1-670)</td>
<td>HmEV1-670</td>
<td>Viral RNA helicase 1 and RNA_dep_RNApol2</td>
<td>BAE94538</td>
</tr>
<tr>
<td>Little cherry virus 2</td>
<td>LChV-2</td>
<td>RNA_dep_RNApol2</td>
<td>NP_891562</td>
</tr>
<tr>
<td>Oryza rufipogon endornavirus</td>
<td>ORV</td>
<td>Viral RNA helicase 1 and RNA_dep_RNApol2</td>
<td>YP_438202</td>
</tr>
<tr>
<td>Oryza sativa endornavirus</td>
<td>OSV</td>
<td>Viral RNA helicase 1 and RNA_dep_RNApol2</td>
<td>BAA06862</td>
</tr>
<tr>
<td>Phytophthora endornavirus 1</td>
<td>PEV1</td>
<td>Viral RNA helicase 1 and RNA_dep_RNApol2</td>
<td>CAI4756</td>
</tr>
<tr>
<td>Vicia faba endornavirus</td>
<td>VFV</td>
<td>Viral RNA helicase 1 and RNA_dep_RNApol2</td>
<td>CAA04392</td>
</tr>
</tbody>
</table>
RESULTS

Host and physicochemical properties of dsRNA

RAMS fingerprint analysis of all 31 isolates (Supplementary Table S1) confirmed them to be G. abietina type B (not shown). We detected three different dsRNA molecules among eight isolates and double infections were apparent in two isolates; E46 contained dsRNAs of 11 and 5 kb, whereas AU58 contained dsRNAs of 11 and 3 kb (not shown). Electrophoretic mobility of the 5 and 3 kb dsRNAs was identical to that of putative fungal totiviruses and mitoviruses from G. abietina type A (Tuomivirta & Hantula, 2005).

Ultracentrifugation of isolate E46 identified four fractions containing dsRNA (not shown). Most of the 11 kb dsRNAs were recovered from the nucleic acid pellet, but small amounts also occurred in the uppermost (1: \(\delta=1.26 \text{ g ml}^{-1}\)) and lowermost (10: \(\delta=1.505 \text{ g ml}^{-1}\)) fractions. The 5 kb dsRNA was found only in fraction 8 (\(\delta=1.387 \text{ g ml}^{-1}\)) and with a similar mean density (\(\delta=1.405 \text{ g ml}^{-1}\)) to a totivirus isolated from G. abietina type A (Tuomivirta & Hantula, 2005).

Using the cellulose-binding protocol, we successfully isolated the 11 kb dsRNA from a mycelial culture of isolate E46 without phenol extraction (Fig. 1). However, we were unable to recover the 5 kb dsRNA using the same approach. The 11 kb dsRNAs, probably of viral origin, were named as Gremmeniella abietina type B RNA virus XL (GaBRV-XL).

Production of cDNA and nucleotide sequence analysis of GaBRV-XL from isolates AU58 and E46

Complete sequences of the 11 kb dsRNAs of isolates AU58 (GaBRV-XL1) and E46 (GaBRV-XL2) were assembled from 109 and 86 sequencing reactions of clones and direct sequencing of RT-PCR products, respectively. Their fully compiled and contiguous lengths were 10375 and 10374 bp, respectively. The sequences were 97% similar and both contained a single, 10287 bp open reading frame (ORF) starting at nt 24 according to a universal translational table. The ORF spanned 99.2% of the dsRNA and encoded a protein of 3249 aa with an approximate molecular mass of 383 kDa. The protein sequences were 98.7% identical and 99.4% similar (Fig. 2).

GaBRV-XL codes for a viral methyltransferase, two helicases and an RNA-dependent RNA polymerase

Based on amino acid sequence analysis, the GaBRV-XL dsRNAs encoded two strains of a novel putative virus. Both CD-Search and InterProScan inferred four conserved regions. The first was a putative viral methyltransferase (CD-Search: pfam01660; expect value 2E-14) region, ranging from aa 265 to 573. The second was a putative DEXDc, DEAD-like helicases superfamily (CD-Search: smart00487; 1E-08) region, aa 1218–1376. The third was a putative viral RNA helicase 1 (CD-Search: pfam01443; 6E-08) region, aa 1908–2025. The last region was RNA_dep_RNApol2 (CD-Search: pfam00978; 2E-14), aa 3090–3254.

The putative methyltransferase region was most similar to sequences of Soil-borne cereal mosaic virus (Diao et al., 1999a; Koenig et al., 1999), Soil-borne wheat mosaic virus (Shirako & Wilson, 1993; Ratti et al., 2004) and Oat golden stripe virus (Diao et al., 1999a) (unassigned members of Furovirus), Fragaria chiloensis latent virus (Tzanetakis & Martin, 2005) (Bromoviridae: Ilarivirus), Chinese wheat mosaic virus (Yang et al., 2001; Diao et al., 1999b) (unclassified Furovirus) and Pepper mild mottle virus (Velasco et al., 2002; GenBank accession no. BAD90599) (unassigned Tobamovirus), and the expect value ranged from 3.5E-06 to 6E-03. A selection of putative methyltransferases was aligned with that of GaBRV-XL.
Putative endornaviruses contain a cysteine-rich region with several CxCC signatures

In the BLAST search, aa 545–1143 of the PEV1 polyprotein matched significantly with a region extending between positions 520 and 1140 (expect value 2E-4) of GaBRV-XL2. While similarities with other putative endornaviruses were not detected in this region, we noted that the cysteine-rich region first reported by Hacker et al. (2005) showed some degree of sequence similarity among putative endornaviruses. The cysteine-rich regions in ORV (aa 856–900; 20% cysteine) and OSV (855–899; 20%) each contained two, whereas VHF (1265–1362; 16.3%), PEV1 (675–763; 21.4%) and HmEV1-670 (1198–1269; 22.2%) and both strains of GaBRV-XL (747–846; 17%) each contained three CxCC signatures. One of the observed signatures was in the form CxCCG in all viruses.

Phylogenetic analysis of the GaBRV-XL genome

The four conserved regions of GaBRV-XL were each separately aligned with sequences from similar viruses and exposed to phylogenetic analysis (Table 1, Fig. 3). The substitution model applied in MrBayes and PhyML was GTR+G+I. Phylogenies produced by neighbour-joining (MEGA 3.1), Bayesian (MrBayes 3.1.2) and maximum-likelihood (PhyML) methods were identical and inferred a distant relationship between GaBRV-XL and other putative endornaviruses. Phylogenies based on the viral RNA helicase 1 and RNA_dep_RNApol2 regions were decisive, fully resolved and well supported by bootstrap and posterior probability values. While we note that taxonomic sampling was different for all alignments, phylogenetic resolution was weak for alignments of the viral methyltransferase regions and motifs Ia1, Ia2 and IIa1 of altoviruses (Rozanov et al., 1992).

The DExDc, DEAD-like helicases superfamily region (DExH box helicase) of GaBRV-XL was most similar to regions from Classical swine fever virus (Grebennikova et al., 1999; Mayer et al., 2003) (Flaviviridae: Pestivirus), Bovine viral diarrhea virus (GenBank accession no. BAD04938) (Flaviviridae: Pestivirus), Hepatitis C virus (Nakao et al., 1996) (Flaviviridae: Hepacivirus), Hepatitis GB virus (De Tomassi et al., 2002) (unclassified member of Flaviviridae), Shallot yellow stripe virus (Chen et al., 2005) (Potyviridae: Potyivirus) and Sorghum mosaic virus (Yang & Mirkow, 1997) (Potyviridae: Potyivirus). The expect value ranged from 2E-05 in flaviviruses to 3.3 in potyviruses. Flaviviruses and potyviruses are members of the flavi- and alphavirus-like supergroups, respectively (van der Heijden & Bol, 2002). An alignment of putative DExH box helicases (Supplementary Fig. S2) contained I, II, III, V and VI motifs of DExH box helicases (Tanner & Linder, 2001).

A BLAST analysis suggested that the viral RNA helicase 1 region of GaBRV-XL2 was most similar to a partially sequenced putative endornavirus of bell pepper (Valverde & Gutierrez, 2007) with an expect value of 3E-4. Other similar sequences were putative triple block proteins (helicases) of Cherry necrotic rusty mottle virus (GenBank accession no. AAFA78211) (expect value 3E-3) and Cherry green ring mottle virus (Gentit et al., 2001) (4E-4), which are unassigned members of the Flexiviridae (Foveavirus). An alignment of the helicase 1 region of these viruses with putative endornaviruses and partially sequenced Phaseolus vulgaris endornavirus (Fukuhara et al., 2005) (Supplementary Fig. S3) contained helicase motifs I, IA, II, III, IV, V and VI of viral superfamily 1 (Koonin & Dolja, 1993). Unfortunately, the helicase of the bell pepper endornavirus could not be aligned unambiguously with these sequences.

The two helicases (DExH box helicase and viral RNA helicase 1) of GaBRV-XL were significantly dissimilar from each other and could not be aligned unambiguously. However, motif I in the two helicases was significantly similar at the amino acid and nucleotide sequence levels. The cDNA (and amino acid) sequence for the four putative endornaviruses and partially sequenced putative endornaviruses (Fukuhara et al., 2005) of 'Sinbis-like supergroup' of the methyltransferase regions from 1E-17 to 8E-3 and for ssRNA viruses Grapevine rootstock stem lesion-associated virus (NCBI reference sequence NP_835244) (an unclassified member of the Closteroviridae, genus Closterovirus) and Little cherry virus 2 (Rott & Jelkmann, 2005) (Closteroviridae: Ampelovirus). Complete or partial polymerase amino acid sequences of GaBRV-XL1-2, DdV-3 and the putative endornaviruses listed above were aligned (Supplementary Fig. S4) and subjected to phylogenetic analysis. The alignment contained all eight conserved motifs of the RNA-dependent RNA polymerases of positive-strand RNA viruses and related double-stranded viruses (Koonin & Dolja, 1993). In addition, a new and unique motif was identified, IXa.
In summary, our phylogenetic analyses suggested GaBRV-XL to be distinct and clearly separated from other recognized viral groups.

**DISCUSSION**

We have reported the occurrence of three different dsRNA molecule types in the fungus *G. abietina* type B. The largest type, GaBRV-XL, is a sexually transmitted, cryptic, monopartite, linear dsRNA virus, somewhat similar to assigned and unassigned members of genus *Endornavirus*. Based on centrifugation trials and dsRNA extraction tests, in line with known endornaviruses, it does not exhibit a protein capsid. However, the putative totivirus infecting the same isolate seems to have one.

The genomic structure of GaBRV-XL appears to be a composite of five conserved regions. While physicochemical properties and sequences of the RNA<sub>dep</sub> RNApol2, viral RNA helicase 1 and cysteine-rich regions resemble those of known endornaviruses, the DExH box helicase and viral methyltransferase regions appear to be restricted to GaBRV-XL. The genome of GaBRV-XL is further distinguished from almost all known endornaviruses in that it lacks the UDP glycosyltransferase motifs (Hacker et al., 2005), and RT-PCR results suggest that the nick in its coding strand is missing.

The putative methyltransferase region in GaBRV-XL is based on sequences deposited in the EBI (InterPro: IPR002588) and is widely found in a range of ssRNA viruses, including hordei-, tobra-, tomoabo-, bromo-, clostero- and caliciviruses. The methyltransferase region is involved in mRNA capping to increase stability. In eukaryotes, mRNA is transcribed in the nucleus, and cytoplasmic viruses therefore encode their own methyltransferase. The methyltransferase region is commonly found in the so-called Alphavirus supergroup and this region has also been detected, e.g. in a fungal reovirus (Hillman et al., 2004) and in a mycovirus infecting *Sclerotinia sclerotiorum* (Xie et al., 2006). The occurrence of the CAP structure in GaBRV-XL was not examined in this study.

The GaBRV-XL genome contained two different helicase regions with unknown roles. Two helicases in a single viral genome have been reported in a group of plant positive-strand ssRNA viruses comprising the potex-, carla-, hordei- and furoviruses, where one helicase is involved in RNA replication and the other in movement of the virus in plant tissue (Kadare & Haenni, 1997).

RNA helicases of the DEAD box and related DExD/H proteins play various roles in RNA metabolism such as nuclear transcription, pre-mRNA splicing, ribosome biogenesis, nucleocytoplasmic transport, translation, RNA decay and organellar gene expression (Tanner & Linder, 2001). In addition, DExD/H box RNA helicases can also displace proteins from RNA (Schwer, 2001).

The viral RNA helicase 1 regions in endornaviruses and foveaviruses are closely related to the alpha-like supergroup (Gibbs et al., 2000; van der Heijden & Bol, 2002) and are thought to be involved in duplex unwinding during viral RNA replication (Gomez de Cedron et al., 1999). A common feature of helicases is that NTP hydrolysis is usually coupled to the unwinding reaction of nucleic acids (Kadare & Haenni, 1997). Motif I is a Walker A NTP-binding motif that binds phosphates on NTP (Tanner & Linder, 2001), and it represents a critical biochemical function. Thus, that motif I is highly similar in the helicase regions of GaBRV-XL may be a convergent consequence of their similar function in the same virus–host system rather than evidence of an evolutionary relationship.

Endornaviruses are generally thought to encode a polyprotein that can be digested by protease. However, protease domains or activity have yet to be reported among endornaviruses. A candidate for a protease domain could
be the somewhat conserved cysteine-rich region. Cysteine is unique among naturally occurring amino acids in that it contains a thiol group which is capable of reversible oxidation/reduction reactions in the active sites of thiol proteases (papain-like proteases). However, the CxxC signature has also been linked to heavy metal binding (Giritch et al., 1998; Lu et al., 2003), and cysteine itself forms disulfide bonds between adjacent residues to stabilize the tertiary structure of functional proteins.

The differences described above combined with the phylogenetic analyses conducted on the putative methyltransferase, DExH box helicase, viral RNA helicase 1 and RNA_dep_RNApol2 regions suggest that GaBRV-XL is related to but distinct from known endornaviruses and distantly related or even unrelated to ssRNA viruses. However, the partial sequence of the RNA_dep_RNApol2 region from Discula destructiva virus 3 suggests that similar viruses may occur in fungi.

GaBRV-XL and putative endornaviruses discussed in this study are somewhat closely related and may have evolved from an alpha-like virus, as suggested by Gibbs et al. (2000) based on their analysis of polymerase and viral RNA helicase regions. However, the distinction of the GaBRV-XL genome from known endornaviruses suggests a complex evolutionary history for these non-enveloped viruses. Complete sequences of Discula destructiva virus 3, other putative endornaviruses (Fukuhara et al., 2005; Coutts, 2005; Valverde & Gutierrez, 2007) and the putative XL virus of G. abietina type A (11 kb in electrophoresis; T. T. Tuomivirta and J. Hantula, unpublished) may help us to understand the evolutionary origins and genomics of this interesting group of viruses.

ACKNOWLEDGEMENTS

Ms Marianne Bostro¨m (isolate AU58), Ms Tiina Nurmi (isolate E46) and Ms Marja-Leena Santanen are acknowledged for their skilful technical assistance. Dr Antti Uotila is thanked for providing isolate AU58. Dr Michael Hardman helped with editing and language. This research was funded by the Academy of Finland.

REFERENCES


