Turkish Journal of Botany

Volume 49 | Number 2 Article 4

3-7-2025

Complete genome characterizations of two novel mitoviruses hosted by the hypogeous, mycorrhizal fungi Statesia pompholyx and Tuber sp.

ILGAZ AKATA

GÜLCE EDİŞ

EDA KUMRU

EMRE KESKİN

ERGİN ŞAHİN

Follow this and additional works at: https://journals.tubitak.gov.tr/botany

Recommended Citation
AKATA, ILGAZ; EDİŞ, GÜLCE; KUMRU, EDA; KESKİN, EMRE; and ŞAHİN, ERGİN (2025) "Complete genome characterizations of two novel mitoviruses hosted by the hypogeous, mycorrhizal fungi Statesia pompholyx and Tuber sp.," *Turkish Journal of Botany*: Vol. 49: No. 2, Article 4. https://doi.org/10.55730/1300-008X.2845
Available at: https://journals.tubitak.gov.tr/botany/vol49/iss2/4

This work is licensed under a Creative Commons Attribution 4.0 International License.
This Research Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Botany by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr

TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2025) 49: 102-110
© TÜBİTAK
doi:10.55730/1300-008X.2845

Complete genome characterizations of two novel mitoviruses hosted by the hypogeous, mycorrhizal fungi *Statesia pompholyx* and *Tuber* sp.

Ilgaz AKATA[1,*], Gülce EDİŞ[2], Eda KUMRU[2], Emre KESKİN[3], Ergin ŞAHİN[4,5]

[1]Department of Biology, Faculty of Science, Ankara University, Ankara, Turkiye
[2]Graduate School of Natural and Applied Sciences, Ankara University, Ankara, Turkiye
[3]Evolutionary Genetics Laboratory (eGL), Department of Fisheries and Aquaculture, Faculty of Agriculture, Ankara University, Ankara, Turkiye
[4]Department of Biology, Faculty of Science, Dokuz Eylül University, İzmir, Turkiye
[5]Fauna and Flora Research and Application Center, Dokuz Eylül University, İzmir, Turkiye

Received: 22.08.2024 • **Accepted/Published Online:** 19.01.2025 • **Final Version:** 07.03.2025

Abstract: The study of viral diversity in uncultivated fungi, including those involved in mycorrhizal relationships, alongside research on viral communities in phytopathogenic and economically important fungi, is crucial for a comprehensive understanding of mycovirus diversity and evolution. Mycorrhizal fungi host various viruses, contributing to our understanding of virus diversity and evolution. Here, we report on the identification and complete genome characterization of two novel mitoviruses that naturally infect the hypogeous, mycorrhizal fungi *Statesia pompholyx* and *Tuber* sp. The identified viruses, denominated as "Statesia pompholyx mitovirus 1" (SpMV1) and Tuber mitovirus 3 (TruMV3), have genome lengths of 2549 and 3292 nucleotides and G+C contents of 39.00% and 34.7%, respectively. Both viruses contain a single open reading frame (ORF) encoding for RNA-dependent RNA polymerase (RdRp). BLASTp analyses showed that the RdRp domains of SpMV1 and TruMV3 share sequence similarities with those of members belonging to the genera *Unuamitovirus* and *Triamitovirus*. Specifically, they exhibit the highest similarities (44.09% and 68.34% identity) to Plasmopara viticola lesion-associated mitovirus 22 and Tuber excavatum mitovirus, respectively. According to phylogenetic analysis, SpMV1 and TruMV3 are classified as members of the genera *Unuamitovirus* and *Triamitovirus* within the *Mitoviridae* family, respectively. SpMV1 represents the first instance of a mitovirus identified in the hypogeous mycorrhizal fungal genus *Statesia*. These novel mitovirus sequences enhance our understanding of the diversity and evolution within the *Mitoviridae* family and contribute to the rapidly expanding dataset of mycovirus sequences.

Key words: Mycoviruses, mitovirus, *Statesia pompholyx*, *Tuber* sp., truffle

1. Introduction

Recently, there has been a surge of research focusing on the viral communities associated with fungal taxa (Akata et al., 2023; Li et al., 2023; Raco et al., 2023; Akata et al., 2024a, 2024b; Bora et al., 2024; Ferilli et al., 2024; Rueda-Maíllo et al., 2024; Sahin et al., 2024). This momentum can be attributed to the advancements in high-throughput sequencing (HTS) technologies (Ayllon and Vainio, 2023). Using HTS analyses, it has become evident that fungal viruses, or mycoviruses, exhibit remarkable diversity and can infect various fungal hosts across various fungal groups. These groups encompass basal lineages and apically diverging divisions, each characterized by unique lifestyles (Hough et al., 2023). Additionally, applying HTS methodologies has allowed researchers to identify numerous novel fungal viruses belonging to unassigned virus groups, as well as previously undocumented members of established viral taxa within the fungal kingdom (Ayllon and Vainio, 2023).

Mycorrhizas are among terrestrial life's oldest and most vital mutualistic symbioses (Genre et al., 2020). Their primary function is facilitating robust growth in fungal and plant partners by efficiently exchanging nutrients. In addition to nourishing both partners, mycorrhizas can link adjacent root systems of individual plants, known as common mycorrhizal networks (CMN). However, our understanding of whether CMNs act as interconnecting networks that facilitate communication and resource sharing among neighbouring plants is limited. Therefore, this hypothesis requires solid evidence to be substantiated (Karst et al., 2023). The collaboration between fungi, plants, and soil microorganisms in mycorrhizal

* Correspondence: akata@science.ankara.edu.tr

This work is licensed under a Creative Commons Attribution 4.0 International License.

associations is crucial for ecosystem dynamics (Boyno et al., 2023; Zhang et al., 2024). The outdated perspective of considering plants, animals, fungi, and microorganisms as separate entities impeded progress in ecology in the 20th century. Consequently, ecologists now acknowledge the importance of mycorrhizas in shaping models for nutrient cycling and energy transfer within ecosystems (Netherway et al., 2021; Bahram and Netherway, 2022).

The genus *Tuber* P. Micheli ex F.H. Wigg., belonging to the *Tuberaceae* family, is a diverse group of ectomycorrhizal hypogean fungi within the order *Pezizales* and division *Ascomycota*. These fungi are highly valued for producing subterranean fruiting bodies known as truffles. The genus comprises approximately 230 species, with ongoing discoveries, particularly in previously unexplored areas (Cseh et al., 2024). *Tuber* species establish symbiotic relationships with various tree species, leading to their broad distribution, primarily across the Northern Hemisphere (Obase et al., 2021). Key identifying features of *Tuber* species include their hypogenous, globose to lobed ascomata, often with a basal mycelial tuft, solid marbled gleba, and a smooth or warted peridium. Their asci, typically containing 1–6 ornamented spores, exhibit structures such as warts, spines, or ridges (Akata et al., 2020; 2022). The evolutionary shift from epigeous to hypogeous fruiting bodies in *Tuber* represents a key adaptation, protecting spores from environmental fluctuations and reducing reliance on wind or water for dispersal (Trappe et al., 2009; Ori et al., 2018). Instead, these fungi rely on animals attracted by volatile compounds to consume and disperse spores packed within their asci, reflecting a specialised evolutionary strategy for subterranean spore dispersal (Bonito et al., 2013; Ori et al., 2021; Kuhar et al., 2024; Rosa–Gruszecka et al., 2024).

In contrast, the genus *Statesia* Castellano, T. Lebel, Davoodian & K. Hosaka, which falls under the family *Hysterangiaceae* E. Fisch. within the order *Hysterangiales* K. Hosaka & Castellano and division *Basidiomycota*, comprises four distinct species forming ectomycorrhizal associations with different plants. Members of this genus are characterized by their underground, typically round basidiomata, which have smooth and finely hairy surfaces. Initially white, these basidiomata mature to shades of brown or red. Notably, *Statesia pompholyx* is recognized for its unique morphological features, which add to the intrigue and fascination of its study. The basidiomata are globose to slightly irregular and undergo a color transition from light to deep reddish-brown as they mature. The gleba darkens with age and drying, featuring elongated locules. A thin, gelatinous, dendroid columella and abundant rhizomorphs are present, with the peridium consisting of a single layer of dark reddish-brown polyhedral cells. The spores are narrowly elliptical to broadly fusiform, thick-walled, and exhibit a rough texture, often found in pairs. *S. pompholyx* is distinct within its genus due to its singularly layered peridium and elongated, slender spores (Lebel et al., 2022).

Ectomycorrhizal fungi's virus communities, despite their ecological importance, have received limited attention, with few to zero studies dedicated to exploring the ecological niches of these viruses in soil environments (Petrzik et al., 2016; Sahin and Akata 2019; Sahin et al., 2020; Sutela and Vainio, 2020; Sahin and Akata, 2021; Sahin et al., 2021a, 2021b; Akata et al., 2023; Sahin et al., 2023). While two studies in the existing literature report on mitoviruses inhabiting hypogeous ectomycorrhizal fungal species such as *Tuber excavatum* Vittad. and *Tuber aestivum* (Wulfen) Spreng. (Stielow et al., 2011; Stielow et al., 2012), no mycovirus has hitherto been reported from Statesia or its closely related genus, *Hysterangium* Vittad.

The *Mitoviridae* family comprises RNA viruses that lack capsids. These viruses possess a positive-sense, single-stranded RNA genome, which varies in size from 2.1 to 4.9 kb (Hillman & Cai, 2013; Koonin et al., 2020) (https://ictv.global/ictv/proposals/2021.003F.R.Mitoviridae_100nsp_4ngen.zip). Their genomes contain a single open reading frame (ORF) that utilises the mitochondrial genetic code. This ORF encodes an RNA-dependent RNA polymerase (RdRp) domain consisting of six conserved amino acid sequence motifs (A–F). Recently, four newly identified genera have been included in the *Mitoviridae* family: *Kvaramitovirus*, *Triamitovirus*, *Duamitovirus*, and *Unuamitovirus* (https://ictv.global/taxonomy). While initially discovered in fungi, *Mitoviridae* members that inhabit plants and insects have been more recently documented (Bruenn et al., 2015; Nibert et al., 2018; Fonseca et al., 2020). However, it is important to note that mitoviruses have not been observed in any species within the genus *Statesia* or its closely related genus *Hysterangium*. In this study, we isolated and characterized two novel mitoviruses inhabiting *S. pompholyx* and *Tuber* sp. These newly discovered viruses were named "Statesia pompholyx mitovirus 1" (SpMV1) and "Tuber mitovirus 3" (TruMV3).

2. Materials and method

2.1. Sampling and molecular characterization of the fungal specimens

During field studies conducted in northwestern Türkiye, specifically on October 10, 2022, in the Demirköy district of Kırklareli province, and on December 6, 2022, in the Enez district of Edirne province, two distinct fungal specimens were carefully sampled. The first specimen, a basidiocarp from a single colony of *Statesia pompholyx* (Figure 1A), grew beneath a beech tree (*Fagus* sp.). The second specimen, an ascocarp from a single colony of a

member of the *Tuber* genus (Figure 1C), was located under a pine tree (*Pinus* sp.). These specimens were carefully collected and have since been cataloged and preserved in the Ankara University Herbarium (ANK), with voucher specimen identifiers Akata TT 159 and Akata TT 347, respectively.

Genomic DNA was extracted from the AKATA TT 159 sample using the CTAB method (Rogers & Bendich, 1994). The DNA was then used for PCR amplification of the Internal Transcribed Spacer (ITS) rDNA and the translation elongation factor 1-alpha (TEF1a) gene regions. ITS amplification employed the ITS1/ITS4 primer set (Martin and Rygiewicz, 2005), while TEF1a was amplified using EF1-983F/EF1-1567R primers (Rehner and Buckley, 2005). PCR products were confirmed via agarose gel electrophoresis, purified using the Expin Clean Up SV Kit (Tribioscience), and sequenced by Sanger sequencing with the BigDye Direct Cycle Sequencing Kit. Sequence analysis, performed on an ABI Prism 3130 Genetic Analyzer, revealed over 99% similarity between the nrITS rDNA and TEF1a sequences of the sample and *S. pompholyx* reference sequences (GenBank IDs: OR223352 and PP622673).

The BLASTn analysis of the specimen identified as AKATA TT 347 revealed a perfect alignment quality (QC: 100%) and a 98.49% sequence similarity to *Tuber lusitanicum* Ant. Rodr. & Muñoz-Mohedano (accession number MT621652.1). The similarity falls short of confirming a species-level match despite this genetic resemblance. Moreover, morphological assessments highlighted discrepancies, as the specimen's macro and micromorphological traits did not correspond with those characteristic of *T. lusitanicum*. The discovery of additional samples with similar molecular and morphological features further supports the possibility of AKATA TT 347 representing an uncharacterised species within the *Tuber* genus. Given the lack of comprehensive taxonomic data, referring to this specimen as *Tuber* sp. is the most appropriate interim classification until further studies provide more definitive insights.

2.2. Sample preparation for high throughput sequencing

The specimens first underwent a surface sterilisation to prepare the collected fungal specimens for subsequent analysis. This involved immersing the specimens in a 2% sodium hypochlorite solution for two min to eliminate potential contaminants, followed by a brief 10-s immersion in 70% ethanol. After these sterilization steps, the specimens were thoroughly rinsed with sterile distilled water to remove residual chemicals. Once sterilized, the specimens were dehydrated and finely ground using a laboratory grinder, ensuring a homogeneous sample.

Double-stranded RNAs (dsRNA) were extracted from the pulverised fruiting bodies of both *S. pompholyx* and the *Tuber* species using a cellulose column-based solid-phase extraction method, which is detailed in previous studies (Morris and Dodds, 1979; Darissa et al., 2010). This protocol used cellulose Type 101 (Sigma) for column preparation instead of the more commonly employed cellulose CF-11, which slightly modified the established method. After extraction, the dsRNAs were subjected to further processing, including DNase I and S1 nuclease treatment, to remove contaminating DNA and single-stranded RNA. This was followed by reverse transcription into complementary DNA (cDNA) using the primer-dN6 (5'-CCTGAATTCGGATCCTCCNNNNNN-3') and the RevertAid First-Strand cDNA Synthesis Kit after a purification step involving a GeneJet PCR purification kit (Thermo Fisher Scientific). To amplify the cDNA products, random PCR amplification (rPCR) was conducted by previously established protocols (Darissa et al., 2010). The amplified products were sequenced using the high-throughput Illumina Novaseq 6000 platform, with sequencing services provided by Novogene in the United Kingdom.

2.3.Bioinformatic analyses of the high throughput sequencing data

For the subsequent bioinformatic analyses, the filtered sequencing reads were de novo assembled using the CLC Genomics Workbench version 20.0.2 software, employing its default settings. This assembly process yielded 38 contigs from the *Statesia pompholyx* specimen and 123 from the *Tuber* specimen, each exceeding 1 kb. These assembled contigs were then subjected to BLASTx analysis against the non-redundant protein database at NCBI GenBank to determine potential viral identities.

Among the contigs, contig 4, which measured 2438 nucleotides in length, derived from the dsRNA extracts of the *Statesia pompholyx* specimen (Akata TT 159), and contig 1, measuring 2816 nucleotides, from the dsRNA extracts of the *Tuber* specimen (Akata TT 347), exhibited the highest sequence similarities to known viruses. Specifically, contig 4 showed a 44.09% identity with the Plasmopara viticola lesion-associated mitovirus 22, while contig 1 displayed a 68.34% identity with the Tuber excavatum mitovirus. These significant sequence similarities strongly suggested the presence of novel viruses belonging to the *Mitoviridae* family within the examined fungal specimens. As a result, these newly identified viral sequences were designated "Statesia pompholyx mitovirus 1" (SpMV1) and "Tuber mitovirus 3" (TruMV3), marking the discovery of previously undocumented members of the *Mitoviridae* family.

2.4. RNA-ligase-mediated rapid amplification of cDNA ends

The 5'- and 3'-terminal sequences of the genomic RNAs of Statesia pompholyx mitovirus 1 (SpMV1) and Tuber

mitovirus 3 (TruMV3) were cloned and sequenced using RNA-ligase-mediated rapid amplification of cDNA ends (RLM-RACE). The process began with the ligation of the 3' ends of the purified double-stranded RNAs (dsRNAs) to an oligonucleotide adapter, RLO (5'p-CATGGTGGCGACCGGTAG-NH2 3'), at 37 °C for six h. This was followed by an overnight treatment at 12 °C with T4 RNA Ligase 1 (New England Biolabs) to ensure efficient ligation. The RNA molecules, now attached to the oligonucleotide, were purified using the GeneJET PCR Purification Kit (Thermo Fisher Scientific).

Subsequently, these purified RNAs were reverse-transcribed into complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) and the RTP primer (5'-CTACCGGTCGCCACCATG-3'), which is complementary to the RLO oligonucleotide. The terminal sequences of the 5' and 3' ends were then separately amplified using specific primers for each virus. For SpMV1, the reverse primer for the 5' end was 5'-GCCGGACAAAACCGTCACAATC-3', and the forward primer for the 3' end was 5'-AGCCAAGAAAGCTCAGGCCAAG-3'. For TruMV3, the reverse primer for the 5' end was 5'-GCCGAGACAGATCGTTATGGTG-3', and the forward primer for the 3' end was 5'-ATCATCGTCTCGATCACGCTTG-3'. Each primer was paired with the RTP primer during the PCR amplification stage.

The PCR amplicons were then inserted into the pGEM-T Easy Vector (Promega) for cloning and sequenced using the standard Sanger Dideoxy Termination method. The genome sequences were assembled using Geneious Prime 2020.2.5 software (Dotmatics). This rigorous and methodical approach ensured the accurate determination of the terminal sequences, providing crucial insights into the genetic structure of these newly identified mitoviruses.

2.5. Phylogenetic analyses of the complete viral genome sequences

To identify single Open Reading Frames (ORFs) in the genomes of Statesia pompholyx mitovirus 1 (SpMV1) and Tuber mitovirus 3 (TruMV3), the ExPASy translate tool (https://web.expasy.org/translate/) was employed, using the mold mitochondrial genetic code. This tool facilitated the translation of nucleotide sequences into their corresponding amino acid sequences. The resultant polypeptide sequences from SpMV1 and TruMV3 were then aligned with sequences from known mitoviruses utilising the Clustal Omega multiple sequence alignment tool, ensuring accurate and comprehensive alignment.

Following the alignment, a phylogenetic tree was constructed using the maximum-likelihood (ML) method with the WAG+G+I model, as implemented in MEGA X software (Whelan and Goldman, 2001; Kumar et al., 2018). This approach provided a robust framework for assessing evolutionary relationships among the viral sequences. To validate the reliability of the phylogenetic tree, the bootstrap method was applied with 1000 bootstrap replicates, offering a high level of confidence in the inferred phylogenetic relationships.

3. Results

The dsRNA segments, the genome replication intermediates of the identified mitoviruses, which were named "Statesia pompholyx mitovirus 1" (SpMV1) and "Tuber mitovirus 3" (TruMV3), were successfully isolated from the specimens AKATA TT 159 and AKATA TT 347 respectively (Figure 1B-D). The genetic makeup of SpMV1 and TruMV3 consist of 2,549 and 3,292 nucleotides (nt) and have G+C contents of 39.00% and 34.7%, respectively. The genomic sequences of SpMV1 and TruMV3 were submitted to the NCBI GenBank database under the accession numbers PP620543.1 and PP620544.1. Using the genetic code for fungal mitochondria (where UGA codes for tryptophan), the analyses revealed that both the SpMV1 and TruMV3 genomes contain a single open reading frame (ORF) (Figure 1E–1F). The predicted polypeptides encoded by these ORFs are composed of 643 and 794 amino acids with molecular weights of 74.24 and 88.11 kDa, respectively, as calculated using the online Protein Molecular Weight tool (https://www.bioinformatics.org/sms/prot_mw.html). The lengths of the 5′ and 3′ untranslated regions (UTRs) are 524 and 93 nt for SpMV1 and 790 and 117 nt for TruMV3 (Figure 1E–1F). The 5′- and 3′-UTRs were analysed by RNA Folding Form V2.3 of the RNA mfold server (http://www.unafold.org/mfold/applications/rna-folding-form-v2.php) and shown to have stem-loop structures with initial ΔG values of –23.91 kcal/mol and –10.27 kcal/mol for SpMV1, and –6.52 kcal/mol and –16.33 kcal/mol for TruMV3, respectively. Additionally, the terminal sequences of SpMV1 were predicted to have a potential cyclization motif forming a panhandle structure with an initial ΔG value of –22.20 kcal/mol (Figure 1G–1H).

Searches in the NCBI Conserved Domains Database (CDD) revealed that the polypeptides encoded by SpMV1 and TruMV3 contain RdRp domains located between amino acid (aa) positions 119 and 497, 206, and 502, respectively (Figure 1E–1F). Further analysis in CDD showed that these conserved RdRp domains of SpMV1 and TruMV3 are part of the mitovirus RdRp family (Accession: cl05469, with E-values of 7.73e-87 and 9.74e-62, respectively). Additionally, BLASTp analyses indicated that the RdRps of SpMV1 and TruMV3 share the highest similarity (44.09% and 68.34% identity, respectively) with those of Plasmopara viticola lesion-associated mitovirus 22 and Tuber excavatum mitovirus.

A phylogenetic tree was created using RdRp sequences from SpMV1, TruMV1, and other mitoviruses to establish the relationship between SpMV1 and TruMV3 with other mycoviruses. The tree revealed that SpMV1 is grouped with different members within the genus *Unuamitovirus*, such as Plasmopara viticola lesion-associated mitovirus 22 and Entomophthora muscae mitovirus 8, which infects a species of entomopathogenic fungus called *Entomophthora muscae* (Cohn) Fresen. (Figure 2A). It was hypothesised that the mitoviruses infecting this entomopathogenic species might have been obtained through horizontal transmission from other distantly related phyla, such as *Basidiomycota* (Nibert et al., 2019). On the other hand, the tree provided evidence that TruMV3 belongs to the *Triamitovirus* genus, along with several other members such as Tuber excavatum mitovirus and Geopora sumneriana mitovirus 1. By conducting a thorough analysis of the RdRp domains of eight different members from the *Unuamitovirus* and *Triamitovirus* genera, it was determined that both SpMV1 and TruMV3 possess all six conserved motifs (F, A, B, C, D, and E) in the N-terminal-to-C-terminal direction. Notably, these motifs include the highly conserved A, B, and C motifs in the catalytic palm subdomain (Figure 2B).

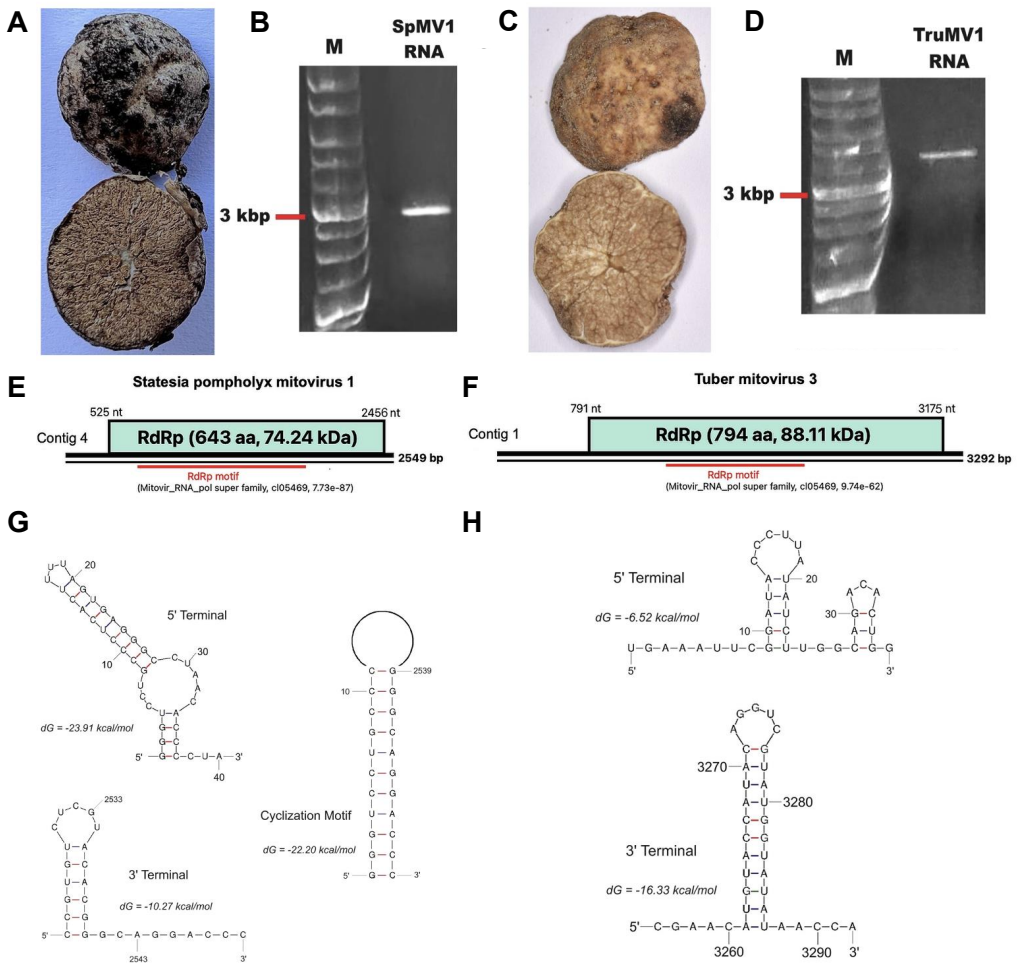


Figure 1. A. Basidiocarps of *Statesia pompholyx* Akata TT 159. B. The agarose gel image of the dsRNA segment represents the genome replication intermediates of SpMV1. C. Ascocarps of *Tuber* sp. Akata TT 347. D. The agarose gel image of the dsRNA segment representing the genome replication intermediates of TruMV3. E–F. Schematic depiction of the SpMV1 and TruMV3 genomes. The open reading frames (ORFs) and the untranslated regions (UTRs) are shown by a rectangular box and single black lines, respectively. The genome portions that encode potential RNA-dependent RNA polymerase (RdRp) domains are indicated with red lines. G. The anticipated secondary structures of the 5′ and 3′ termini of SpMV1. H. The anticipated secondary structures of the 5′ and 3′ termini of TruMV3 genomes are shown. Additionally, a predicted cyclization motif (panhandle structure) formed by the complementary nucleotides at the 5' and 3' terminals of the SpMV1 genome is illustrated.

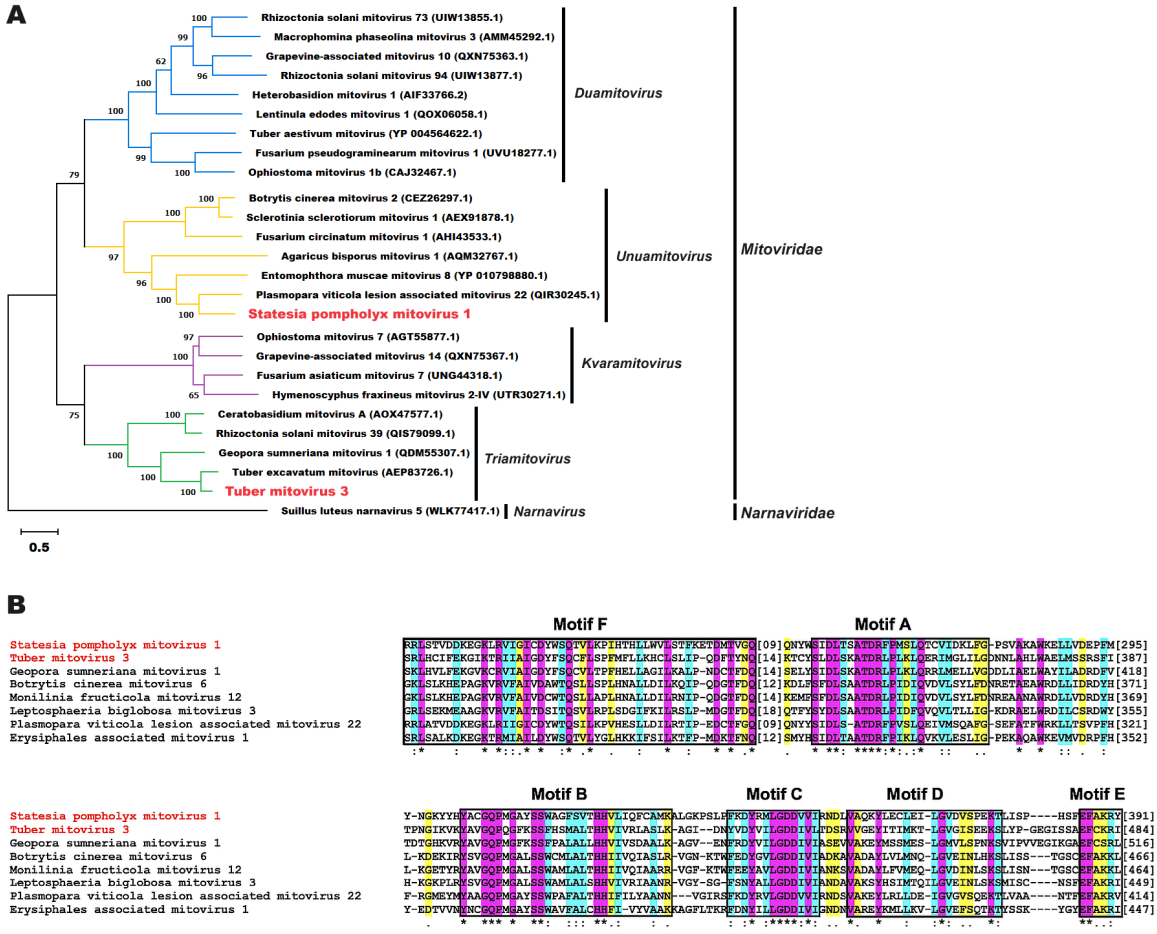


Figure 2. A. The phylogenetic tree demonstrating the evolutionary relatedness of SpMV1 and TruMV3 RdRps with their counterparts from various members of the *Mitoviridae* family. The tree was calculated using the maximum-likelihood method, which involved bootstrapping analysis performed 1000 times within MEGA X software. The calculated percent bootstrap values are shown in branch nodes. The scale bar represents a genetic distance of 0.5, with the GenBank accession numbers provided in parentheses for each mitovirus RdRp sequence. B. The comparison of the RdRp conserved motifs (F, A, B, C, D, and E in the order from N-terminal to C-terminal) among SpMV1, TruMV3, and closely related viruses within the *Unuamitovirus* and *Triamitovirus* genera is shown. Matching amino acid residues are highlighted in magenta boxes, while amino acid residues sharing similar chemical properties are depicted in blue and yellow boxes.

4. Discussion

Considerable variation in length and significant sequence diversity are observed in the untranslated regions (UTRs) of mitoviruses, even within the same species. Although translation factors are believed to interact with these regions, the precise host factors (proteins and/or RNAs) that may also engage with them are still unidentified. Moreover, the functions of these non-coding regions and whether they have any epitranscriptomic modifications influencing the host's physiology have not been experimentally investigated. Currently, it is believed that the terminal sequences of each UTR region serve as cis-elements, assisting in their interaction with the viral RdRp while mitovirus genome replication takes place. Comparative genomic analyses utilizing advanced deep learning models hold promise in gaining deeper insights into these regulatory elements. However, it is essential to accumulate substantial sequence data before employing these models effectively.

In evolutionary biology, the relationships between viruses and their hosts are intricate and can stem from different evolutionary mechanisms. These mechanisms include "codivergence," where the evolutionary trees of viruses and hosts are similar in structure; "switching,"

where a virus is transferred to a new host that is significantly different from the previous one, and pathogen "duplication," where the parasite diversifies within the same host species, leading to several parasite groups that share the same host range (Göker et al., 2011).

In this context, considering the currently known closest relative of SpMV1, which is hosted by an oomycete species, *Plasmopara viticola*, we could speculate that the evolutionary driving forces act on SpMV1 appears to at least involve a host-switching event. On the other hand, the emergence of TruMV1 most likely resulted from strict codivergence with the host and duplication events (adaptive radiation) within the same host species.

SpMV1 and TruMV3 have been identified as new additions to distinct mitovirus species, as per the demarcation threshold of 70% RdRp sequence similarity within the *Mitoviridae* family (https://ictv.global/ictv/proposals/2021.003F.R.Mitoviridae_100nsp_4ngen.zip).

In summary, based on the analysis of genomic structures, comparisons of RdRp sequences, and phylogenetic evaluations, we propose that SpMV1 belongs to the genus *Unuamitovirus*. In contrast, TruMV3 belongs to the genus *Triamitovirus* within the *Mitoviridae* family. Consequently, considering the existing literature, this study represents the first documentation of the complete genome sequence of a mitovirus associated with the hypogeous, ectomycorrhizal fungus *S. pompholyx*. We also present the genome characterisation of the third mitovirus identified in the *Tuber* genus.

Acknowledgement
This study was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) under Grant Number 121Z924. The authors thank TÜBİTAK for their support.

References

Akata I, Sevindik M, Şahin E (2020). *Tuber fulgens* Quél., a new record for Turkish truffles. Turkish Journal of Agriculture-Food Science and Technology 8 (11): 2472-2475. https://doi.org/10.24925/turjaf.v8i11.2472-2475.3884

Akata I, Şen İ, Sevindik M, Kabaktepe Ş (2022). Truffle Checklist of Turkey II with A New Record. Turkish Journal of Agriculture-Food Science and Technology 10: 1913-1920. https://doi.org/10.24925/turjaf.v10i10.1913-1920.5482

Akata I, Edis G, Keskin E, Sahin E (2023). Diverse partitiviruses hosted by the ectomycorrhizal agaric *Hebeloma mesophaeum* and the natural transmission of a partitivirus between phylogenetically distant, sympatric fungi. Virology 581: 63-70. https://doi.org/10.1016/j.virol.2023.03.002

Akata I, Edis G, Kumru E, Sahin E (2024a). Identification and full-length genome characterization of a novel mitovirus hosted by the truffle species *Tuber rufum*. VirusDisease 35 (3): 531-536. https://doi.org/10.1007/s13337-024-00887-6

Akata I, Edis G, Ozbey BG, Keskin E, Sahin E (2024b). Complete genome analyses of a novel flexivirus with unique genome organization and three endornaviruses hosted by the mycorrhizal fungus *Terfezia claveryi*. Current Microbiology 81 (7): 1-11. https://doi.org/10.1007/s00284-024-03745-2

Ayllon MA, Vainio EJ (2023). Mycoviruses as a part of the global virome: Diversity, evolutionary links and lifestyle. Advances in Virus Research 115: 1-86. https://doi.org/10.1016/bs.aivir.2023.02.002

Bahram M, Netherway T (2022). Fungi as mediators linking organisms and ecosystems. FEMS Microbiology Reviews 46 (2): fuab058. https://doi.org/10.1093/femsre/fuab058

Bonito G, Smith ME, Nowak M, Healy RA, Guevara G et al. (2013). Historical biogeography and diversification of truffles in the Tuberaceae and their newly identified southern hemisphere sister lineage. PLoS One 8 (1): e52765. https://doi.org/10.1371/journal.pone.0052765

Bora E, Akata I, Keskin E, Sahin E (2024). Molecular characterization and comparative genomic analysis of two triamitovirus isolates hosted by the hypogean fungus *Tuber excavatum* Vittad. Trakya University Journal of Natural Sciences, (Online First). https://doi.org/10.23902/trkjnat.1478899

Boyno G, Rezaee Danesh Y, Demir S, Teniz N, Mulet JM et al. (2023). The Complex ınterplay between arbuscular mycorrhizal fungi and strigolactone: Mechanisms, sinergies, applications and future directions. International Journal of Molecular Sciences 24 (23): 16774. https://doi.org/10.3390/ijms242316774

Bruenn JA, Warner BE, Yerramsetty P (2015). Widespread mitovirus sequences in plant genomes. PeerJ 3: e876. https://doi.org/10.7717/peerj.876

Cseh P, Merényi Z, Bóna L, Varga T, Bóka K et al. (2024). Taxonomic characterisation of the Regianum clade (genus *Tuber*) and the trait evolution of spore size among true truffles. Mycological Progress 23: 11. https://doi.org/10.1007/s11557-024-01949-1

Darissa O, Willingmann P, Adam G (2010). Optimized approaches for the sequence determination of double-stranded RNA templates. Journal of Virological Methods 169: 397-403. https://doi.org/10.1016/j.jviromet.2010.08.013

Ferilli F, Lione G, Gonthier P, Turina M, Forgia M (2024). First detection of mycoviruses in *Gnomoniopsis castaneae* suggests a putative horizontal gene transfer event between negative-sense and double-strand RNA viruses. Virology 594: 110057. https://doi.org/10.1016/j.virol.2024.110057

Fonseca P, Ferreira F, da Silva F, Oliveira LS, Marques JT et al. (2020). Characterization of a novel mitovirus of the sand fly *Lutzomyia longipalpis* using genomic and virus-host interaction signatures. Viruses 23;13 (1): 9. https://doi.org/10.3390/v13010009

Genre A, Lanfranco L, Perotto S, Bonfante P (2020). Unique and common traits in mycorrhizal symbioses. Nature Reviews Microbiology 18(11): 649-660. https://doi.org/10.1038/s41579-020-0402-3

Göker M, Scheuner C, Klenk HP, Stielow JB, Menzel W (2011). Codivergence of mycoviruses with their hosts. PLoS One 6 (7): e22252. https://doi.org/10.1371/journal.pone.0022252

Hillman BI, Cai G (2013). Chapter Six - The family *Narnaviridae*: simplest of RNA viruses. Advances in Virus Research 86: 149-176. https://doi.org/10.1016/B978-0-12-394315-6.00006-4

Hough B, Steenkamp E, Wingfield B, Read D (2023). Fungal viruses unveiled: A comprehensive review of mycoviruses. Viruses 19;15 (5): 1202. https://doi.org/10.3390/v15051202

Karst J, Jones MD, Hoeksema JD (2023). Positive citation bias and overinterpreted results lead to misinformation on common mycorrhizal networks in forests. Nature Ecology & Evolution 7 (4): 501-511. https://doi.org/10.1038/s41559-023-01986-1

Koonin EV, Dolja VV, Krupovic M, Varsani A, Wolf YI et al. (2020). Global organization and proposed megataxonomy of the virus world. Microbiology and Molecular Biology Reviews 84 (2): 10-1128. https://doi.org/10.1128/mmbr.00061-19

Kuhar F, Tejedor-Calvo E, Sequeira A, Pelissero D, Cosse M et al. (2024). Comprehensive characterization of *Tuber maculatum*, New in Uruguay: Morphological, molecular, and aromatic analyses. Journal of Fungi 10 (6): 421. https://doi.org/10.3390/jof10060421

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 1;35 (6): 1547-1549. https://doi.org/10.1093/molbev/msy096

Lebel T, Davoodian N, Bloomfield M, Syme K, May T et al. (2022). A mixed bag of sequestrate fungi from five different families: *Boletaceae*, *Russulaceae*, *Psathyrellaceae*, *Strophariaceae*, and *Hysterangiaceae*. Swainsona 36: 33-65.

Li X, Li S, Yin W, Sossah FL, Song B et al. (2023). Complete genome sequence of a novel mycovirus from *Pleurotus citrinopileatus*. Archives of Virology 168 (2): 66. https://doi.org/10.1007/s00705-022-05668-4

Martin KJ, Rygiewicz PT. (2005). Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiology 5: 1-11. https://doi.org/10.1186/1471-2180-5-28

Morris TJ, Dodds JA (1979). Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. Phytopathology 69 (8): 854-858. https://doi.org/10.1094/Phyto-69-854

Netherway T, Bengtsson J, Krab EJ, Bahram M (2021). Biotic interactions with mycorrhizal systems as extended nutrient acquisition strategies shaping forest soil communities and functions. Basic and Applied Ecology 50: 25-42. https://doi.org/10.1016/j.baae.2020.10.002

Nibert ML, Vong M, Fugate KK, Debat HJ (2018). Evidence for contemporary plant mitoviruses. Virology 518: 14-24. https://doi.org/10.1016/j.virol.2018.02.005

Nibert ML, Debat HJ, Manny AR, Grigoriev IV, De Fine Licht HH (2019). Mitovirus and mitochondrial coding sequences from basal fungus *Entomophthora muscae*. Viruses 11 (4): 351. https://doi.org/10.3390/v11040351

Obase K, Yamanaka S, Kinoshita A, Tamai Y, Yamanaka T (2021). Phylogenetic placements and cultural characteristics of *Tuber* species isolated from ectomycorrhizas. Mycoscience 62 (2); 124-131. https://doi.org/10.47371/mycosci.2020.12.001

Ori F, Trappe J, Leonardi M, Iotti M, Pacioni G (2018). Crested porcupines (*Hystrix cristata*): mycophagist spore dispersers of the ectomycorrhizal truffle *Tuber aestivum*. Mycorrhiza 28: 561-565. https://doi.org/10.1007/s00572-018-0840-1

Ori F, Menotta M, Leonardi M, Amicucci A, Zambonelli A et al. (2021). Effect of slug mycophagy on *Tuber aestivum* spores. Fungal Biology 125 (10): 796-805. https://doi.org/10.1016/j.funbio.2021.05.002

Petrzik K, Sarkisova T, Stary J, Koloniuk I, Hrabakova L et al. (2016). Molecular characterization of a new monopartite dsRNA mycovirus from mycorrhizal *Thelephora terrestris* (Ehrh.) and its detection in soil oribatid mites (Acari: Oribatida). Virology 489: 12-19. https://doi.org/10.1016/j.virol.2015.11.009

Raco M, Jung T, Horta Jung M, Chi NM, Botella L et al. (2023). Sequence and phylogenetic analysis of a novel alphaendornavirus, the first virus described from the oomycete plant pathogen *Phytophthora heveae*. Archives of Virology 168 (6): 158. https://doi.org/10.1007/s00705-023-05786-7

Rehner SA, Buckley E (2005). A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97 (1): 84-98. https://doi.org/10.1080/15572536.2006.11832842

Rueda-Maíllo F, Garrido-Jurado I, Kotta-Loizou I, Quesada-Moraga E (2024). A mycoviral infection drives virulence and ecological fitness of the entomopathogenic fungus *Beauveria bassiana*. Journal of Invertebrate Pathology 108251. https://doi.org/10.1016/j.jip.2024.108251

Sahin E, Akata I (2019). Complete genome sequence of a novel mitovirus from the ectomycorrhizal fungus *Geopora sumneriana*. Archives of Virology 164: 2853-2857. https://doi.org/10.1007/s00705-019-04367-x

Sahin E, Akata I, Keskin E (2020). Novel and divergent bipartite mycoviruses associated with the ectomycorrhizal fungus *Sarcosphaera coronaria*. Virus Research 286: 198071. https://doi.org/10.1016/j.virusres.2020.198071

Sahin E, Akata I (2021). Full-length genome characterization of a novel alphapartitivirus detected in the ectomycorrhizal fungus *Hygrophorus penarioides*. Virus Genes 57: 94-99. https://doi.org/10.1007/s11262-020-01814-9

Sahin E, Akata I, Keskin E (2021a). Molecular characterization of a new endornavirus inhabiting the ectomycorrhizal fungus *Hygrophorus penarioides*. Brazilian Journal of Microbiology 52: 1167-1172. https://doi.org/10.1007/s42770-021-00500-8

Sahin E, Keskin E, Akata I (2021b). Novel and diverse mycoviruses co-inhabiting the hypogeous ectomycorrhizal fungus *Picoa juniperi*. Virology 552: 10-19. https://doi.org/10.1016/j.virol.2020.09.009

Sahin E, Ozbey Saridogan BG, Keskin E, Akata I (2023). Identification and complete genome sequencing of a novel betapartitivirus naturally infecting the mycorrhizal desert truffle *Terfezia claveryi*. Virus Genes 59: 254-259. https://doi.org/10.1007/s11262-023-01972-6

Sahin E, Edis G, Keskin E, Akata I (2024). Molecular characterization of the complete genome of a novel ormycovirus infecting the ectomycorrhizal fungus *Hortiboletus rubellus*. Archives of Virology 169 (5): 110. https://doi.org/10.1007/s00705-024-06027-1

Stielow B, Klenk HP, Winter S, Menzel W (2011). A novel *Tuber aestivum* (Vittad.) mitovirus. Archives of Virology 156: 1107-1110. https://doi.org/10.1007/s00705-011-0998-8

Stielow JB, Bratek Z, Klenk HP, Winter S, Menzel W (2012). A novel mitovirus from the hypogeous ectomycorrhizal fungus *Tuber excavatum*. Archives of Virology 157: 787-790 https://doi.org/10.1007/s00705-012-1228-8

Sutela S, Vainio EJ (2020). Virus population structure in the ectomycorrhizal fungi *Lactarius rufus* and *L. tabidus* at two forest sites in Southern Finland. Virus Research 285: 197993. https://doi.org/10.1016/j.virusres.2020.197993

Whelan S, Goldman N (2001). A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Molecular Biology and Evolution 18: 691-699. https://doi.org/10.1093/oxfordjournals.molbev.a003851

Zhang C, van der Heijden MGA, Dodds BK, Nguyen TB, Spooren J et al. (2024). A tripartite bacterial-fungal-plant symbiosis in the mycorrhiza-shaped microbiome drives plant growth and mycorrhization. Microbiome 12:13. https://doi.org/10.1186/s40168-023-01726-4