



Molecular characterization of a new monopartite dsRNA mycovirus from mycorrhizal *Thelephora terrestris* (Ehrh.) and its detection in soil oribatid mites (Acari: Oribatida)



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ABSTRACT

A novel dsRNA virus was identified in the mycorrhizal fungus *Thelephora terrestris* (Ehrh.) and sequenced. This virus, named *Thelephora terrestris* virus 1 (TtV1), contains two reading frames in different frames but with the possibility that ORF2 could be translated as a fusion polyprotein after ribosomal -1 frameshifting. Picornavirus 2A-like motif, nudix hydrolase, phytoeovirus S7, and RdRp domains were found in a unique arrangement on the polyprotein. A new genus named Phlegivirus and containing TtV1, PgLV1, RfV1 and LeV is therefore proposed. Twenty species of oribatid mites were identified in soil material in the vicinity of *T. terrestris*. TtV1 was detected in large amounts in *Steganacarus (Tropacarus) carinatus* (C.L. Koch, 1841) and in much smaller amounts in *Nothrus silvestris* (Nicolet). This is the first description of mycovirus presence in oribatid mites.

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Introduction

Mycorrhizal symbioses are fundamental to tree growth, increasing the availability of water and mineral nutrients through extramatrical mycelia (Smith and Reed, 1997). *Thelephora terrestris* (Ehrh.) (earthfan fungus; family *Thelephoraceae* Chevall.) is a pioneer mycobiont which occurs in a wide variety of soils, including both non-fertilized and highly fertilized nursery soils, as well as mineral or peaty soils (Colpaert, 1999). *Pinus* spp. are the most frequent hosts of *T. terrestris*, but the fungus can form mycorrhizas with a wide range of plant genera including alder (*Alnus*), birch (*Betula*), chestnut (*Castanea*), beech (*Fagus*), poplar (*Populus*), oak (*Quercus*), willows (*Salix*), and others (Colpaert, 1999). More than 21% of screened roots of a Norway spruce (*Picea abies*) forest were colonized with *T. terrestris* (Johnsson et al., 1999). Cytokinins of the zeatin riboside-type produced by the fungus are enhanced by the

presence of host roots (Kraigher et al., 1991). Furthermore, cytokinins have been held to influence the morphological characteristics and distribution of mycorrhizal roots, resistance to root pathogens, and longevity of roots and seedlings. The presence of *T. terrestris* has been observed to be highly correlated with presence of copper (Cu) and iron (Fe) as foliar nutrients (Smaill and Walbert, 2013), and a high content of aluminum has indicated bioconcentration of this trace element by the fungus (Rudawska and Leski, 2005). *T. terrestris* has been observed to enhance the growth of Japanese poplar (*Populus maximowiczii*) seedlings in an N-deficient substrate (Obase et al., 2009). The fungus is described as tolerant of high nitrogen availability, and therefore it has recently become a common ectomycorrhizal symbiont in conifer tree nurseries across the world.

The exploration of viruses in nonpathogenic mycorrhizal fungi has been widely neglected, and little information has been obtained regarding such fungi due to their obligate biotrophic nature. The presence of viruses in fungi should not be surprising, however, as diverse viruses have been described in *Tuber aestivum* and *Tuber excavatum* ectomycorrhizal fungi: *T. aestivum* mitovirus (Stielow et al., 2011a), *T. excavatum* mitovirus (Stielow et al., 2012), *Tuber aestivum* endornavirus (Stielow et al., 2011b), and *T. aestivum*

Abbreviations: PgLV, *Phlebiopsis gigantea* large virus; LeV, *Lentinula edodes* virus; RfV, *Rhizoctonia fumigata* virus; NUDIX hydrolase, a hydrolase acting upon a substrate of general nucleoside diphosphate structure linked to another moiety

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virus 1 (totivirus) (Stielow and Menzel, 2010). A unique mitovirus has been described in *Rhizophagus clarus* arbuscular mycorrhizal fungus (Kitahara et al., 2015), and a 4.5 kbp dsRNA element (GRF1V-M) was found in a *Glomus* sp. fungus (Ikeda et al., 2012).

Mites of the suborder Oribatida comprise one of the most diverse and abundant arthropod groups in the upper layers of soil and litter in natural and agricultural systems. Most species are generalists that feed on decomposing plant debris and fungi (Schneider et al., 2004), while others feed on lichens or soil algae (Seyd and Seaward, 1984). They are dominant in forest ecosystems but they occur worldwide in any type of soil, where they are important decomposers. Microphytophagous and panphytophagous species may be important short-distance dispersers of fungal spores, which could be dispersed on the body surface or in the digestive tract of oribatid mites (Renker et al., 2005).

As true of other organisms, mites may be subject to viral disease. Surprisingly, however, only in the citrus red mite, *Panonychus citri*, the European red mite, *P. ulmi*, (both Order Prostigmata, Family Tetranychidae), and an ectoparasitic mite of honeybees *Varroa destructor* (order Mesostigmata, Varroidae) are devastating viruses known in more detail (Van Der Geest et al., 2000; Yue and Genersch, 2005). The presence of other viruses has been documented by microscopy examination where virus-like particles of different sizes have been observed in the predatory gamasid mites *Metaseiulus occidentalis*, *Neoseiulus cucumeris*, and *Phytoseiulus persimilis* (all Mesostigmata, Phytoseiidae) (Poinar and Poinar, 1998). Mites are also involved in the transmission of viruses. The phytophagous mites *Aceria tulipae* (Prostigmata, Eriophyidae), *Brevipalpus phoenicis*, and *Brevipalpus californicus* (Prostigmata, Tenuipalpidae) are known to transmit plant viruses. *A. tulipae* transmits the *Garlic mite-borne filamentous virus* (Allexivirus) (Van Dijk and Van der Vlugt, 1994), while rhabdovirus-like viruses have been transmitted by *B. phoenicis* and *B. californicus* (Kitajima et al., 2003; Kondo et al., 2003; Rodrigues et al., 2003). *West Nile virus*, *Fowl pox virus*, and *Tick-borne encephalitis virus* have been isolated from mites of the superfamily Dermanssoidea (Mesostigmata), which may play an important role in the transmission of these viruses (Moro et al., 2005; Van der Geest, 2010).

In addition to the pathogenic effects of viruses on higher life forms, both the role of viruses in wild host populations and details about vector–virus–host relationships pointing to long-standing interactions among partners remain fragmentary (Roossinck, 2015). In this paper, we describe a new virus found in the mycorrhizal fungus *T. terrestris* and its presence in *Steganacarus (Tropacarus) carinatus* oribatid mite from the vicinity of that fungus.

Results

Description of the *T. terrestris* virus 1 genome

In dsRNA isolation from asymptomatic fruiting bodies of *T. terrestris* fungus, which shared 100% nucleotide identity with the *T. terrestris* voucher BB64_301_Oh_Pi_150506 18 S rRNA gene, GenBank AC:HM189958.1, a single band of about 10 kbp was visible on agarose gel (Fig. 1).

A single contig 10,316 nt long was assembled and found to contain two open reading frames (ORFs) in different frames on the genomic plus strand: ORF1 in frame 2 encodes a 202 kDa (1843 aa) protein and ORF2 in frame 1 encodes a 174 kDa (1548 aa) protein. Similarly as in *Phlebiopsis gigantea* large virus 1 (PgL1) and 2 (Kozlakidis et al., 2009), there are no in-frame stop codons upstream from the ORF2 start codon for 195 nt but ribosomal -1 frameshifting sequence AAAAAAA was found at nt 5492–5498 (56 nt upstream from the stop codon of ORF1). This raises the possibility that ORF2 could be translated as a fusion protein with ORF1 in similar way as it was described in *Rosellinia necatrix* megabirnavirus 1 and its host *Cryphonectria parasitica* (Salaipeth et al., 2014). In contrast to PgL1, no pseudoknot structure was predicted downstream of the shifty site in the HPknotter program (Huang et al., 2005), but stable stem-loop structures (results not shown) that could assist in pausing translating ribosomes were predicted upstream as well as downstream of the slippery site in RNAfold (Gruber et al., 2008). Polyprotein processing is intrinsic for many viruses. In picornaviruses, proteinase 2A^{Pro} cleaves between 2A and the N-terminal proline of 2B or the C-terminal NPGP motif of 2A promotes ribosome skipping during translation (Palmenberg et al., 2010). The 2A-like motif comprises the seven aa residues G/DxExNPGP and N-terminal proline of 2B protein (underlined). These sequences had been found also in insect ssRNA iflaviruses, tetraviruses, and dicistroviruses, in dsRNA rotaviruses, cypoviruses, and in non-fungal totiviruses (Donnelly et al., 2001; Nibert, 2007). In our PSI-BLAST screening of the GenBank database, the motif was found in mycoviruses *Rosellinia necatrix* mycovirus 2-W 1032/S6 (Alphapartitivirus), *Fusarium graminearum* hypoviruses 1 and 2 (FgHV1 and 2, Hypoviridae), PgL1, as well as the newly sequenced virus from *T. terrestris* (TtV1) (Fig. 2a). In *Rosellinia necatrix* mycovirus 2-W 1032/S6, the 2A-like motif was found after the RdRp domain, in FgHV1 twice (at aa position 749 and 879, after putative peptidase C7), in FgHV2 once at position 242 and once in PgL1, and in TtV1 close to the N-terminus of ORF1 (Fig. 1). Based on expected activity of 2A^{Pro}, the N-terminal part of TtV1 polyprotein 90 aa long could be released from the polyprotein.

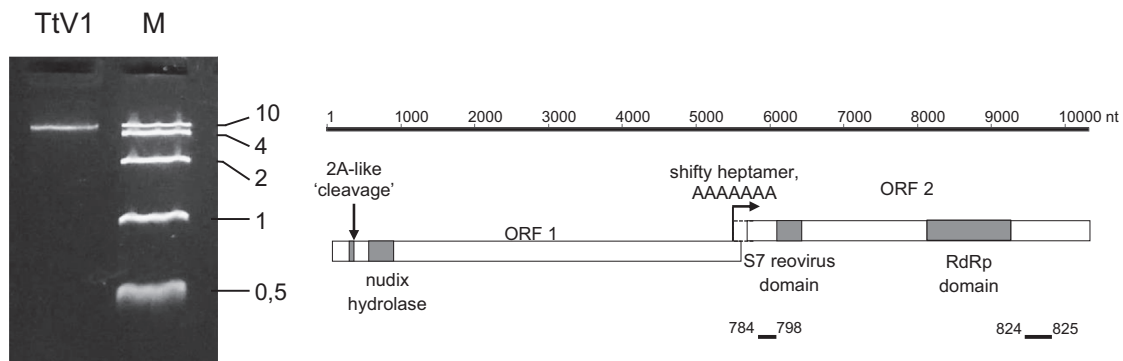


Fig. 1. Agarose gel electrophoresis of dsRNA isolated from *T. terrestris*, analysis of nucleic acids. M –DNA Ladder (10 kbp, 4 kbp 2 kbp, 1 kbp, 500 bp). Genome arrangement and motifs of TtV1. Schematic representation of the genomic organization of TtV1 shows the presence of two ORFs (ORF1 and ORF2). The dotted-line box indicates a possible extension of ORF1 via a translational frameshift mechanism. Position of 2A-like motif, nudix hydrolase, S7 phyteoreovirus domain, and RdRp conserved domain are indicated by boxes. Regions used for RT-PCR detection with primer numbers are indicated by thick lines.

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