http://dergipark.org.tr/trkjnat
Trakya University Journal of Natural Sciences, 25(2): xx-xx, 2024
ISSN 2528-9691
DOI: 10.23902/trkjnat.1478899

Research Article

Molecular characterization and comparative genomic analysis of two triamitovirus isolates hosted by the hypogean fungus *Tuber excavatum* Vittad.

Elifnaz Bora[1], Ilgaz Akata[2], Emre Keskin[3], Ergin Sahin[4,5*]

[1] Ankara University, Graduate School of Natural and Applied Sciences, 06110, Dışkapı, Ankara, TÜRKİYE
[2] Ankara University Faculty of Science, Department of Biology 06100 Tandogan, Ankara, TÜRKİYE
[3] Ankara University, Faculty of Agriculture Department of Fisheries and Aquaculture, Evolutionary Genetics Laboratory (eGL), 06110, Dışkapı, Ankara, TÜRKİYE
[4] Dokuz Eylul University, Faculty of Science, Department of Biology, 35390 Buca, İzmir, TÜRKİYE
[5] Dokuz Eylul University, Fauna and Flora Research and Application Center, 35390 Buca, İzmir, TÜRKİYE

Cite this article as:

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 Univ J Nat Sci*, 25(2): xx-xx, DOI: 10.23902/trkjnat.1478899

Received: 05 May 2024, Accepted: 12 September 2024, Online First: 24 September 2024

Edited by:
Boris Assyov

***Corresponding Author:**
Ergin Sahin
ergin.sahin@deu.edu.tr

ORCID iDs of the authors:
EB. 0000-0009-6047-6776
IA. 0000-0002-1731-1302
EK. 0000-0002-7279-313X
ES. 0000-0003-1711-738X

Key words:
Mycovirus
Mitovirus
Virus evolution
Truffle
Tuber excavatum

Abstract: The connections between viruses and their hosts are complex and can arise from any combination of different evolutionary events including “codivergence”, “switching”, and “duplication” of the pathogen. Mycoviruses, a diverse virus group whose members specifically infect fungal hosts, are subject to similar evolutionary processes. In this study, we present the identification and complete genome characterization of the second isolate of a mitovirus, commonly known as *Tuber excavatum* mitovirus, officially named *Triamitovirus tuex1*. This mycovirus infects the hypogean, ectomyrrhizal fungus *Tuber excavatum* Vittad.. Both *Triamitovirus tuex1* isolates, Tekirdağ (identified by us) and Lammspringe, were found in the fruiting bodies of *T. excavatum* isolates collected from Türkiye and Germany, respectively. Comparative genomic analyses revealed that the two virus isolates share 85.33% sequence similarity in their whole genomes, with their protein encompassing RNA-dependent RNA polymerase (RdRp) domain showing an identity rate of 94.60%. The most diverse part of the viral genomes was found to be the 5’ untranslated regions (UTRs), with a sequence similarity of 78.53%, while the 3’ UTRs were the most conserved, with 91.53% sequence similarity. Considering the shared host species, the emergence of these *Triamitovirus tuex1* isolates appears to reflect a duplication pattern (intra-host divergence) resulting from adaptive radiation.

Özet: Virüsler ile konakları arasındaki bağlantılar karmaşıktır ve “kodivergens (birlikte ıraksama)”, “değişim” ve “patojenin çoğaltılması” gibi farklı evrimsel olayların herhangi bir kombinasyonundan kaynaklanabilir. Mikovirüsler, özel olarak mantar konaklarını enfekte eden, çeşitlilik gösteren bir virüs grubudur ve benzer evrimsel süreçlere tabidir. Bu çalışmada, *Tuber excavatum* mitovirüsü olarak bilinen ve resmi olarak *Triamitovirus tuex1* olarak adlandırılan bir mitovirüsün ikinci izolatının tanımlanması ve tüm genom nitelemesi sunulmaktadır. Bu mikovirüs, hipogean, ektomikorizal mantar *Tuber excavatum* Vittad.'ı enfekte eder. Sırasıyla Türkiye ve Almanya'dan toplanan Tekirdağ (bizim tarafımızdan tanımlanan) ve Lammspringe *Triamitovirus tuex1* izolatlarının her ikisi de, *T. excavatum* meyvelerinde tanımlandı. Karşılaştırmalı genom analizleri, her iki virüs izolatının da tüm genomlarında %85,33'lük bir dizi benzerliği paylaştığını ve RNA bağımlı RNA polimeraz (RdRp) alanını (domain) içeren proteinlerinin %94,60'lık bir benzerlik oranına sahip olduğunu ortaya koymuştur. Viral genomların en çok farklılık gösteren kısmının %78,53'lük bir dizi benzerliği gösteren 5' translasyonu yapılmayan bölgeler (UTR’ler) olduğu, 3' UTR’lerin ise %91,53'lük bir dizi benzerliği ile en çok korunmuş kısımlar olduğu bulunmuştur. Konak türlerin ortak olması göz önünde bulundurulduğunda, bu *Triamitovirus tuex1* izolatlarının ortaya çıkışları, adaptif radyasyondan kaynaklanan bir çoğaltma modelini (konak-içi çeşitlenmesi) yansıtıyor gibi görünmektedir.

Introduction

Research on fungal-associated viral communities has experienced a significant increase, propelled by the advancements in high-throughput sequencing (HTS) technologies (Ayllon & Vainio 2023). HTS analyses have unveiled the remarkable diversity of fungal viruses (mycoviruses) and their wide-ranging infectivity across

diverse fungal groups, spanning from basal lineages to highly divergent divisions, each with unique lifestyles (Hough *et al.* 2023). Moreover, HTS methodologies have facilitated the discovery of numerous novel fungal viruses within unassigned virus groups, as well as the identification of various established viral taxa previously undocumented in the fungal kingdom (Ayllon & Vainio 2023) (https://talk.ictvonline.org/ictv-reports/ictv_online_report/n.

Currently, the International Committee on the Taxonomy of Viruses (ICTV) classifies mycoviruses into more than 30 families (https://ictv.global/). The majority of mycoviruses typically possess genomes comprised of double-stranded RNA (dsRNA) or positive-sense single-stranded RNA (+ssRNA) (Ghabrial *et al.* 2015). Additionally, mycoviruses with genomes containing negative-sense single-stranded RNA (−ssRNA) have been identified and are classified within the family *Mymonaviridae* (Wang *et al.* 2018, Lin *et al.* 2019, Walker *et al.* 2020, Guo *et al.* 2021). Recently, several single-stranded DNA (ssDNA) mycovirus species have been discovered, with only two classified within the recognized mycoviral family *Genomoviridae*. These include the monopartite *Gemycircularvirus sclero1* infecting *Sclerotinia sclerotiorum* and the tripartite *Gemytripvirus fugra1* infecting *Fusarium graminearum* (Yu *et al.* 2010, Varsani & Krupovic 2021).

In a single host species, multiple factors can impact the development of diverse genotypes within a virus species. These factors could be mutation rates and genetic recombination (e.g. genome segment reassortment), as well as host factors (e.g. antiviral status and genetic background) and ecological factors (e.g. climate and habitat disruption) that exert selection pressure on viruses (Elena & Sanjuin 2007, Parvez & Parveen 2017, LaTourrette & Garcia-Ruiz 2022). In evolutionary terms, the interactions between viruses and their hosts are complex and can arise from various evolutionary processes. These include "codivergence", where the phylogenies of viruses and hosts show topological congruence, "switching" involving lateral transfer of the virus to a new host that is phylogenetically distant from the previous one, and pathogen "duplication" where the parasite undergoes adaptive radiation within the same host species, resulting in multiple parasite groups with an identical host range (Göker *et al.* 2011).

The *Mitoviridae* family consists of RNA viruses that lack capsids and have a positive-sense, single-stranded RNA (ssRNA) genome, which ranges from 2.1 to 4.9 kb in length (Hillman & Cai 2013, Koonin *et al*. 2020). These viruses feature a single open reading frame (ORF) that employs the mitochondrial genetic code, encoding an RNA-dependent RNA polymerase (RdRp) domain characterized by six conserved protein motifs each denoted with single letters (A-F). The family *Mitoviridae* now encompasses four newly identified genera: *Kvaramitovirus*, *Triamitovirus*, *Duamitovirus*, and *Unuamitovirus* (https://ictv.global/taxonomy). Although these viruses were initially discovered in fungi, more recent findings have identified *Mitoviridae* members in plants and insects (Bruenn *et al.* 2015, Nibert *et al*. 2018, Fonseca *et al*. 2020).

The genus *Tuber* comprises ectomycorrhizal fungi known for their subterranean ascomata (Akata *et al*. 2020). Among these, *Tuber excavatum* Vittad. is distinguished by its underground fruit bodies known with transition from a pale yellowish brown to a reddish brown hue upon maturation. These ascomata are generally spherical or slightly lobed and feature a pronounced cavity. The gleba within begins as white or straw-colored and gradually darkens to reddish brown, with a network of branching yellowish veins. This species typically flourishes in calcareous soils, where it forms symbiotic relationships with both deciduous trees and conifers (Castellano & Türkoğlu 2012, Fan *et al*. 2013).

Despite their ecological importance, virus communities hosted by ectomycorrhizal fungi have received limited attention, with few studies focusing on the ecological roles of these viruses in soil environments (Petrzik *et al.* 2016, Sahin & Akata 2019, Sahin *et al.* 2020, Sutela & Vainio 2020, Sahin & Akata 2021, Sahin *et al.* 2021a, Sahin *et al.* 2021b, Akata *et al*. 2023, Sahin *et al.* 2023). Existing literature reports only two studies on mitoviruses found in hypogeous ectomycorrhizal fungal genus *Tuber* (Stielow *et al.* 2011, Stielow *et al.* 2012). These viruses designated as Tuber excavatum mitovirus (TeV) and Tuber aestivum mitovirus (TaV) were officially classified as member of the virus species *Triamitovirus tuex1* isolate Lammspringe and *Duamitovirus tuae1*, respectively.

In this study, we characterize the full-length genome sequence of the second member (isolate) of *Triamitovirus tuex1* (TeV isolate Tekirdağ) identified in a *Tuber excavatum* isolate. We further make evolutionary inferences about the two *Triamitovirus tuex1* isolates in light of the comparative genomic analyses.

Materials and Methods

Sampling of T. excavatum ascocarp

During a field survey conducted on August 18, 2021, a single ascocarp of *Tuber excavatum* (Fig. 1a) was collected under an oak tree (*Quercus* sp.) in the Tekirdağ province of Türkiye. This specimen was subsequently deposited at the ANK Ankara University Herbarium under the voucher specimen, with identifier Akata & Sahin TT 002.

dsRNA Isolation, in vitro Reverse Transcription, and Polymerase Chain Reaction Amplification

The ascocarp sample was sterilized by submerging it in a 2% sodium hypochlorite solution for one minute, then rinsing it thoroughly with sterile distilled water. After dehydration, the sample was finely milled into a powder. This powdered sample was used to enrich dsRNA with the Viral dsRNA Extraction Mini Kit (iNtRON Biotechnology, South Korea). The dsRNA obtained was

treated with S1 nuclease and DNase I according to the manufacturer's guidelines (Promega). The sample was purified with the GeneJET PCR Purification Kit (Thermo Fisher Scientific) and converted to cDNA using the primer-dN6 (5'-CCTGAATTCGGATCCTCCNNNNNN-3') and the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). This cDNA was then randomly amplified with the rPCR primer (5'-CCTGAATTCGGATCCTCC-3') and DreamTaq DNA Polymerase (Thermo Fisher Scientific), following the method outlined previously (Darissa *et al*. 2010). The rPCR amplicons were then cleaned up using the PureLink™ PCR Purification Kit (Thermo Fisher Scientific). About 1µg of the purified PCR products was sent to the Agrigenomics Hub (Ankara University, Türkiye) for library preparation. Sequencing of 150 bp paired ends, at a minimum depth of 100x, was performed on an Illumina Novaseq 6000 platform.

Sequence Data Analysis and Phylogenetic Study

Raw reads from high-throughput sequencing (HTS) were assembled into contigs *de novo* using CLC Genomic Workbench version 20.0.2 (Qiagen). *De novo* assembly analyses included a word size of 26, a default bubble size of 50, automatic estimation of paired distances, and a minimum contig length of 200 nucleotides (nt). The resulting contigs and their amino acid (aa) sequences, translated using the Swiss Institute of Bioinformatics' (SIB) online tool (https://web.expasy.org/translate/), were analyzed via BLASTx and BLASTp to identify viral sequences with an e-value < 1. Viral protein domains, such as RNA-dependent RNA polymerase, were identified using the Pfam protein family database (https://pfam.xfam.org/). Evolutionary analysis involved aligning the RdRp aa sequences of *Triamitovirus tuex1* isolates Tekirdağ and Lammspringe with other mitovirus species within the *Mitoviridae* family using the ClustalW multiple sequence alignment tool (Madeira *et al*. 2019). Phylogenetic trees were generated using MEGA X software, applying the maximum-likelihood (ML) method and the JTT+G+I substitution model (Kumar *et al*. 2018). The reliableness of the tree branches was tested with 1000 bootstrap replicates.

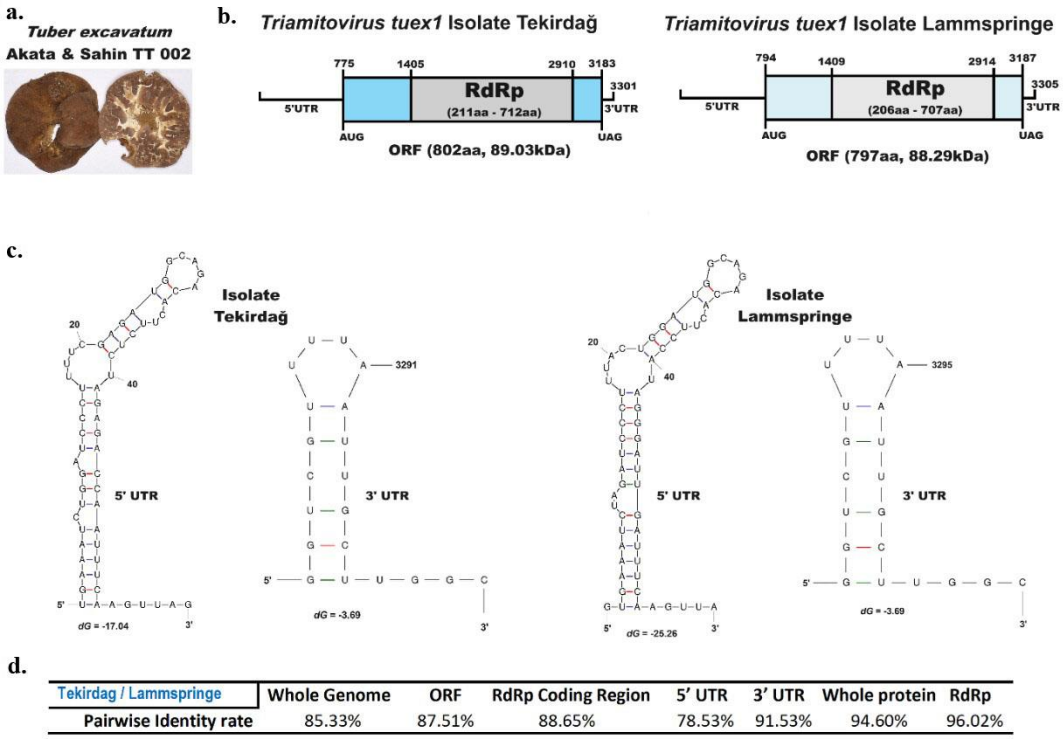
Determining the Sequences of 5' and 3' Termini with RLM-RACE

To sequence the 5'- and 3'-termini, the 3' ends of the extracted dsRNA were tagged with the short DNA oligo RLO (5'p-CATGGTGGCGACCGGTAG-NH2 3') using T4 RNA ligase 1 (New England Biolabs). The tagged dsRNA was cleaned up using the PureLink™ PCR Purification Kit (Thermo Fisher Scientific) and reverse transcribed into cDNA with the primer RTP (5'-CTACCGGTCGCCACCATG-3') and the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The terminal sequences were PCR amplified using the sequence specific reverse and forward oligonucleotide primers, TeV-5RACE1 (5'-ATCCTGTTGCGTCTCACATG-3') and TeV-3RACE1 (5'-TCAGTTGGGTTGGGTAGAGG-3'), respectively, with the RTP primer included in the PCR. The resulting PCR products were inserted into the pGEM-T Easy Vector (Promega) and sequenced using the conventional Sanger seqeuncing with universal M13 oligonucleotides at Agrigenomics Hub (Ankara University, Türkiye).

Results

BLASTx analyses of the assembled contigs derived from *T. excavatum* Akata & Sahin TT 002 (Fig. 1a) revealed a contig exhibiting 94.60% aa sequence similarity to the RdRp of a mitovirus, previously identified in a *T. excavatum* isolate collected from the Lammspringe village located in Lower Saxony, Germany (Stielow *et al.* 2012). This predefined virus was designated as "Tuber excavatum mitovirus (TeV) isolate Lammspringe" at that time. Later, it was officially defined as an exemplar virus of a mitovirus species *Triamitovirus tuex1* by the ICTV. Condsidering the 70% RdRp sequence similarity as the species demarcation threshold for mitovirids (https://ictv.global/ictv/proposals/2021.003F.R.Mitoviridae_100nsp_4ngen.zip), the mitovirus we identified was defined as an isolate of *Triamitovirus tuex1*. We, therefore, used the isolate name "Tekirdağ" to define the mitovirus we identified in the *T. excavatum* specimen Akata & Sahin TT 002. The genetic makeup of TeV isolate Tekirdağ and isolate Lammspringe consist of 3,301 and 3,305 nucleotides (nt) and have G+C contents of 38.17% and 37.70%, respectively (Supplementary Material). The genome sequences of TeV isolate Tekirdağ was kept in the NCBI GenBank database with the accession number OR157964.1. Using the fungal mitochondrial genetic code, where the opal stop codon UGA codes for tryptophan, the analysis showed that TeV isolate Tekirdağ genome harbors a single open reading frame (ORF) as similar to the genome of TeV isolate Lammspringe (Fig. 1b). The predicted polypeptides encoded by the ORFs of isolates Tekirdağ and Lammspringe composed of 802 and 797 aa with the molecular weights of 89.03 kDa and 88.29 kDa as calculated using the online Protein Molecular Weight tool (https://www.aatbio.com/tools/calculate-peptide-and-protein-molecular-weight-mw). The lengths of the 5′ and 3′ untranslated regions (UTRs) of isolate Tekirdağ are 774 and 118 nt, and the sizes of the corresponding regions in isolate Lammspringe are 793 and 118 nt (Fig. 1b). The 5′- and 3′- terminals of both isolates were analyzed 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 similar stem-loop secondary structures (Fig. 1c). No potential cyclization motif forming a panhandle structure which is often present in genomes of mitovirids was predicted in TeV isolates Tekirdağ and Lammspringe.

Searches in the Conserved Domains Database (CDD) of NCBI showed that the polypeptides encoded by TeV isolates Tekirdağ and Lammspringe contain RdRp domains located between aa positions 211 and 712, and 206 and 707, respectively (Fig. 1b).



d.

Tekirdag / Lammspringe	Whole Genome	ORF	RdRp Coding Region	5' UTR	3' UTR	Whole protein	RdRp
Pairwise Identity rate	85.33%	87.51%	88.65%	78.53%	91.53%	94.60%	96.02%

Fig. 1. a. Ascocarp of the *Tuber excavatum* Akata & Sahin TT 002, **b.** schematic representation of the genome organizations of *Triamitovirus tuex1* isolates Tekirdağ and Lammspringe. The UTRs and the ORFs encoding for RdRp were shown for each genome. The portion of the ORFs encoding for the RdRp domains are also specified, **c.** Predicted secondary structures of the 5' and 3' UTRs of both *Triamitovirus tuex1* isolates. The initial free energy values calculated for the secondary structures were stated, **d.** the percentage of sequence similarity rates observed between the *Triamitovirus tuex1* isolates Tekirdağ and Lammspringe at both the nucleotide and protein levels.

Further analysis in CDD showed that these conserved RdRp domains of both isolates are part of the mitovirus RdRp family (Accession: cl05469, with E-values of 3.09586e-67 and 9.95093e-67, respectively). Additionally, BLASTp analyses indicated that the RdRps of TeV isolates Tekirdağ and Lammspringe exhibit 94.60% sequence similarity to each other and the RdRp of Tuber mitovirus 3 (NCBI GenBank accession: WZN15221.1) was found to be their second best hit with 68.09% and 68.34% identities, respectively.

After comparing the genome sequences of both isolates, we found that the 3' UTRs displayed the highest similarity, with 91.53% nt sequence identity. Following this, the RdRp domain encoding regions exhibited 88.65% nt identity, while the ORFs showed 87.51% nt identity. The overall similarity across the entire genomes was 85.33%. Conversely, the most diverse regions were the 5' UTRs, with a nt sequence similarity of 78.53% (Fig. 1c). At the protein level, the isolates share 94.60% aa sequence similarity in their entire protein encoded by their single ORFs. Additionally, the RdRp domains of both isolates have a 96.02% aa sequence overlap (Fig. 1c).

To elucidate the relationship between TeV isolates Tekirdağ and Lammspringe and other mycoviruses, a phylogenetic tree was generated using the RdRp sequences from both isolates along with those from various mitoviruses. The resulting tree revealed that both isolates are grouped within the genus *Triamitovirus*, clustering with several members such as Tuber mitovirus 3, Geopora sumneriana mitovirus 1, Rhizictonia solani mitovirus 39, and Ceratobasidium mitovirus A (Fig. 2a). A multiple sequence alignment analysis of the RdRp domains from ten different *Triamitovirus* genus members showed that the RdRp domains of TeV isolates Tekirdağ and Lammspringe encompass all six conserved motifs (F, A, B, C, D, and E, arranged from the N-terminal to the C-terminal). Especially, this includes the most conserved motifs A, B, and C, which are located in the catalytic palm subdomain (Fig. 2b).

Discussion

Taking into account the species demarcation criteria for mitoviruses, which sets a 70% RdRp sequence identity threshold, along with the high sequence similarity rates exceeding 85% and 94% at the genome and protein levels respectively, the two TeV isolates, infecting the same host species *Tuber excavatum*, are considered as two representative members (strains, genotypes, or variants) of the mitovirus species *Triamitovirus tuex1*.

Within a single host species, various factors can influence the emergence of different genotypes of a virus species. In the context of the emergence of various mycovirus strains, these factors include, but are not limited to, 1) the viral mutation rate, 2) genetic recombination events, 3) specific host factors, and 4) ecological differences.

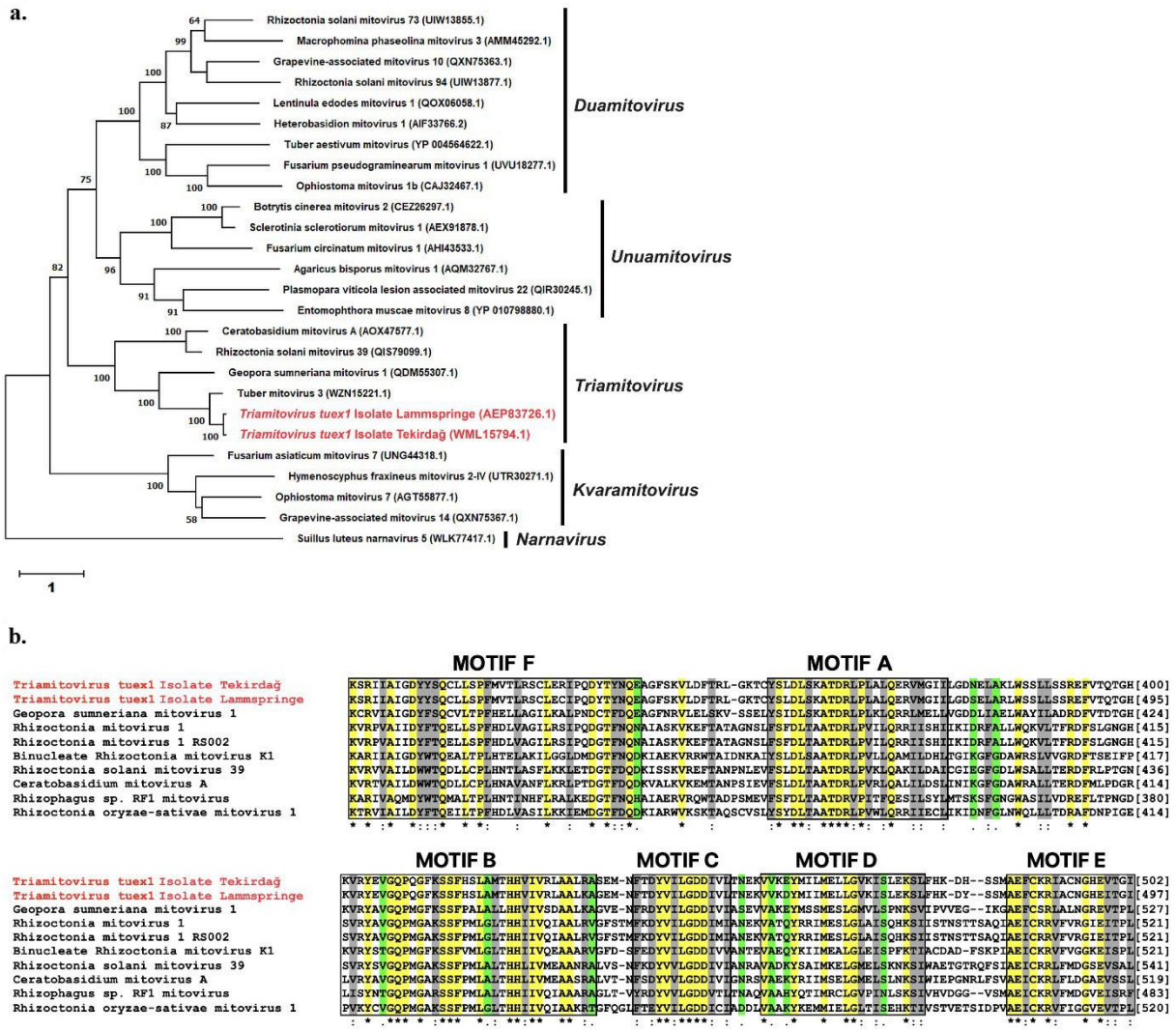


Fig. 2. a. Unrooted maximum-likelihood tree of the RdRps of both *Triamitovirus tuex1* isolates and related mitoviruses. Bootstrap values (>50%) are shown for each branch. GenBank accession numbers are also stated for each virus. Suillus luteus narnavirus 5 was selected as the outgroup sequence in the phylogenetic tree. The bars (lower left) show a genetic distance of 1 for the phylogenetic tree, **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 both *Triamitovirus tuex1* isolates and closely related viruses within the genus *Triamitovirus* is shown. Matching amino acid residues are highlighted in yellow boxes, while amino acid residues sharing similar chemical properties are shown in grey and green boxes.

If we briefly mention each of these factors; 1) Viruses mutate rapidly due to their high replication rates and lack of proofreading mechanisms in their replication machinery. Thus, mutations can occur randomly during viral genome replication and lead to the emergence of new virus strains. This phenomenon can generally be observed in mitoviruses as well. 2) Mitoviruses have RNA genomes that can undergo genetic recombination (for instance via intermolecular template switch or via non-replicative recombination involving strand break and ligation) when multiple mitovirus strains infect the same host cell. In fact, multiple mycovirus infections are commonly observed in fungal hosts of diverse origin. This infection state of the host can result in the creation of novel viral genotypes with combinations of genetic material from different viral strains. 3) Variability in host factors such as antiviral status and genetic background, can influence which viral genotypes are more successful in establishing infection and spreading within a host population. It is plausible that at least some of the genetic variations in TeV isolates might be the result of the accumulation of host adaptive mutations 4) Environmental factors such as climate, habitat disruption, and interspecies interactions can impact the distribution and prevalence of viruses, potentially creating selection pressure on viruses. As a result, certain genotypes/variants may have advantages in specific environments, leading to their propagation and survival. Overall, the interplay of these factors can result in the diversification of viral genotypes even within a single host species, contributing to the ongoing evolution and adaptation of viruses.

A fundamental question in evolutionary biology concerns the extent to which the evolution of parasites is tied to the evolution of their hosts (Klassen 1992, Johnson

et al. 2003). If viruses are specific to their hosts and transmitted solely vertically (for instance, without natural vectors), their phylogeny should be congruent with that of their hosts, adhering to Fahrenholz' rule of strict codivergence (de Vienne *et al.* 2013). However, a combination of events such as host switching, duplication (intra-host divergence), and parasite extinction can result in incongruence between the phylogenies of viruses and their hosts (Göker *et al.* 2011). In this context, "switching" denotes the lateral transfer of the parasite and its successful establishment in a new host that is phylogenetically distant from the previous one. If this transfer leads to parasite speciation, it is termed a "complete switch"; otherwise, it is an "incomplete switch." "Duplication" refers to the parasite's adaptive radiation within the same host species, producing multiple parasite groups with an identical host range.

In a prior investigation, advanced statistical methods were employed to evaluate the hypothesis that mycoviruses from different lineages codiverge/coevolve with their hosts (Göker *et al.* 2011). With a dataset limited to 25 mitovirus-related sequences, the researchers observed that the evolutionary patterns of mitoviruses closely resembled, though not precisely mirrored, the duplication-switching pattern rather than codivergence. In this sense, the emergence of *Triamitovirus tuex1* isolates within the same host species appears to reflect a duplication (intra-host divergence) event resulting from adaptive radiation.

It is important to highlight that various genomic regions of *Triamitovirus tuex1* members evolve at different rates. For example, comparative analyses revealed that the 3' UTRs of both isolates are highly conserved (91.53% similarity), whereas the 5' UTRs show the most diversity (78.53%). The relatively high sequence similarity observed in the 3' UTRs of the virus isolates could be attributed to their widely acknowledged crucial roles in synthesizing the minus (-) strand during the genome replication of positive (+) ssRNA viruses.

The untranslated regions (UTRs) of mitoviruses show considerable variability in length and sequence diversity, even within the same species. Although it is believed that translation factors interact with these sites, the exact host factors (non-coding RNAs and/or proteins) that participate in these interactions have yet to be identified. Additionally, the functions of these non-coding regions, and whether they undergo any epitranscriptomic modifications affecting the host's physiology, have yet to be experimentally explored. It is currently theorized that the terminal sequences of each UTR region function as cis-elements, aiding in their interaction with the viral RdRps during the replication of the mitovirus genome. To enhance the understanding of these regulatory cis elements, comparative genomic analyses using advanced deep learning models could be advantageous, provided that a substantial amount of sequence data is available.

In conclusion, our objective was to enhance the expanding mycovirus sequence database by sequencing and characterizing the complete genome of a mitovirus isolate. Additionally, through comparative genomic analyses with closely related mitovirus isolates, we aimed to provide a deeper understanding of the evolutionary processes influencing mitovirus genome and protein

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: All data are available within the study and in the Supplementary Material.

Author Contributions: Concept: E.Ş., Design: E.Ş., Execution: E.B., Material supplying: I.A, Data acquisition: E.B., E.Ş., Data analysis/interpretation: E.B., I.A., E.K., E.Ş., Writing: I.A., E.Ş., Critical review: E.B., I.A., E.Ş.

Conflict of Interest: The authors have no conflicts of interest to declare.

Funding: The study was supported by the The Turkish Scientific and Technological Research Council (TÜBİTAK) with project number 121Z924.

References

1. 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
2. 2. 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
3. 3. Ayllon, M.A. & 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
4. 4. Bruenn, J.A., Warner, B.E. & Yerramsetty, P. 2015. Widespread mitovirus sequences in plant genomes. *PeerJ*, 3: e876. https://doi.org/10.7717/peerj.876
5. 5. Castellano, M.A. & Türkoğlu, A. 2012. New Records of Truffle Taxa in *Tuber* and *Terfezia* from Turkey. *Turkish Journal of Botany*, 36: 295-298. https://doi.org/10.3906/bot-1106-10
6. 6. Darissa, O., Willingmann, P. & Adam, G. 2010. Optimized approaches for the sequence determination of double-stranded RNA templates. *Journal of Virological Methods*, 169(2): 397-403. https://doi.org/10.1016/j.jviromet.2010.08.013
7. 7. De Vienne, D.M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M.E. & Giraud, T.J.N.P. 2013. Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist*, 198(2): 347-385. https://doi.org/10.1111/nph.12150
8. 8. Elena, S.F. & Sanjuán, R. 2007. Virus evolution: insights from an experimental approach. *Annual Review of Ecology*,

Evolution, and Systematics, 38: 27-52. https://doi.org/10.1146/annurev.ecolsys.38.091206.095637

9. 9. Fan, L., Cao, J.Z. & Li, Y. 2013. A reassessment of excavated *Tuber* species from China based on morphology and ITS rDNA sequence data. *Mycotaxon*, 124(1): 155-163. https://doi.org/10.5248/124.155

10. 10. Fonseca, P., Ferreira, F., da Silva, F., Oliveira, L.S., Marques, J.T., Goes-Neto, A., Aguiar, E. & Gruber, A. 2020. Characterization of a novel mitovirus of the sand fly *Lutzomyia longipalpis* using genomic and virus-host interaction signatures. *Viruses*, 13(1): 9. https://doi.org/10.3390/v13010009

11. 11. Ghabrial, S.A., Castón, J.R., Jiang, D., Nibert, M.L. & Suzuki, N. 2015. 50-plus years of fungal viruses. *Virology*, 479: 356-368. https://doi.org/10.1016/j.virol.2015.02.034

12. 12. Göker, M., Scheuner, C., Klenk, H.P., Stielow, J.B. & Menzel, W. 2011. Codivergence of mycoviruses with their hosts. *PLoS One* 6(7): e22252. https://doi.org/10.1371/journal.pone.0022252

13. 13. Guo, M., Shen, G., Wang, J., Liu, M., Bian, Y. & Xu, Z. 2021. Mycoviral diversity and characteristics of a negative-stranded RNA virus LeNSRV1 in the edible mushroom *Lentinula edodes*. *Virology*, 555: 89-101. https://doi.org/10.1016/j.virol.2020.11.008

14. 14. Hillman, B.I. & Cai, G. 2013. 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

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

16. 16. Johnson, K.P., Adams, R.J., Page, R.D. & Clayton, D.H. 2003. When do parasites fail to speciate in response to host speciation? *Systematic Biology*, 52(1): 37-47. https://doi.org/10.1080/10635150390132704

17. 17. Klassen, G.J. 1992. Coevolution: a history of the macroevolutionary approach to studying host-parasite associations. *The Journal of Parasitology*, 78: 573-587. https://doi.org/10.2307/3283532

18. 18. Koonin, E.V., Dolja, V.V., Krupovic, M., Varsani, A., Wolf, Y. I., Yutin, N., Zerbini, F.M. & Kuhn, J.H. 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

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

20. 20. LaTourrette, K. & Garcia-Ruiz, H. 2022. Determinants of virus variation, evolution, and host adaptation. *Pathogens*, 11(9): 1039. https://doi.org/10.3390/pathogens11091039

21. 21. Lin, Y.H., Fujita, M., Chiba, S., Hyodo, K., Andika, I.B., Suzuki, N. & Kondo, H. 2019. Two novel fungal negative-strand RNA viruses related to mymonaviruses and phenuiviruses in the shiitake mushroom (*Lentinula edodes*). *Virology*, 533: 125-136. https://doi.org/10.1016/j.virol.2019.05.008

22. 22. Madeira, F., Park, Y.M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A.R.N., Potter, S.C., Finn, R.D. & Lopez, R. 2019. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Research*, 47(W1): W636-W641. https://doi.org/10.1093/nar/gkz268

23. 23. Nibert, M.L., Vong, M., Fugate, K.K. & Debat, H.J. 2018. Evidence for contemporary plant mitoviruses. *Virology*, 518: 14-24. https://doi.org/10.1016/j.virol.2018.02.005

24. 24. Parvez, M.K. & Parveen, S. 2017. Evolution and emergence of pathogenic viruses: past, present, and future. *Intervirology*, 60(1-2): 1-7. https://doi.org/10.1159/000478729

25. 25. Petrzik, K., Sarkisova, T., Starý, J., Koloniuk, I., Hrabáková, L. & Kubešová, O. 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

26. 26. 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

27. 27. Sahin, E. & Akata, I. 2021. Full-length genome characterization of a novel alphapartitivirus detected in the ectomycorrhizal fungus *Hygrophorus penarioides*. *Virus Genes*, 57(1): 94-99. https://doi.org/10.1007/s11262-020-01814-9

28. 28. 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

29. 29. Sahin, E., Akata, I. & Keskin, E. 2021a. Molecular characterization of a new endornavirus inhabiting the ectomycorrhizal fungus *Hygrophorus penarioides*. *Brazilian Journal of Microbiology*, 52(3): 1167-1172. https://doi.org/10.1007/s42770-021-00500-8

30. 30. 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

31. 31. Sahin, E., Ozbey Saridogan, B.G., 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(2): 254-259. https://doi.org/10.1007/s11262-023-01972-6

32. 32. Stielow, B., Klenk, H.P., 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

33. 33. Stielow, J.B., Bratek, Z., Klenk, H.P., 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

34. 34. Sutela, S. & Vainio, E.J. 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

35. 35. Varsani, A. & Krupovic, M. 2021. Family *Genomoviridae*: 2021 taxonomy update. *Archives of Virology*, 166: 2911-2926. https://doi.org/10.1007/s00705-021-05183-y

36. 36. Walker, P.J., Siddell, S.G., Lefkowitz, E.J., Mushegian, A.R., Adriaenssens, E.M., Dempsey, D.M., Dutilh, B.E., Harrach, B., Harrison, R.L., Hendrickson, R.C., Junglen, S., Knowles, N.J., Kropinski, A.M., Krupovic, M., Kuhn, J.H., Nibert, M., Orton, R.J., Rubino, L., Sabanadzovic, S., Simmonds, P., Smith, D.B., Varsani, A., Zerbini, F.M. & Davison, A.J. 2020. Changes to virus taxonomy and the statutes ratified by the International Committee on Taxonomy of Viruses (2020). *Archives of Virology*, 165: 2737-2748. https://doi.org/10.1007/s00705-020-04752-x

37. 37. Wang L., He, H., Wang, S., Chen, X., Qui, D., Kondo, H. & Guo, L. 2018. Evidence for a novel negative-stranded RNA mycovirus isolated from the plant pathogenic fungus *Fusarium graminearum. Virology*, 518: 232-240. https://doi.org/10.1016/j.virol.2018.03.008

38. 38. Yu, X., Li, B., Fu, Y., Jiang, D., Ghabrial, S.A., Li, G., Peng, Y., Xie, J., Cheng, J., Huang, J. & Yi, X. 2010. A geminivirus-related DNA mycovirus that confers hypovirulence to a plant pathogenic fungus. *Proceedings of the National Academy of Sciences of the U.S.A."*, 107(18): 8387-8392. https://doi.org/10.1073/pnas.0913535107