



# Discovery of Cucumis melo endornavirus by deep sequencing of human stool samples in Brazil

Antonio Charlys da Costa<sup>1,2</sup> · Elcio Leal<sup>3</sup> · Danielle Gill<sup>1,2</sup> · Flavio Augusto de Pádua Milagres<sup>4,5,6</sup> · Shirley Vasconcelos Komninakis<sup>7,8</sup> · Rafael Brustulin<sup>4,5,6</sup> · Maria da Aparecida Rodrigues Teles<sup>5,6</sup> · Márcia Cristina Alves Brito Sayão Lobato<sup>5,6</sup> · Rogério Togisaki das Chagas<sup>5,6</sup> · Maria de Fátima Neves dos Santos Abrão<sup>5,6</sup> · Cassia Vitória de Deus Alves Soares<sup>5,6</sup> · Xutao Deng<sup>9,10</sup> · Eric Delwart<sup>9,10</sup> · Ester Cerdeira Sabino<sup>1,2</sup> · Adriana Luchs<sup>11</sup>

Received: 15 September 2018 / Accepted: 8 February 2019 / Published online: 26 March 2019  
© Springer Science+Business Media, LLC, part of Springer Nature 2019

## Abstract

The nearly complete genome sequences of two Cucumis melo endornavirus (CmEV) strains were obtained using deep sequencing while investigating fecal samples for the presence of gastroenteritis viruses. The Brazilian CmEV BRA/TO-23 (aa positions 116–5027) and BRA/TO-74 (aa positions 26–5057) strains were nearly identical to the reference CmEV CL-01 (USA) and SJ1 (South Korea) strains, showing 97% and 98% of nucleotide and amino acid identity, respectively. Endornaviruses are not known to be associated with human disease and their presence may simply reflect recent dietary consumption. Metagenomic analyses offered an opportunity to identify for the first time in Brazil a newly described endornavirus species.

**Keywords** Virus discovery · Gastroenteritis · Metagenomic · Plant viruses · Endornavirus

---

Communicated by Dr. Seung-Kook Choi.

---

The virus genome sequences were deposited in GenBank under the accession numbers MH365458 (BRA/TO-23) and MH365459 (BRA/TO-74).

---

Eric Delwart and Ester Cerdeira Sabino jointly supervised this work.

---

✉ Antonio Charlys da Costa  
charlysbr@yahoo.com.br

✉ Adriana Luchs  
driluchs@gmail.com

<sup>1</sup> Institute of Tropical Medicine, University of São Paulo, Av. Dr. Enéas de Carvalho Aguiar, no 470, São Paulo, São Paulo 05403-000, Brazil

<sup>2</sup> LIM/46, Faculty of Medicine, University of São Paulo, São Paulo, Brazil

<sup>3</sup> Institute of Biological Sciences, Federal University of Pará, Belém do Pará, Brazil

<sup>4</sup> Federal University of Tocantins, Palmas, Brazil

<sup>5</sup> Public Health Laboratory of Tocantins State (LACEN/TO), Palmas, Brazil

The family *Endornaviridae* (two genus: *Betaendornavirus* and *Alphaendornavirus*) includes viruses infecting plants, fungi, and oomycetes. Endornaviruses have a linear double-stranded RNA (dsRNA) molecule of 9.8–17.6 kb, depending on the virus, encoding a single long open reading frame (ORF), and most of them have a discontinuity (nick) near the 5' end of the plus strand [1, 2]. Endornaviruses are endogenous viral elements not encapsulated to form virions and without an extracellular phase. Virtually, the infection

<sup>6</sup> Secretary of Health of Tocantins, Palmas, Brazil

<sup>7</sup> Postgraduate Program in Health Science, Faculty of Medicine of ABC, Santo André, Brazil

<sup>8</sup> Retrovirology Laboratory, Federal University of São Paulo, São Paulo, São Paulo, Brazil

<sup>9</sup> Blood Systems Research Institute, San Francisco, USA

<sup>10</sup> Department Laboratory Medicine, University of California San Francisco, San Francisco, USA

<sup>11</sup> Enteric Disease Laboratory, Virology Center, Adolfo Lutz Institute, Av. Dr Arnaldo, no 355, São Paulo, São Paulo 01246-902, Brazil

caused by endornaviruses is persistent and symptomless. They have frequently been found in various healthy plants, ranging from algae to higher plants, not affecting the host phenotype [2–6]. *Vicia faba* endornavirus [7] and *Helicobasidium mompa* endornavirus 1 [6] are the two exceptions reported to be associated with altered host phenotypes.

Endornaviruses are known to be unconventional viruses: no evidence of cell-to-cell movement; the dsRNA occurs in every tissue and at every developmental stage at relatively consistent concentrations [2, 8]. Although the dsRNA is found in the cytoplasm of host cells, it is transmitted very efficiently (> 98%) to progeny plants via pollen and ova [2]. Recently, Hao et al. [9] demonstrated endornavirus ability for horizontal transmission between hosts in necrotrophic fungus *Botrytis cinerea*. Endornaviruses have been reported to infect economically important crops such as avocado, barley, bell pepper, common bean, fava bean, melon, and rice, except for isolates of *Vicia faba endornavirus* [5] their overall effect on plants is not known.

Next-generation sequencing (NGS) techniques are transforming the study of viruses and opening a new path for viral discovery [10]. Consequently, knowledge of endornaviruses has been expanding [11, 12]. The aim of the present study is to describe the completely sequenced genomes of two *Cucumis melo* endornavirus (CmEV) strains, which were identified during an NGS investigation conducted using human stool samples collected in Brazil. CmEV was recently reported in *Cucumis melo* in the USA (CL-01) [8] and South Korea (SJ1) and is being considered as a possible novel species in the genus *Endornavirus*. Currently, only two complete genome sequences (CL-01 and SJ1 strains) and three partial sequences of CmEV are available in the GenBank database.

During the period September of 2010–February of 2016, a total of 250 fecal samples were collected from patients with acute gastroenteritis in the state of Tocantins, Northern Brazil. The specimens were screened for enteric viruses by NGS. CmEV was detected in only two (0.8%; 2/250) samples: BRA/TO-23 and BRA/TO-74, in which group A rotavirus was also detected. The BRA/TO-23 sample was collected in August of 2014 from a 2-year-old male child; the BRA/TO-74 sample was obtained in December of 2010 from a 6-month-old female infant. Both patients were experiencing acute gastroenteritis symptoms. The collected stool samples were sent to the Central Laboratory of Public Health of Tocantins State (LACEN/TO), a regional reference center for gastroenteritis surveillance. The state of Tocantins is characterized by cerrado vegetation (dry grassland and scrub forests), vast rivers, and soybean plantations. The patients were inhabitants of Araguaína, which is the second largest city in the state and is located 384 km from the state capital, Palmas.

The procedure used to perform deep sequencing is a combination of several previously described protocols that have been applied to viral metagenomics and/or virus discovery [13, 14]. In summary, 50 mg of the human BRA/TO-23 and BRA/TO-74 fecal samples were diluted in 500 µl of Hanks' buffered salt solution, added to a 2 ml impact-resistant tube containing lysing matrix C (MP Biomedicals, USA), and homogenized in a FastPrep-24 5G Homogenizer (MP bio-medicals, USA). The homogenized samples were centrifuged at 12,000×g for 10 min, and approximately 300 µl of the supernatant was then percolated through a 0.45 µm filter (Merck Millipore, Billerica, MA, USA) in order to remove eukaryotic and bacterial cell-sized particles. Approximately, 100 µl, roughly equivalent to one-fourth of the volume of the tube of cold PEG-it Virus Precipitation Solution (System Biosciences, CA, USA) was added to the obtained filtrate, and the contents of the tubes were gently mixed then incubated at 4 °C for 24 h. After the incubation period, the mixtures were centrifuged at 10,000×g for 30 min at 4 °C. Following centrifugation, the supernatants (~350 µl) were discarded. The pellets rich in viral particles were treated with a combination of nuclease enzymes (TURBO DNase and RNase Cocktail Enzyme Mix—Thermo Fischer Scientific, CA, USA; Baseline-ZERO DNase-Epicentre, WI, USA; Benzonase-Darmstadt, Germany; and RQ1 RNaseFree DNase and RNase A Solution-Promega, WI, USA) in order to digest unprotected nucleic acids. The resulting mixtures were subsequently incubated at 37 °C for 2 h.

After incubation, viral nucleic acids were extracted using ZR & ZR-96 Viral DNA/RNA Kit (Zymo Research, CA, USA) according to the manufacturer's protocol. The cDNA synthesis was performed with AMV Reverse transcription (Promega, WI, USA). A second strand of cDNA synthesis was performed using DNA Polymerase I Large (Klenow) Fragment (Promega, WI, USA). Subsequently, a Nextera XT Sample Preparation Kit (Illumina, CA, USA) was used to construct a DNA library, identified using dual barcodes. For size range selection, Pippin Prep (Sage Science, Inc.) was used to select a 300 bp insert (range 200–400 bp). The library was deep-sequenced using the HiSeq 2500 Sequencer (Illumina, CA, USA) with 126 bp ends. Bioinformatics analysis was performed according to the protocol described by Deng et al. [15]. Contigs that shared a percent nucleotide identity of 95% or less were assembled from the obtained sequence reads by de novo assembly. Based on the bioinformatics pipeline used [15], no reads related to human, plant, fungal, or bacterial sequences were obtained. Figure 2 displays types (or species) and amount of the detected viruses in the BRA/TO-23 and BRA/TO-74 samples.

Totals of 88,074 and 1,600,771 paired-end reads were obtained from the BRA/TO-23 and BRA/TO-74 samples, respectively. Of the total reads, 5.9% ( $n = 5228$ ) from BRA/TO-23 and 1.03% ( $n = 16,444$ ) from BRA/TO-74 showed

BLASTx score (coverage of 4,8925× and 7,64×, respectively) to CmEV, represented by the complete dsRNA genome sequences of the CL-01 (KT727022) [8] and SJ1 (KX641269) strains. At nucleotide level (BLASTn) identity was 97% with 99% of coverage and e-values were ~1E-110–1E-120 (Fig. 2).

The final genome analysis was performed using the Geneious software v9.1.8 (Biomatters Ltd., Auckland, New Zealand). ORF was predicted with the Geneious ORF finder. The BRA/TO-23 and BRA/TO-74 strains were nearly identical to the CL-01 and SJ1 strains, showing 97% nucleotide identity. At the amino acid level, the Brazilian CmEV BRA/TO-23 and BRA/TO-74 strains aligned 98% to the polyproteins of CL-01 [Cucumis melo alphaendornavirus] (YP\_009222598) [8] and SJ1 [Cucumis melo alphaendornavirus] (ARI71634) strains.

The genomes obtained from the BRA/TO-23 and BRA/TO-74 samples were determined to be 14,737 and 15,004 bp in size, respectively, and contains a single large ORF as reported to *Endornaviridae* family. The CDD search (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) of the deduced gene products produced from the deep sequencing of the dsRNA of the BRA/TO-23 and BRA/TO-74 strains revealed the presence of several domains with enzymatic functions. The most conserved domain, viral RNA-dependent RNA polymerase 2 (RdRp\_2) (CL03049; *E*-value = 1.44e-14 for BRA/TO-23 and 1.56e-14 for BRA/TO-74), has been recognized as the C-terminus of the polyprotein belonging to the pfam00978 family. The polyprotein always carries a conserved RdRp domain (pfam00978), specifying its affiliation to the alphavirus-like superfamily [11]. Other typical conserved replicase motifs, such as those encoding for methyltransferase, helicase, and glycosyltransferase, were also identified (Table 1). Phylogenetic analyses were conducted using MEGA 6.0 [16]. Clade support was obtained by bootstrap after 500 replications. For the amino

acid coding sequences, the JTT matrix-based model was used and the evolutionary models were selected according to the likelihood ratio test. Maximum clade credibility trees were annotated with TreeAnnotator and viewed with FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). The two Brazilian CmEV strains demonstrated by the present study form a separate clade together with the reference strains CL-01 and SJ1. This group also shows a distant relationship to other *Endornaviridae* family members, reinforcing the conclusion that the Brazilian (BRA/TO-23 and BRA/TO-74), American (CL-01) and Korean (SJ1) strains belong to the same CmEV species (Fig. 1).

Plant viral content has already been described in human feces. Fecal samples have significant dietary content, and plant viral nucleic acid is expected to appear in large amounts in the resulting sequences [17]. Endornaviruses form a well-known group of endogenous plant viruses and have a worldwide distribution [8]; therefore, their presence in feces may simply reflect recent dietary consumption. Endornaviruses have also been detected in Australian Muscovy duck fecal samples [18]. A plethora of plant viruses should be expected in BRA/TO-23 and BRA/TO-74 samples besides CmEV. However, these data were not observed. Figure 2 shows the number of reads plotted against *E*-values. It is possible to observe that a high quantity of CmEV reads (large pink circle) could be obtained compared to other viruses detected (small blue circles), including the plant viruses. It is noteworthy that only two of 250 fecal samples screened displayed CmEV reads.

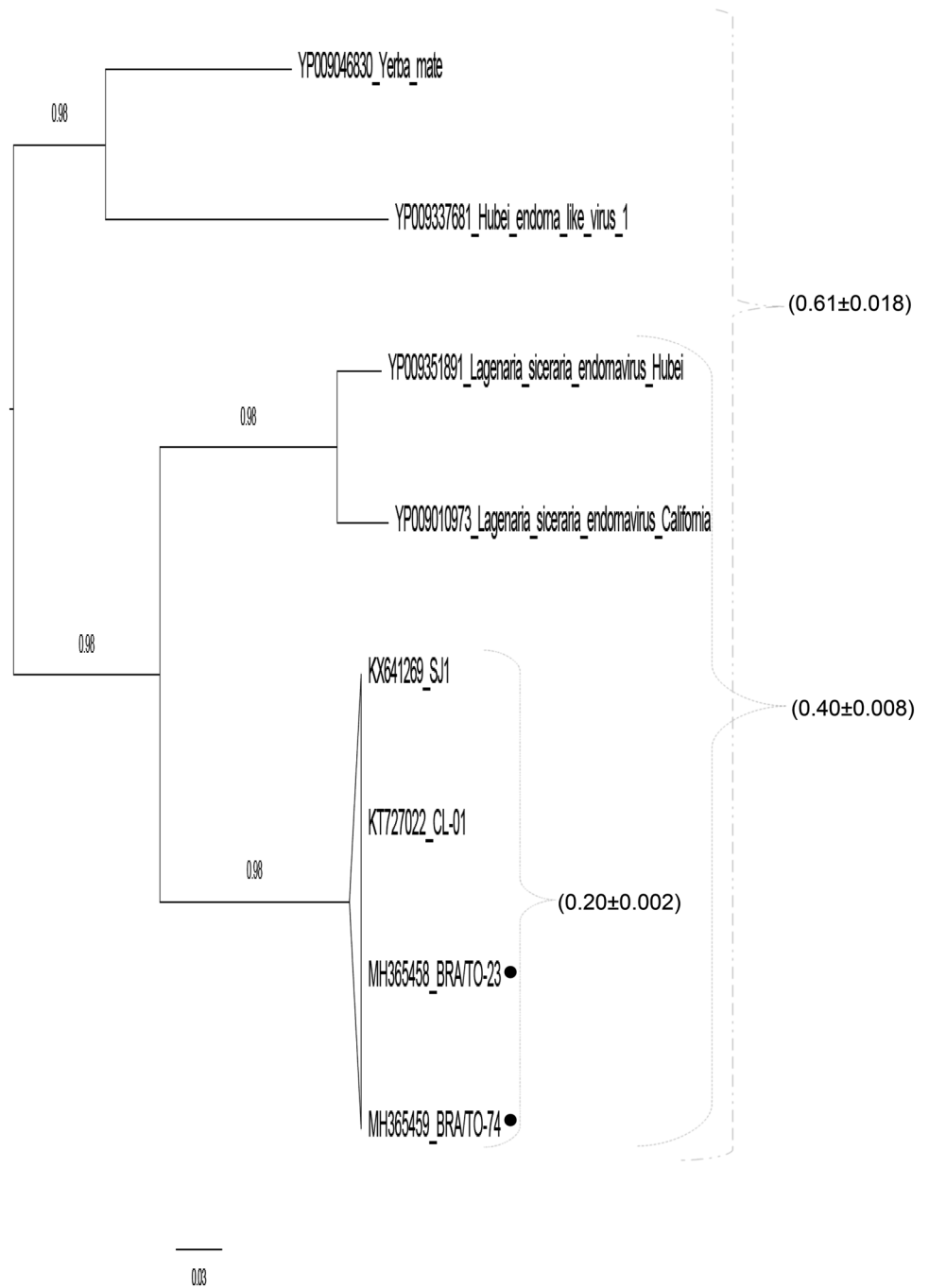
The high proportion of CmEV reads in these two particular samples might reflect the different levels of reduction and condensation of mass from food to feces through absorption during digestion, once both patients were presenting acute diarrhea. Zhang et al. [17] demonstrated that fluctuations of the Pepper Mild Mottle Virus RNA viral amount in human feces could be associated with the kinds

**Table 1** Motifs positions in the polyproteins of the CmEV BRA/TO-23 and BRA/TO-74 strains compared to CL-01 and SJ1 reference strains

Motif	Position in BRA/TO-74 and BRA/TO-23	Position in CL-01 and SJ1	Pfam ID*	Description
Caps_synth	2728–2889 3140–3291	2729–2875 3141–3292	PF05704	Capsular polysaccharide synthesis protein
RdRpol2	4570–4843	4571–4844	PF00978	RNA-dependent RNA polymerase
Gly_transf_sug	2750–2820 3173–3240	2751–2821 3174–3241	PF04488	Glycosyltransferase sugar-binding region containing DXD motif
Helicase1	1380–1624	1381–1625	PF01443	Viral (superfamily 1) RNA helicase
RdRP_3	4740–4823	4741–4824	PF00998	Viral RNA-dependent RNA polymerase
AAA_30	1378–1472	1379–1473	PF13604	AAA domain
AAA_14	1378–1482	1379–1483	PF13173	AAA domain
Glyco_tran_28_C	3723–3792	3724–3791	PF04101	Glycosyltransferase family 28 C-terminal domain
UDPGT	3743–3779	3744–3785	PF00201	UDP-glucuronosyl and UDP-glucosyl transferase

\*Pfam motifs were identified using online server at: <https://www.genome.jp>

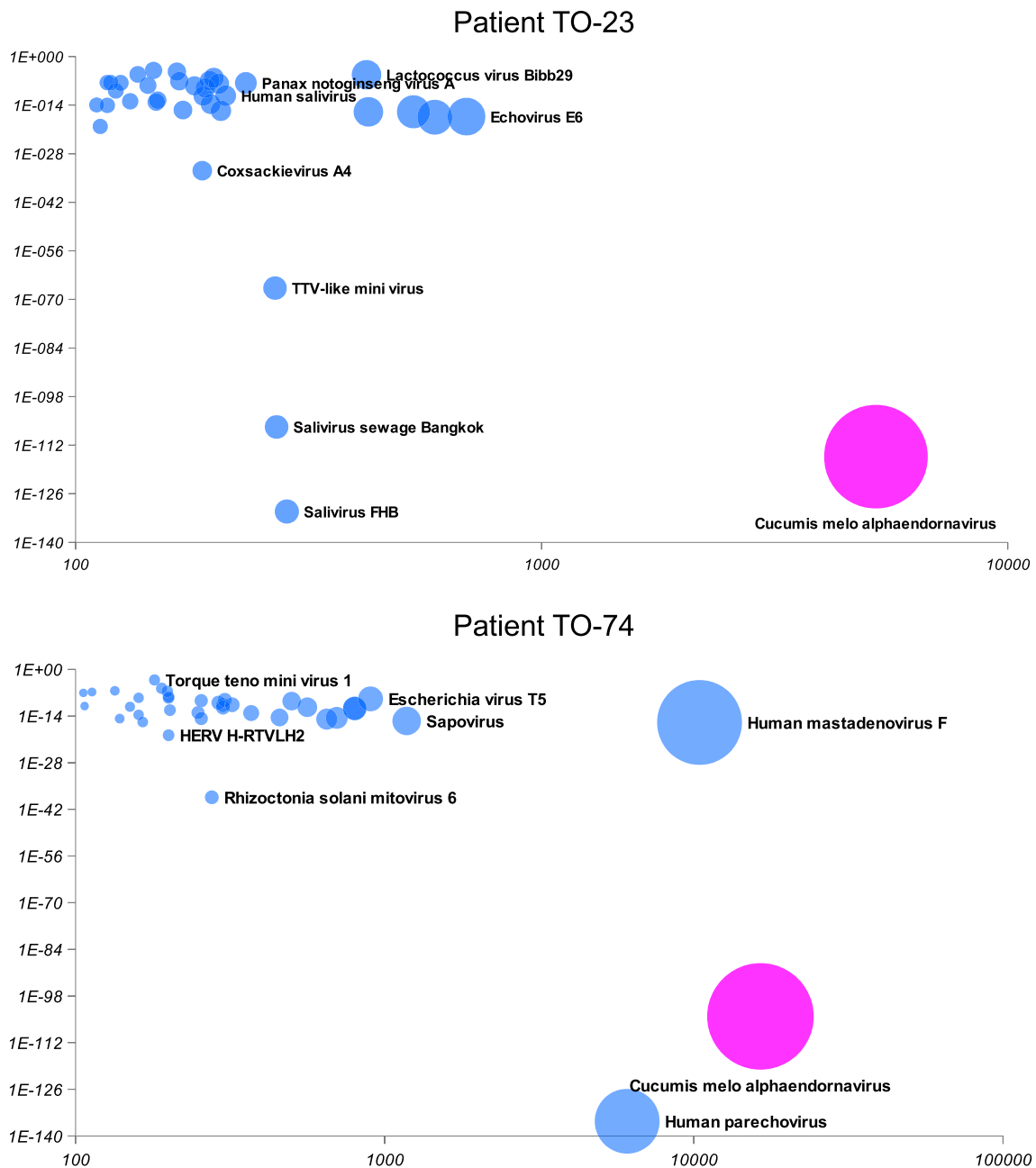
**Fig. 1** Phylogenetic tree of Brazilian CmEV strains. CmEV genome references SJ01 and CL-01 and the nearly complete genomes of the Brazilian BRA/TO-23 and BRA/TO-74 strains were used to infer a maximum likelihood tree inferred using the polyprotein of endornaviruses. References used to infer the tree were the best-hit sequences obtained using Blast search. Values above branches are statistical support based of the tree. Hatched brackets indicate genetic divergence (pairwise distances and standard errors) between clades on the tree. The tree was constructed using the JTT model and composite likelihood method implemented in the Mega6.0



of food consumed, methods of food preparation, and idiosyncratic conditions in the gastrointestinal tract. On the other hand, earlier studies showed that plant viral particles could be assembled in *Escherichia coli* cells [19, 20], which leads to the speculation that some feces-borne plant viruses might be capable of interacting with microbes in human guts [17]. However, the evidence for active replication of CmEV in human gut is currently lacking. Further research efforts are needed to determine whether and how

plant viruses may interact with intestinal cells or microorganisms in the human gastrointestinal tract [17].

A common feature of virome analysis is the frequent unavailability of epidemiological background information on potential consumption of contaminated food and/or patient medical records. The present study is no exception, and the epidemiological connection between the patient and the virus is lacking. Endornaviruses are not known to be associated with human disease [8], and the gastroenteritis



**Fig. 2** Plethora of other viruses present in samples BRA/TO-23 and BRA/TO-74. Number of reads ( $x$ -axis and size of circles) was plotted against  $E$ -values ( $y$ -axis,  $E$ -values were expressed in log scale).

CmEV reads are represented by large pink circles and other detected viruses, including the plant viruses by small blue circles

symptoms observed in the patients BRA/TO-23 and BRA/TO-74 are probably linked to one (or more) of the enteric viruses detected in their fecal samples (i.e., Sapovirus, Human Mastadenovirus F or Salivirus) (Fig. 2). However, it was an inquisitive finding the detection of CmEV in a fecal sample collected from a 6-month-old child (BRA/TO-74). Food diversification occurs at the transition between exclusive breast or formula feeding and the introduction of regular and significant quantities of non-milk foods [21]. In Brazil,

parents are advised to begin food diversification between 4 and 12 months, generally starting with the introduction of fruit/vegetable purées [22]. It can be speculated that the child was undergoing food diversification; in fact, some members of the *Cucurbitaceae* family, such as melon and watermelon, are among the eligible groups of fruits that can be introduced at this particular age (<http://www.who.int>). However, it is important to emphasize that no cultural-epidemiological data were obtained from the patients analyzed.

Melons are members of the gourd family (*Cucurbitaceae*) and are important crops cultivated for fresh consumption [8]. The number of commercial fields of *Cucumis melo* in the state of Tocantins has increased rapidly in the last decade (<https://sidra.ibge.gov.br/home/ipca15/brasil>). However, investigations addressing the identification and genome characterization of endornavirus in Brazil are virtually absent [1]. Therefore, prediction of any association between the results found here and the cultivated plants in the state of Tocantins is hampered by a lack of genome sequencing data. It worth to mentioned that CmEV is not associated with any leaf symptoms; therefore, possible effects of the CmEV on yield and fruit quality remain to be studied [23].

Although plant virus discovery was not included in the original aims of the NGS surveillance proposed to study enteric viruses, metagenomic analyses offered an opportunity to identify for the first time in Brazil the endornavirus species, CmEV. The full genetic diversity of endornavirus genomes remains to be described [12], especially in Brazil. The data obtained from this investigation will contribute to the growing database of the geographic distribution and molecular diversity of endornaviruses, as well as contribute to the growing body of knowledge concerning the ecology, epidemiology, and evolution of these viruses.

**Acknowledgements** We thank Luciano Monteiro da Silva, Renan Alves, Vanessa Silva for support. We also thank the Illumina Inc, Sage Science Inc, MP Biomedicals Inc, Zymo Research Corporation, Promega Biotecnologia do Brasil, for the donation of reagents for this project.

**Author Contributions** ACC and AL conceived the study; AL, ACC, and SVK designed the study protocol; FAPM, RB, MCABSL, RTC, MFNSA, and CVDAS participated in the conduct of the study, collection, and screening of the specimens; SVK and DG performed the deep-sequencing assays; ACC, EL, SVK, XD, and ED analyzed the big data; ACC, ECS, and AL analyzed and interpreted the data; ED and ECS supervised the study; ACC and AL drafted the manuscript; All authors critically revised the manuscript for intellectual content and approved the final version. ACC and AL are guarantors of the paper.

**Funding** Adriana Luchs is funded by FAPESP #2015/12944-9, and Antônio Charlys da Costa by FAPESP #2017/00021-9. This work was also supported by FAPESP #2016/01735-2 and CNPq #400354/2016-0.

## Compliance with ethical standards

**Conflict of interest** All authors declare no conflict of interest.

**Ethical approval** Previous Ethics Committee approval was granted by Adolfo Lutz Institute, São Paulo, Brazil (CEP 965.723; CTC 45G-2014), Faculdade de Medicina da Universidade de São Paulo (CAAE: 53153916.7.0000.0065), and Centro Universitário Luterano de Palmas - ULBRA (CAAE 53153916.7.3007.5516). This was an anonymous unlinked study and informed consent was not required according to resolution 466/12 concerning research involving humans (Conselho Nacional de Saúde/Ministério da Saúde, Brasília, 2012).

## References

- Nordenstedt N, Marcenaro D, Chilagane D, Mwaipopo B, Rajamäki ML, Nchimbi-Msolla S, Njau PJR, Mbanzibwa DR, Valkonen JPT (2017) Pathogenic seedborne viruses are rare but *Phaseolus vulgaris* endornaviruses are common in bean varieties grown in Nicaragua and Tanzania. *PLoS ONE* 12(5):e0178242
- Fukuhara T, Koga R, Aoki N, Yuki C, Yamamoto N, Oyama N, Udagawa T, Horiuchi H, Miyazaki S, Higashi Y, Takeshita M, Ikeda K, Arakawa M, Matsumoto N, Moriyama H (2006) The wide distribution of endornaviruses, large double-stranded RNA replicons with plasmid-like properties. *Arch Virol* 151(5):995–1002
- Lim S, Kim KH, Zhao F, Yoo RH, Igori D, Lee SH, Moon JS (2015) Complete genome sequence of a novel endornavirus isolated from hot pepper. *Arch Virol* 160(12):3153–3156
- Liu H, Fu Y, Xie J, Cheng J, Ghabrial SA, Li G, Yi X, Jiang D (2012) Discovery of novel dsRNA viral sequences by in silico cloning and implications for viral diversity, host range and evolution. *PLoS ONE* 7(7):e42147
- Pfeiffer P (1998) Nucleotide sequence, genetic organization and expression strategy of the double-stranded RNA associated with the '447' cytoplasmic male sterility trait in *Vicia faba*. *J Gen Virol* 79(10):2349–2358
- Osaki H, Nakamura H, Sasaki A, Matsumoto N, Yoshida K (2006) An endornavirus from a hypovirulent strain of the violet root rot fungus, *Helicobasidium mompa*. *Virus Res* 118(1–2):143–149
- Grill LK, Garger SJ (1981) Identification and characterization of double-stranded RNA associated with cytoplasmic male sterility in *Vicia faba*. *Proc Natl Acad Sci USA* 78(11):7043–7046
- Sabanadzovic S, Wintermantel WM, Valverde RA, McCreight JD, Aboughanem-Sabanadzovic N (2016) *Cucumis melo* endornavirus: genome organization, host range and co-divergence with the host. *Virus Res* 214:49–58
- Hao F, Ding T, Wu M, Zhang J, Yang L, Chen W, Li G (2018) Two novel hypovirulence-associated mycoviruses in the phytopathogenic fungus *Botrytis cinera*: molecular characterization and suppression of infection cushion formation. *Viruses* 10(5):254
- Fernandez-Cassi X, Timoneda N, Martínez-Puchol S, Rusiñol M, Rodríguez-Manzano J, Figuerola N, Bofill-Mas S, Abril JF, Girones R (2018) Metagenomics for the study of viruses in urban sewage as a tool for public health surveillance. *Sci Total Environ* 618:870–880
- Bartholomäus A, Wibberg D, Winkler A, Pühler A, Schlüter A, Varrelmann M (2016) Deep sequencing analysis reveals the mycoviral diversity of the virome of an avirulent isolate of *Rhizoctonia solani* AG-2-2 IV. *PLoS ONE* 11(11):e0165965
- Ong JW, Li H, Sivasithamparam K, Dixon KW, Jones MGK, Wylie SJ (2016) Novel endorna-like viruses, including three with two open reading frames, challenge the membership criteria and taxonomy of the *Endornaviridae*. *Virology* 499:203–211
- Li L, Deng X, Mee ET, Collot-Teixeira S, Anderson R, Schepelmann S, Minor PD, Delwart E (2015) Comparing viral metagenomics methods using a highly multiplexed human viral pathogens reagent. *J Virol Methods* 213:139–146
- Charlys da Costa A, Thézé J, Komninakis SCV, Sanz-Duro RL, Felinto MRL, Moura LCC, Barroso IMO, Santos LEC, Nunes MAL, Moura AA, Lourenço J, Deng X, Delwart EL, Guimarães MRDAS, Pybus OG, Sabino EC, Faria NR (2017) Spread of chikungunya virus East/Central/South African genotype in Northeast Brazil. *Emerg Infect Dis* 23(10):1742–1744
- Deng X, Naccache SN, Ng T, Federman S, Li L, Chiu CY, Delwart EL (2015) An ensemble strategy that significantly improves de novo assembly of microbial genomes from metagenomic next-generation sequencing data. *Nucleic Acids Res* 43(7):e46

16. Tamura K, Stecher G, Peterson D, Filipinski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30(12):2725–2729
17. Zhang T, Breitbart M, Lee WH, Run JQ, Wei CL, Soh SW, Hibberd ML, Liu ET, Rohwer F, Ruan Y (2006) RNA viral community in human feces: prevalence of plant pathogenic viruses. *PLoS Biol* 4(1):e3
18. Vibin J, Chamings A, Collier F, Klaassen M, Nelson TM, Alexandersen S (2018) Metagenomics detection and characterisation of viruses in faecal samples from Australian wild birds. *Sci Rep* 8(1):8686. <https://doi.org/10.1038/s41598-018-26851-1>
19. Jagadish MN, Huang D, Ward CW (1993) Site-directed mutagenesis of a potyvirus coat protein and its assembly in *Escherichia coli*. *J Gen Virol* 74(Pt 5):893–896
20. Hwang DJ, Roberts IM, Wilson TM (1994) Expression of tobacco mosaic virus coat protein and assembly of pseudovirus particles in *Escherichia coli*. *Proc Natl Acad Sci USA* 91(19):9067–9071
21. Longfier L, Soussignan R, Reissland N, Leconte M, Marret S, Schaal B, Mellier D (2016) Emotional expressiveness of 5–6 month-old infants born very premature versus full-term at initial exposure to weaning foods. *Appetite* 107:494–500
22. Mello CS, Barros KV, de Moraes MB (2016) Brazilian infant and preschool children feeding: literature review. *J Pediatr* 92(5):451–463
23. Quito-Avila DF, Peralta EL, Martin RR, Ibarra MA, Alvarez RA, Mendonza A, Insuasti M, Ochoa J (2014) Detection and occurrence of melon yellow spot virus in Ecuador: an emerging threat to cucurbit production in the region. *Eur J Plant Pathol* 140(2):193–197

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.