HHS Public Access

Author manuscript

Infect Genet Evol. Author manuscript; available in PMC 2022 September 17.

Published in final edited form as:

Infect Genet Evol. 2022 September ; 103: 105315. doi:10.1016/j.meegid.2022.105315.

Canine picornaviruses detected in wastewater in Arizona, USA 2019 and 2021

Temitope O.C. Faleye[a], **Peter T. Skidmore**[b], **Amir Elyaderani**[b], **Abriana Smith**[b], **Nicole Kaiser**[b], **Sangeet Adhikari**[a,c], **Allan Yanez**[a], **Tyler Perleberg**[a], **Erin M. Driver**[a], **Rolf U. Halden**[a,c,d], **Arvind Varsani**[e], **Matthew Scotch**[a,b,*]

[a]Biodesign Center for Environmental Health Engineering, Biodesign Institute, Arizona State University, Tempe, AZ 85287, USA

[b]College of Health Solutions, Arizona State University, Phoenix, AZ 85004, USA

[c]School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ 85287, USA

[d]OneWaterOneHealth, Nonprofit Project of the Arizona State University Foundation, Tempe, AZ 85287, USA

[e]The Biodesign Center for Fundamental and Applied Microbiomics, School of Life Sciences, Center for Evolution and Medicine, Arizona State University, Tempe, Arizona, AZ 85287, USA

Abstract

Virus surveillance by wastewater-based epidemiology (WBE) in two Arizona municipalities in Maricopa County, USA (~700,000 people), revealed the presence of six canine picornavirus (CanPV) variants: five in 2019 and one in 2021. Phylogenetic analysis suggests these viruses might be from domestic dog breeds living within or around the area. Phylogenetic and pairwise identity analyses suggest over 15 years of likely enzootic circulation of multiple lineages of CanPV in the USA and possibly globally. Considering <10 CanPV sequences are publicly

*Corresponding author at: Biodesign Center for Environmental Health Engineering, Biodesign Institute, Arizona State University, Tempe, AZ 85287, USA. matthew.scotch@asu.edu (M. Scotch).

Declaration of interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: E.M.D and R.U.H. are cofounders of AquaVitas, LLC, 9260 E. Raintree, Ste 130, Scottsdale, AZ 85260, USA, an ASU start-up company providing commercial services in wastewater-based epidemiology. R.U. H. is the founder of OneWaterOneHealth, a non-profit project of the Arizona State University Foundation.

CRediT authorship contribution statement

Temitope O.C. Faleye: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Peter T. Skidmore:** Investigation, Writing – review & editing. **Amir Elyaderani:** Investigation, Writing – review & editing. **Abriana Smith:** Investigation, Writing – review & editing. **Nicole Kaiser:** Investigation, Writing – review & editing. **Sangeet Adhikari:** Writing – review & editing. **Allan Yanez:** Investigation, Writing – review & editing. **Tyler Perleberg:** Investigation, Writing – review & editing. **Erin M. Driver:** Supervision, Writing – review & editing. **Rolf U. Halden:** Supervision, Writing – review & editing, Funding acquisition. **Arvind Varsani:** Writing – review & editing, Funding acquisition. **Matthew Scotch:** Supervision, Writing – review & editing, Funding acquisition.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2022.105315.

available in GenBank as of June 2, 2022, the results provided here constitute an increase of current knowledge on CanPV diversity and highlight the need for increased surveillance.

Keywords

Virus surveillance; Wastewater-based epidemiology; Canine picornavirus; Arizona; USA

Canine picornaviruses (CanPVs) are positive-sense, single-stranded RNA viruses in Supergroup 3 (subfamily *Ensavirinae*) of the family *Picornaviridae* (Woo et al., 2016) but have not been assigned any species classification (Zell et al., 2021). Between 2008 (first positive sample detected) and 2019, CanPVs have been detected in dogs (feces, nasopharynx, and urine) and red foxes (feces and liver) in the United Arab Emirates (UAE), China, Hong-Kong and Australia (Woo et al., 2012, 2016; Campbell et al., 2020; Li et al., 2021). Their ~8 kb genome encodes a single polyprotein flanked on both sides by untranslated regions. In-silico analysis of the polyprotein suggests it is cleaved into twelve proteins: four structural proteins (VP1-VP4) and eight nonstructural proteins (L, 2A-2C and 3A-3D) (Woo et al., 2012, 2016; Li et al., 2021). There is, however, paucity of CanPV nucleotide sequence data with <10 sequences publicly available in GenBank as of June 2, 2022. Thus, the results we provide here increase our knowledge of CanPV variant diversity.

The CanPV variants described here were recovered from four aggregated wastewater (WW) samples (Table 1) collected as part of an ongoing wastewater-based virus surveillance study in a population of ~700,000 people in two municipalities in Maricopa County, Arizona, Southwestern United States. Each WW sample is a two liter (2 L) pool made from ten (Kumar et al., 2018) time- or flow-weighted 200 mL samples collected over 24 h from 10 different sites (Table 1). The samples were filtered using a 450 nM membrane filter (Thermo Fisher Scientific, Waltham, MA, USA) and virus in both the filtrate and filter-trapped solids were independently concentrated to a final volume of 2 mL using centrifugal filters as previously discussed (Faleye et al., 2021a; Fontenele et al., 2021).

For each sample, 140 μL of concentrate from filtrate and filter-trapped solids were independently subjected to RNA extraction and two one-step reverse-transcriptase polymerase chain reaction assays (RT-PCR) as previously described (Majumdar and Martin, 2018; Faleye et al., 2021b). Amplicons from both RT-PCR assays were pooled, cleaned, and used for library preparation (KAPA Hyperplus Library Kit) and paired-end sequencing (2 × 250 bp) on an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA) at the Biodesign Institute, Arizona State University. Raw reads/sample (from both filtrate and filter-trapped solids) were merged, trimmed and *de novo* assembled using Merge-Reads-Libraries v1.0.1, Trimmomatic v0.36, and metaSPAdes v3.15.3 (using default parameters), respectively on the KBase platform (Arkin et al., 2018). Viral contigs were identified using a BLASTn (Altschul et al., 1990) search of the GenBank nucleotide database (Sayers et al., 2021). In order to ascertain depth of coverage, the raw reads were mapped to individual CanPV contigs using Bowtie2 v2.3.2 on the KBase platform (Arkin et al., 2018).

The CanPV contigs detected in this study were used as query in a BLASTn search of the GenBank nucleotide database and hits of previously described CanPV genomes

were downloaded and used for multiple sequence alignments (MSA) alongside those detected in this study. MSA was done using ClustalW in MEGAX (Kumar et al., 2018) while maximum-likelihood (ML) phylogenetic tree was generated in MEGAX using 1000 bootstrap replicates. Pairwise Identity analysis was performed using SDT v1.2. (Muhire et al., 2014).

Six CanPV contigs (>2000 nt spanning 5-UTR to VP1; GC% = 41%–43%) were recovered from the four samples. Between 108,000 and 1,386,276 reads/sample mapped to CanPVs and depth of coverage ranged from 5632× to 123,910× (Table 1). Phylogenetic analysis showed the six contigs belonged to three independent clusters with 100% bootstrap support (Fig. 1). Pairwise identity analysis showed members of each cluster were > 95% identical and members of different clusters were at least >5% (sometimes >15%) divergent (Table S1). It is important to note that the two variants recovered from December 2019 were > 15% divergent (Table 2).

CanPV detection as described here was consequent of an “off-target” amplification due to homologous binding sites for the Enterovirus complete capsid amplification assay primers in CanPV genomes as previously described (Faleye et al., 2022). To confirm CanPVs were in our samples, we further subjected the amplicons to a CanPV specific PCR assay (Fig. 2a) targeting an ~945 bp region of the genome spanning the VP2-VP3 junction (Faleye et al., 2022). All four samples were positive for the assay (Fig. 2b) and Sanger sequencing of the amplicons confirmed all four samples contained CanPVs. Sanger sequencing result for the December sample however had multiple peaks (Fig. 2c) further confirming the presence of more than one CanPV variant (Fig. 1) in the concentrate.

Considering the CanPV sequences described here (from WW) and those recently found in Kentucky, USA in 2020 (from sludge) (Fig. 1) (Faleye et al., 2022) are the only ones described in the USA (based on data publicly available in GenBank), it is difficult to categorically state the host organism(s) of CanPVs in the USA. However, since all the CanPVs detected in this study (and in Kentucky) phylogenetically cluster with CanPVs previously found in domestic dogs (*Canis lupus familiaris*), it is likely these viruses are from various dog breeds living within or around human residents. Should CanPV capsid genomic region evolve as fast as that of Polioviruses (the type and best studied member of *Picornaviridae)* which is one of the fastest evolving RNA viruses and evolves at a rate of 1×10^{-2} substitutions per site per year (~1% nucleotide divergence per year) (Jorba et al., 2008; Peck and Lauring, 2018) then a > 15% divergence in capsid sequence suggest predecessors of both variants recovered in December 2019 from wastewater in Arizona diverged from a common ancestor around 15 years ago and accumulated mutations to result in the variants we describe here. On the other hand, should CanPV capsid genomic region evolve at a slower rate (for example closer to the evolutionary rate of Influenza virus *i.e.* 0.5–2.5 $\times 10^{-3}$ substitutions per site per year [Nobusawa and Sato, 2006]), then our estimated divergence dates (of around 15 years for the December 2019 variants) might be significantly underestimated. However, if the CanPV variants we describe here are indigenous to the USA, our data suggests there might have been >15 years of undescribed enzootic CanPV circulation in the USA. We understand that the values stated above are ‘rough estimates’. However, considering there are <20 CanPV sequences in GenBank (those described in this

study included), it might be difficult to make statistically robust time to the most recent comment ancestor (tMRCA) estimation. Effort is therefore ongoing to detect and describe more CanPV genomes (or at least complete capsids) in an effort to make a more accurate tMRCA estimate of variants in the USA and globally. Increased surveillance of CanPVs in the environment, archived samples and domestic dog breeds (and possibly other canids) is needed to unambiguously define CanPV host(s) in the USA and improve our understanding of its evolution and diversity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the City of Tempe wastewater management authorities for help with sample collection from the city sewers. They also thank the Genomics Core at Biodesign Institute, Arizona State University for help with library preparation, Illumina and Sanger sequencing.

Funding

The research reported in this publication was supported by the National Library of Medicine of the National Institutes of Health under Award Number U01LM013129 to RUH, MS and AV. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Data availability

The mapped reads, Illumina and Sanger sequencing consensus CanPV contigs described in this study have been deposited in SRA and GenBank under accession numbers PRJNA797388, OM243093-OM243098 and OM953762-OM953764, respectively.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990 Oct 5. 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410. 10.1016/S0022-2836(05)80360-2. [PubMed: 2231712]

Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, Sneddon MW, Henderson ML, Riehl WJ, Murphy-Olson D, Chan SY, Kamimura RT, Kumari S, Drake MM, Brettin TS, Glass EM, Chivian D, Gunter D, Weston DJ, Allen BH, Baumohl J, Best AA, Bowen B, Brenner SE, Bun CC, Chandonia JM, Chia JM, Colasanti R, Conrad N, Davis JJ, Davison BH, DeJongh M, Devoid S, Dietrich E, Dubchak I, Edirisinghe JN, Fang G, Faria JP, Frybarger PM, Gerlach W, Gerstein M, Greiner A, Gurtowski J, Haun HL, He F, Joachimiak Jain RMP, Keegan KP, Kondo S, Kumar V, Land ML, Meyer F, Mills M, Novichkov PS, Oh T, Olsen GJ, Olson R, Parrello B, Pasternak S, Pearson E, Poon SS, Price GA, Ramakrishnan S, Ranjan P, Ronald PC, Schatz MC, Seaver SMD, Shukla M, Sutormin RA, Syed MH, Thomason J, Tintle NL, Wang D, Xia F, Yoo H, Yoo S, Yu D, 2018. KBase: the United States department of energy systems biology knowledgebase. Nat. Biotechnol. 36 (7), 566–569. 10.1038/nbt.4163, 2018 Jul 6. [PubMed: 29979655]

Campbell SJ, Ashley W, Gil-Fernandez M, Newsome TM, Di Giallonardo F, Ortiz-Baez AS, Mahar JE, Towerton AL, Gillings M, Holmes EC, Carthey AJR, Geoghegan JL, 2020. Red fox viromes in urban and rural landscapes. Virus Evol. 6 (2) 10.1093/ve/veaa065, 2020 Aug 25. veaa065.

Faleye TOC, Bowes DA, Driver EM, Adhikari S, Adams D, Varsani A, Halden RU, Scotch M, 2021a. Wastewater-based epidemiology and long-read sequencing to identify enterovirus circulation in three municipalities in Maricopa County, Arizona, Southwest United States between June and October 2020. Viruses 13 (9), 1803. 10.3390/v13091803, 2021a Sep 10. [PubMed: 34578384]

Faleye TOC, Driver E, Bowes D, Adhikari S, Adams D, Varsani A, Halden RU, Scotch M, 2021b. Pan-enterovirus amplicon-based high-throughput sequencing Detects the complete capsid of a EVA71 genotype C1 variant via wastewater-based epidemiology in Arizona. Viruses 13 (1), 74. 10.3390/v13010074, 2021b Jan 7. [PubMed: 33430521]

Faleye TOC, Driver EM, Bowes DA, Holm RH, Talley D, Yeager R, Bhatnagar A, Smith T, Varsani A, Halden RU, Scotch M, 2022. Detection of human, porcine and canine picornaviruses in municipal sewage sludge using pan-enterovirus amplicon-based long-read Illumina sequencing. Emerg, Microb. Infect. 11 (1), 1339–1342. 10.1080/22221751.2022.2071173. Dec.

Fontenele RS, Kraberger S, Hadfield J, Driver EM, Bowes D, Holland LA, TOC Faleye, Adhikari S, Kumar R, Inchausti R, Holmes WK, Deitrick S, Brown P, Duty D, Smith T, Bhatnagar A, Yeager RA, Holm RH, von Reitzenstein NH, Wheeler E, Dixon K, Constantine T, Wilson MA, Lim ES, Jiang X, Halden RU, Scotch M, Varsani A, 2021. High-throughput sequencing of SARS-CoV-2 in wastewater provides insights into circulating variants. Water Res. 205, 117710. 10.1016/j.watres.2021.117710, 2021 Oct 15. Epub 2021 Sep 25. [PubMed: 34607084]

Jorba J, Campagnoli R, De L, et al. , 2008. Calibration of multiple poliovirus molecular clocks covering an extended evolutionary range. J. Virol. 82 (9), 4429–4440. Available from. https://ncbi.nlm.nih.gov/pubmed/, 18287242. [PubMed: 18287242]

Kumar S, Stecher G, Li M, Knyaz C, Tamura K, 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35 (6), 1547–1549. 10.1093/molbev/msy096, 2018 Jun 1. [PubMed: 29722887]

Li C, Li Y, Li H, Chen Z, Zhou J, Liu G, Wang Y, 2021. Genomic characterization and phylogenetic analysis of a new canine picornavirus variant in the mainland of China. Virus Res. 296, 198351. 10.1016/j.virusres.2021.198351, 2021 Apr 15. Epub 2021 Feb 24. [PubMed: 33639222]

Majumdar M, Martin J, 2018. Detection by direct next generation sequencing analysis of emerging enterovirus D68 and C109 strains in an environmental sample from Scotland. Front. Microbiol. 9, 1956. 10.3389/fmicb.2018.01956, 2018 Aug 21. [PubMed: 30186268]

Muhire BM, Varsani A, Martin DP, 2014. SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9 (9). 10.1371/journal.pone.0108277, 2014 Sep 26. e108277.

Nobusawa E, Sato K, 2006. Comparison of the mutation rates of human influenza A and B viruses. J. Virol. 80 (7), 3675–3678. 10.1128/JVI.80.7.3675-3678.2006, 2006 Apr. [PubMed: 16537638]

Peck KM, Lauring AS, 2018. Complexities of viral mutation rates. J. Virol. 92 (14) 10.1128/JVI.01031-17, 2018 Jun 29. e01031–17.

Sayers EW, Cavanaugh M, Clark K, Pruitt KD, Schoch CL, Sherry ST, Karsch-Mizrachi I, 2021. GenBank. Nucleic Acids Res. 49 (D1), D92–D96. 10.1093/nar/gkaa1023, 2021 Jan 8. [PubMed: 33196830]

Woo PC, Lau SK, Choi GK, Yip CC, Huang Y, Tsoi HW, Yuen KY, 2012. Complete genome sequence of a novel picornavirus, canine picornavirus, discovered in dogs. J. Virol. 86 (6), 3402–3403. 10.1128/JVI.07228-11, 2012 Mar. [PubMed: 22354943]

Woo PCY, Lau SKP, Choi GKY, Huang Y, Sivakumar S, Tsoi HW, Yip CCY, Jose SV, Bai R, Wong EYM, Joseph M, Li T, Wernery U, Yuen KY, 2016 Jul. 2016. Molecular epidemiology of canine picornavirus in Hong Kong and Dubai and proposal of a novel genus in Picornaviridae. Infect. Genet. Evol. 41, 191–200. 10.1016/j.meegid.2016.03.033. Epub 2016 Apr 3. [PubMed: 27051044]

Zell R, Knowles NJ, Simmonds P, 2021. A proposed division of the family Picornaviridae into subfamilies based on phylogenetic relationships and functional genomic organization. Arch. Virol. 166 (10), 2927–2935. 10.1007/s00705-021-05178-9, 2021 Oct. Epub 2021 Aug 4. [PubMed: 34350513]

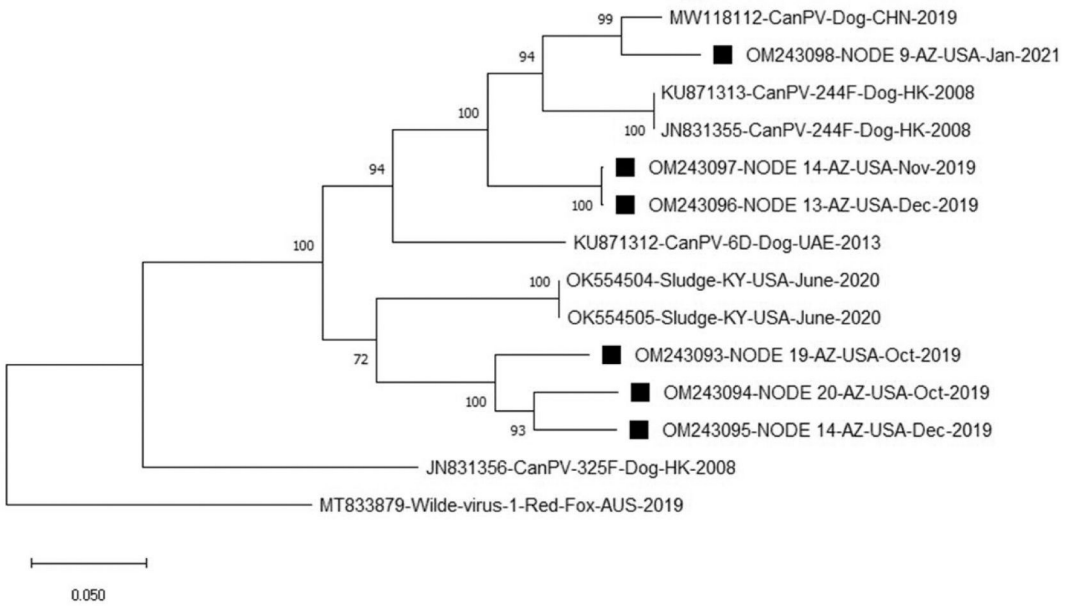

Fig. 1.

Maximum likelihood phylogenetic tree of CanPV using an alignment of P1 nucleotide sequences (~2040 nt) of all the CanPV sequences available in GenBank and those described in this study (black square). Bootstrap values are indicated if >50%. HK=Hong Kong, AUS = Australia, USA = United States of America, CHN=China, KY=Kentucky, AZ = Arizona.

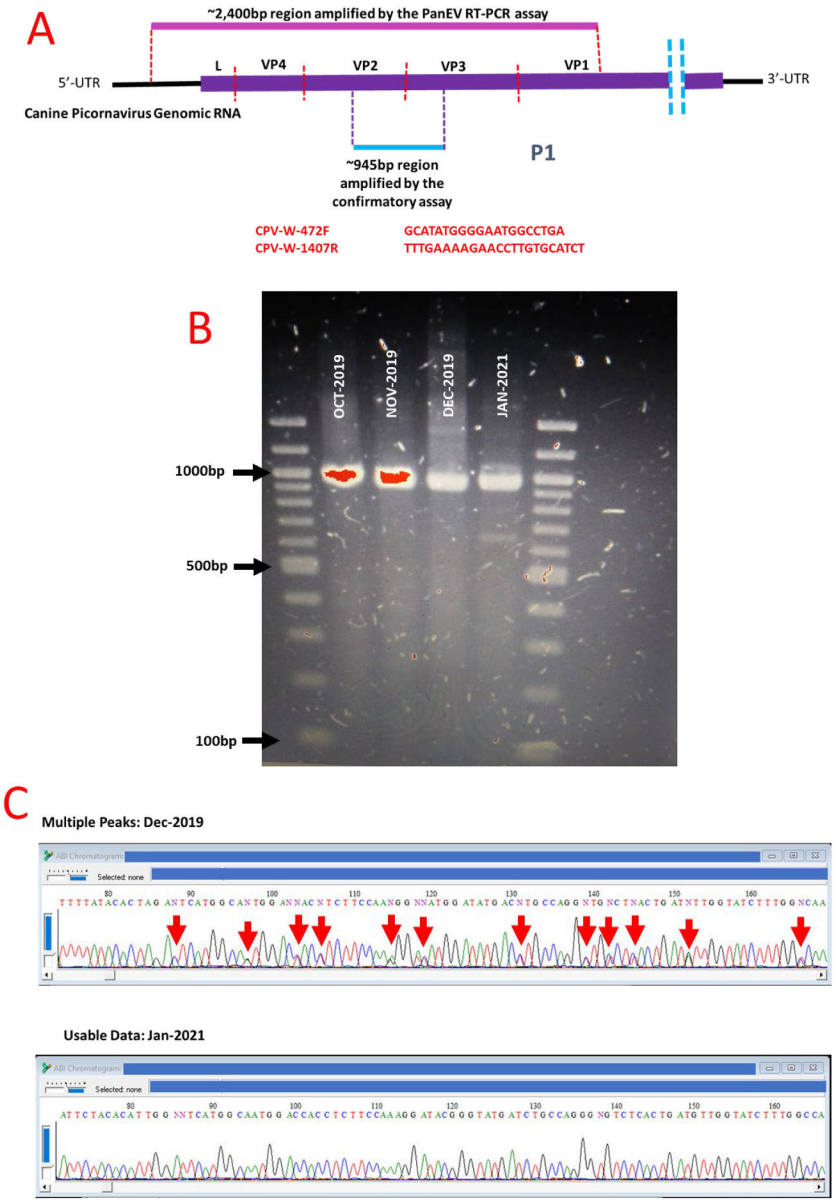

Fig. 2.

A) Schematic representation of CanPV genomic regions amplified by the Enterovirus complete capsid and CanPV specific assays, respectively. B) Gel electrophoresis picture showing amplification products of the CanPV specific assay. Gel visualized using BioRad Gel Doc XR+ system running Image lab 4.1 software with option to “highlight saturated pixels” enabled. C) Samples of Sanger sequencing data showing multiple (red arrows) and clean individual peaks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Details of CanPVs recovered from wastewater in this study.

S/N	Sample ID	Total Reads	Mapped Reads (%)	Node-ID	Accession #	Size (nt)	Mean Depth	GC%
1	Oct-2019	3,653,404	108,844 (2.98)	Node-19	OM243093	2112	5632×	43%
				Node-20	OM243094	2110	6349×	42%
2	Nov-2019	3,682,288	281,552 (7.65)	Node-14	OM243095	2366	30,577×	41%
3	Dec-2019	5,066,940	1,386,276 (27.36)	Node-13	OM243096	2349	24,358×	41%
				Node-14	OM243097	2153	123,910×	41%
4	Jan-2021	3,392,898	724,563 (21.36)	Node-9	OM243098	2465	81,028×	43%

Table 2

Pairwise Identity analysis of CanPV types detected in Nov-Dec 2019 detailed in Fig. 1. Please see Table S1 for complete pairwise identity of all sequences in Fig. 1.

First Sequence	Second Sequence	Pairwise Identity %	Pairwise Divergence %
OM243095NODE_14AZUSADec2019	OM243097NODE_14AZUSANov2019	84.52	15.48
OM243095NODE_14AZUSADec2019	OM243096NODE_13AZUSADec2019	84.57	15.43
OM243097NODE_14AZUSANov2019	OM243096NODE_13AZUSADec2019	99.76	0.24