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GENETIC VARIATION AMONG CULTIVATED SELECTIONS OF MAMEY SAPOTE (*POUTERIA* SPP. [SAPOTACEAE])

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Abstract. **Mamey sapote (*Pouteria* spp., Sapotaceae) is a tree fruit of economic and cultural importance in South Florida, Central America, Mexico, and the Caribbean. This study is among the first to analyze genetic variability among cultivated selections of mamey sapote. The Amplified Fragment Length Polymorphism (AFLP) technique was used to estimate levels of genetic diversity in the germplasm collections of Fairchild Tropical Botanic Garden and University of Florida. Although the collections overall represent a low level of genetic diversity, higher levels of genetic diversity were found among selections from Central America. This indicates future plant collection in that region could capture greater genetic diversity. This information can be applied to the management and expansion of the germplasm collections by identifying duplicate selections, mislabeled plants, and locating geographical areas for future collection.**

Knowledge of, and access to, the full range of a crop's genetic resources is vitally important to the continuing development of agriculture. Genetic diversity, the total number of different alleles present in a species, is an important component of a plant's genetic resources. The distribution and scale of a crop's genetic diversity must be understood before effective germplasm collection can be undertaken. Comprehensive genetic information allows curators of living collections to optimize the genetic diversity in their collections, plan for efficient collection enlargement, and ultimately furnish a wider selection of plants to growers.

Mamey sapote is a well known crop in South Florida, and has been described by Balerdi et al. (1996) and Léon (2000). Fairchild Tropical Botanic Garden (FTBG) holds one of the most representative and genetically diverse collections in the USA. Yet, a greater range of morphological traits can be observed in mamey sapote's native or cultivated range than exist in the FTBG germplasm collection. In addition, changing ideas about the differentiation between the three closely related species which produce the fruit commonly called "mamey sapote" have the potential to enlarge the range of traits that should be represented in such a collection.

This paper aims to facilitate mamey sapote germplasm collection expansion and management by identifying geographic areas of high genetic diversity for future collection. This is accomplished by analyzing the genetic diversity of selections held by FTBG and the University of Florida's Tropical Research and Education Center (TREC) using the Amplified Fragment Length Polymorphism (AFLP) technique.

The mamey sapote selections in this study can be grouped into three main categories based on the region in which they were collected: Cuba, the Caribbean coast of Central America (specifically the Yucatán Peninsula, the Petén of Guatemala, and Belize), and the Pacific coast of Central America (Guatemala, El Salvador, Nicaragua, and Costa Rica at elevations from sea level to 1000 m) (Fig. 1). Genetic diversity of selections from the Yucatán peninsula were hypothesized to be low due to their morphological similarity and to the land clearing that occurred there during the Spanish conquest only 500 years ago (Landa 1978).

Concurrently, selections from the Pacific coast population were hypothesized to have greater variation because there are three species present in the region whose fruit is considered mamey sapote. *Pouteria sapota* is currently cultivated throughout Central America and the Caribbean in a range of soil types at altitudes up to 1,200 m. *Pouteria viridis* shares the same geographical range as *P. sapota*, but tends to grow at a higher altitude (1000-1500 m). *Pouteria fossicola* is distributed further south than *P. viridis* and *P. sapota* with a range extending from Nicaragua through Costa Rica to Panama between 0 and 800 m above sea level (Pennington 1990).

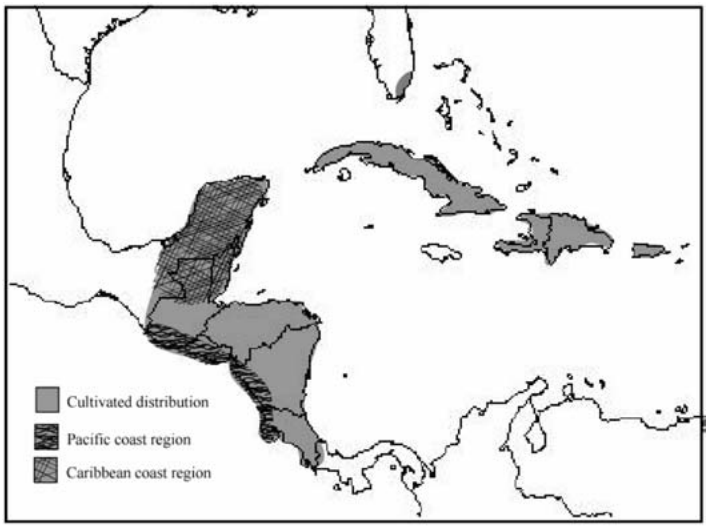

Fig. 1. Map indicating the Pacific and Caribbean regions from which mamey sapote selections in the germplasm collections of Fairchild Tropical Botanic Garden (FTBG) and the University of Florida's Tropical Research and Education Center (TREC) were collected.

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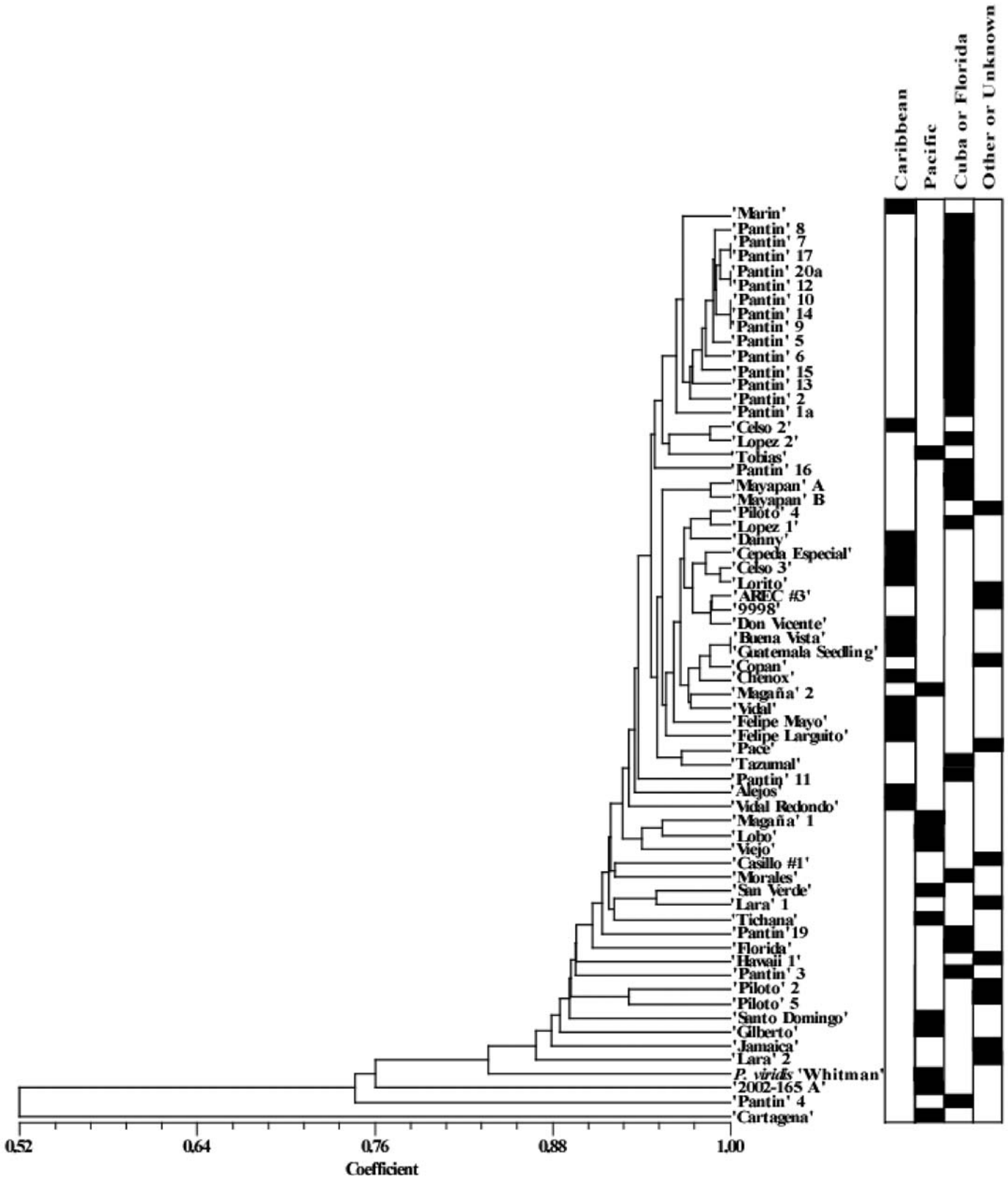

Fig. 2. UPGMA dendrogram of cultivated selections of mamey sapote in Fairchild Tropical Botanic Garden (FTBG) and the University of Florida's Tropical Research and Education Center (TREC) germplasm collections in South Florida, USA.

Table 1. Mamey sapote sample material used in AFLP analysis of genetic diversity present in the Fairchild Tropical Botanic Garden (FTBG) and the University of Florida's Tropical Research and Education Center (TREC) germplasm collections in South Florida, USA.

Population Group	FTBG	TREC	Total
Caribbean	13	1	14
Pacific	10	3	13
Cuba	2	2	4
Florida	2	0	2
Cuba or Florida	2	0	2
Other	2	0	2
Unknown	0	6	6
Total	31	12	43

Materials and Methods

A total of 41 unique mamey sapote selections were analyzed, including 29 individual selections from FTBG and 12 from TREC (Table 1). Appendix 1 specifies collection location and replicate information for all selections included in this study.

Laboratory procedures were carried out in the USDA-ARS-SHRS Plant Sciences Laboratory using the ethanol-precipitation based Epicentre MasterPure™ Plant Leaf DNA Purification Kit (Epicentre, Madison, Wis.). AFLP markers were generated using Applied Biosystem's AFLP™ Ligation/Preselective Amplification Module (Applied Biosystems, 2000). The protocol was modified by reducing reaction volumes by one half to reduce costs and repeated twice for each selection. The resulting fragments were separated by capillary electrophoresis and fragment sizes were analyzed with Genotyper version 3.7 (Applied Biosystems, Foster City, Calif.).

From the multitude of fragments produced, AFLP markers were selected based on their consistency over two AFLP replicates of each sample. Fragment sizes identified by Genotyper were manually verified. Mismatches between replicates were accepted as missing data, for a total of 1.4% missing data over a total of 104 markers.

Pairwise similarity between samples was estimated using Nei and Li's (1979) similarity coefficient, also known as the Dice coefficient, using NTSYS (Exeter Software, Setauket, N.Y.). Dendrograms were produced using the unweighted pair group method of analysis (UPGMA) (Sneath and Sokal, 1973). Confidence levels were placed on the dendrograms using 2000 bootstrap replications with the program WinBoot (Yap and Nelson, 1996). Finally, principle component analysis (PCA) performed by SPSS (SPSS, Inc., Chicago, Ill.) was used as another visualization of the data. Analysis of Molecular Variance (AMOVA) was used to assess differentiation between the Pacific and Caribbean populations (Excoffier et al., 1992).

Results and Discussion

Some grouping of the selections according to their collection location was observed in the UPGMA dendrogram but was not supported by the bootstrapping procedure, perhaps due to the high overall genetic similarity of the samples. However, several trends can be noted. The majority of selections collected from the Yucatán and Cuba showed a high level of similarity. In addition, the selections collected in the Pacific region show greater dissimilarity from each other than do those from the Caribbean (Fig. 2).

Table 2. Analysis of Molecular Variance (AMOVA) for cultivated selections of mamey sapote in Fairchild Tropical Botanic Garden (FTBG) and the University of Florida's Tropical Research and Education Center (TREC) germplasm collections in South Florida, USA.

Source of variation	df	Variance component	Percentage
Caribbean versus Pacific regions			
Between region	1	1.244	13%
Within regions	27	8.216	87%
Control: same individuals distributed randomly into regions			
Between regions	1	0.167	2%
Within regions	27	8.773	98%

Principal component analysis (PCA) supported the UPGMA-based cluster analysis. The first three axes summarized 20.6, 7.5 and 6.3% of the data set's variability, respectively. A scatterplot of the first three principal components shows a tight cluster including the majority of the study selections, with only the more diverse selections separating out (Fig. 3). By removing outliers, the distribution can be seen more clearly (Fig. 4).

AMOVA results indicate 13% of the variance was attributable to genetic variation between the regions. This is higher than the between-region variance of control groups randomly assigned to populations (Table 2). Unfortunately, F statistics are not relevant for use with AFLP data.

Selection '2002-165 A', which originated from a seed collected in a fruit market in northern Costa Rica, is one of the more distinct selections. It was collected because the fruit had morphological characteristics intermediate between *P. sapota* and *P. fossicola*: a green skin with brown scruff on the nose. Added to the morphological ambiguity between the two spe-

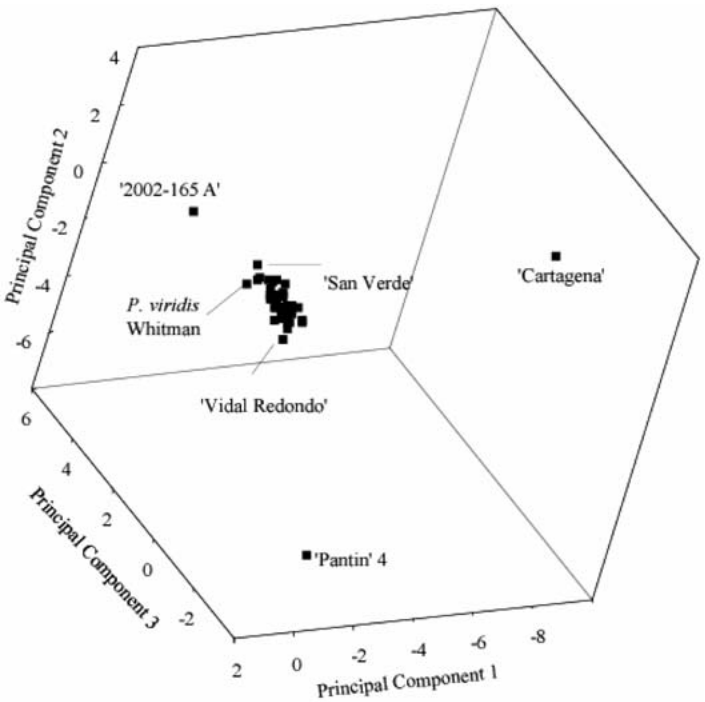

Fig. 3. Principal component analysis of mamey sapote data based on AFLP fingerprints of 65 cultivated selections of mamey sapote. The first three principal components account for 20.6, 7.5 and 6.3% of the data set's variability, respectively.

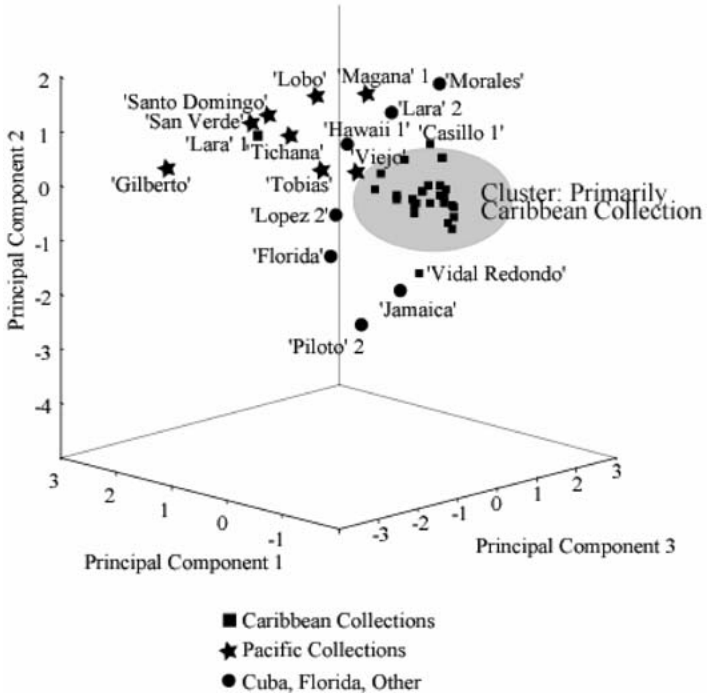

Fig. 4. Principal component analysis of mamey sapote data based on AFLP fingerprints. Outliers have been removed.

cies, these results suggest that the traditional taxonomic differentiation between *P. sapota* and *P. fossicola* may need reexamination. Because the selections from northern Costa Rica and Nicaragua appear to be more dissimilar from each other than do selections from any other location, future collecting in this region would increase the genetic diversity present in the FTBG collection more than would collecting from Yucatán.

This study indicates that the range of genetic diversity present in the FTBG and TREC germplasm collections is distributed unevenly. The majority of selections form a cluster with low overall genetic diversity, while a few selections increase the level of genetic diversity. Field observations of mamey sapote prior to this study have found greater morphological variation throughout Central America than is currently present in the germplasm collection. In addition, AFLP analysis of those individuals in the germplasm collection with intermediate characteristics of *P. sapota*, *P. viridis* and/or *P. fossicola* indicate that these morphological variants represent greater genetic variation. Inclusion of these variants would increase the genetic diversity represented in the germplasm collection.

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Appendix 1. List of mamey sapote selections analyzed with AFLP markers. Population group was only assigned to selections collected from the Pacific or Caribbean regions. Collection location was not known for all selections

Selection	Number of DNA extractions	Germplasm location	Population group	Collection location	Notes
2002-165 A	1	FTBG	Pacific	Nicaragua/Costa Rica	Intermediate fruit characteristics
'9998'	1	TREC			Selected for rootstock (consistently high seed number).
'Alejos'	1	FTBG	Caribbean	Campeche, Mexico	Red, sweet fruit, little fiber
'AREC 3'	1	TREC	Not applicable	Isle of Pines, Cuba	Selected for use as rootstock
'Buena Vista'	1	FTBG	Caribbean	Belize	Large fruit
'Cartagena'	1	FTBG	Not applicable	Cartagena, Colombia	Deep red, fibrous fruit
'Casillo #1'	1	TREC			
'Celso 2'	1	FTBG	Caribbean	Campeche, Mexico	Seedling of 'Alejos'
'Celso 3'	1	FTBG	Caribbean	Campeche, Mexico	Seedling of 'Alejos'
'Cepeda Especial'	1	FTBG	Caribbean	Campeche, Mexico	Synonym of 'Adelantado 2'?
'Chenox'	1	FTBG	Caribbean	Belize	
'Copan'	1	TREC	Not applicable	Cuba	
'Danny'	1	FTBG	Caribbean	Mexico or Belize	Came from Gary Zill
'Don Vicente'	1	FTBG	Caribbean	Campeche, Mexico	
'Felipe Larguito'	1	FTBG	Caribbean	Campeche, Mexico	Fruits in March (Campeche)
'Felipe Mayo'	1	FTBG	Caribbean	Campeche, Mexico	Hard exoderm, resistant to fruit flies, fruits in May in Campeche
'Florida'	1	TREC			
'Gilberto'	1	FTBG	Pacific	Orotina, Costa Rica	Large fruit
Guatemala Seed	1	TREC	Caribbean	Guatemala	
'Hawaii 1'	1	FTBG	Not applicable	Hawaii	
'Jamaica'	1	TREC			
'Lara' 1	1	TREC			Collected by Julian Lara
'Lara' 2	1	TREC			Collected by Julian Lara
'Lara' 3	1	TREC			Collected by Julian Lara
'Lobo'	1	FTBG	Pacific	Jesus Maria, C. R.	Small fruit and seed. Some green.
'Lopez 1'	1	FTBG	Not applicable	FL or Cuba	Collected by Pedro Lopez
'Lopez 2'	1	FTBG	Not applicable	FL or Cuba	Collected by Pedro Lopez
'Lorito'	1	FTBG	Caribbean	Campeche, Mexico	Small fruit, strong red
'Magaña' 1	1	TREC	Pacific	El Salvador	Large fruit
'Magaña' 2	1	TREC	Pacific	El Salvador	Reputed scion of original
'Magaña' 3	1	FTBG	Pacific	El Salvador	Reputed scion of original
'Marin'	1	FTBG	Caribbean	Campeche, Mexico	Large fruit, red, little fiber
'Mayapan'	2	TREC	Not applicable	Isle of Pines, Cuba	
'Morales'	1	FTBG	Not applicable	FL	Collected by Dr. Morales
P. viridis Whitman	1	FTBG	Pacific		
P. viridis Gray Australia	1	FTBG	Pacific		
'Pace'	1	FTBG			
'Pantin' 1	3	TREC	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 2	1	TREC	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 3	1	TREC	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 4	1	TREC	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 5	1	TREC	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 6	1	TREC	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 7	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 8	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 9	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 10	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar

Appendix 1. (Continued)List of mamey sapote selections analyzed with AFLP markers. Population group was only assigned to selections collected from the Pacific or Caribbean regions. Collection location was not known for all selections

Selection	Number of DNA extractions	Germplasm location	Population group	Collection location	Notes
'Pantin' 11	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 12	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 13	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin'14	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 15	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 16	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 17	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 18	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 19	1	Greenland Nursery	Not applicable	Cuba	S. Florida commercial cultivar
'Pantin' 20	3	FTBG	Not applicable	Cuba	S. Florida commercial cultivar
'Piloto' 1	1	TREC			Collected by Julian Lara
'Piloto' 2	1	TREC			Collected by Julian Lara
'Piloto' 3	1	TREC			Collected by Julian Lara
'Piloto' 4	1	TREC			Collected by Julian Lara
'Piloto' 5	1	TREC			Collected by Julian Lara
'San Verde'	1	FTBG	Pacific	Jesus Maria, C. R.	Green skinned fruit
'Santo Domingo'	1	FTBG	Pacific	Omotepe, Nicaragua	Remnant tree, 200+ years
'Tazumal'	1	TREC	Pacific	El Salvador	
'Tichaná'	1	FTBG	Pacific	Omotepe, Nicaragua	Tree 100+ years old
'Tobias'	1	FTBG	Pacific	Geronimo, Costa Rica	Giant, old remnant tree
'Vidal'	1	FTBG	Caribbean	Campeche, Mexico	Possibly 'Vidal'
'Vidal Redondo'	1	FTBG	Caribbean	Campeche, Mexico	Round, red fruit
'Viejo'	1	TREC	Pacific	El Salvador	