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Guava SSR Analysis: Diversity Assessment in U.S. and Similarity to Accessions Associated with Reducing Citrus Huanglongbing in Vietnam

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Abstract

Closely interplanting citrus (*Citrus sp.* L) and guava (*Psidium guajava* L.), in Vietnam were reported to greatly slow progression of huanglongbing (HLB). It was hypothesized that volatiles in the guava may repel the Asian citrus psyllid (ACP, *Diaphorina citri* Kuwayama) which vectors the HLB causal bacterial agent. To date, field confirmation of ACP being repelled by guava has not been demonstrated in the U.S., but it was not known whether the guava selections used were the same as those reported to be effective in Vietnam. In this study, SSR (simple sequence repeat) analysis was conducted on all readily accessible U.S. guava accessions, as well as material from three Vietnamese citrus/guava orchards, to determine whether Vietnamese guava genotypes reported to suppress ACP were already available in the U.S. and to assess diversity of guava available in the U.S. Accessions included 60 distinct sources, and multiple samples were collected from 10 of these as an internal check. Ten SSR primer pairs were used in the analysis. Alleles per locus ranged from 4 to 8, with an average of 6.2. Forty different genotypes were identified, as several accessions appeared to be synonymous based on this analysis. The cluster analysis using the neighbor-joining method revealed five distinct affinities. The Vietnamese accessions were placed in two of the five major clusters. Several guava varieties in Florida are in the same clusters as the Vietnamese cultivars. However, the sweet pink-fleshed varieties that have predominated in the western hemisphere did not cluster with the accessions from Vietnam.

The guava (*Psidium guajava*) is an evergreen tree in the Myrtaceae family. It is native to tropical America, likely originating in the area of Southern Mexico into Central America (Morton, 1987). It has been widely dispersed by man, and is now grown throughout the tropics and subtropics of the world, where it is enjoyed as a fresh fruit and processed into juice, jelly and paste. Guava has been a dooryard fruit, minor commercial product, and significant invasive threat in Florida for many years (Crane and Balerdi, 2005). Introduction of huanglongbing (HLB) into Florida compelled renewed interest in guava, since closely interplanting citrus and guava in Vietnam was reported to greatly slow progression of HLB (Beattie et al., 2006; Gottwald et al., 2010). It was hypothesized that volatiles from guava plants may repel the Asian citrus psyllid (ACP, *Diaphorina citri*) which vectors the HLB causal bacterial agent. Laboratory studies by Silva et al. (2016) confirmed that mature guava leaves and essential oils from guava exhibited repellency to the psyllid. To date, field confirmation of ACP being repelled by guava

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has not been demonstrated in the U.S., but it was not known whether the guava selections used were the same as those reported to be effective in Vietnam.

In this study, SSR analysis was conducted on all readily accessible U.S. guava accessions, as well as material from three Vietnamese citrus/guava orchards, to determine whether Vietnamese guava genotypes were already available in the U.S. and to assess diversity of guava available in the U.S.

Materials and Methods

Plant material studied included all available guava accessions from the USDA-ARS, National Clonal Germplasm Repository (NCGR), in Hilo, Hawaii and the University of Florida, Tropical Research and Education Center (TREC) in Homestead, Florida. Samples were also collected and imported under regulatory permit from three Vietnamese citrus/guava plantings where psyllids were reported to be virtually absent despite being abundant in nearby citrus-only plantings (Hall and Gottwald, unpublished) and also included some additional guava cultivars available from commercial nurseries in South Florida. Accessions numbered 60 distinct sources, and multiple samples were collected from 10 of these as an internal check. Accessions in this study are described in Table 1.

Young leaves from near the shoot-tip of rapidly growing guava trees were collected and rapidly dried between blotting paper in sealed, labeled envelopes, which were placed in plastic bags with approximately 20 grams of Drierite (W.A Hammond Drierite Company LTD., Xenia, OH, USA). Total DNA was extracted from approximately 20 mg of dried leaf tissue using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol.

Table 1. Guava accessions, along with source, tested in this study

Sample ID	Germplasm	Direct source	Original source and notes on accession	Flesh color	Cluster
FL02	Tree 1,'Xaly nghi'	Collected in Vietnam	Collected interplanted with citrus Vietnam	white	1a
FL04	*Tree 2 sample 1,'Xaly nghi'* [z]	Collected in Vietnam	Collected interplanted with citrus Vietnam	white	1a
FL05	*Tree 2 sample 2, 'Xaly nghi'*	Collected in Vietnam	Collected interplanted with citrus Vietnam	white	1a
FL06	*Tree 2 sample 3,'Xaly nghi'*	Collected in Vietnam	Collected interplanted with citrus Vietnam	white	1a
FL07	*Tree 2 sample 4,'Xaly nghi'*	Collected in Vietnam	Collected interplanted with citrus Vietnam	white	1a
FL10	*Tree 3 sample 1,'Xaly nghi'*	Collected in Vietnam	Collected interplanted with citrus Vietnam	white	1a
FL11	*Tree 3 sample 2,'Xaly nghi'*	Collected in Vietnam	Collected interplanted with citrus Vietnam	white	1a
HPSI-50	Alahabad Safeda	USDA ARS NCGR Hilo, HI	Collected Australia	white	1b
IFAS19	White Indonesian Seedless (Fla.)	Collection U of Florida, TREC	Unknown introduction	white	1b
IFAS25	3-2 [y]	Collection U of Florida, TREC	Old map says "RxS?", (presumably Ruby x Supreme)	lost	1b
FL19	Tree 1, White Seedless	South Florida Nurseries	Pine Island Nursery, Miami, FL	white	1c
FL20	*Tree 2, White Seedless* [z]	South Florida Nurseries	Pine Island Nursery, Miami, FL	white	1c
HPSI-61	Pearl Guava [y]	USDA ARS NCGR Hilo, HI	Taiwan Nat. Pingtung Polytech. Inst	white	1c
IFAS15	Thai Maroon	Collection U of Florida, TREC	Donated 1995 from Tenom, Malaysia	purple	1c
HPSI-33	Allahabad Safeda	USDA ARS NCGR Hilo, HI	U HI, collected in India	white	1d
HPSI-44	Bon Dov	USDA ARS NCGR Hilo, HI	U HI, acquired as seed from Israel	white	1d
HPSI-47	Uma	USDA ARS NCGR Hilo, HI	Donated from San Diego, CA	white	1d
IFAS10	Indonesian Red	Collection U of Florida, TREC	Unknown introduction	dark pink	1d
HPSI-06	Patillo	USDA ARS NCGR Hilo, HI	U HI, indirectly from Ruehle TREC	pink	2a
HPSI-34	Fan Retief	USDA ARS NCGR Hilo, HI	U HI, selected in South Africa	pink	2a
HPSI-35	Ka Hua Kula	USDA ARS NCGR Hilo, HI	U HI, OP seedling of Beaumont	pink	2a
HPSI-37	Beaumont	USDA ARS NCGR Hilo, HI	U HI, OP seedling of Beaumont	pink	2a
HPSI-42	Diminuitive [y]	USDA ARS NCGR Hilo, HI	Local donation HI	pink	2a
HPSI-16	Puerto Rico 2	USDA ARS NCGR Hilo, HI	U HI, collected Puerto Rico	pink	2b
IFAS04	Blitch	Collection U of Florida, TREC	Grafted from seedling in West Palm Beach, FL 1945	pink	2b
IFAS06	Giant Vietnamese	Collection U of Florida, TREC	From CA Trop. Fruit Nursery, Vista, CA	white	2b
IFAS17	TREC 53-6550 [x]	Collection U of Florida, TREC	Seedling of 31-18 x Queen made at TREC 1953	lost	2b

Table 1. Guava accessions, tested in this study, continued

Sample ID	Germplasm	Direct source	Original source and notes on accession	Flesh color	Cluster
HPSI-17	138-T [y]	USDA ARS NCGR Hilo, HI	U HI	pink	2c
HPSI-19	Kona 1 [y]	USDA ARS NCGR Hilo, HI	U HI	pink	2c
IFAS03	Blanca	Collection U of Florida, TREC		pink	2c
IFAS12	Patillo	Collection U of Florida, TREC	Air-layer seedling in Deland, FL 1953	pink	2c
IFAS26	4-1 [x]	Collection U of Florida, TREC	Old map says Patillo	pink	2c
IFAS27	4-2 [x]	Collection U of Florida, TREC	Old map says Patillo	pink	2c
IFAS28	4-3 [x]	Collection U of Florida, TREC	Old map says Patillo	pink	2c
FL08	Tree 1 sample 1, 'Bom'	Collected in Vietnam	Collected interplanted with citrus Vietnam	white	3a
FL09	*Tree 1 sample 2, 'Bom'* [z]	Collected in Vietnam	Collected interplanted with citrus Vietnam	white	3a
HPSI-27	J.B. White [y]	USDA ARS NCGR Hilo, HI	U HI, seed collected Singapore farmer's orchard	white	3a
HPSI-51	Khao Niyom	USDA ARS NCGR Hilo, HI	U HI, collected Thailand	white	3a
HPSI-60	Klom Toonklao	USDA ARS NCGR Hilo, HI	U HI, collected Thailand	white	3a
FL01	Tree 1, Thai White, source 1	South Florida Nurseries	Khemara Farms, Homestead	white	3b
FL03	Tree 2, Thai White, source 1	South Florida Nurseries	Khemara Farms, Homestead	white	3b
FL17	Tree 1, Thai White, source 2	South Florida Nurseries	Khemara Farms, Homestead	white	3b
FL18	Tree 2, Thai White, source 2	South Florida Nurseries	Khemara Farms, Homestead	white	3b
HPSI-53	Klom Sa Lee	Germplasm repository- Hilo, HI	U HI, collected Thailand	white	3b
HPSI-55	Khao Sawaive	Germplasm repository- Hilo, HI	U HI, collected Thailand	white	3b
IFAS02	Asian White	Collection U of Florida, TREC	Selection from Lara Farm and Nursery, Homestead, FL	white	3b
HPSI-32	Lucknow 49	USDA ARS NCGR Hilo, HI	U HI, collected in India	white	4
HPSI-38	Poamoho Pink [y]	USDA ARS NCGR Hilo, HI	U HI selection	pink	4
IFAS01	Alhabari White	Collection U of Florida, TREC	From CA Trop. Fruit Nursery, Vista, CA	white	4
IFAS11	Mexican Crème	Collection U of Florida, TREC	From CA Trop. Fruit Nursery, Vista, CA	off-white	4
IFAS20	Barbie Pink	Collection U of Florida, TREC	Selection Florida Colors Nursery, Homestead, FL	pink	4
IFAS21	Asian-2	Collection U of Florida, TREC		white?	4
FL13	Tree 1, Barbie Pink	South Florida Nurseries	Pine Island Nursery, Miami, FL	pink	5
FL14	*Tree 2, Barbie Pink* [z]	South Florida Nurseries	Pine Island Nursery, Miami, FL	pink	5
FL15	Tree 1, Ruby Supreme	South Florida Nurseries	Pine Island Nursery, Miami, FL	pink	5
FL16	*Tree 2, Ruby Supreme* [z]	South Florida Nurseries	Pine Island Nursery, Miami, FL	pink	5
HPSI-14	Ruby x Supreme	USDA ARS NCGR Hilo, HI	U HI, indirectly from Ruehle TREC	pink	5
HPSI-15	Hong Kong Pink	USDA ARS NCGR Hilo, HI	U HI, collected Hong Kong, 1958	pink	5
HPSI-20	Waiakea	USDA ARS NCGR Hilo, HI	U HI selection	pink	5
HPSI-26	Gushiken Sweet [y]	USDA ARS NCGR Hilo, HI	BioEnergy Dev. Co.	pink	5
IFAS07	Hawaiian White Indonesian	Collection U of Florida, TREC	From Plant It, Hilo, HI	white	5
IFAS08	Homestead (Ruby x Supreme 6-29)	Collection U of Florida, TREC	Cross of Ruby x Supreme made at TREC 1945, released as 'Homestead'	pink	5
IFAS09	Hong Kong Pink	Collection U of Florida, TREC	Donated 1995 from Tenom, Malaysia	pink	5
IFAS13	Queen	Collection U of Florida, TREC	Unknown introduction	pink	5
IFAS14	Ruby x Supreme 10-30	Collection U of Florida, TREC	Cross of Ruby x Supreme made at TREC 1945	pink	5
IFAS16	TREC 51-4967 [x]	Collection U of Florida, TREC	Introduced from Saharanpur Fruit Res. Station India in 1951	pink	5
IFAS22	1-1 [x]	Collection U of Florida, TREC	Old map says RxS? (possible Ruby x Supreme in notes)	pink	5
IFAS23	2-1 [x]	Collection U of Florida, TREC	Old map says RxS? (possible Ruby x Supreme in notes)	pink	5
IFAS24	3-1 [x]	Collection U of Florida, TREC	Old map says RxS? (possible Ruby x Supreme in notes)	pink	5
IFAS29	5-1 [x]	Collection U of Florida, TREC	Old map says RxS? (possible Ruby x Supreme in notes)	pink	5

[z] italicized samples were blind replicates of preceding accessions
[y] unverified as a cultivar name
[x] numbered accession in collection

PCR amplifications were performed using GeneAmp PCR system Thermal Cycler (model 9700, Thermo-Fisher Scientific, Waltham, MA) in total 20-µl volume reactions following typical protocols (Dangl et al., 2005). Each sample was analyzed at ten SSR loci: mPgCIR05, mPgCIR07, mPgCIR09, mPgCIR10, mPgCIR11, mPgCIR13, mPgCIR19, mPgCIR22, mPgCIR25, and mPgCIR26 (Risterucci et al., 2005). Forward primers were labeled with one of four fluorescent dyes. Fragment amplifications were verified on 2% agarose gels.

Samples were prepared for capillary electrophoresis by diluting 1.0 µl of amplified product and 0.4 µl of the internal size standard 400HD ROX (ABI) in 12 µl of formamide. Typically, products from four loci labeled with different fluorescent dyes were multiplexed in PCR and thus also in electrophoresis. Amplified fragments were separated by electrophoresis using a Genetic Analyzer (ABI Prism 3100, Thermo-Fisher Scientific, Waltham, MA) using 22 cm capillary with 3100 POP-4 as the matrix, (Dangl et al., 2005).

Genescan (Version 3.1, Thermo-Fisher Scientific, Waltham, MA) and Genotyper (Version 2.5, Thermo-Fisher Scientific, Waltham, MA) were used to assemble data as microsatellite genotypes as well as in binary format.The Nei and Li distance (Nei and Li, 1979) were calculated on the binary data based on proportion of alleles shared between two accessions for all possible pair-wise combinations. The matrices generated were used for cluster analysis using the neighbor-joining (NJ) method (Saitou and Nei, 1987) producing an unrooted additive phenetic tree. From the results of the NJ cluster analysis, multilocus SSR genotype data were pooled into groups and analyzed for within-group genetic variability such as mean number of alleles per locus and observed and expected levels of heterozygosities. The heterogeneity among groups was determined using contingency χ^2 analysis. F-statistics (Wright, 1965) were used to determine genetic differentiation within and among groups.

Results and Discussion

Marker data were collected for the all accessions, including the guava accessions from Vietnam and the phenotypically-similar accessions already in Florida (‘Thai White’ and ’White Seedless’ which should not be considered cultivar names, but groups of similar cultivars with phenotypic fruit characteristics in common). Forty different genotypes were identified, as several accessions appeared to be synonymous based on this analysis. The cluster analysis using the neighbor-joining method revealed five distinct affinities. The genetic differentiation within and among the five groups showed marked differentiation (F_{ST} = 0.325) and inbreeding was slight (F_{IS} = 0.154). For the ten accessions where we ran multiple samples, the samples had a perfect match, except that one differed by one allele at one locus compared to its duplicate, reflecting a very low error rate. All further discussion of genotypic similarity focus on the dendrogram of genetic distance in Fig. 1.

Comparison to Vietnamese accessions. The guava accessions directly from Vietnam, where they were reported to suppress HLB when interplanted with citrus, are similar to some accessions already in the US, but not identical. It is reported that the material collected directly from Vietnam are the cultivars ‘Xaly nghi’ and ‘Bom’. ‘Xaly nghi’ was in Cluster 1 of the dendrogram (three separate trees with tree 1 labelled FL02, multiple samples of tree 2 labelled FL04 through FL07, and two samples from tree 3 labelled FL10 and FL11), and were in the same cluster as material obtained from a South Florida nursery and labelled ‘White Seedless’ (FL19 and duplicated as FL20). The second Vietnamese genotype, ‘Bom’, was in Cluster 3 of the dendrogram and was most closely related to three accessions at the Hilo repository, J.B. White, ‘Khao Niyom’, and ‘Klom Toonklao’ (HSPI 27, 51, and 60 respectively). This Vietnamese accession was fairly similar (different subgroups of cluster 3) to the material obtained from South Florida nurseries that was designated ‘Thai White’ (FL1, FL2, FL17, and FL18).

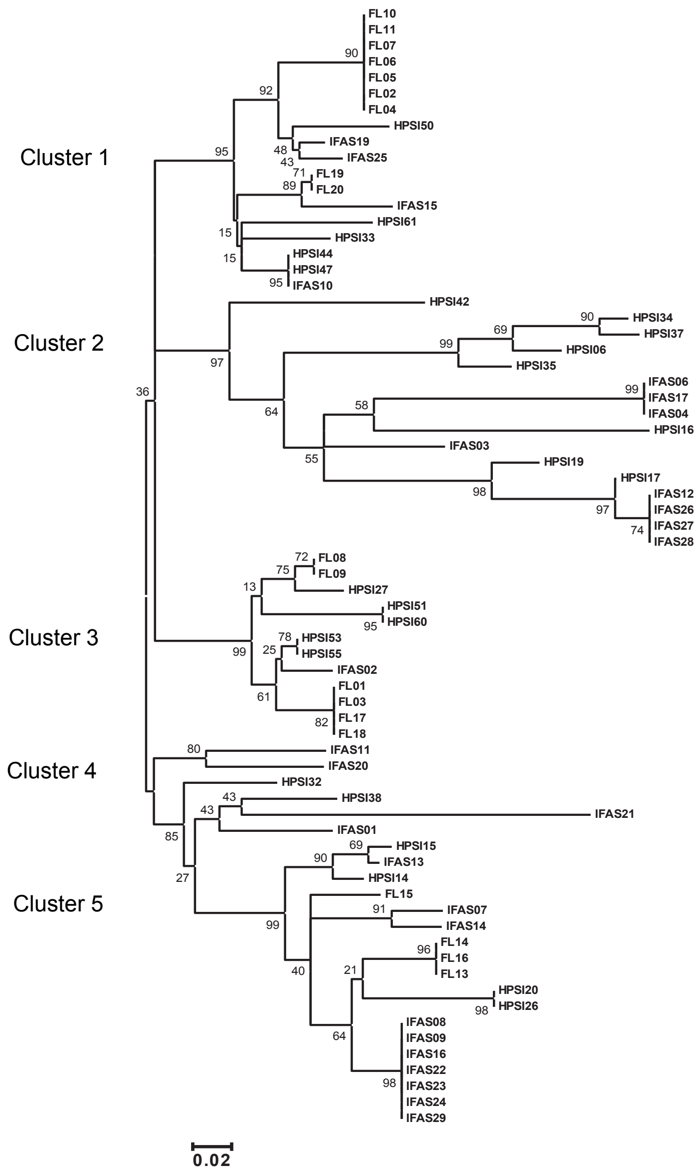

Figure 1. Unrooted neighbor joining tree with Nei and Li (1979) genetic distance designations, for ten guava SSR markers. Identity of guava accessions tested are indicated in Table 1.

Association of SSR fingerprints with pulp color. Some dendrogram clusters correlated with flesh pigmentation. Clusters 1 and 3 were virtually entirely white fleshed accessions, except that the highly pigmented 'Thai Maroon' (IFAS15), with red peel, flesh, and leaves and 'Indonesian Red' (IFAS10) which has dark pink flesh which are included cluster 1. In contrast, cluster 2 and cluster 5 are almost entirely pink-fleshed (exception is IFAS06 in cluster 2 and IFAS07 in cluster 5) and cluster 4 is a mixture of flesh colors. Nimisha et al. (2013) reviewed guava breeding and cited several Indian papers indicating that white flesh color is recessive to red, is monogenically inherited, and white flesh is linked to seedlessness. Chromosomal segments containing the white-fleshed gene may be associated with several SSRs which would contribute to the observed association between clustering and flesh pigmentation. There are earlier reports that SSR dendrogram clusters showed association with flesh color (Kanupriya et al., 2011; Sitther et al., 2014).

Apparent mis-naming of accessions. When multiple accessions with the same name were compared, a number of seeming discrepancies in identity, as well as potential solutions to mysteries, were found in this study. Two accessions are maintained at NCGR-Hilo under the name 'Alahabad Safeda'. The dendrogram places both of these accessions in the same cluster but with different fingerprints, and the curator of the collection had noted that the fruit had different characteristics. 'Patillo' from the Florida and Hawaii collections were different, but again in the same cluster. It has been noted that 'Patillo' in Hawaii produces an acid fruit, while in Florida it is subacid (Morton, 1987). This reported difference may largely reflect differing genotypes. 'Patillo' was selected from a seedling population in Deland, Florida, and the two accessions in this study may reflect different selections from the same population. 'Hong Kong Pink', which Brooks and Olmo (1997) reported as a Hawaiian seedling selection of seeds from Hong Kong while Lim and Khoo (1990) reported as being a cultivar from Hong Kong, was evaluated from collections in both Florida and Hawaii. The material from Florida (sourced in Malaysia) was different from the Hawaiian 'Hong Kong Pink', and was identical to a group of Florida accessions, including the released cultivar 'Homestead'.

Four accessions recently collected in Thailand are actually two genotypes with two names for each: 'Klom Toonklao' matched 'Khao Niyom' and 'Klom Sa Lee' matched 'Khao Sawaive'. These are all large, crispy white fleshed dessert types grouping in cluster 3.

Unexpected synonymy was also observed. 'Bon Dov' and 'Uma' from the NCGR-Hilo were identical in fingerprint and also matched 'Indonesian Red' from Florida. The two NCGR-Hilo selections are white fleshed and similar in appearance (NCGR-Hilo, 2016), and 'Bon Dov' is reported to be true from seed (GRIN Global), while 'Indonesian Red is dark pink. Three accessions from Florida ('Blitch', 'Giant Vietnamese', and TREC 53-6550) shared a fingerprint with 'Puerto Rico-2' from Hawaii. 'Waiakea 11-26' matched Gushiken Sweet from NCGR-Hilo.

Seven Florida accessions had identical fingerprints: 'Homestead', 1-1, 2-1, 3-1, and 5-1 are all known 'Ruby' x 'Supreme' seedlings. Surprisingly TREC 51-4967 and IFAS 09 'Hong Kong Pink' also had identical fingerprints to accessions in this group. The red-fleshed 'Ruby' mother originated from seed brought from Peru by Wilson Popenoe (P.I. 81849) and the white-fleshed 'Supreme' is a seedling selection made by George Ruehle at the University of Florida (Ruehle, 1946). Ruehle crossed 'Ruby' x 'Supreme' in 1945 and the resulting seedlings were tested for decades before the release of 'Homestead' in 1989 (Campbell 1989). A majority of the trees produced large crops of high quality fruit, and another seedling tree from the original cross, labelled 10-30, was also considered for release. Campbell indicated (1989) "Planting material was also distributed to persons at many other locations in Florida and other states and countries, where the trees proved to be highly productive and were considered to have fruit of excellent quality". Prior to the official release, nurseries were selling 'Homestead' as 'Ruby' x 'Supreme'. Campbell specifically indicated that 'Homestead' does not come true from seed. It seems likely that the accessions from IFAS labelled 1-1, 2-1, 3-1, and 5-1 were clonal propagations of 'Homestead'. TREC 51-4967 is likely 'Homestead' from budwood reimported from material transferred to India, and 'Hong Kong Pink' reimported from Malaysia, or material was mixed up at some point. The Hilo repository accession of 'Ruby' x 'Supreme', differs from the IFAS 'Ruby' x 'Supreme' 10-30 by only one allele at one locus (one allele missing, homozygous rather than heterozygous) and may reflect a read error or mutation. The two different 'Ruby' x 'Supreme', purchased from a commercial nursery, also differ by one allele at 1-2 loci from both 'Homestead' and 'Ruby' x 'Supreme' 10-30. They may be other seedlings from the original cross. The University of Florida guava collection has received little attention in the last twenty years, due to funding issues. However during the last 15-20 years, a small but vibrant commercial pink- and white-pulped fresh guava industry has existed in Florida (~250 ha valued at $7.7 million) (Garcia et al., 2016). Even actively maintained germplasm collections have misnamed material, so this is a very real possibility in this guava collection.

Another potential explanation for these abundant synonyms is that apomixis may occur in guava in some conditions. To our knowledge, there is no published report of apomixis in *Psidium guajava*. There are anecdotal reports that 'Bon Dov' (HPSI-44, collected as seed from Israel) is true from seed (indicated in GRIN global 2017). From the Hilo accessions the fingerprints are identical for 'Bon Dov' and 'Uma' (HPSI-47 collected as vegetative material from San Diego, CA, USA), and the fruit are very similar in appearance. The related *Syzygium jambos* and some other members of the Myrtaceae are reported to display apomixis (reviewed in Lughadha and Proenca, 1996). However, Ruehle (1948) noted that in the 1940s "practically all of the commercial (guava) orchards in Florida are of seedling trees" though "choice varieties can be increased only by some vegetative means".

Other reports on genotyping NCGR-Hilo guavas. Another project on SSR relationship of the NCGR-Hilo guava accessions was conducted shortly after the work reported here, but published several years ago (Sitther et al., 2014). This study used primers for 20 SSRs identified by Risterucci et al. (2005), encompassing the ten SSRs selected for this study. Our results are largely similar where we found identical fingerprints for 'Bon Dov' and 'Uma', Gushiken Sweet and 'Waiakea', 'Khao Sawaive' and 'Klom Sa Lee', but Sitther et al. (2014) found the pairs nearly identical. Most clustering is also similar although in our study Pearl Guava did not cluster with the recent Thai accessions, unlike in Sitther et al. (2014) but did with 'Bon Dov' and 'Uma' which agrees with the earlier report. The additional SSR markers may have contributed to some differences in grouping but seemingly not in finding accessions nearly identical.

Possible relevance to citrus protection. This study was initiated following initial positive reports from plantings in Vietnam, that citrus interplanted with guava displayed greatly slowed HLB development (Beattie et al., 2006). There have been many subsequent reports, largely indicating variable responses in the field and consistently showing ACP repellence in the laboratory. In field trials in Vietnam (Ichinose et al., 2012) an orchard of citrus interplanted with guava was uninfected by CLas at 12-16 months, while the comparable guava-free citrus trees had 20% infection. However, almost all trees were infected at 30 months.

In a Florida field study, citrus interplanted with the pink 'Beaumont' showed suppressed ACP infestation, but no reduction in HLB development, while citrus trees interplanted with the white Vietnamese guava 'Xaly nghi' showed no reduction in ACP infestation or HLB development (Gottwald et al., 2014). In the same report, citrus nursery trees closely interplanted with guava displayed lower ACP infestation and lower percentage of trees CLas positive than in a nearby nursery of citrus only. The authors concluded that the suppression observed was not sufficient to merit commercial use. They also noted that guava cultivars used may be different from those shown to be effective in suppressing HLB in Vietnam, and thus may differ in volatiles proposed to suppress ACP infestation. An additional concern for field implementation is that both pink and white-fleshed guavas tested in Florida were highly susceptible to freeze and root-knot-nematode damage (Hall et al., 2013). Several efforts have been reported to verify ACP avoidance from guava (Barman et al., 2016; Zaka et al., 2010; Hall et al., 2008; Silva et al., 2016;) and attempts to identify the volatile(s) associated (Mann et al., 2011; Onagbola et al., 2011; Zaka et al., 2015). Several of these studies compared two guava selections and one compared oil from five guava cultivars for ACP repellency, showing they were all effective but not different from each other (Silva et al., 2016). The evidence seems to overwhelmingly support that guava does have some degree of ACP repellence. Given the diversity in guava and likely variability in volatile production, it may merit greater attention to identify selections that are particularly effective at repelling ACP.

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Correction

On the inside cover of the April issue (volume 72, number 2), the name of an Advisory Committee member was misspelled and should read Sara Serra rather than Sera Serra.