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ANNOTATED SEQUENCE RECORD

A novel maize-infecting mastrevirus from La Réunion Island

Daniel Pande · Simona Kraberger · Pierre Lefeuvre · Jean-Michel Lett · Dionne N. Shepherd · Arvind Varsani · Darren P. Martin

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Abstract Despite extensive sampling, only one virus belonging to the genus Mastrevirus of the family *Geminiviridae*, maize streak virus (MSV), has until now been detected in maize with maize streak disease (MSD) symptoms. Here, we report for the first time a second, highly divergent, mastrevirus isolated from two maize plants displaying characteristic MSD-like symptoms, sampled on the South-west Indian Ocean Island, La Réunion. The two isolates shared <57 % genome-wide identity with all other known mastreviruses. We propose calling the new species Maize streak Réunion virus.

The genus *Mastrevirus* of the family *Geminiviridae* contains species with circular single-stranded DNA (ssDNA) genomes of approximately 2.7 kb. Mastreviruses infect a wide variety of monocotyledonous and dicotyledonous hosts and are transmitted by leafhopper vectors. Monocot-infecting mastreviruses have previously been found throughout Africa (including the South-west Indian Ocean islands: SWIO), Europe, Japan, Southeast Asia, the island of Vanuatu, and Australia. Mastreviruses previously found on SWIO islands such as La Réunion and Mauritius include various maize streak virus (MSV) strains, sugarcane streak Réunion virus (SSRV) and sugarcane streak virus (SSV) [13, 19, 25, 26].

Here, we report the genome sequences of two isolates (PR50 and PR52) belonging to a divergent mastrevirus species from La Réunion. Leaves were sampled in Saint Pierre (21.6161 S, 55.45784 E and 21.3206 S, 55.48536 E) from two maize plants displaying chlorotic streaks along leaf veins similar to those seen in MSV infections of maize.

GenBank accession numbers: MSRV-[RE-StP-PR52-2009]: JQ624879. MSRV-[RE-StP-PR50-2009]: JQ624880

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D. Pande
Department of Botany and Horticulture, Maseno University, P.O. Box 333, Maseno, Kenya

S. Kraberger · A. Varsani (✉)
School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
e-mail: arvind.varsani@canterbury.ac.nz

P. Lefeuvre · J.-M. Lett
CIRAD, UMR 53 PVBMT CIRAD-Université de la Réunion, Pôle de Protection des Plantes, Ligne Paradis, 97410 Saint Pierre, La Réunion, France

D. N. Shepherd
Department of Molecular and Cell Biology, University of Cape Town, Rondebosch, Cape Town 7701, South Africa

A. Varsani
Biomolecular Interaction Centre, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand

A. Varsani
Electron Microscope Unit, University of Cape Town, Rondebosch, Cape Town 7701, South Africa

D. P. Martin
Computational Biology Group, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Observatory, Cape Town 7925, South Africa

Circular viral DNA molecules were amplified from a crude total DNA extract from the two maize samples using Phi29 DNA polymerase (TempliPhi^{TM}, GE Healthcare, USA) as described previously [15, 16, 18] and cut with *Kpn*I (PR50) and *Bam*HI (PR52) to yield ~2.7-kb DNA fragments, which were subsequently ligated to the *Kpn*I and *Bam*HI sites of pGEMZf+ (Promega Biotech, USA). The clones were fully sequenced by primer walking at Macrogen Inc. (Korea).

The two viral isolates share 97.3 % nucleotide sequence identity. A NCBI BLASTn [28] analysis of the assembled 2882-bp sequence of the virus isolates from both plants indicated that they shared significant degrees of similarity with mastreviruses in general and in particular with panicum streak virus (PanSV) isolates in the capsid protein (CP) and replication-associated protein (Rep) open reading frames (ORFs; maximum identity of 72 % with 30 % genome-wide coverage E value 6×10^{-49} to PanSV-A [ZM-Nya-g180-2007], GenBank # EU224263). The novel viral genomes were aligned using MUSCLE [6] with representative sequences of mastrevirus species and major strain groupings using MEGA version 5 [24]. Pairwise identity analysis (Hamming- or p-distance with pairwise deletion of gaps) indicated that, with the exception of Eragrostis minor streak virus (EMSV), the novel viral isolates shared only 53-57 % genome-wide identity with all other known African streak viruses. The novel maize viruses shared only 48.7 % genome-wide identity with EMSV (Fig. 1). It is clear that these new viral genomes represent a new mastrevirus species (based on the currently accepted International Committee on Taxonomy of Viruses 75 % identity mastrevirus species demarcation threshold [4, 7]), and we propose the name Maize streak Réunion virus (MSRV) (Fig. 2).

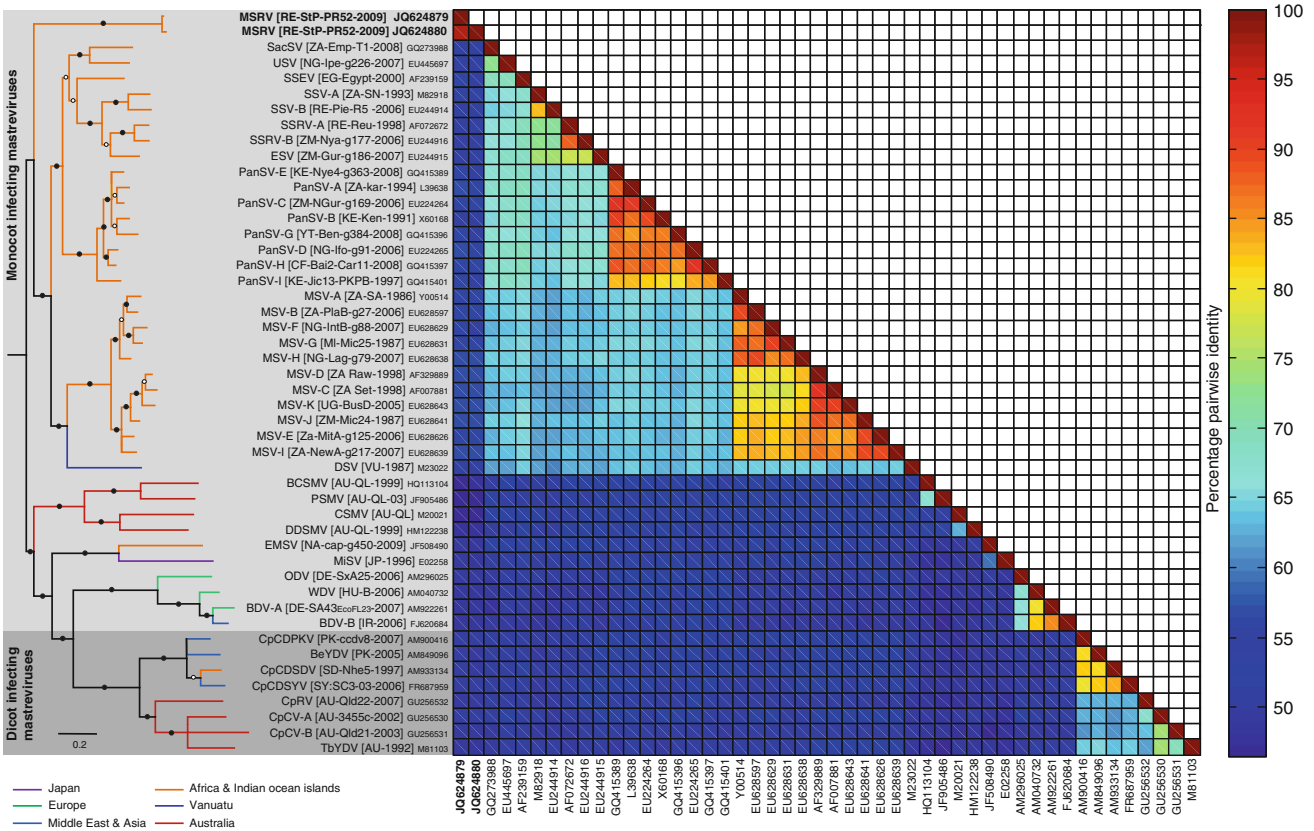
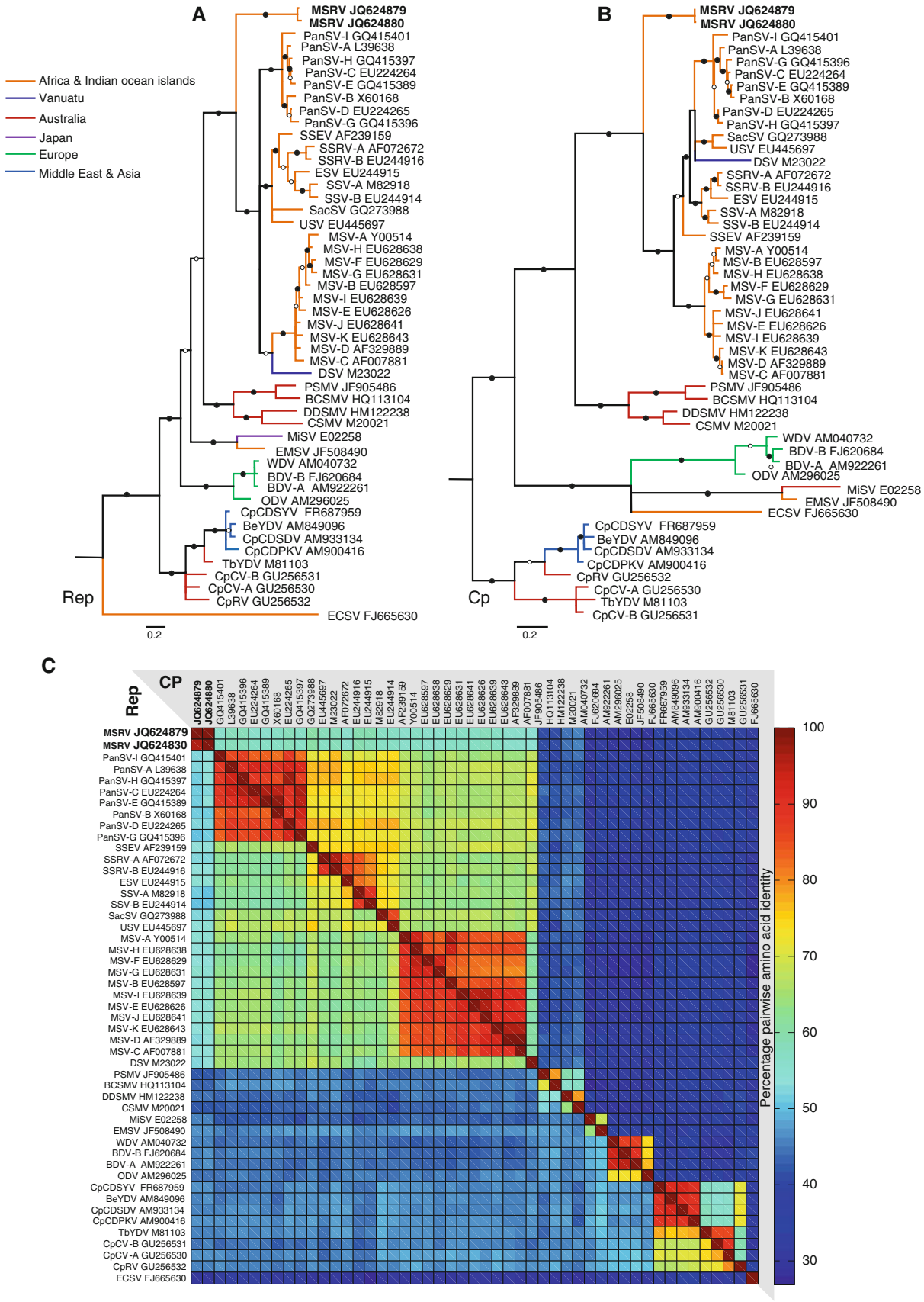


Fig. 1 Maximum-likelihood phylogenetic tree (constructed with GTR + I + G_4 selected as the best-fit model by RDP3) and pairwise sequence similarity matrix of 48 virus isolates representing each known mastrevirus species and major strain. Tree branches are coloured according to the geographical origins of the viruses. Branches marked with filled and open circles were supported in >90 % and 60-89 % of bootstrap replicates respectively, and branches with <50 % bootstrap support have been collapsed. The phylogenetic tree is rooted using the full genome sequence of Eragrostis curvula streak virus (ECSV; not shown)

Fig. 2 Maximum-likelihood phylogenetic tree of Rep (**A**), and CP (**B**) proteins (constructed using the LG amino acid substitution model) along with the pairwise distance matrix of Rep and CP. Branches marked with filled and open circles were supported in >90 % and 60-89 % of bootstrap replicates respectively, and branches with <50 % bootstrap support have been collapsed. Tree branches are coloured according to the geographical origins of the viruses. Eragrostis curvula streak virus (ECSV) is included in both trees despite not being a mastrevirus because it has a very mastrevirus-like capsid protein gene. Beet curly top Iran virus was used to root both the trees


A
B
C
Africa & Indian ocean islands
Vanuatu
Australia
Japan
Europe
Middle East & Asia
Rep
Cp
CP
0.2
MSRV JQ624879
MSRV JQ624880
PanSV-I GQ415401
PanSV-A L39638
PanSV-H GQ415397
PanSV-C EU224264
PanSV-E GQ415389
PanSV-B X60168
PanSV-D EU224265
PanSV-G GQ415396
SSEV AF239159
SSRV-A AF072672
SSRV-B EU244916
ESV EU244915
SSV-A M82918
SSV-B EU244914
SacSV GQ273988
USV EU445697
DSV M23022
MSV-A Y00514
MSV-H EU628638
MSV-F EU628629
MSV-G EU628631
MSV-B EU628597
MSV-I EU628639
MSV-E EU628626
MSV-J EU628641
MSV-K EU628643
MSV-D AF329889
MSV-C AF007881
PSMV JF905486
BCSMV HQ113104
DDSMV HM122238
CSMV M20021
MiSV E02258
EMSV JF508490
WDV AM040732
BDV-B FJ620684
BDV-A AM922261
ODV AM296025
CpCDSYV FR687959
BeYDV AM849096
CpCDSDV AM933134
CpCDPKV AM900416
TbYDV M81103
CpCV-B GU256531
CpCV-A GU256530
CpRV GU256532
ECSV FJ665630
Percentage pairwise amino acid identity
100
90
80
70
60
50
40
30

Recombination analysis using RDP3 (with default settings; [12]) failed to reveal any evidence that the virus was recombinant. A maximum-likelihood (ML) phylogenetic tree was therefore constructed from the full alignment using PHYML (version 3 with GTR+I+G_4 chosen as the best-fit model by RDP; [9, 12]; Fig. 1). The ML tree clearly indicated that the new virus is a mastrevirus that is most closely related to the virus clade including Digitaria streak virus (DSV) from Vanuatu and the various known African streak viruses (with the notable exception of EMSV, which is more closely related to the Asian streak virus Miscanthus streak virus from Japan [5]).

As with almost all known geminiviruses, MSRV contains the nonanucleotide sequence TAATATTAC, which marks its origin of virion-strand DNA replication. The MSRV genomes contain (1) two probable intergenic regions (one short and one long), (2) two probable virion-strand genes (likely encoding a movement protein [MP] and a CP), and (3) an intron-containing complementary-sense gene (likely expressing both a replication-associated protein (Rep) from the spliced transcript and a Rep derivative, RepA, from the unspliced transcript (Supplementary Fig. 1). Various nucleotide sequence motifs that are probably functional within the full-genome nucleotide and ORF sequences of MSRV are shown in Supplementary Figs. 1 and 2, respectively.

While the MP of MSRV shares the highest amino acid sequence identity (42.9 %) with chickpea chlorosis virus B (CpCV-B–[AU:Qld21:2003]-GU256531), its CP is most similar to that of sugarcane streak Egypt virus (60.6 %; SSEV; SSEV-[EG:Egypt:2000]-AF239159). Since it is known that the African streak viruses MSV, PanSV, SSRV, SSEV and SSV are all transmitted by *Cicadulina* species [1–3, 17, 20–23], it is likely that the other viruses in the main African streak virus group are also transmitted by the same species. It is, however, not certain that MSRV is also transmissible by *Cicadulina* species since the CP is the determinant of geminivirus transmission specificity, and the MRSV CP sequence branches basal to the African streak virus group sequences.

The Rep of MSRV shares 56.3 % amino acid identity with MSV-I (MSV-I [ZA-NewA-g217-2007]-EU62863) and 47-55 % identity with those of other African streak virus Reps. Within the MSRV Rep we identified various amino acid motifs previously identified in other mastreviruses including (1) a likely rolling-circle replication (RCR) motif I sequence (FLTYP [11]), (2) a possible metal ion coordination domain (HLHVLLQ, corresponding to RCR motif II [11]), (3) a possible GRS motif (YHPNIQASR [14]), (4) a possible RCR motif III sequence (YILKSP [11]), (5) a probable dNTP-binding domain sequence ($VGX_4GKTTW_{30}DD$ [8]), (6) a retinoblastoma-related protein binding motif (LHCYE [27]), and (7) a potential oligomerisation domain (SAERLFPTLPSPFV [10]; Supplementary Fig. 2).

MSRV is particularly interesting because despite extensive sampling and analysis of maize displaying maize streak disease symptoms, to date, the only other mastrevirus ever sampled from maize is MSV. Given that maize is the most important food crop in Africa and the possibility that this new virus might already be circulating throughout Africa or that it could be introduced to the continent at some time in the future, it is imperative that we actively pursue the biological characterization of MSRV. Just as members of multiple geminivirus species form a disease complex affecting Africa's other important food crop, cassava, it remains a possibility that MRSV and MSV might together form a disease complex that has a far-reaching impact on African maize production. We are currently in the process of obtaining infectious MSRV clones, which will be used to determine both the capacity of this virus to infect different maize genotypes and whether, as is the case with other African streak viruses, it is transmissible by *Cicadulina* sp.

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