Complete genome sequence of a novel mitovirus detected in *Colocasia esculenta*

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Abstract

A novel mitovirus was detected in taro (*Colocasia esculenta*) growing in Ningbo, China. The complete sequence of *Colocasia esculenta* associated mitovirus 1 (CeaMV1) was determined by next-generation sequencing combined with RT-PCR and RACE. The genome is 2921 nucleotides long and contains a single ORF encoding a putative RNA-dependent RNA polymerase. Homology searches and phylogenetic analysis suggested that CeaMV1 is a member of a new species in the genus *Duamitovirus*. This is the first report of a member of the family *Mitoviridae*associated with taro.

Full Text

Taro (*Colocasia esculenta* L. Schott) is a perennial root crop in the family *Araceae*, which is widely planted in tropical and sub-tropical regions. Like other vegetatively propagated plants, viruses easily accumulate in the corms, resulting in serious degradation of varieties. About eight viruses have been found to infect taro, often in mixed infections, of which the most common in China are dasheen mosaic virus (DsMV), cucumber mosaic virus and taro bacilliform CH virus [1-4].

Ningbo city in Zhejiang province is one of the main areas of taro production in China (~1800ha annually). During 2020-2022, a virus survey was conducted among local taro varieties in Ningbo. One sample with virus-like symptoms of mosaic and feather mottle in the leaves was collected at random for identification by high-throughput sequencing (HTS). Total RNA was extracted from the leaves using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and a cDNA library was constructed using a TruSeq RNA Sample Preparation Kit (Illumina) according to the manufacturer's instructions. An Illumina NovaSeq 6000 platform with PE150bp and CLC Genomic Workbench 11 (QIAGEN) were used for sequencing and data analysis with default parameters. A total of 37,767,580 paired-end reads were obtained, and 148,128 contigs (49-31,520 bp) were generated *de novo* and compared with sequences in the GenBank database using BLASTn or BLASTx. In addition to the commonly-occurring DsMV, 3 contigs mapped to viruses in the family *Mitoviridae*: 1 contig of 1210nt mapped to cannabis sativa mitovirus 1 (37.3% aa identity; 68% query coverage) and 2 contigs of 719 and 456nt mapped to paris mitovirus 1 (respectively 50.0 and 54.9% aa identity; 99% and 93% query coverage).

The complete genomic sequence of the novel mitovirus was then determined using specific primers to amplify three fragments while the 5'- and 3'-terminal sequences were determined using RACE (Tiosbio, Beijing, China) (Supplementary Table S1). PCR products were then cloned into the pEASY-T5 Zero Cloning Vector (TransGen Biotech, Beijing), and at least 5 clones of each product were sent for Sanger sequencing (Ykang, Hangzhou, China). The complete sequence was assembled using DNAMAN 8.0 software (Lynnon Biosoft, Canada) and indicated the presence of a single mitovirus. The complete 2921nt of this novel virus, named *Colocasia esculenta* associated mitovirus 1 (CeaMV1) was deposited in the GenBank database under accession OR134096. CeaMV1 has a single ORF (nt 307-2592), which is predicted to encode an 87.2 kDa RNA-dependent RNA polymerase (RdRp) of 761aa, and has 5' and 3' untranslated regions (UTRs) of 306bp and 329bp, respectively (Fig. 1a).

A conserved Mitovir_RNA_pol domain (pfam05919) at aa 250-547 of the CeaMV1 RdRp was confirmed by a conserved domain database search. The aa sequence was then aligned with those of all reported plant mitoviruses and some mitoviruses from fungi using MAFFT version 7, employing the L-INS-i algorithm [5]. The five highly conserved motifs (I to V) of mitoviruses were all identified in CeaMV1 (Fig. 1b). In a BLASTp search, the CeaMV1 RdRp was most closely related to those of the plant-infecting mitoviruses paris mitovirus 1 (48.0%, 95% query coverage), solanum chacoense mitovirus 1 (44.6%, 98% query coverage) and cannabis sativa mitovirus 1 (45.4%, 95% query coverage). These aa identity values are well below the demarcation criteria currently used to establish new species in the family (<70% aa identity) [6,7].

Phylogenetic analysis based on a multiple sequence alignment of the RdRp domain sequences demonstrated that CeaMV1 is closely related to ParMV1 (Fig. 2), and clusters with other plant-infecting viruses as a distinct clade in the genus *Duamitovirus*. No fungal infection was detected in the leaf sample in which CeaMV1 was found, further indicating that CeaMV1 is a plant mitovirus. In tests of taro plants from different areas of Ningbo, CeaMV1 was detected in 17 out of the 34 samples suggesting that it is widespread. Further work is needed to determine whether this mitovirus has any direct impact on the growth of taro and to evaluate its economic importance. To our knowledge, this is the first report of the complete genome sequence of a mitovirus associated with *C. esculenta*.

Declarations

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Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Additional information

The nucleotide sequence reported in this manuscript has been deposited in the GenBank database under accession number OR134096.

Electronic supplementary material

Below is the link to the electronic supplementary material.

References

1. Yusop, M. S. M., Saad, M. F. M., Talip, N., Baharum, S. N., & Bunawan, H. (2019). A Review on Viruses Infecting Taro (*Colocasia esculenta* (L.) Schott). *Pathogens (Basel, Switzerland)*, *8*(2), 56. https://doi.org/10.3390/pathogens8020056
2. Zettler F. W..(1987).Dasheen Mosaic Virus Infecting Taro in People's Republic of China. Plant Disease(9). https://doi.org/10.1094/pd-71-0837
3. Gao, L. L., Jiang, S. S. , Bin, W. U. , Zhang, M. , Wang, S. J. , & Xin, X. Q. (2017). Field investigation and identification of dasheen mosaic virus on taro in Shandong province. China Vegetables. 2017(7):68-72. https:// doi.org/10.19928/j.cnki.1000-6346.2017.07.014
4. Kazmi, S. A., Yang, Z., Hong, N., Wang, G., & Wang, Y. (2015). Characterization by Small RNA Sequencing of Taro Bacilliform CH Virus (TaBCHV), a Novel Badnavirus. *PloS one*, *10*(7), e0134147. https://doi.org/10.1371/journal.pone.0134147
5. Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010
6. Botella L, Manny AR, Nibert ML, Vainio E (2021). Create 100 new species and four new genera (Cryppavirales: Mitoviridae). https://ictv.global/ictv/proposals/2021.003F.R.Mitoviridae_100nsp_4ngen.zip
7. Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Alfenas-Zerbini P, Dempsey DM, Dutilh BE, García ML, Curtis Hendrickson R, Junglen S, Krupovic M, Kuhn JH, Lambert AJ, Łobocka M, Oksanen HM, Orton RJ, Robertson DL, Rubino L, Sabanadzovic S, Simmonds P, Smith DB, Suzuki N, Van Doorslaer K, Vandamme AM, Varsani A, Zerbini FM (2022). Recent changes to virus taxonomy ratified by the International Committee on Taxonomy of Viruses. Arch Virol. 2022 Nov;167(11):2429-2440. https://dor.org/10.1007/s00705-022-05516-5

Figures

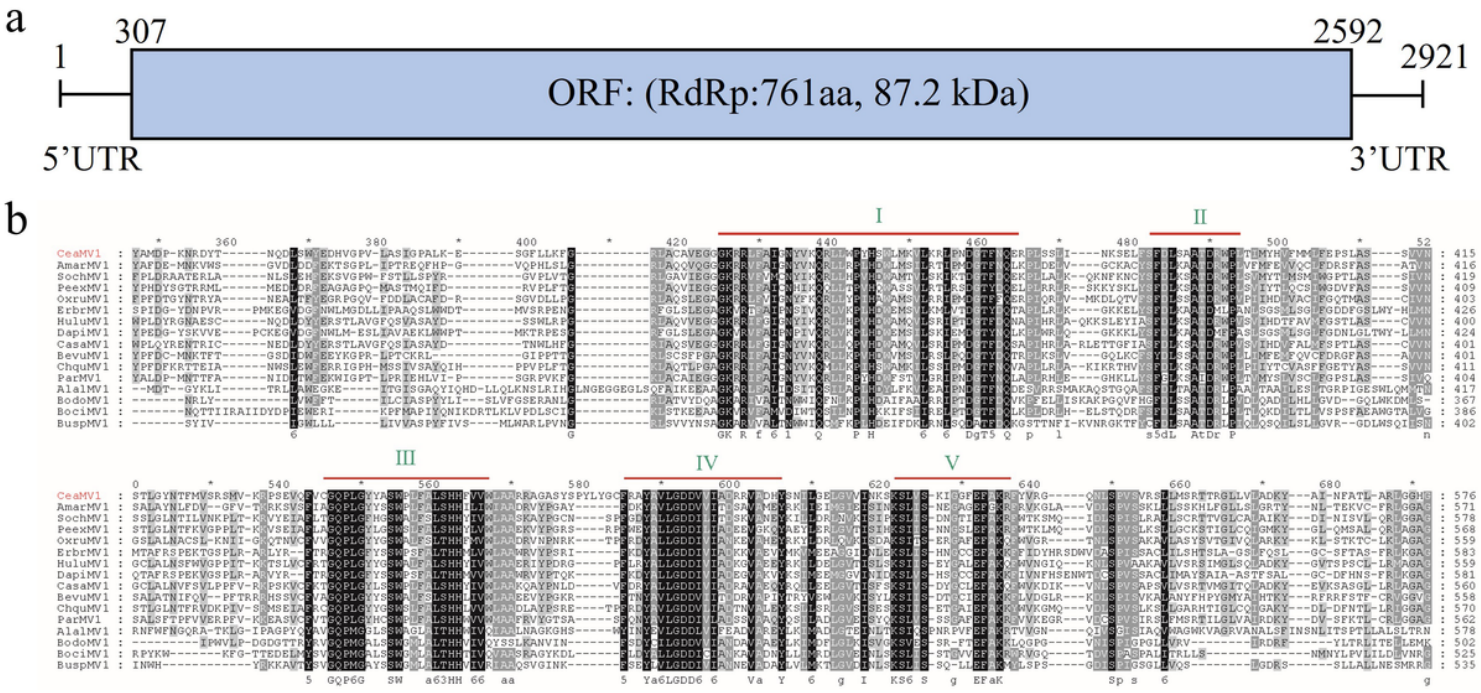

Figure 1

Multiple alignment of the amino acid (aa) sequences of RdRPs encoded by CeaMV1 and other viruses of the family *Mitoviridae*. Abbreviations of virus names: CeaMV1, Colocasia esculenta associated mitovirus 1; AmarMV1, Ambrosia artemisifolia mitovirus 1; SochMV1, Solanum chacoense mitovirus 1; PeexMV1, Petunia exserta mitovirus 1; OxruMV1, Oxybasis rubra mitovirus 1; ErbrMV1, Erigeron breviscapus mitovirus 1; HuluMV1, Humulus lupulus mitovirus 1; DapiMV1, Dahlia pinnata mitovirus 1; CasaMV1, Cannabis sativa mitovirus 1; BevuMV1, Beta vulgaris mitovirus 1; ChquMV1, Chenopodium quinoa mitovirus 1; ParMV1, Paris mitovirus 1; AlalMV1, Alternaria alternata mitovirus 1; BodoMV1, Botryosphaeria dothidea mitovirus 1; BociMV1, Botrytis cinerea mitovirus 1; BuspMV1, Buergenerula spartinae mitovirus 1. The five conserved RdRP motifs are indicated by the Roman numerals I to V. See Fig. 2 for GenBank accession numbers.

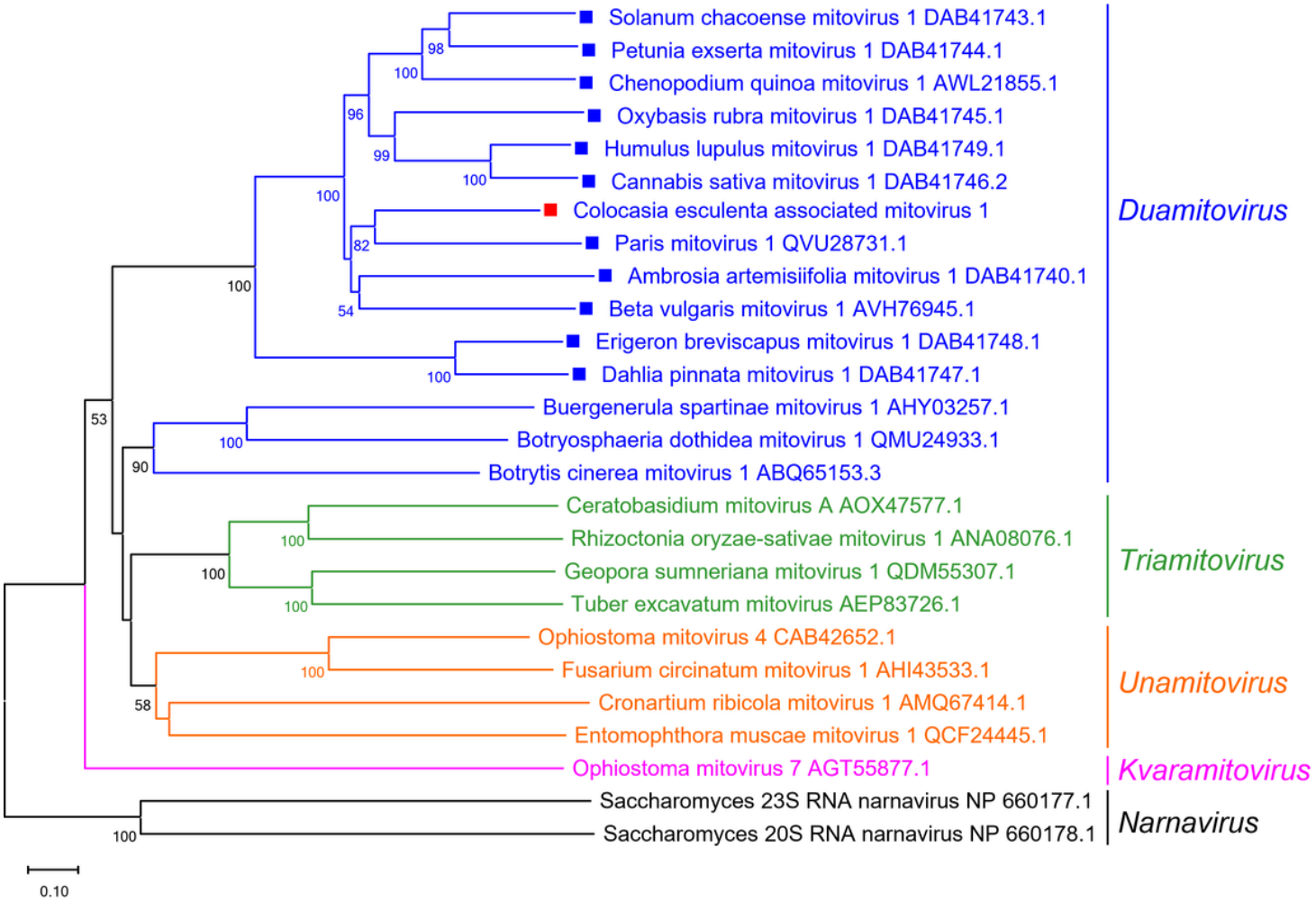

Figure 2

Phylogenetic analysis of the RdRP of CeaMV1 and related viruses (mitoviruses and members of the genus *Narnavirus*) using the Neighbor-Joining method with 1000 bootstrap replications in MEGA11. The scale bar indicates the number of amino acid substitutions per site.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CeaMV1completeseq.txt
- SupplementaryTableS1.docx