Trakya University Journal of Natural Sciences, 27(1), 115–120, 2026
DOI: 10.23902/trkjnat.2025122
www.tujns.org

Research Article

Complete genome characterization of a novel mitovirus from *Morchella* sp.

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Cite this article as: Saif, H., Şahin, E., Ediş, G., & Akata, I. (2026). Complete genome characterization of a novel mitovirus from *Morchella* sp. *Trakya University Journal of Natural Sciences, 27*(1), 115–120. https://doi.org/10.23902/trkjnat.2025122

Abstract

Background: Investigating viral diversity in non-cultivated fungi is essential for achieving a comprehensive understanding of mycoviral evolution and ecology, as current knowledge is largely derived from studies on phytopathogenic and commercially important species.

Aims: This study aimed to identify and characterize mycoviruses associated with wild-collected fungi and to expand existing knowledge on mitovirus diversity in underexplored fungal hosts.

Methods: A specimen assigned to *Morchella* sp. was screened for mycoviruses using molecular detection and genome sequencing. The complete viral genome was sequenced and annotated; bioinformatic techniques were employed to determine organization, nucleotide composition, and coding regions. Sequences were compared using BLASTp, and phylogenetic relationships were inferred based on RNA-dependent RNA polymerase (RdRp) sequences.

Results: A novel mitovirus, designated *Morchella mitovirus 1* (MMV1), was identified. MMV1 possesses a linear RNA genome consisting of 3,167 nucleotides with a G + C content of 41.00% and contains a single open reading frame encoding an RdRp. BLASTp revealed that the MMV1 RdRp shares the highest amino acid sequence identity (40.29%) with *Tuber mitovirus 3*, a member of the *Triamitovirus* genus of the *Mitoviridae* family. Phylogenetic analysis confirmed the placement of MMV1 within *Triamitovirus*.

Conclusion: This study reports the first mitovirus identified from *Morchella* and expands the known genetic diversity and evolutionary

Özet

Dayanak: Kültüre alınmamış mantarlardaki viral çeşitliliğin araştırılması, mikovirüs evrimi ve ekolojisinin kapsamlı biçimde anlaşılması açısından gereklidir; çünkü mevcut bilgiler büyük ölçüde fitopatojenik ve ticari açıdan önemli türler üzerinde yapılan çalışmalardan elde edilmiştir.

Amaçlar: Bu çalışma, doğadan toplanmış mantarlarla ilişkili mikovirüsleri tanımlamayı ve karakterize etmeyi, ayrıca yeterince araştırılmamış mantar konaklarda mitovirüs çeşitliliğine ilişkin mevcut bilgiyi genişletmeyi amaçlamıştır.

Yöntemler: *Morchella* sp. olarak tanımlanan bir örnek, moleküler tespit ve genom dizileme yöntemleri kullanılarak mikovirüsler açısından taranmıştır. Viral genomun tamamı dizilenmiş ve anotasyon yapılmış; organizasyon, nükleotid bileşimi ve kodlama bölgelerini belirlemek için biyoinformatik teknikler uygulanmıştır. Diziler BLASTp kullanılarak karşılaştırılmış ve filogenetik ilişkiler RNA'ya bağımlı RNA polimeraz (RdRp) dizilerine dayanarak çıkarılmıştır.

Bulgular: *Morchella mitovirus 1* (MMV1) olarak adlandırılan yeni bir mitovirüs tanımlanmıştır. MMV1, 3.167 nükleotitten oluşan lineer bir RNA genomuna sahiptir, G + C içeriği %41,00'dır ve RdRp kodlayan tek bir açık okuma çerçevesi içerir. BLASTp analizine göre MMV1'in RdRp'si, *Mitoviridae* familyasının *Triamitovirus* cinsine ait *Tuber mitovirus 3* ile en yüksek amino asit dizi benzerliğini (%40,29) göstermektedir. Filogenetik analiz, MMV1'in *Triamitovirus* içinde konumlandığını doğrulamıştır.

Edited by: *Boris Assyov*

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Received: 13 January 2026, **Accepted:** 14 April 2026, **Published:** 24 April 2026

landscape of mitoviruses, emphasizing the importance of investigating mycoviruses in non-cultivated fungal hosts.

Sonuç: Bu çalışma, *Morchella* cinsinden tanımlanan ilk mitovirüsü rapor etmekte ve mitovirüslerin bilinen genetik çeşitliliğini ve evrimsel dağılımını genişletmektedir; ayrıca kültüre alınmamış mantar konaklarda mikovirüslerin araştırılmasının önemini vurgulamaktadır.

Keywords: Mycovirus, mitovirus, *Triamitovirus*, *Morchella*, RNA-dependent RNA polymerase

Introduction

Growing interest in mycovirome research is exemplified by numerous recent investigations exploring virus populations across diverse fungal taxa (Akata et al., 2023; Bora et al., 2024; Ferilli et al., 2024; Li et al., 2023; Raco et al., 2023; Rueda-Maíllo et al., 2025; Sahin et al., 2024). This expanding focus is largely driven by improved accessibility and capability of high-throughput sequencing (HTS) platforms. HTS-based approaches have revealed an extensive array of mycoviruses inhabiting phylogenetically varied fungal hosts—spanning early-diverging lineages to more recently evolved clades—each exhibiting distinct ecological adaptations (Hough et al., 2023). Furthermore, HTS screening can detect many unknown viral species, including unclassified groups and novel representatives of established families within the fungal kingdom (Ayllon & Vainio, 2023).

Members of the family *Mitoviridae* are non-encapsidated viruses with uncapped, positive-sense, single-stranded RNA (ribonucleic acid) genomes ranging from 2.1 to 4.9 kb in length (Hillman & Cai, 2013; Koonin et al., 2020). They features a single open reading frame (ORF) that is translated via mitochondrial codon usage and encodes an RNA-dependent RNA polymerase (RdRp) harboring six characteristic conserved motifs (A–F). *Mitoviridae* has been recently expanded to include four new genera: *Kvaramitovirus*, *Triamitovirus*, *Duamitovirus*, and *Unuamitovirus* (International Committee on Taxonomy of Viruses [ICTV], 2024). Although first described in fungi, mitoviruses have since been identified in plants and insects (Bruenn et al., 2015; Nibert et al., 2018; Fonseca et al., 2020). Notably, no mitoviruses have been reported in the fungus *Morchella*. This study isolated and molecularly characterized a novel mitovirus from *Morchella* sp., designated "*Morchella mitovirus 1*" (MMV1).

Based on previous literature, viruses documented within the true morel genus *Morchella* are three endornaviruses, one fusarivirus, one unclassified RNA virus isolated from *M. importuna*, one endornavirus from *M. sextelata*, and two fusariviruses from *M. esculenta* (Gilbert et al., 2019; Lyu et al., 2025; Sahin et al., 2021). Given the ecological and economic importance of *Morchella* and the limited knowledge of its associated virome, further investigation of viruses infecting this genus is warranted. We hypothesized that underexplored *Morchella*-related viromes may include undescribed mitoviruses. Thus, this study aimed to identify and characterize a novel mitovirus from *Morchella* sp. and to determine its genomic and phylogenetic relationships within *Mitoviridae*.

Materials and Methods

Sampling and Molecular Characterization of Fungal Specimens

Fungal specimens were collected during field surveys in northwestern Türkiye. On April 21, 2023, an ascocarp of a *Morchella* specimen (Figure 1a) was collected from the edge of a dirt road in Kaynaklar village, Buca district, İzmir province. The specimen was deposited in the Ankara University Herbarium (ANK) with the accession code ANK Akata & Saif 012, for future reference. Total genomic DNA was isolated from a small piece of the specimen ANK Akata & Saif 012 employing CTAB (Rogers & Bendich, 1994). The nuclear ribosomal internal transcribed spacer (ITS) regions were subsequently amplified by polymerase chain reaction (PCR) using the primers ITS1/ITS4 (Martin & Rygiewicz, 2005). The amplicon was visualized *via* agarose gel electrophoresis, purified with the GeneJET PCR Purification Kit (Thermo Fisher Scientific, MA, USA), and Sanger sequenced utilizing the BigDye™ Direct Cycle Sequencing Kit (Thermo Fisher Scientific, MA, USA). Sequences were resolved on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, MA, USA) and compared against the National Center for Biotechnology Information (NCBI) GenBank reference database (https://www.ncbi.nlm.nih.gov/) using BLASTn. The ITS sequence showed 100% query coverages and identities to multiple *Morchella* spp., including *M. esculenta*, *M. elata*, *M. importuna*, *M. conica*, *M. costata*, *M. vulgaris*, and *M. hortensis*. Given the overlap among sequences and the known taxonomic complexity of this genus, the specimen was conservatively retained as *Morchella* sp.

Sample Preparation for HTS

For surface sterilization, the fungal samples were sequentially immersed in 2% NaOCl (2 min) and 70% ethanol (10 s). Following disinfection, all residual chemicals were removed through extensive rinsing with sterile distilled water. The material was then lyophilized and pulverized into a fine, homogeneous powder with a mechanical grinder to facilitate downstream molecular analysis.

Viral dsRNA was isolated from powdered fungal fruiting bodies *via* cellulose-based affinity chromatography, following previously described solid-phase extraction techniques (Darissa et al., 2010; Morris & Dodds, 1979). The protocol was adapted by substituting cellulose CF-11 with Type 101 (Merck, Sigma-Aldrich, Darmstadt, Germany) for column packing. Purified dsRNA extracts were then treated with DNase I (New England Biolabs, MA, USA) and S1 nuclease (Thermo Fisher Scientific,

MA, USA) to degrade any co-extracted DNA. The resulting dsRNA was reverse-transcribed into cDNA using a dN6 primer, 5′-CCTGAATTCGGATCCTCCNNNNNN-3′, and the RevertAid First-Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA) after initial cleanup with a GeneJet™ PCR purification Kit (Thermo Fisher Scientific, MA, USA). cDNA was amplified by random PCR (rPCR), as described by Darissa et al. (2010). The amplicons were sequenced on an Illumina NovaSeq 6000 system (Novogene, UK).

Bioinformatic Analyses of HTS Data

Bioinformatic processing of quality-filtered reads involved *de novo* assembly in CLC Genomics Workbench 20.0.2 using standard parameters; it generated 106 contigs >1.5 kb in length. All contigs were analyzed utilizing BLASTx against the NCBI non-redundant protein database to screen for viral sequences. It identified a contig 13 of notable length—3,089 nt—assembled from 1,664 reads, exhibiting marked homology to established mitoviruses. BLASTx revealed that it shared 40.29% amino acid identity with *Tuber mitovirus 3*. Such substantial sequence alignment indicates the detection of a novel mitovirus, which we have named MMV1, thereby expanding the diversity of the *Mitoviridae*.

RNA-Ligase-Mediated Rapid Amplification of cDNA Ends

To determine the terminal sequences of the MMV1 genomic RNA, we employed RNA-ligase-mediated rapid amplification of cDNA ends (RLM-RACE). The 3′ termini of the purified viral dsRNA were first ligated to a synthetic adapter oligonucleotide—RLO: 5′p-CATGGTGGCGACCGGTAG-NH2 3′—catalyzed by T4 RNA Ligase 1 (New England Biolabs, MA, USA), via a 6-h incubation at 37°C, followed by a 12-h extension at 12°C to maximize efficiency. Ligated RNAs were subsequently purified with a GeneJET PCR Purification Kit (Thermo Fisher Scientific, MA, USA) prior to downstream amplification.

First-strand cDNA was synthesized using purified RNA, the RevertAid First-Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA) with the universal RTP primer (5′-CTACCGGTCGCCACCATG-3′), designed to bind an RLO adapter. Following reverse transcription, the 5′ and 3′ termini were PCR-amplified independently employing gene-specific primers in conjunction with RTP. The 5′ end was targeted with a reverse primer: 5′-CCGAAGACTTTGTTTTGTTAACG-3′, and the 3′ end with a forward primer: 5′-TTGGTCGTCTGGATCGTGTAG-3′. Following PCR amplification, the products were cloned into a pGEM-T Easy Vector system (Promega, WI, USA) and Sanger sequenced. The final genome was assembled using Geneious Prime 2020.2.5 (Dotmatics, Bishop's Stortford, UK). Such a comprehensive strategy enabled precise characterization of the terminal regions of the viral genome, yielding essential information about its architecture in a novel mitovirus.

Phylogenetic Analyses of Complete Viral Genome Sequences

A potential ORF within the MMV1 genome was predicted using the ExPASy Translate tool (https://web.expasy.org/translate/), applying the mold mitochondrial genetic code for nucleotide-to-amino acid translation. The resulting putative protein sequences were aligned against reference mitovirus proteins using Clustal Omega to ensure a robust and accurate comparison. Evolutionary relationships were inferred through maximum likelihood phylogeny in MEGA 11, implementing the WAG + G + I + F substitution model (Tamura et al., 2021; Whelan & Goldman, 2001). The phylogenetic framework obtained was statistically validated using 1000 bootstrap replicates to assess node support and ensure confidence in topological conclusions.

Results

The dsRNA segment, which serves as a genome replication intermediate for MMV1, was effectively isolated from the specimen labeled ANK Akata & Saif 012, as represented in Figure 1b. The genomic composition of MMV1 encompassed 3,167 nucleotides and demonstrated a G + C content of 41.00%. The complete sequence of the MMV1 genome has been deposited in GenBank under the accession number PX516901.1. By employing the fungal mitochondrial genetic code, in which "UGA" encodes tryptophan, the MMV1 genome was found to have a single ORF (Figure 1c). The amino acid sequence predicted from this ORF consisted of 802 residues and a calculated molecular weight of 87.59 kDa, as determined by the Protein Molecular Weight calculator tool (bioinformatics.org). The 5′ and the 3′ UTRs (untranslated regions) were 627 and 131 nucleotides long, respectively, depicted in Figure 1d. These UTRs were structurally characterized *via* the RNA Folding Form V2.3 interface of the RNA mfold web server (https://www.unafold.org/mfold/applications/rna-folding-form-v2.php), which demonstrated that both UTRs adopted stem-loop secondary structures exhibiting initial Gibbs free energy (ΔG) values of −9.20 and −7.80 kcal/mol for the 5′ and 3′ UTRs, respectively (Figure 1d).

Analysis using the NCBI Conserved Domains Database (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) indicated that the MMV1-encoded polypeptide included an RdRp domain, which spanned amino acid residues 203–538 (Figure 1d). Subsequent evaluation within the Conserved Domains Database (CDD) framework confirmed this domain to belong to the mitovirus RdRp family (Accession: cl05469; E-value: 6.04e-78). Moreover, comparisons employing the protein Basic Local Alignment Search Tool (BLASTp) demonstrated that the MMV1 RdRp had the highest sequence identity (41.00%) with the corresponding polymerase from *Tuber mitovirus 3*.

To elucidate the evolutionary position of MMV1 among other mitoviruses, phylogeny was reconstructed based on a comparison of RdRp sequences. The resulting tree demonstrated that MMV1 formed a well-supported clade with recognized members of the genus *Triamitovirus*, including *Tuber mitovirus 3* and Geopora sumneriana mitovirus 1 (Figure 2a). Furthermore, multiple sequence alignment of RdRp domains from ten mitoviruses confirmed the presence of six conserved motifs—F, A, B, C, D, and E—arranged sequentially from the N- to C-termini of the MMV1 polymerase. Notably, this suite includes the catalytically essential A, B, and C motifs situated within the palm subdomain (Figure 2b).

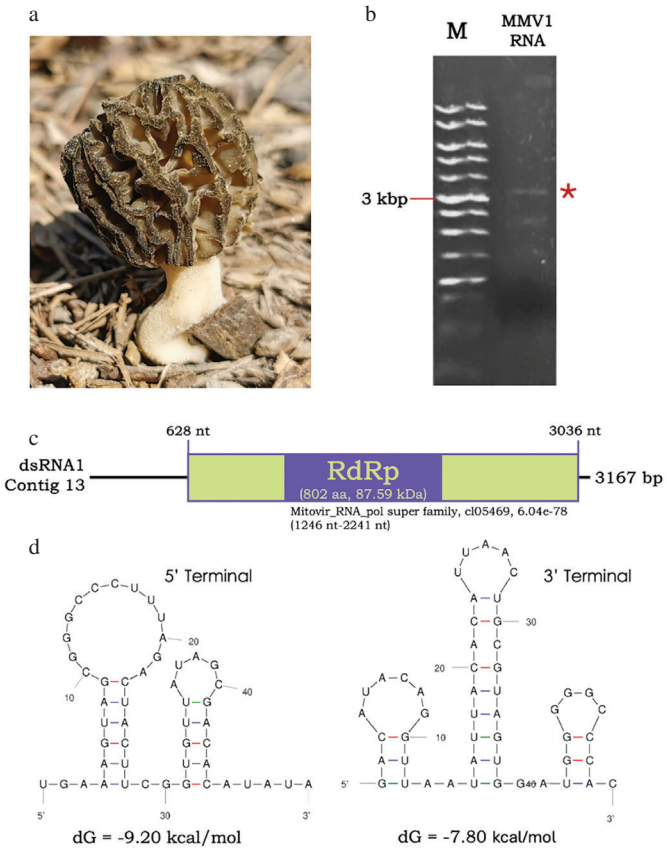

Figure 1. (a) Fruiting body (ascocarp) of *Morchella* specimen ANK Akata & Saif 012. (b) Electrophoretic profile of the double-stranded RNA (dsRNA) segment corresponding to the MMV1 viral genome. (c) Genomic organization of MMV1. The single ORF is represented by a rectangle flanked by UTRs (solid black lines). The region encoding the predicted RNA-dependent RNA polymerase domain is highlighted in purple. (d) Predicted secondary structures for the 5′ and 3′ termini of the MMV1 genome.
MMV1 = Morchella mitovirus 1; ORF = open reading frame; UTRs = untranslated regions.

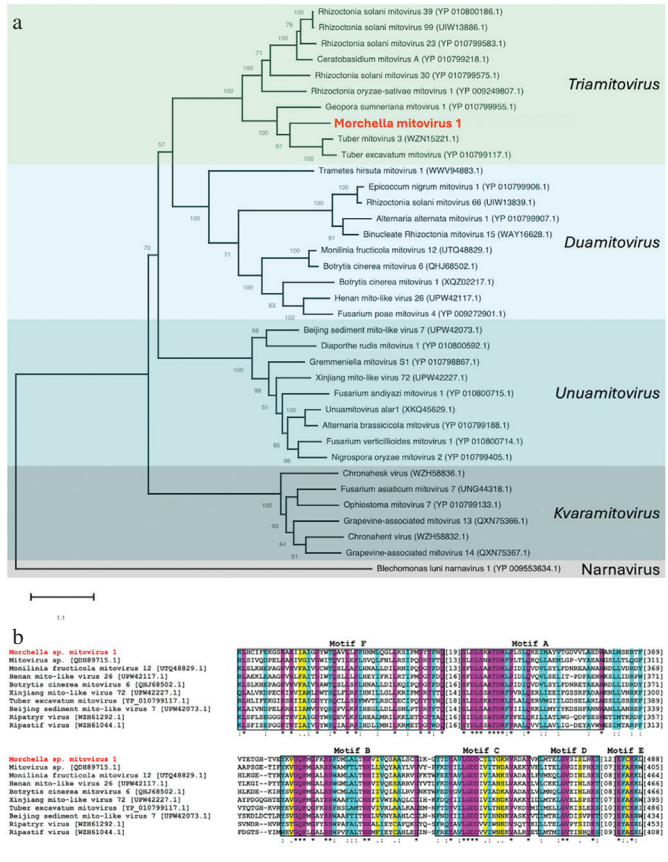

Figure 2. (a) Maximum likelihood phylogenetic tree illustrating the evolutionary relationships between the RNA-dependent RNA polymerase (RdRp) from MMV1 and homologs from other mitoviruses. It was constructed in MEGA 11 with 1000 bootstrap replicates; support values are indicated at branch nodes. The scale bar corresponds to a genetic distance of 0.5. GenBank accession numbers are provided in parentheses. (b) Multiple sequence alignment of the conserved RdRp motifs—F, A, B, C, D, and E, from the N-terminus of the MMV1 genome and those of phylogenetically related mitoviruses. Identical residues are highlighted in magenta, and those with similar biochemical properties are shaded in blue and yellow.
MMV1 = Morchella mitovirus 1.

Discussion

Although morphological characteristics have long served as the primary method for species identification, such an approach has proven inadequate for *Morchella,* as its external features can vary substantially depending on environmental conditions. Thus, species differentiation based solely on morphology often yields unreliable results. The introduction of molecular methods has marked a crucial turning point in *Morchella* taxonomy; however, these alone are also insufficient to fully resolve species boundaries. Although the combined evaluation of morphological and molecular data enhances taxonomic resolution within the *Morchella* group, the ITS region alone is insufficient for accurate species delimitation. This region has been recognized as the universal barcode for fungi since 2012 (Schoch et al., 2012). Nevertheless, pronounced ITS polymorphism among *Morchella* spp. indicates that relying solely on this region may result in misidentifications. Therefore, concatenated analyses that combine multiple gene regions provide more robust and reliable species-level results (Loizides et al., 2022). Indeed, in a study encompassing 45 *Morchella* specimens, a combination of EF1-α, RPB1, RPB2, and ITS regions yielded the greatest resolution in species delimitation, whereas analyses involving only two or three regions produced lower taxonomic accuracy (Sa et al., 2022).

To evaluate the taxonomic position of the current specimen, only the ITS region was sequenced. BLASTn analysis of the obtained sequence showed similarity to those of several *Morchella* spp. Considering the variability and sequence overlap known among species within this genus, as well as the morphological ambiguity observed in the specimen, assigning a specific epithet was not

deemed reliable. Therefore, to avoid potential misidentifications within such a taxonomically complex group, the specimen was conservatively designated as *Morchella* sp.

GenBank contains >4,000 complete, near-complete, and partial mitovirus genomes. A phylogenetic analysis of 2,000 randomly selected mitovirus RdRp sequences revealed distinct lineages that cluster, either branching at the base or apex of the four established mitovirus genera (data not shown). This pattern suggests that these lineages represent novel, yet-to-be-classified genera within *Mitoviridae* and may be evaluated by the ICTV study groups.

The UTRs of mitoviruses display substantial heterogeneity in length and nucleotide sequence, a feature observed even among intra-species isolates. While these genomic segments are hypothesized to serve as binding sites for the translation initiation machinery, specific host-derived molecules—including proteins or non-coding RNAs—that potentially associate with them remain largely uncharacterized. Furthermore, the functional roles of these UTRs and their possible regulation through RNA modifications (epitranscriptomics) that could alter host cellular functions have not been directly experimentally validated. A prevalent hypothesis suggests that their terminal segments function as cis-acting regulatory elements that facilitate recognition and binding by viral RdRp during genomic replication. The application of sophisticated computational approaches, such as deep learning-based comparative genomics, offers a promising avenue for elucidating the properties of these regulatory regions.

The evolutionary dynamics shaping virus–host associations are complex and can originate from multiple distinct processes. Key among these are: 1) codivergence, characterized by topological congruence between the phylogenetic trees of viruses and their hosts; 2) host switching, involving cross-species transmission of a virus to a phylogenetically distant host; and 3) duplication, a process wherein a parasite lineage diversifies within a single host species, resulting in multiple lineages that occupy an identical host range (Göker et al., 2011). In this context, as evolutionary virologists continue to elucidate the sequence diversity of mitoviruses across diverse fungal hosts, they can be better positioned to estimate the evolutionary forces that predominantly govern mitovirus evolution. Following the established species demarcation criteria for *Mitoviridae* (ICTV, 2021), MMV1 constitutes a new species, as its RdRp shares <70% sequence similarity with all known mitoviruses.

MMV1 shared the typical genomic features of *Mitoviridae*, including a linear RNA genome, a single ORF encoding the RdRp, and protein translation based on the fungal mitochondrial genetic code. In addition, the predicted terminal stem-loop structures were consistent with characteristics reported for other mitoviruses. A comparative analysis showed that MMV1 was most closely related to *Tuber mitovirus 3*; however, the relatively low RdRp amino acid identity indicated that it represented a distinct virus. Its phylogenetic placement within *Triamitovirus* further supported such an interpretation. Although the biological impacts of MMV1 on *Morchella* remain unknown, its detection expands the diversity of mitoviruses known to infect wild fungi and underlines the importance of further studies on host–virus interactions and mitovirus evolution in morels.

Conclusion

In conclusion, the genomic features, RdRp sequence similarity, and phylogenetic placement of MMV1 support its assignment to the genus *Triamitovirus* within the family *Mitoviridae*. To our knowledge, this study is the first to report a complete mitovirus genome from a fungal host assigned to *Morchella* sp. Although the findings expand the current dataset on mitoviruses associated with non-cultivated fungi, they should be interpreted with caution as the study is based on a single specimen, and the host could not be identified with confidence at the species level.

Acknowledgements

This study was derived from the MSc thesis of Hatice Saif completed at Ankara University.

Ethics

Ethics Committee Approval: Not required.

Data Sharing Statement: All data are available within the study.

Footnotes

Authorship Contributions:

Conceptualization: E.Ş., G.E., and I.A.; Design/methodology: E.Ş., G.E., and I.A.; Execution/investigation: H.S., E.Ş., and G.E.; Resources/materials: H.S., E.Ş., and G.E.; Data acquisition: H.S., E.Ş., G.E., and I.A.; Data analysis/interpretation: H.S., E.Ş., and G.E.; Writing – original draft: E.Ş., G.E., and I.A.; Writing – review & editing/critical revision: E.Ş. and I.A.

Conflict of Interest: The author(s) have no conflicts of interest to declare.

Funding: This study was funded by Ankara University, Coordinatorship of Scientific Research Projects, under project number FYL-2023-2976.

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