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Exploring the virome of *Vasconcellea x heilbornii:* the first step towards a sustainable production program for babaco in Ecuador

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Abstract The virome of babaco (*Vasconcellea x heilbornii*) —a non-traditional fruit crop native to Ecuador— was investigated by high-throughput sequencing (HTS) on plants obtained from a commercial nursery. Six virus-like sequences were detected, including the full length of papaya ringspot virus (PRSV) and an RNA-dependent-RNA-polymerase (RdRp) sequence with homology to papaya virus Q. Three RNA sequences were found with homology, respectively, to apple latent spherical virus (genus *Cheravirus,* 71% nt identity), cherry leaf roll virus (genus *Nepovirus*, 54% nt identity) and Citrullus lanatus cryptic virus (genus *Deltapartitivirus*, 66% nt identity); whereas a DNA pararetrovirus-like sequence with homology to citrus endogenous pararetrovirus (58% nt identity) was also detected. RT-PCR-based virus surveys on a total of 284 samples collected from three provinces revealed that the partitivirus- and pararetrovirus-like sequences were present in 100% of tested plants; whereas the other virus sequences were detected in up to 68% of plants and were associated with different symptoms. This work provides information on the occurrence and prevalence of PRSV and five additional virus-like sequences in babaco, a vegetatively propagated crop, supporting the need for a virus-free certification program.

Keywords Vasconcellea x heilbornii · Virus · Babaco · Papaya · Ecuador

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Babaco (*Vasconcellea x heilbornii*) is a natural hybrid in the family *Caricaceae* found natively in temperate areas of southern Ecuador. The plant is commonly known as "mountain papaya" due to its resemblance to papaya (*Carica papaya* L.) (Kyndt et al., 2005). Its fruits are elongated pentagon-like shaped, with a unique low-sugar tart flavor suitable for making juice and marmalade. The production of this crop, however, has been

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hampered by pathogen-associated diseases that reduce both fruit quality and yield (Robles-Carrión et al., 2016). Babaco is a sterile hybrid propagated vegetatively using stem cuttings. This type of propagation poses the risk of substantial dissemination of pathogens, especially viruses, that might compromise the sustainability of babaco production.

Babaco mosaic virus (BabMV) is the only virus reported and fully characterized in babaco to date. The virus is commonly detected in plants showing mild leaf mosaic (Alvarez-Quinto et al. 2017). However, a range of symptoms including severe mottling, leaf deformation and necrosis, is frequently observed in nurseries and production orchards, suggesting that additional viruses affect this crop. The objectives of this work were twofold: i) to study the virome of babaco plants obtained from a commercial nursery in Azuay province, and ii) to determine the prevalence of the viruses in representative nurseries and production orchards of Ecuador.

For the first objective, six BabMV-free plants, which are part of our greenhouse collection from a previous study (Alvarez-Quinto et al. 2017), were subjected to high-throughput sequencing (HTS) using total RNA as input. Plants were labeled according to their absence or presence of symptoms as follows: plant 1 = no symptoms, plants 2 and 3 = mild leaf mosaic, plant 4 = leaf mosaic and mottle, plant 5 = vein clearing and leaf deformation, and plant 6 = leaf deformation and necrosis. To validate the sensitivity and quality of HTS in pooled samples, one of the six plants (plant number 2) was mechanically inoculated with an isolate of BabMV (Acc. number MF978248).

Once BabMV infection was confirmed in plant 2 (30 days post inoculation, dpi), total RNA was individually extracted from 100 mg of leaf samples from each of the six plants as described by Halgren et al. (2007) and treated with Turbo™ DNase (Invitrogen, USA). The purified RNA was quantified and approximately 830 ng per sample were combined to a total of 5 μg. The pooled RNA was subjected to plant ribosomal RNA removal using the Illumina Ribo-Zero rRNA kit (Illumina, USA). Complementary DNA (cDNA) was generated by the Illumina TrueSeq RNA library preparation kit, followed by 100 bp paired-end sequencing on a Hiseq4000 Illumina platform at Macrogen (South Korea). Sequence reads were de novo assembled into contigs (word size = 24, bubble size = 50, minimum contig length = 200) using CLC Workbench 9.0 (Qiagen, USA) and the resulting contigs were compared against a database containing all plant virus proteins in RefSeq using BLASTx (BLOSUM 62 using default parameters) (Altschul et al. 1990).

HTS yielded approximately 35 million raw reads, from which about 800,000 formed six contigs that mapped to plant virus proteins. These virus-mapping contigs, with sizes ranging from 658 to 10,317 nucleotides (nt) and an average coverage of 2453.7 x per site, were compared to GenBank nt database using BLASTn. A 6371 nt long contig, assembled from 3125 reads, corresponded to the genome of BabMV, with 95% of coverage and 99% identity to the isolate used to inoculate plant 2. This result validated the effectiveness of HTS for virus detection in pooled samples.

Two contigs of 10,317 nt and 4649 nt, showed 97% identity (99% coverage) with papaya ringspot virus (PRSV, Acc. number MH974110) and 63% identity (100% coverage) with papaya virus Q (PpVQ, Acc. number KP165407), respectively (Table 1).

PpVQ is an umbra-like virus, which has not been formally assigned to a genus (Quito-Avila et al. 2015; Sá Antunes et al. 2016; Cornejo-Franco et al. 2018). Therefore, the species demarcation criteria for umbraviruses (less than 70% overall nucleotide identity; Ryabov et al. 2012; Adams et al. 2015), were provisionally used and suggested that the newly found PpVQ homologue from babaco represents a distinct virus, for which the name babaco virus Q (BabVQ) is proposed tentatively.

In addition, a 6806 nt long contig showed homology to RNA 1 of apple latent spherical virus (genus *Cheravirus,*71% nt identity, 97% coverage); whereas a 5635 nt long contig showed homology to RNA 1 of cherry leaf roll virus (genus *Nepovirus*, 54% nt identity, 80% coverage) (Table 1). Chera- and nepoviruses belong to the family *Secoviridae* and are characterized by having bipartite genomes, with RNA 1 encoding the polymerase and RNA 2 coding for the movement (MP) and the capsid proteins (CP) (Thompson et al. 2017). Interestingly, sequences with identity to RNA 2 for each homologous virus were not assembled. It appears that the titer of these RNA segments was too low to be detected by our HTS approach.

Sequence identity criteria for species demarcation in the *Secoviridae* call for <80% aa identity in the protease - polymerase (pro-pol) region and < 75% aa identity in the coat protein (Thompson et al. 2017). Pairwise aa identity of the pro-pol region between the new chera- and nepo-like sequences from babaco and their closest

Table 1 Results summary from high throughput sequencing. Pol: viral polymerase, nt: nucleotides, CP: viral coat protein, N.A. not applicable

Contig length (nt)	Number of reads in the assembly (% of total virus reads)	Homology information			Tentative name for new virus (abbreviation)	Genbank Acc. number for new virus sequences from babaco
		Blast homologue (Genbank acc. number)	Overall nucleotide sequence identity	Amino acid sequence identity (protein)		
10,317	2694 (0.34)	Papaya ringspot virus (MH974110)	97%	98% (polyprotein)	N.A.	MH974109
6371	3125 (0.39)	Babaco mosaic virus (MF978248)	99%	100% (pol)	N.A.	N.A.
4649	788,774 (99.04)	Papaya virus Q (KP165407)	63%	73% (pol)	Babaco virus Q (BabVQ)	MN648673
6806	239 (0.03)	Apple latent spherical virus (NC003787)	68%	70% (pol)	Babaco cheravirus-1 (BabChV-1)	MN648671
5635	452 (0.06)	Cherry leaf roll virus (FR851461)	54%	41% (pol)	Babaco nepovirus −1 (BabNV-1)	MN648672
658	255 (0.03)	Citrullus lanatus cryptic virus (KY081284)	66%	55% (CP)	Babaco cryptic virus-1 (BabCV-1)	MN648674
1060	894 (0.11)	Citrus endogenous pararetrovirus (KF800043)	58%	51% (polyprotein)	Babaco endogenous pararetrovirus (BabEPV)	MN626638

relatives was 79% and 49%, respectively; suggesting two distinct species in the genera *Cheravirus* and *Nepovirus*. This was supported by a Bayesian phylogenetic analysis inferred by BEAST 1.8.4 using the LG + G + I evolution model for the pro-pol region of representative members of the *Secoviridae* (Fig. 1). The analysis was done with two runs of 5,000,000 MCMC and burn-in of 10% using a strict molecular clock with constant size tree prior and default prior assumptions. The names babaco cheravirus-1 (BabChV-1) and babaco nepovirus −1 (BabNV-1) are tentatively proposed until a full genome characterization is possible (Table 1).

A short contig of 658 nt was also assembled. This contig had homology (66% nt identity, 45% coverage) to RNA 2 of Citrullus lanatus cryptic virus (CiLCV, Acc. number KY081284), a double-stranded RNA virus in the genus *Deltapartitivirus*, family *Partitiviridae* (Xin et al. 2017).

Partitiviruses usually are considered cryptic due to their indefinite persistence in plant cells (Nibert et al. 2014). Partitivirus genomes are segmented with RNA 1 encoding the polymerase and RNA 2 the CP. In babaco, sequences corresponding to RNA 1 were not assembled for this putative partitivirus. The deduced amino acid sequence of the partial CP showed 48% identity to its counterpart from CiLCV. This amino acid sequence identity suggests the existence of a new babaco-infecting partitivirus (species demarcation criteria < than 80% in the CP, Nibert et al. 2014), for which the name babaco cryptic virus-1 (BabCV-1) is proposed tentatively. A Bayesian phylogenetic analysis using the partial CP under the LG evolution model was inferred as described above. The analysis clustered BabCV-1 with CiLCV and other members of the genus *Deltapartitivirus* (Fig. 2 A).

Lastly, a 1060 nt long contig with homology to the ribonuclease H (RNaseH) of several plant endogenous pararetroviruses was assembled making up approximately 15% of average pararetrovirus genome length. Endogenous pararetroviruses are DNA virus sequences integrated into the plant genome, which may or may not become infectious (Hull et al. 2000). Phylogenetic analyses using pararetrovirus-like RNaseH sequences from different hosts, showed that the babaco pararetrovirus-like sequence clustered most closely (58% nt identity) with endogenous pararetroviruses from *Cajanus cajan* and *Citrus sinensis* (Fig. 2 B). Based on sequence identity and associated host (Yu et al. 2019), the name babaco endogenous pararetrovirus (BabEPV) is proposed for this new tentative integrated pararetrovirus. Table 1 summarizes virus sequences, reads abundance and accession numbers for each new contig obtained by HTS.

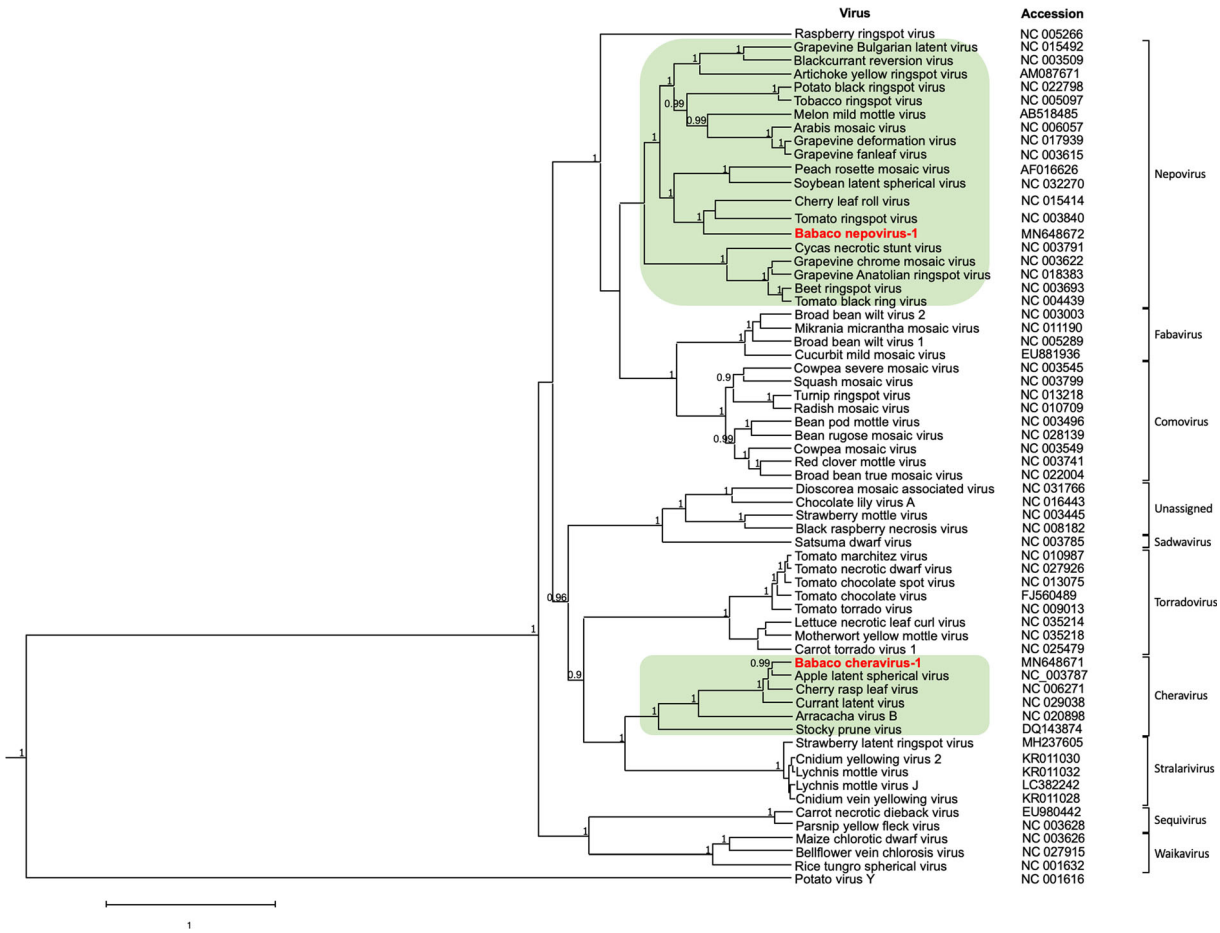

Fig. 1 Phylogenetic relationships across representative members of the *Secoviridae* and the new babaco chera- and nepo-like sequences based on the protease-polymerase (pro-pol) amino acid region. Bayesian tree built under the LG + G + I evolution model using strict molecular clock, constant size tree prior, and default tree prior assumptions. Posterior probability values above 0.9, are shown above the nodes. NCBI accession numbers of the sequences used for the phylogenetic analysis are shown on the right

Detection primers were designed for each new virus sequence and used to validate and determine the occurrence of each virus in the six babaco plants. Virus detection was done using total RNA, followed by reverse-transcription (RT) and PCR as described (Quito-Avila et al. 2015; Alvarez-Quinto et al. 2017; Cornejo-Franco et al. 2018). Primer information and the size of the amplification product is shown in Table 2.

All six plants tested positive for BabCV-1 and BabEPV; whereas, BabVQ was found in plants 3 and 4; BabNV-1 and BabChV-1 were detected, respectively, in plants 4 and 5; and plants 5 and 6 were infected by PRSV. Symptoms observed in each plant are shown in Fig. 3.

Considering the symptomless nature of plant 1, which was only positive for BabCV-1 and BabEPV, the babaco isolate of PRSV, from plant 5, was mechanically inoculated onto rooted cuttings ($n = 3$) of plant 1. Infection of PRSV, but not BabChV-1, in inoculated plants was confirmed by RT-PCR at 30 dpi. Plants exhibited symptoms similar to those observed in the naturally infected plant 5, suggesting that PRSV induces severe symptoms in babaco, independently from co-infecting BabChV-1 (Fig. 3).

The second objective of this study tested a total of 284 leaf samples for each of the newly discovered viruses. Samples were obtained from nurseries in Paute, Azuay province ($n = 108$) and from production orchards in Patate, Tungurahua province ($n = 127$). In addition, a mix of naturally growing and cultivated plants in Loja province ($n = 49$), the most probable diversity center of *Vasconcellea* species (Scheldeman et al. 2007), was included. Leaf samples were collected from both

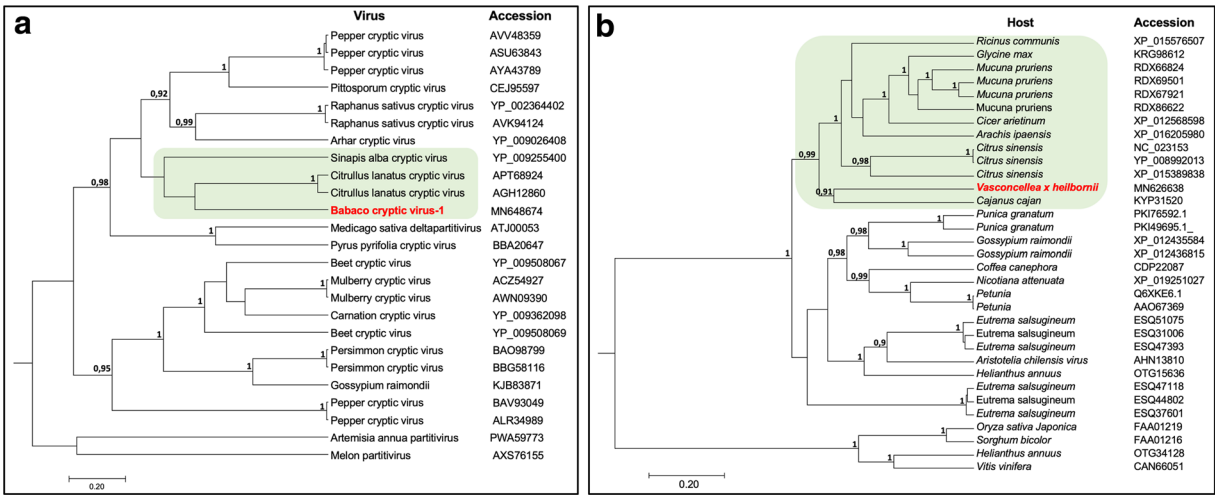

Fig. 2 Phylogenetic inferences for the new pararetro- and deltapartitivirus-like sequences from babaco. Bayesian trees inferred using a strict molecular clock, constant size tree prior, and default prior assumptions. Posterior probabilities above 0.9 are shown. A) Tree built under the LG model showing the evolutionary relationships of selected partitiviruses. B) Tree built under the WAG model showing genetic relationships across several endogenous pararetroviruses, annotated as part of their host genomes. NCBI accession numbers of the sequences used for the phylogenetic analysis are shown on the right

symptomatic (80%) and asymptomatic (20%) plants. Total RNA extraction and RT-PCR was done as described above.

The virus survey revealed that BabCV-1 and BabEPV were present in 100% of the samples, including asymptomatic ones; whereas BabVQ and PRSV were detected, respectively, in 68% and 36% of the samples. The incidence of BabNV-1 and BabChV-1 was lower (Table 3).

The ubiquitous presence of BabCV-1 and BabEPV in babaco plants, suggests that these two viruses are not associated to babaco diseases. Accordingly, their presumed cryptic and/or integrated nature supposes that plants infected with those viruses be considered 'healthy', as observed in the greenhouse plants (Fig. 3).

In general, field plants infected with PRSV, regardless of the co-infecting virus, showed the most conspicuous symptoms including severe leaf mosaic,

Table 2 Primers information and size of amplified products for RT-PCR detection of viruses studied in this work

Primer information					
Virus	**Forward**	**Reverse**	**Annealing temperature °C**	**Amplification product**	**Reference**
Papaya ringspot virus	GAGARGTAYATGCC GCGGTATGG	CGCATACCCAGGAG AGAGTGC	55	263	Quito-Avila et al. 2015
Babaco mosaic virus	GGATGCACTCATTA CATCCAAGC	CCACTCCAAGGCTT CCATGAGC	57	647	Alvarez-Quinto et al. 2017
Babaco virus Q	CGTGTGCTTGCTGG TTTTCGTTC	CAACGGGAAACCCA TACACCTGG	55–57	1055	This study
Babaco cheravirus-1	GCTTGTCATTAGCA CGGCTAAC	GCAGGAAAGAGCGT CTGATCA	55–57	447	This study
Babaco nepovirus −1	GGTATGCTCGACAG AGCATTGT	CCCTTCTACATTCC ACAACCAC	55–57	269	This study
Babaco cryptic virus-1	GGACTAGTACACCC TACCAACG	CCATAGGGTACCAT GCACAAAC	55–57	469	This study
Babaco endogenous pararetrovirus	CACCCTTGGCGAAT ATATGCAG	TGCTCAACATGTCC TGAAGC	55–57	407	This study

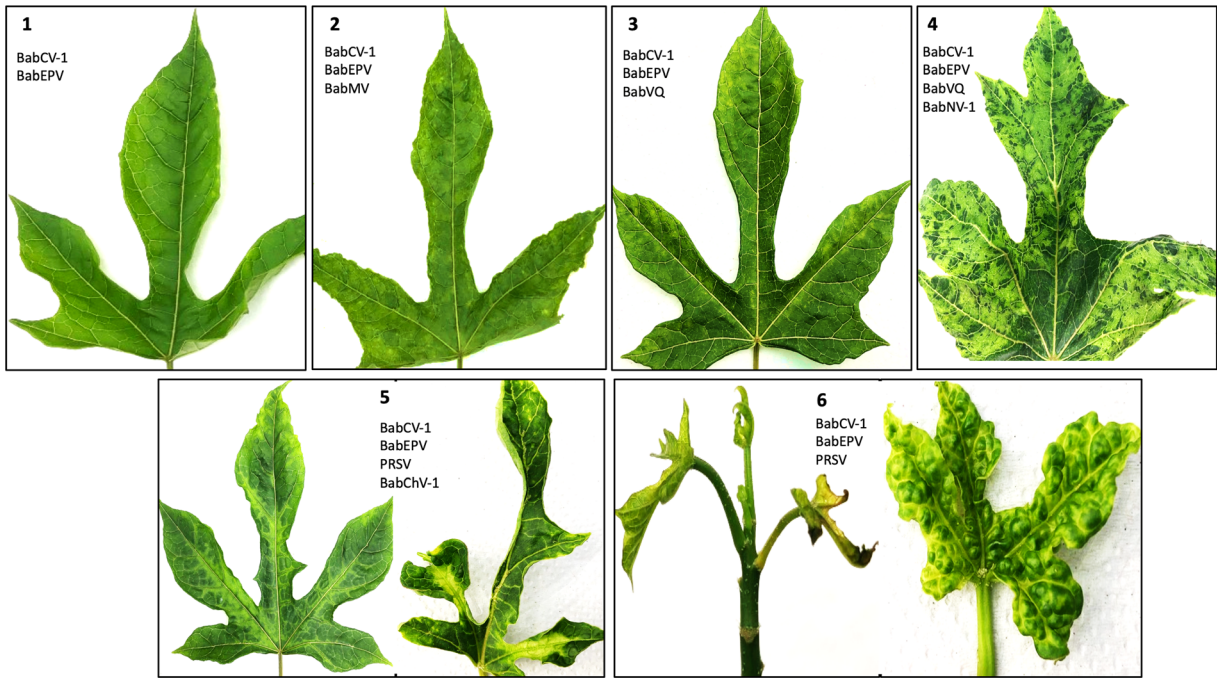

Fig. 3 Symptom expression in babaco plants under greenhouse conditions. Representative samples from each of the six plants subjected to the study are shown. For practical purposes, viruses found in each plant are listed below the number corresponding to the plant. Virus abbreviations: babaco cryptic virus 1 (BabCV-1), babaco pararetrovirus (BabPrV), papaya ringspot virus (PRSV), babaco nepovirus 1 (BabNV-1), babaco cheravirus-1 (BabChV-1), babaco meleira-associated virus (BabMelaV) and babaco virus Q (BabVQ)

deformation, blistering, and necrosis. Plants that were infected with BabMV in co-infections with BabNV-1 or PRSV displayed more pronounced leaf mosaic compared to plants singly infected with BabMV. All plants infected with BabNV-1 and BabChV-1 were found co-infecting with either BabMV or PRSV. Therefore, further studies are needed to determine whether the new tentative nepovirus (BabNV-1) and cheravirus (BabChV-1) can cause symptoms when in single infections. Plants infected by BabVQ did not show leaf symptoms. However, the potential impact of this virus on babaco fruit remains to be investigated.

Table 3 Occurrence and prevalence of new viruses in field collected samples from three provinces. Virus abbreviations: papaya ringspot virus (PRSV), babaco virus Q (BabVQ), babaco cheravirus-1 (BabChV-1), babaco nepovirus-1 (BabNV-1), babaco cryptic virus 1 (BabCV-1), babaco endogenous pararetrovirus (BabEPV)

	Occurrence (% positives)		
Virus	**Paute (Azuay province) Nurseries (n = 108)**	**Patate (Tungurahua province) Production orchards (n = 127)**	**Saraguro (Loja province) Wild conditions (n = 49)**
PRSV	11%	36%	22%
BabVQ	23%	68%	0
BabChV-1	4%	10%	6%
BabNV-1	8%	29%	0
BabCV-1	100%	100%	100%
BabEPV	100%	100%	100%

In summary, this work expands the current knowledge of the virome of babaco (*Vasconcellea x heilbornii*), a crop for which only a single virus (BabMV) was formally reported (Alvarez-Quinto et al. 2017). HTS allowed for the identification of PRSV and five partial virus genome sequences, which according to sequence identities, belong to five distinct virus species. Babaco virus Q, babaco cheravirus-1, babaco nepovirus −1 and babaco cryptic virus-1 are RNA viruses with sequence similarities to the genera *Umbravirus, Cheravirus, Nepovirus* and *Deltapartitivirus*, respectively; whereas babaco

endogenous pararetrovirus groups with several members of the *Caulimoviridae,* with the integrated citrus endogenous pararetroviruses as closest relatives.

Although, the complete genomes for the newly found viruses were not determined, RT-PCR based diagnostic assays were developed and confirmed the occurrence and prevalence of each virus in field collected samples.

This study revealed that PRSV induces more severe symptoms in babaco compared to BabMV. PRSV, a well-known potyvirus transmitted by several aphid species, represents the major threat to the papaya production worldwide and could have the same impact on the babaco sustainability in Ecuador.

Although a clear implication of the new viruses in symptoms was not determined, their potential synergistic interactions with additional babaco infecting viruses should be monitored. Being tentative new members of the *Chera-* and *Nepovirus*, both BabChV-1 and BabNV-1 are most likely transmitted by nematodes. Hence, a full characterization of these viruses, including the identification of natural vectors, is warranted to implement management strategies.

Partial sequence corresponding to a putative novel deltapartitivirus (BabCV-1) and an endogenous pararetro-like virus (BabEPV) were found in every plant tested in this study. Considering the sequence identity to the corresponding homologues, it is reasonable to hypothesize they are cryptic and integrated, as these are typical features in members of the *Partitiviridae* (Xin et al. 2017) and the endogenous plant pararetrovirus group (Yu et al. 2019), respectively (Fig. 2).

Lastly, the use of HTS for plant virus discovery has proven a powerful tool in several different systems (Adams and Fox, 2016; Villamor et al. 2019). To our knowledge, this is the first HTS-based virome study in babaco, which reports the presence of several potentially yield-limiting viruses. Hence, a virus free certification program is warranted to contribute to the sustainability of this vegetatively propagated crop.

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Compliance with ethical standards

Conflict of interest The authors declare that no conflict of interest exists.

Ethical approval This work did not involve any human and/ or animal participants.

References

Adams, M. J., Lefkowitz, E. J., King, A. M. Q., Bamford, D. H., Breitbart, M., Davison, A. J., Ghabrial, S. A., Gorbalenya, A. E., Knowles, N. J., Krell, P., Lavigne, R., Prangishvili, D., Sanfaçon, H., Siddell, S. G., Simmonds, P., & Carstens, E. B. (2015). Ratification vote on taxonomic proposals to the international committee on taxonomy of viruses (2015). *Archives of Virology, 160*, 1837–1850. https://doi.org/10.1007/s00705-015-2425-z.

Adams, I., & Fox, A. (2016). Diagnosis of plant viruses using next-generation sequencing and metagenomic analysis. In A. Wang & X. Zhou (Eds.), *Current research topics in plant virology* (pp. 323–335). Cham: Springer International Publishing.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403–410.

Alvarez-Quinto, R. A., Cornejo-Franco, J. F., & Quito-Avila, D. F. (2017). Characterization of a not so new potexvirus from babaco (Vasconcellea x heilbornii). *PLoS One, 12*(12), e0189519.

Cornejo-Franco, J. F., Alvarez-Quinto, R. A., & Quito-Avila, D. F. (2018). Transmission of the umbra-like papaya virus Q in Ecuador and its association with meleira-related viruses from Brazil. *Crop Protection, 110*, 99–102.

Fuentes, G., & Santamaría, J. M. (2014). Papaya (Carica papaya L.): Origin, domestication, and production. In R. Ming & P. H. Moore (Eds.), *Genetics and genomics of papaya* (pp. 3–15). Springer New York: New York, NY.

Halgren, A., Tzanetakis, I. E., & Martin, R. R. (2007). Identification, characterization, and detection of black raspberry necrosis virus. *Phytopathology, 97*(1), 44–50.

Hull, R., Harper, G., & Lockhart, B. (2000). Viral sequences integrated into plant genomes. *Trends in Plant Science, 5*, 362–365.

Kyndt, T., Romeijn-Peeters, E., Van Droogenbroeck, B., Romero-Motochi, J. P., Gheysen, G., & Goetghebeur, P. (2005). Species relationships in the genus Vasconcellea (Caricaceae) based on molecular and morphological evidence. *American Journal of Botany, 92*(6), 1033–1044.

Nibert, M. L., Ghabrial, S. A., Maiss, E., Lesker, T., Vainio, E. J., Jiang, D. H., & Suzuki, N. (2014). Taxonomic reorganization of family *Partitiviridae* and other recent progress in partitivirus research. *Virus Research, 188*, 128–141.

Quito-Avila, D. F., Alvarez, R. A., Ibarra, M. A., & Martin, R. R. (2015). Detection and partial genome sequence of a new umbra-like virus of papaya discovered in Ecuador. *European Journal of Plant Pathology, 143*(1), 199–204.

Robles-Carrión, A. R., Herrera Isla, L., & Torres, G. R. (2016). El babaco (Vasconcellea heilbornii var. pentagona Badillo). Principales agentes fitopatógenos y estrategias de control. *Centro Agrícola, 43*, 83–92.

Ryabov, E. V., Taliansky, M. E., Robinson, D. J., Waterhouse, P. M., Murant, A. F., de Zoeten, G. A., Falk, B. W., Vetten, H. J., & Gibbs, M. J. (2012). Genus *Umbravirus*. In A. King, M. J. Adams, E. C. Carstens, & E. J. Lefkowitz (Eds.), *Virus taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses* (pp. 1191–1195). New York: Elsevier.

Sá Antunes, T. F., Amaral, R. J., Ventura, J. A., Godinho, M. T., Amaral, J. G., Souza, F. O., et al. (2016). The dsRNA virus papaya Meleira virus and an ssRNA virus are associated with papaya sticky disease. *PLoS One, 11*(5), e0155240.

Scheldeman, X., Willemen, L., Coppens d'Eeckenbrugge, G., Romeijn-Peeters, E., Restrepo, M. T., Romero Motoche, J., et al. (2007). Distribution, diversity and environmental adaptation of highland papayas (Vasconcellea spp.) in tropical and subtropical America. In D. L. Hawksworth & A. T. Bull (Eds.), *Plant conservation and biodiversity* (pp. 293–310). Dordrecht: Springer Netherlands.

Thompson, J. R., Dasgupta, I., Fuchs, M., Iwanami, T., Karasev, A. V., Petrzik, K., Sanfaçon, H., Tzanetakis, I., Van der Vlugt, R., Wetzel, T., Yoshikawa, N., Lefkowitz, E. J., Davison, A. J., Siddell, S. G., Simmonds, P., Adams, M. J., Smith, D. B., Orton, R. J., & Knowles, N. J. (2017). ICTV virus taxonomy profile: *Secoviridae*. *The Journal of General Virology, 98*, 529–531.

Villamor, D. E. V., Ho, T., Al Rwahnih, M., Martin, R. R., & Tzanetakis, I. E. (2019). High throughput sequencing for plant virus detection and discovery. *Phytopathology, 109*(5), 716–725.

Xin, M., Cao, M., Liu, W., Ren, Y., Lu, C., & Wang, X. (2017). The genomic and biological characterization of Citrullus lanatus cryptic virus infecting watermelon in China. *Virus Research, 232*, 106–112.

Yu, H., Wang, X., Lu, Z., Xu, Y., Deng, X., & Xu, Q. (2019). Endogenous pararetrovirus sequences are widely present in Citrinae genomes. *Virus Research, 262*, 48–53.