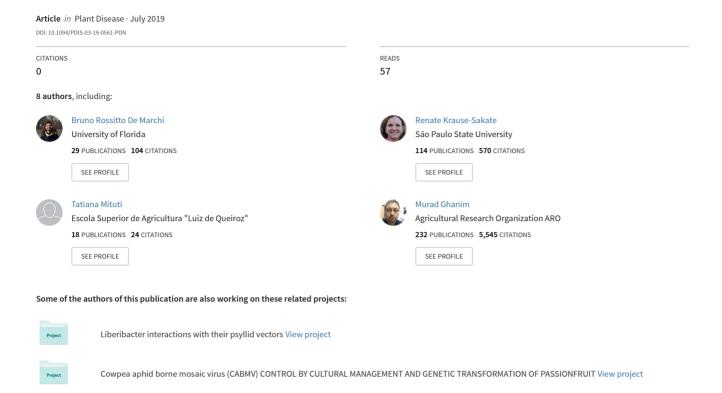
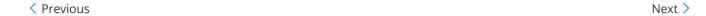
First Report of a Putative New Pepper Vein Yellows Virus Species Associated with a Vein Yellows Disease of Bonnet Pepper Plants in Brazil







DISEASE NOTES



First Report of a Putative New Pepper Vein Yellows Virus Species Associated with a Vein Yellows Disease of Bonnet Pepper Plants in Brazil

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Published Online: 4 Sep 2019 https://doi.org/10.1094/PDIS-03-19-0561-PDN

The bonnet pepper (*Capsicum chinense*) is among the most important vegetables in Brazil. Although it is grown in all Brazilian states, the production is more intense in the northern states of the country. In June 2017, around 80 pepper plants (cv. Cumari-do-Pará) showing symptoms of interveinal yellowing, leaf blade size reduction of 80%, and shortening of the internodes were observed in a small farm (1.0 ha) near Altamira County, Pará State. Suspecting that the symptoms might be owing to a viral disease, two plants were collected and analyzed for virus detection by next generation sequencing (NGS). Initially, symptomatic leaves collected from two infected pepper plants (cv. Cumari-do-Pará) were used as a pooled sample for partial virus purification (*Cali and Moyer 1981*) followed by total RNA

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extraction with the RNeasy Plant Mini Kit (Qiagen). Then, a complementary DNA library was constructed from the RNA using the Complete ScriptSeq Kit (Epicenter, Illumina) and submitted for a transcriptome sequencing with the Illumina HiSeg2500 platform. The 27,290,912 raw reads (average length 101 bp) were trimmed, to remove low-quality bases and adapters, followed by de novo assembly using CLC Genomics Workbench software version 7.0.3. Subsequently, the 1,693 contigs (ranging from 503 to 12,470 bp) obtained were submitted to a BLASTX search with Geneious software version 9.1.5. against NCBI's viral database, which revealed contig identities higher than 89% with Pepper vein yellows virus (PeVYV) (Polerovirus, Luteoviridae). No other viruses were detected in this analysis. The trimmed reads, as well as the contigs, were mapped to a PeVYV reference sequence (AB594828), and the consensus sequence (mean coverage of 53.6×) was extracted to generate a 6,203-bp draft genome of PeVYV isolate Altamira (MK184554). In addition, total RNA was extracted from leaves collected from two symptomatic pepper plants using the Total RNA Purification Kit (Norgen Biotek Corp.) followed by RT-PCR with the newly designed primers PeVYV 3682F (5'-GGTGGATCACGTAACACCCG-3') and PeVYV 4261R (5'-ATTTCGGGTTGTGCAGTTGC-3'), which amplifies a 580-bp amplicon of ORF 3/4. The expected size amplicon was obtained for one sample and directly sequenced (GenBank accession MK883752), showing 100% nucleotide identity with the NGS-derived sequence. Afterward, a Bayesian phylogenetic analysis revealed a clustering of the draft genome of PeVYV-Altamira with other four complete genome sequences of other species of the complex. Presently, six species of Pepper vein yellows virus (PeVYV-1 to PeVYV-6) are recognized (ICTV 2018b release), with recent description of PeVYV-7 from Saudi Arabia (Kamran et al. 2018). Moreover, the recent report of Pepper whitefly borne vein yellows virus, a whitefly-transmitted recombinant polerovirus with PeVYVs and African eggplant yellows virus as its parents (Ghosh et al. 2019) makes this complex much interesting. The draft genome of the PeVYV isolate Altamira was most closely related to PeVYV-1 isolate from Japan (89.72%, AB594828) (Murakami et al. 2011), PeVYV-3 from China (89.54%, KP326573) (Liu et al. 2016), PeVYV-4 isolate from Australia (89.42%, KU999109) (Maina et al. 2016), and PeVYV-5 from Spain (84.4%, KY523072) (Fiallo-Olivé et al. 2018). Additionally, a comparison among the open reading frames of the Brazilian isolate with other poleroviruses was performed and revealed identities of 89.53% in the amino acid sequence identity of the ORF-1, with the closest relatives PeVYV-3 and PeVYV-7. These data support the assignment of the PeVYV isolate Altamira as a new species in the PeVYV complex. We propose to name the new species as *Pepper vein yellows virus 8* (PeVYV-8). This is the first report of a polerovirus infecting bonnet pepper in Brazil. The distribution, transmission, and impacts of PeVYV-8 in Brazil in bonnet pepper and other crops is still unknown and need to be further evaluated to develop management strategies to avoid the spread of this virus throughout the country.

The author(s) declare no conflict of interest.

Funding: Funding was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico and Norte Energia SA.



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