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RESEARCH ARTICLE

Discovery of Brassica Yellows Virus and Porcine Reproductive and Respiratory Syndrome Virus in *Diaphorina citri* and Changes in Virome Due to Infection with '*Ca*. L. asiaticus'

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ABSTRACT Detection of new viruses or new virus hosts is essential for the protection of economically important agroecosystems and human health. Increasingly, metatranscriptomic data are being used to facilitate this process. Such data were obtained from adult Asian citrus psyllids (ACP) (*Diaphorina citri* Kuwayama) that fed solely on mandarin (*Citrus* ×*aurantium* L.) plants grafted with buds infected with '*Candidatus* Liberibacter asiaticus' (*C*Las), a phloem-limited bacterium associated with the severe Asian variant of huanglongbing (HLB), the most destructive disease of citrus. Brassica yellows virus (BrYV), the causative agent of yellowing or leafroll symptoms in brassicaceous plants, and its associated RNA (named as BrYVaRNA) were detected in ACP. In addition, the porcine reproductive and respiratory syndrome virus (PRRSV), which affects pigs and is economically important to pig production, was also found in ACP. These viruses were not detected in insects feeding on plants grafted with *C*Las-free buds. Changes in the concentrations of insect-specific viruses within the psyllid were caused by coinfection with *C*Las.

IMPORTANCE The cross transmission of pathogenic viruses between different farming systems or plant communities is a major threat to plants and animals and, potentially, human health. The use of metagenomics is an effective approach to discover viruses and vectors. Here, we collected buds from the *C*Las-infected and *C*Las-free mandarin (*Citrus* ×*aurantium* L. [Rutaceae: Aurantioideae: Aurantieae]) trees from a commercial orchard and grafted them onto *C*Las-free mandarin plants under laboratory conditions. Through metatranscriptome sequencing, we first identified the Asian citrus psyllids feeding on plants grafted with *C*Las-infected buds carried the plant pathogen, brassica yellows virus and its associated RNA, and the swine pathogen, porcine reproductive and respiratory syndrome virus. These discoveries indicate that both viruses can be transmitted by grafting and acquired by ACP from *C*Las+ mandarin seedlings.

KEYWORDS Asian citrus psyllid, virus, brassica yellows virus, porcine reproductive and respiratory syndrome, metatranscriptomics

Plants are infected with a diverse range of microbes that interact with their host and with each other. The majority of these microbes are not causal agents of disease (1), and many promote plant growth (2, 3) while others facilitate resistance to various plant pathogens (4). In addition to these endosymbiotic organisms, coinfections of multiple pathogens are common (5), and citrus trees in orchards are no exception often containing mixed infections of various pathogens, including viruses, many of which can be transmitted by arthropod vectors (6). The extent of these multiple infections

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and their interactions with each other and with their insect hosts is slowly being revealed due to the development of new molecular tools, especially next generation sequencing (7). After long-term, continuous interactions, once these viruses can colonize an insect's gut, there is the possibility of phyllosphere transmission by insect vectors (8, 9).

Brassica yellows virus (BrYV) is a recently discovered virus that mainly infects cruciferous vegetables in which it causes mottling, yellowing, or leaf roll symptoms (10, 11). It infects a range of brassicaceous plants, including members of the genera *Brassica*, *Raphanus,* and *Sinapis* (12). BrYV was first identified in China in 2011 (10) and is tentatively placed in the genus *Polerovirus* (Solemoviridae); it has since been reported in Korea (11), Japan (13) and Australia (14). Molecular and phylogenetic studies show BrYV is closely related to turnip yellows virus (TuYV: Luteoviridae) (10, 14, 15), which has been reported throughout Europe, South Africa, China, Iran, Egypt, South Africa, Egypt, Morocco, and Australia, and infects crops and weeds, including members of the Brassicaceae, Fabaceae, Amaranthaceae and Asteraceae (see references in [14]). Poleroviruses are mostly transmitted by aphids in a circulative and nonreplicative mode (16–19); one known polerovirus is transmitted by whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (20). A recent study showed that the expression of polerovirus proteins mediates changes in plant-aphid interactions and inhibits aphid induction of jasmonic acid signaling in plant hosts (21).

Porcine reproductive and respiratory syndrome was first recognized in the United States in 1987 and has become one of the most important diseases affecting the global pig industry (22, 23). The causative agent of the syndrome, porcine reproductive and respiratory syndrome virus (PRRSV: Nidoviridales: Arteriviridae), is a single-stranded, positive-sense RNA virus, and possesses a genome *ca*. 15 kb in length (24). Two genotypes of PRRSV can be found, the European (type 1) and North American (type 2) types that share only approximately 60% nucleotide similarity (25). The virus was first isolated in China in 1996 (26) and has evolved quickly and spread widely during the last 2 decades, causing large economic losses (27–29). To date, it has mainly been detected in domesticated pigs and wild boars (30). However, PRRSV can be acquired by mosquitoes (*Aedes vexans* (Meigen) (Diptera: Culicidae) and houseflies (*Musca domestica* L. [Diptera: Muscidae]) from infected pigs and in which it can be retained for a short period; the virus did not replicate within the insects (31–34). It can be mechanically transmitted by mosquitoes and houseflies, but the insects could not serve as biological vectors (31, 32).

Diaphorina citri Kuwayama (Asian citrus psyllid, ACP) (Hemiptera: Sternorrhyncha: Psyllidae) is an economically important pest of citrus. It is the major vector of the pathogenic bacterium '*Candidatus* Liberibacter asiaticus' (*C*Las), which is the putative causal agent of the most severe form of huanglongbing (HLB), the most serious disease of citrus (35). Besides spreading HLB, ACP harbors phytopathogenic viruses such as *Citrus* tristeza virus (CTV: Closteroviridae) (36) and several putative insect-specific viruses, including *Diaphorina citri*-associated C virus (DcACV: family not assigned), *Diaphorina citri* bunyavirus (DcBV: Bunyaviridae), *Diaphorina citri* cimodo-like virus (DcCLV: Reoviridae), *Diaphorina citri* densovirus (DcDNV: Parvoviridae), *Diaphorina citri* flavi-like virus (DcFLV: Flaviviridae), *Diaphorina citri* picorna-like virus (DcPLV: Picorna-like virus), and *Diaphorina citri* reovirus (DcRV: Rheoviridae) (37–40).

Traditional viral screening often uses PCR and qPCR (38, 41), which depend on prior knowledge of the viral sequences. The technology of metatranscriptomics, based on high-throughput sequencing, can accurately and efficiently determine the RNA viromes present in tissue and environmental samples (42), including plants (43), mammals (44), and arthropods (39). In this study, metatranscriptomics and transmission electron microscopy were used to discover to uncover the composition of the viromes of ACP that may have been acquired from bud grafts taken from *C*Las+ and *C*Las– plants, to determine if the virome composition differs due to coinfection with *C*Las. Two viruses, the brassica yellows virus and the porcine reproductive and respiratory syndrome virus, are the first reports in ACP.

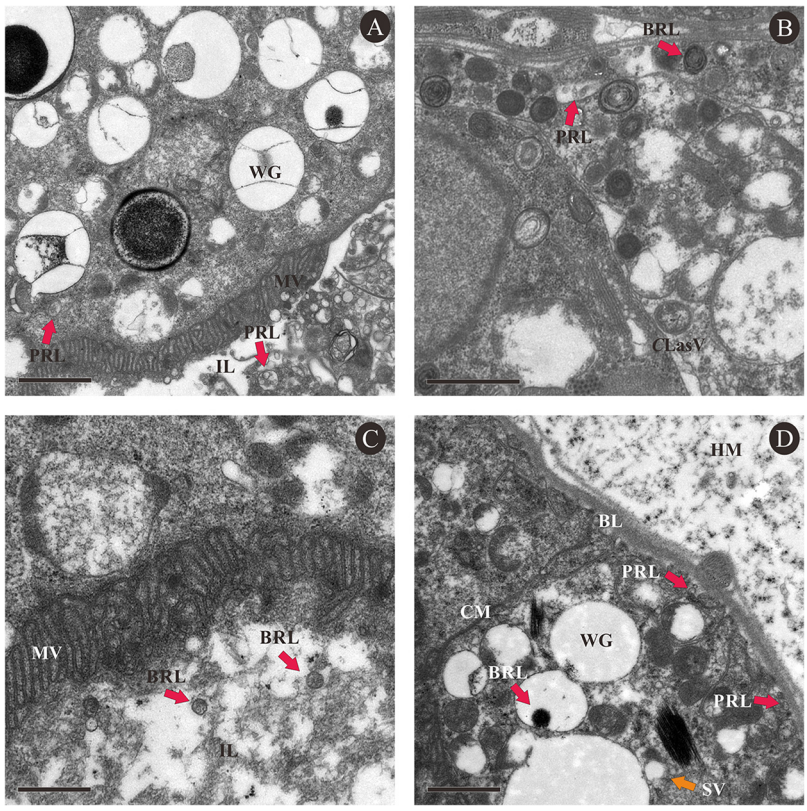

FIG 1 Transmission electron micrographs of cross section of gut tissues of *C*Las infected *D. citri*. A and B, cross section of filter chamber; C and D, cross section of midgut loop. Red arrows indicated viruses. MV, microvilli; WG, White secretory granule; ML, Muscle layer; MT, mitochondrion; IL, Intestinal lumen; CM, Cell membrane; HM, Hemocoel; SV, Secretory vesicle; BRL, BRYV-like; PRL, PRRSV-like; *C*LasV, *C*Las vertical section; *C*Las oblique section; *C*LasO, *C*Las oblique section; *C*LasC, *C*Las cross section. Scale bar 1000 nm.

RESULTS

TEM shows that viruses colonized in the filter chamber and midgut loop of the *C*Las+ ACP and passed into intestinal epithelial cells by incorporation of secretory granules (Fig. 1 A, D). Large accumulations of BrYV-like and PRRSV-like particles can be observed in the apical region close to microvilli (MV) in the intestinal lumen (Fig. 1C), close to the basal lamina (BL) (Fig. 1D), or between these locations (Fig. 1A, B). In addition, degradation of the intestinal microvilli was detected (Fig. 1A, C).

A total of 38,676,951 and 34,843,908 clean reads were obtained from the *C*Las– and *C*Las+ samples, respectively. The presence of viral sequences from over 13 different viral families were identified from the Kaiju classification (Fig. 2) and a BLAST search, including DcDNV, DcPLV, DcRV, and citrus tristeza virus (CTV: Closteroviridae) (Table S1). The highest proportion of reads derived from both the *C*Las– and *C*Las+ samples came from the Microviridae. However, distinct differences in the proportions of reads from the other virus family were detected between the two sets of samples. Most notably, the *C*Las+ samples contained a high proportion of reads from the Parvoviridae and a smaller proportion from the Phycodnaviridae. In addition, two viruses, BrYV and porcine reproductive and PRRSV, were only detected in the *C*Las+ samples (Table S1).

Putative insect-specific viruses. First, DcDNV sequences were identified in *C*Las+ (2 contigs) and *C*Las– (3 contigs) samples of the Guangdong population. These sequences ranged from 326 to 2,339 nucleotides (nt) in length and showed >98% similarity to an isolate from a Taiwan population. Second, a BLAST search showed that contigs of 9,951 and 9,920 nt amplified from *C*Las+ and *C*Las– samples both showed 96.0% similarity to DcPLV found in an ACP population in Brazil. Lastly, a total of 111 contigs from

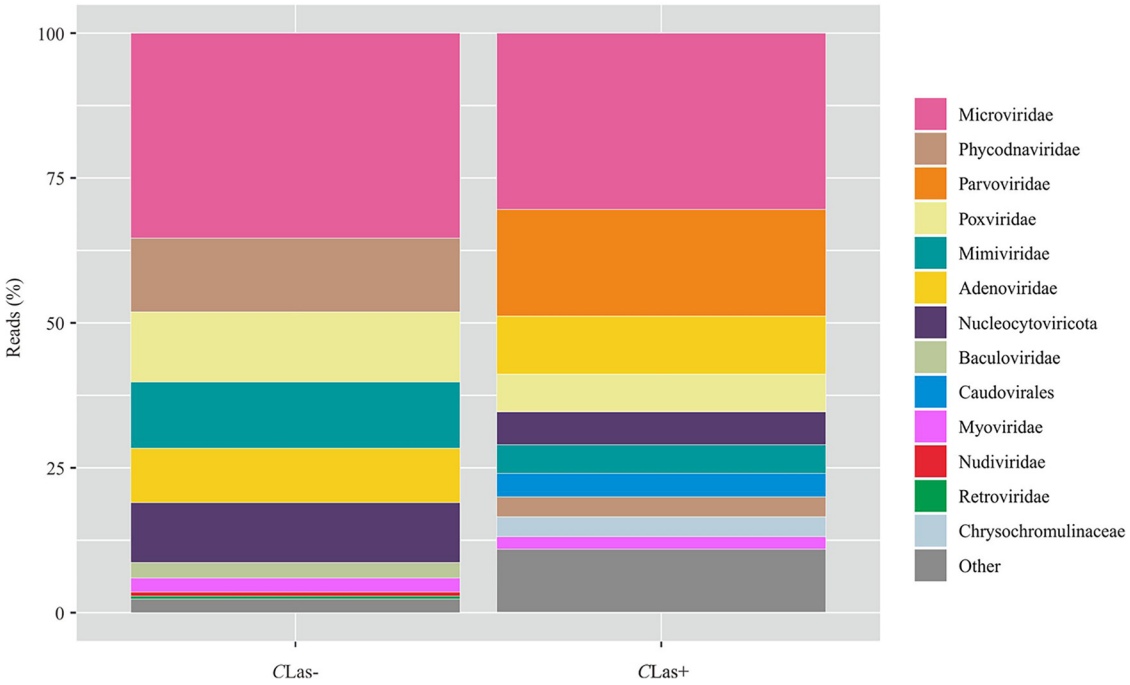

FIG 2 Viral composition of *C*Las-and CLas+ samples of *D. citri*. Relative proportions were determined by the number of reads classified to each virus clades using Kaiju.

*C*Las+ samples and 10 contigs from *C*Las– samples were identified as DcRV and showed >99% similarity to the DcRV-FL isolate recorded in Florida.

Citrus tristeza virus. The Kaiju classification and BLAST searches confirmed that 5 and 15 contigs from *C*Las+ and *C*Las– ACP samples, respectively, as CTV sequences. The contigs ranged from 260 to 3,997 nt in length and had a close relation to the CTV-FN08 isolate from Guangdong, China.

Brassica yellows virus sequence. A contig of 5,465 nt found in the *C*Las+ sample was named as BrYV-DC; this virus was not detected in the *C*Las– samples. It has the typical genomic organization of the BrYV genome, including 6 ORFs (ORF0–ORF5; Fig. 3A). The 5′ untranslated region (UTR) region was missing, while the 3′-UTR and ORF0 were incomplete.

To compare the new BrYV isolate obtained from ACP to previously characterized BrYV isolates (Table S2), a phylogenetic analysis using maximum likelihood (ML) was performed. The analysis showed most of the BrYV isolates being in the same monophyletic clade. BrYV-DC clustered with BrYV-CC and BrYV-CR, which were both associated with the plant host *Raphanus raphanistrum* L. (wild radish; Brassicales: Brassicaceae) (Fig. 3B).

An additional contig of 2,404 nt was amplified from the *C*Las+ sample and shares 95.1% nt identity with a partial sequence of the Turnip yellows virus (TuYV)-associated RNA (TuYVaRNA; MN497833) and 94.9% nt identity with the complete sequence of beet western yellows virus (BWYV)-associated RNA (BWYVaRNA: KF533709). It has a typical genomic organization of Polerovirus-associated RNAs, encoding overlapping ORF1a and ORF1b (Fig. 4A). Compared with other TuYVaRNAs and BWYVaRNAs (Table S3), the contig from ACP forms a unique lineage in the phylogenetic tree (Fig. 4B). Thus, we propose the name "brassica yellows virus-associated RNA" (BrYVaRNA) for this newly identified RNA.

Porcine reproductive and respiratory syndrome virus. A total of 11 contigs from the *C*Las+ sample (defined as PRRSV-DC in this study), ranging from 301 to 1,664 nt in length, were identified as PRRSV type 2 (Table S1). To infer the phylogenetic position of PRRSV-DC, we obtained 1,198 PRRSV type 2 and one PRRSV type 1 isolates from NCBI (Table S4). Based on the sequences of ORF5, phylogenetic analysis showed that

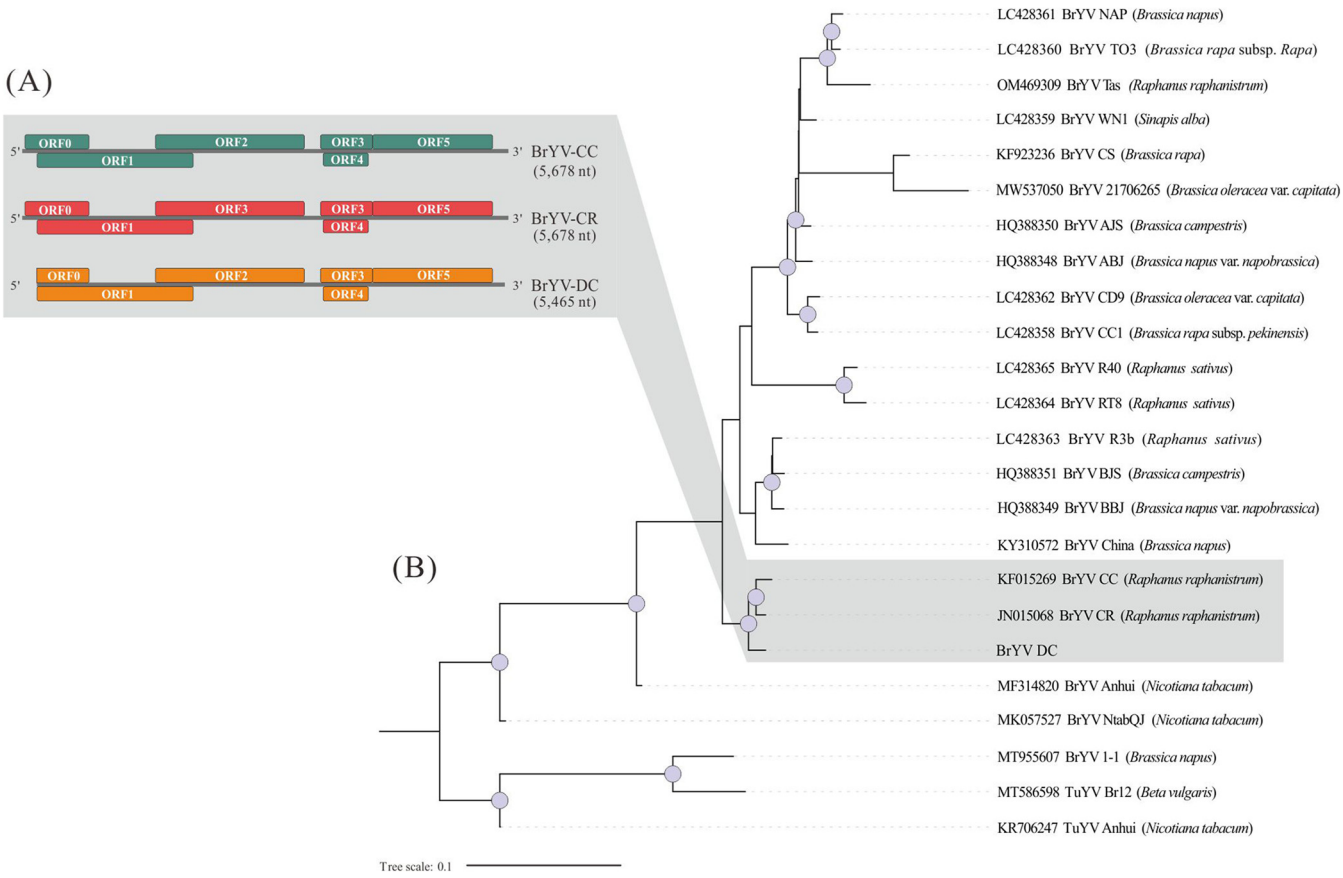

FIG 3 (A) Gene organization of BrYV-DC and its close related isolates; (B) Phylogenomic inference of BrYV using maximum likelihood method and a TIM2+F+R3 model. Circles on the nodes indicate bootstrap values ≥95.

PRRSV-DC belongs to the North American type (type 2) and grouped with isolates rV63, rV68, YN-2011, BJ4, GS2002, GS2003, and GS2004 (Fig. 5).

DISCUSSION

Brassica yellows virus sequences. BrYV usually infects cruciferous crops; however, the most recent studies of this virus have found it in tobacco (*Nicotiana tabacum* L. [Solanales: Solanaceae]) (45) and strawberry (*Fragaria* ×*ananassa* Duchesne [Rosales: Rosaceae]) (46). In this study, we report a novel BrYV isolate associated with ACP that had fed on plants grafted with *C*Las+ buds. Our findings expand the insect and plant host range of BrYV; the virus has never been reported in members of the Rutaceae nor in ACP. In addition, this is the first report of the genomic sequence of BrYVaRNA in any plant; its original host plant(s) including orchard species and roadside weeds, need (s) to be determined. Some Polerovirus-associated RNAs cause severe of symptoms when in combination with a helper virus, e.g., BWYV ST9-associated RNA and Carrot red leaf virus-associated RNA (47). In addition, associated RNAs may help viruses avoid plant immunity and enhance infection. For example, the βC1 protein encoded by the satellite tomato yellow leaf curl China virus (TYLCCNV)-associated betasatellite (TYLCCNB) interacts with DEMETER in *Arabidopsis thaliana* (L.) Heynh. (Brassicales: Brassicaceae), which facilitates DNA glycosylase activity to decrease viral DNA methylation thereby promoting viral virulence (48). This is different from our previous understanding that viruses usually evolve in the direction of reduced pathogenicity. Therefore, the exploration of the mechanisms of BrYV transmission from its usual host is needed to facilitate crop biosecurity, particularly as host substitution often leads to virus mutation and the occurrence of new strains.

Our study suggests that BrYV can be transmitted by grafting, exist in *C*Las+ plants, and be acquired by ACP. The successful colonization of BrYV in mandarin in orchards is

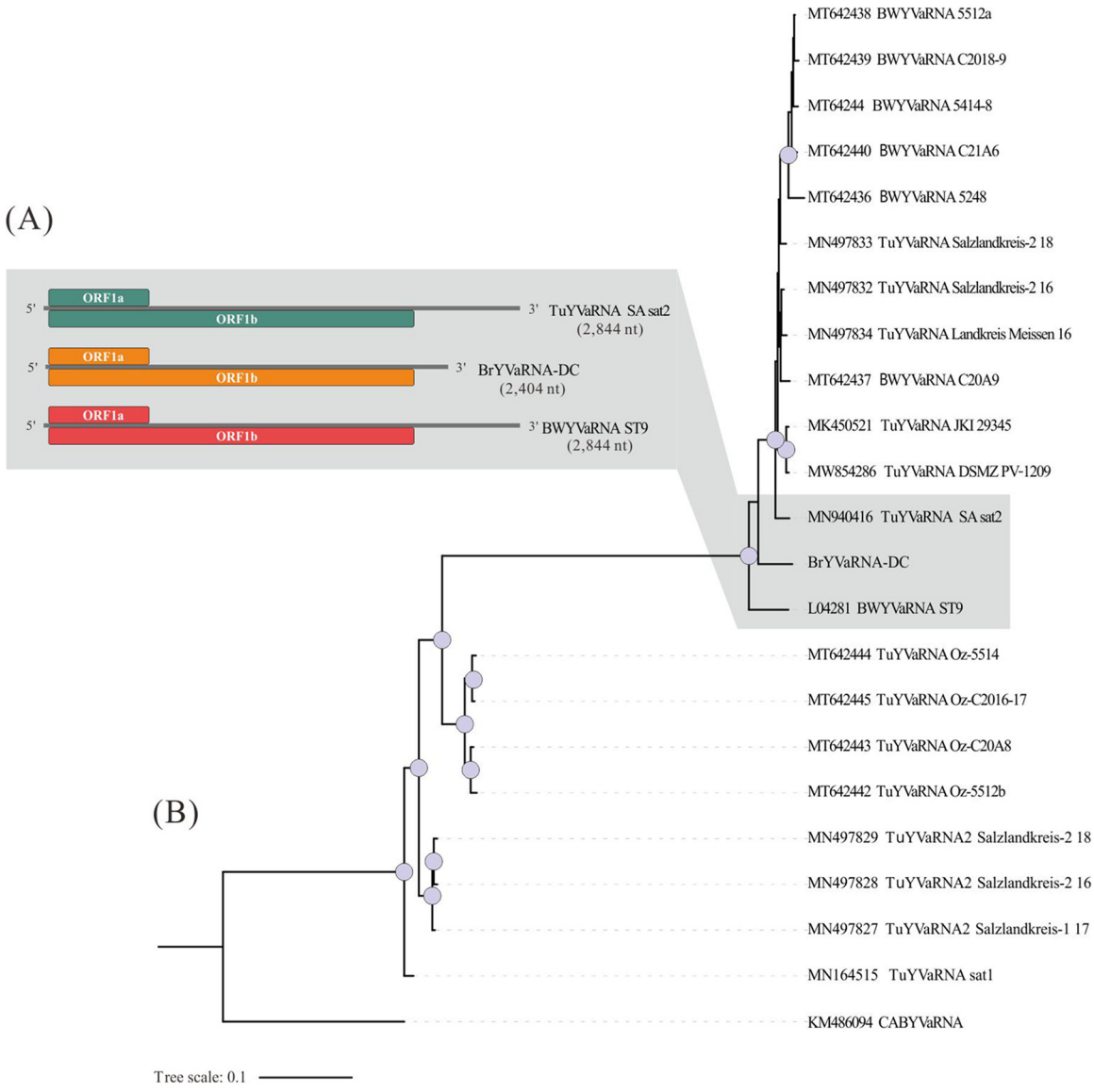

FIG 4 (A) Gene organization of BrYVaRNA-DC and its close related isolates; (B) Phylogenetic inference of virus-associated RNAs sequences using maximum likelihood method and a TVMe+R2 substitution model. Circles on the nodes indicate bootstrap values ≥95.

most likely related to transmission by other insect vectors—particularly phloem-feeding insects other than ACP—commonly found on citrus. There is also a possibility that organisms in the soil rhizosphere, phyllosphere fungi or microbial biofilms could be involved in transmission. For example, COVID-19 can be transmitted through biofilms, aerosols and wastewater (49–51). Moreover, the burning of biomass (straw and wood) in rural areas increases the black carbon, which increases the risk of COVID-19 spreading with aerosols (52). In addition, there is a strong fungal network in the rhizosphere of plants in the soil, which helps plants recruit microorganisms (53). Many viruses can be acquired by fungi and their spores (54). It has been shown that viral genomic sequences account for 0.02% of the citrus rhizosphere microbiome composition (55). The complex microbial community in the rhizosphere of plants can also protect plants from diseases (56). Whether BrYV has evolved to be transmitted by fungi needs further research. BrYV is transmitted by aphids (57) and five species, *Aphis* (*Toxoptera*) *aurantii* Boyer de Fonscolombe, *Aphis* (*Toxoptera*) *citricidus* (Kirkaldy), *Aphis gossypii* Glover, *Aphis spiraecola* Patch, and *Myzus persicae* (Sulzer) (Sternorrhyncha: Aphidae), associated with plant pathogens of citrus in China (58). Of these, *Aphis gossypii* is known to

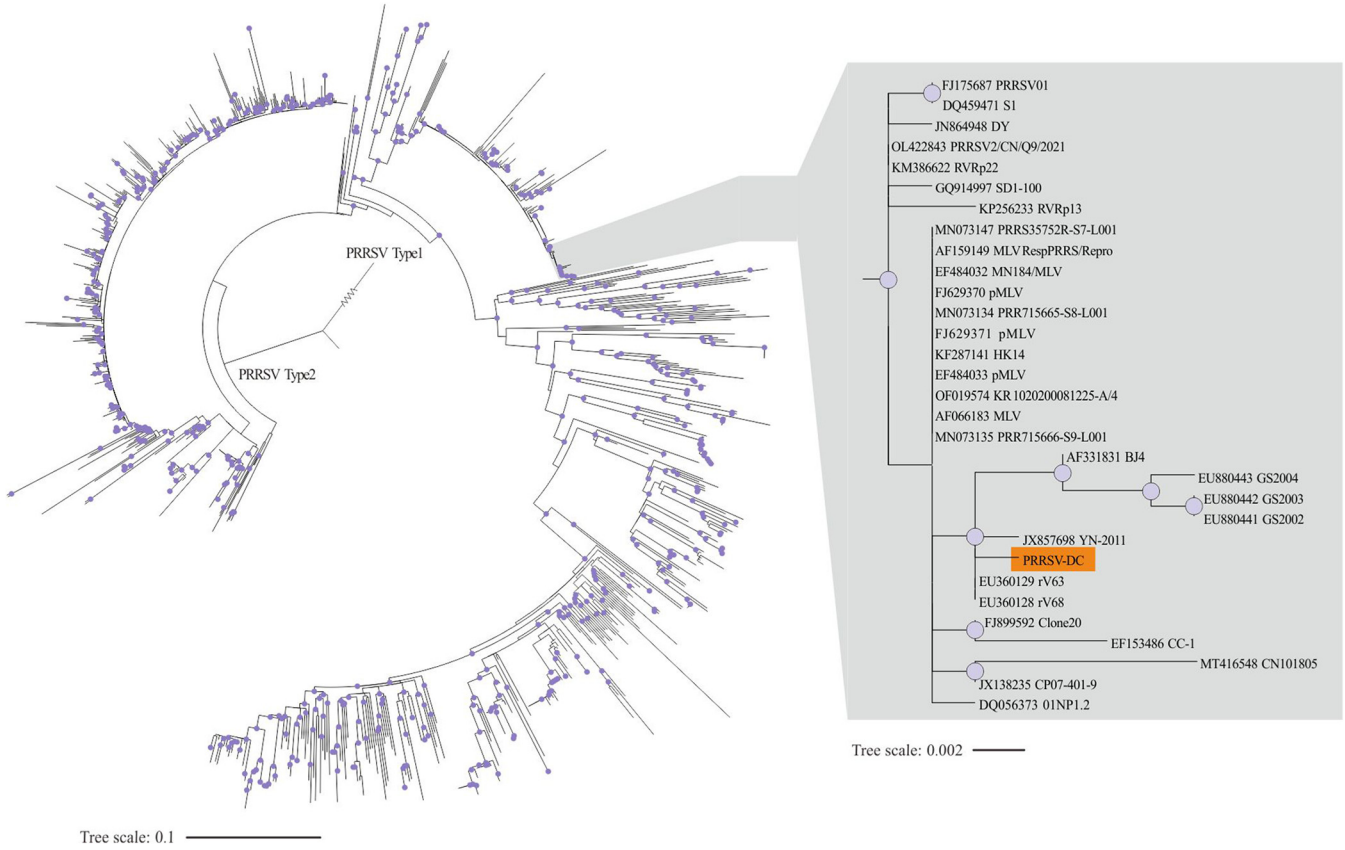

FIG 5 Phylogenetic inference of Porcine respiratory and reproductive syndrome virus (PRRSV) based on the ORF5 sequences using maximum likelihood method and a SYM+R6 substitution model. Circles on the nodes indicate bootstrap values ≥95.

feed on *Brassica rapa* L. (syn. *B. campestris* L.) and from which it can transmit turnip mosaic virus and cucumber mosaic virus 2 (59). *Aphis spiraecola* has a broad host range, feeding on plants from over 90 families (60), and can spread a range of plant viruses (61). *Myzus persicae* feeds on more than 400 plant species from over than 40 families, including various brassica species and can transmit over 100 virus diseases (62, 63) including BWYV and four other poleroviruses (13). Hence, aphids, especially *M. persicae*, should be assessed as potential vectors of BrYV from infected nonrutaceous plants to citrus; however, other phloem-feeding insects that feed on citrus should also be examined.

Our results suggest that BrYV is able to colonize the filter chamber and midgut loop of ACP. Intestinal colonization implies the potential for persistent transmission, which plays a significant role in determining transmission of the virus (64); the acquisition and transmission of this virus by ACP needs further study. The virus can colonize *C.* ×*aurantium* and the intestinal channel of ACP together with *C*Las. Further exploration of interactions between BrYV and *C*Las is needed, as virus infection can affect plant resistance to other diseases. For example, the C4 protein of TLCYnV can inhibit mitogen-activated protein kinase (MAPK) cascades and the subsequent induction of defense responses (65). Four MAPK cascades genes (CsMAP9, CsMAP9-like, CsPR12, and CsPR14) are associated with *C*Las infection (66).

Porcine reproductive and respiratory syndrome virus. Our study showed that PRRSV can be transmitted by grafting and acquired by ACP from *C*Las+ mandarin seedlings. Thus, the virus not only survives in pigs and insects (31–34), but also survives in plants. There are few reports as to how animal viruses escape from plant immunity. In the present study, PRRSV was not detected in ACP that fed on plants grafted with *C*Las-budwood. Whether infection with *C*Las influences the colonization of host plants by viruses needs to be examined. How PRRSV entered the *C*Las+ plants was not

determined, and this also requires further study. We propose three hypotheses with regard to co-colonization of citrus by *C*Las and PRRSV: (1) PRRSV can spread through a biofilm involving *C*Las in the crown and rhizosphere of plants; (2) *C*Las infection leads to increased susceptibility of citrus roots permitting entry via root damage caused by other organisms and (3) a boar or other unknown animals chewed the roots of citrus.

Biofilm production is a common trait associated with soil microorganisms (67)—viruses can attach to these structures (68) and gain protection from environmental stresses (69). PRRSV may persist in the soil due to interactions with biofilms. A species of *Chryseobacterium* was found to be the most abundant member of the rhizosphere microbiome of citrus plants (70, 71), many species of which have the ability to form biofilms and cause denitrification *in vitro* (72). Evidence for a role of biofilms in transmission comes from the fact that *C*Las can be successfully cultured with other microorganisms in a mixed-community biofilm with neutral to alkaline pH *in vitro* (pH 7.0 to 8.0) (70), and the biofilm may aid the persistence of both PRRSV and BrYV. The low concentration of *C*Las in biofilms indicates a strict dependence on the culture environment and the composition of associated bacteria (70). In the field, aerosol ammonium might facilitate biofilm formation through increasing pH—a large amounts of ammonia will be discharged around the pig farms (73). In addition, aerosols with particulate matter formed due to the burning of biomass increase the possibility of microorganisms that are adapted to neutral or alkaline humid environments (74). The complex interactivity between cloud feedback and aerosol-clouds may affect temperatures and humidity in orchards, which can be beneficial to the dispersal of viruses into plants mediated by plant transpiration (75). Furthermore, nitrogen deposition induces eutrophication and neutralizes the pH of the water, including groundwater and that in escape canals in the limestone mountains, which could increase the risk of microbial aggregation through bridging bacteria (74, 76). Due to the lack of nutrition data required by different plants at different growth stages in different climatic regions and the absence of biosensors, even intelligent water and fertilizer integration facilities often cause excessive water and fertilizer irrigation. Excessive irrigation not only wastes resource, but also provides a hotbed for pathogenic microorganisms colonization in the orchard depressions and ditches biofilm (77, 78). In addition, too much nitrogen in the soil often leads to acidification, which could be adjusted by *Chryseobacterium* colonization in citrus rhizosphere and might be beneficial to *C*Las colonization. Soil conditioners and compound fertilizers containing calcium carbonate, aluminum sulfate or iron sulfate used in groves will alter the pH of acidic soil. Additionally, calcium and magnesium ions can increase the ability of bridging bacteria to co-aggregate in biofilms in soil or wastewater (74). Hence, microbial biofilm formation in soil and groundwater might increase the risk of the co-transmission of PRRSV, BrYV and *C*Las. In addition, smelting and mining not only pollutes soil, causing heavy metal toxicity, but also increases the risk of transmission viruses and bacteria though reducing soil microbial diversity (79).

In the hypothesis of susceptibility caused by *C*Las, weakness of trees and poor immune balance of plants caused by *C*Las may lead to susceptibility to viruses. For example, sec-delivered effectors (SDE15) secreted by *C*Las can inhibit multiple members of papain-like cysteine proteases (PLCPs), which are extracellular broad-spectrum defense proteins (80, 81).

In China, pig farms are often located close to mountains, and orchards are often distributed in the mountains or at the foot of mountains; the orchard used in our study and a pig farm are located on a hillside. A route via the soil may be possible and facilitated by long-term fertilization with manure and mediated by root damage caused by soilborne pathogens. In addition, orchards are often attacked by wild boars or mice, which may also be a source of PRRSV.

Host substitution often leads to virus mutation and the occurrence of new strains. A partial genome was obtained in this study, with three situations: the virus is not fully adapted to the ACP and cannot replicate completely; the virus exists in ACP at a low concentration, suggesting that the virus cannot not replicate in insects (32, 33). The

low concentration of the virus did not permit the *de novo* assembly of the virus, but the sequence data suggest parts are mutated which could lead to the formation of a new strain.

Citrus tristeza virus. Citrus tristeza virus was detected in the ACP populations feeding of plants grafted with both *C*Las+ and *C*Las– buds. It is probable, therefore, that this virus was widely distributed in the orchard from which the buds were sourced. Only a partial genome sequence of CTV was obtained from these two ACP populations, suggesting that the virus is maintained at a low titer. Our results show that BrYV and PRRSV could colonize in the filter chamber and midgut loop of ACP that carry CTV. In Florida, presence of CTV widespread in ACP sourced throughout the state (82). This study found a relatively low abundance of CTV sequences compared to those of DcACV, as was also found in this current study in China and that of Britt et al. (82) suggest that CTV is likely a part of the citrus phloem contents consumed during ACP-feeding. Various interactions occur between liberibacters and CTV within their plant hosts. In Florida, coinfection of *C*Las with five strains of CTV did not result in any synergistic effects within different citrus cultivars as judged by symptoms or accumulation of the pathogens (83). However, a study by Fu et al. (84) found that coinfection of sweet orange with a mild-strain of CTV gave limited protection against *C*Las whereas the interaction of a severe strain and *C*Las was synergistic. Working with the '*Ca*. L. africanus' in South Africa, van Vuuren et al. (85) found that some protection against the African form of HLB was conferred by infection with CTV. Therefore, the interactions between different strains of CTV and *C*Las needs further study.

Conclusion. This study using metatranscriptomic data have shown differences the viromes of ACP that had fed on *C*Las– and *C*Las+ plants. In addition, this study is the first to find BrYV in ACP and to find its associated Polerovirus-associated RNAs, BrYVaRNA, in any host. The study also found PRRSV in the psyllid. The whole genome of BrYV and partial genome of PRRSV were *de novo* assembled. To determine the source of these viruses, further metagenomic studies should be undertaken that incorporate the plants, soils and insects that occur in and around the citrus grove. In addition, due to the potential for persistent transmission by insect vectors, transmission studies of psyllids infected with these viruses urgently need to be undertaken.

MATERIALS AND METHODS

Plant and insect material. *C*Las-infected 'Shatangju' potted mandarin trees were collected from Boluo County (23°30′50.19″ N, 114°37′3.05″ E) in east-central Guangdong province, China, and buds from them were cut and grafted onto *C*Las–free mandarin plants. Buds from the *C*Las-free plants were also budded onto *C*Las–free mandarin plants to act as controls. All the grafted citrus plants were kept in a small, insect-proof net house. After 6 months, each plant was assayed by qPCR for the presence of *C*Las with the primer pair, HLBas/HLBr, targeting the 16S rRNA gene (86). The infected (*C*Las+) 'Shatangju' plants with CT values <25, and the uninfected (*C*Las-) control with CT values >36 were chosen for further experimentation.

A laboratory strain of ACP collected from *Murraya paniculata* (L.) Jack (Aurantieae) at South China Agricultural University, Guangzhou, Guangdong, in 2013, was used in the present study. The strain had been maintained on *M. paniculata* in controlled environment chambers under a 14 h: 10 h light: dark cycle at 28 ± 1°C and 60% relative humidity for more than 25 generations.

Third to fourth instar psyllid nymphs were selected and reared on the *C*Las+ and uninfected (*C*Las–) 'Shatangju' plants for 14 days under a 14 h: 10 h light: dark cycle at 28 ± 1°C and 50–60% relative humidity. After this period, the heads of individual adult psyllids were dissected for DNA extraction using a Tiangen DNA extraction kit (Tiangen, Beijing, China) following which the *C*Las status of each individual was determined by qPCR as above.

Transmission electron microscopy (TEM). The abdomen of a *C*Las-infected ACP adult was dissected then fixed in 2.5% glutaraldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.2) at 4°C overnight. The tissue was then rinsed in 0.1 M PBS, secondarily fixed with 2% osmium tetroxide in 0.1 M PBS at room temperature for 4 h then embedded in Spurr's resin. Sections (100 nm) of abdomen tissue were cut with a diamond knife, stained with 2% uranyl acetate and alkaline lead citrate. Sections were then examined using a transmission electron microscope (Advanced Microscopy Techniques Corp., Danvers, MA, United States) and photographed using a digital camera (Morgagni 268, FEI Company, Hillsboro, OR, United States).

RNA extraction, RNA-seq and sequences assembly. Two aliquots of 20 adult *C*Las+ and two aliquots 20 *C*Las– ACP were each pooled to constitute a sample to give two samples of each psyllid type and the thoraxes and abdomens ground in liquid nitrogen. RNA was extracted from each sample using TRIzol. Three biological replicates were made from every sample to give 12 extracts in total and used for

RNA-seq. The resulting Illumina reads were trimmed using Trimmomatic v0.33 (87) to remove the adapters and low-quality sequences (Q ≤ 25). Trinity v2.13.0 (88) was then used for the *de novo* assembly of contigs from the trimmed RNA-seq reads. All data from each psyllid type were combined for analysis.

Sequence analysis. The reads and assembled contigs were preliminarily classified using the Kaiju virus database (89). Then, targeted contigs were subjected to a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Viral sequences were aligned using MAFFT v7.471 with the L-INS-I strategy (90) and maximum likelihood (ML) trees were constructed using IQ-TREE 2.0 (91) to infer the phylogenetic relationships.

Data availability. All the RNA samples are deposited in −80°C refrigerator in the College of Natural Resource and Environment of China Agricultural University. And all the raw data and clean data of metatranscriptome sequencing are deposited in the private account of corresponding author of data storage center of College of Advanced Agricultural Science of Zhejiang A&F University. The newly generated viral sequences were deposited in https://github.com/ljmhaha/data.of.ACP.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.05 MB.

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Yanjing Wang conceived the ideas and designed methodology; Lixia Zeng and Yanjing Wang collected the data; Jinming Lu, Yanjing Wang, and Paul Holford analyzed the data; Jinming Lu, Paul Holford, George A. C. Beattie, and Yanjing Wang led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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