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SPECIES RELATIONSHIPS IN THE GENUS *VASCONCELLEA* (CARICACEAE) BASED ON MOLECULAR AND MORPHOLOGICAL EVIDENCE[1]

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Validity of the taxa currently recognized in the genus *Vasconcellea* was analyzed by investigating morphological and molecular data from 105 specimens of this genus and six specimens of the related genus *Carica*. Taxon identification of these specimens was compared with clustering in two phenetic dendrograms generated with 36 morphological characters and 254 amplified fragment length polymorphic (AFLP) markers. Moreover, cytoplasmic haplotypes were assessed using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) of one mitochondrial and two chloroplast DNA regions. Results show that the morphological data set, containing mainly vegetative characteristics, merely reveals external resemblance between specimens, which is not directly associated with genetic relationships and taxon validity. Phenotypic plasticity and intercompatibility between several species are likely to confuse morphological delimitation of the taxa. Based on the results of our study, several specimens that could not be identified with the currently used identification key (1) could be attributed to a known taxon, which should be extended to include a higher range of morphological variability or (2) could be hypothesized to be of hybrid origin. Because of the high intraspecific variation within *V. microcarpa* and *V.* × *heilbornii*, revision of these taxa is recommended.

Key words: AFLP; *Carica*; hybridization; morphological variability; PCR-RFLP; phenetic relationships; species separation; *Vasconcellea*.

Vasconcellea Saint-Hilaire is by far the largest genus of the Caricaceae Dumortier, uniting 21 of the 35 taxa described for this dicotyledonous plant family (Badillo, 1971, 1993, 2001). In the current classification (Badillo, 2001) *Vasconcellea* comprises 20 species and 1 hybrid, *Vasconcellea* × *heilbornii*. In spite of the frequent absence of sexual reproduction, this hybrid is usually considered as a species.

Species of *Vasconcellea* are commonly referred to as highland papayas or mountain papayas (National Research Council, 1989) because of their resemblance with papaya (*Carica papaya*) and their typical ecological preference for higher altitudes. Until recently (Badillo, 1971, 1993), *Vasconcellea* (also spelled as *Vasconcella*) was considered a section, sister to the section *Carica*, within the genus *Carica* L. Badillo (2000) separated the monospecific section *Carica* (containing only *Carica papaya*) from section *Vasconcellea*, based on morphological and genetic (Aradhya et al., 1999) evidence, by rehabilitating the section on generic level. *Vasconcellea* species are distributed throughout South America, with a concentration of diversity in the Andean valleys of Ecuador, where 16 of the 21 described species appear up to 3500 m a.s.l. (Badillo, 1993, 1997, 1999; Romeijn-Peeters, 2004). Five species of this genus have been placed on the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species: *V. horovitziana*, *V. omnilingua*, *V. palandensis*, *V. pulchra*, and *V. sprucei* (IUCN, 2003). Personal observations in Ecuador suggest that even more species of *Vasconcellea* are endangered. Most significant threats are habitat destruction resulting from deforestation and conversion of forests into croplands or grasslands (IUCN, 2003).

The most comprehensive review of the genus *Vasconcellea*, as section *Vasconcella*, is the monograph of Caricaceae by Badillo (1971, 1993). This monograph contains an identification key, mainly based on characters of the staminate flowers, followed by comprehensive morphological circumscriptions of the different taxa. Species of *Vasconcellea* are wild, semi-domesticated, or domesticated plants, with a shrub- or treelike and pachycaulous habit. Plants are usually dioecious but sometimes monoecious or polygamous. The medullar stem is mostly simple or scarcely branched, in some species it is covered with spiny stipules, whereas the leaves are concentrated in a terminal crown. Leaves are large to very large and vary extensively in shape, from entire to compound. All organs produce a white latex, containing cysteine endopeptidases. Flowers are pentamerous with white, green, yellow, orange, or pink petals. The fruit is a berry with varying shape, dimension, and color (Badillo, 1993).

In the course of previous ethnobotanical inventories of wild and semi-domesticated edible plants in southern Ecuador, an

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unrecognized variability among and within some species of the genus *Vasconcellea* was observed (Jiménez et al., 1998; Scheldeman, 2002). This high diversity is probably partly caused by the intercompatibility between several species (Jiménez and Horovitz, 1957; Horovitz and Jiménez, 1967; Mekako and Nakasone, 1975) leading to the production of hybrids with varying degrees of fertility, which have been shown to occur spontaneously in areas where species distributions overlap (Badillo, 1971). Interspecific hybridization can lead to fertile hybrids, which may cross with parental or nonparental species (Badillo, 1971). Such complex hybrid populations in the so-called hybrid zone are a cline of morphological and genetic variability (Barton and Hewitt, 1985).

Two naturally occurring hybrids of *Vasconcellea* with high introgressive potential have already been described by Horovitz and Jiménez (1967) and Badillo (1971, 1993): (1) *V.* × *heilbornii*, a taxon abundantly present in southern Ecuador and (2) an occasionally occurring hybrid between *V. monoica* and *V. cundinamarcensis*. Within *V.* × *heilbornii*, Badillo (1993) recognizes the cultivar Babaco and the varieties *chrysopetala* and *fructifragrans*.

During our expeditions in Ecuador, we realized that many *Vasconcellea* specimens could not be identified at the specific level with the dichotomous key of Badillo (1993) and that the high morphological variability within the genus is insufficiently understood (J. P. Romero-Motochi, E. Romeijn-Peeters, B. Van Droogenbroeck, and T. Kyndt, personal observation). Taxon identification is hard or even impossible when only vegetative plant parts are present, which is often the case during collection. Additional information about the genotype of the plants is very much needed to resolve taxonomical problems in this genus. Because the genotype is not influenced by environmental factors, evolution of closely related taxa can be investigated from an objective point of view with molecular techniques (Hillis, 1987). In addition, some molecular marker assays, e.g., amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD), reveal a large amount of characters per reaction, i.e., a high multiplex ratio, making them very useful in the assessment of botanical relationships and diversity (Karp et al., 1996; McLenachan et al., 2000). Recently, some molecular analyses have been performed in the Caricaceae family to clarify interspecific and intergeneric phenetic and phylogenetic relationships with the aid of fingerprinting techniques, such as RAPD (Jobin-Decor et al., 1997), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Aradhya et al., 1999; Van Droogenbroeck et al., 2004), and AFLP (Kim et al., 2002; Van Droogenbroeck et al., 2002). Their results verify the large genetic distance between the genera *Carica* and *Vasconcellea*, thereby validating their recent rehabilitation. Moreover, these studies revealed relationships between some *Vasconcellea* species on molecular level. The most extensive analysis, involving eight species of *Vasconcellea*, two species of *Jacaratia* A. DC. (Caricaceae) together with *Carica papaya* (Van Droogenbroeck et al., 2002), suggested a close genetic relationship between the following species pairs: (1) *V. stipulata* and its putative hybrid *V.* × *heilbornii*, (2) *V. weberbaueri* and *V. parviflora*, and (3) *V. palandensis* and *V. goudotiana*. Aradhya et al. (1999) described intraspecific variation in chloroplast PCR-RFLP haplotypes of *V. microcarpa*, *V. quercifolia*, and *V.* × *heilbornii*. *Vasconcellea* × *heilbornii* surprisingly did not share its haplotype with either one of its putative parent species, *V. stipulata* and *V. cundinamarcensis*. These results were confirmed and extended by the PCR-RFLP analysis of Van Droogenbroeck et al. (2004). *Vasconcellea microcarpa* and the hybrid *V.* × *heilbornii* again showed intraspecific variability, with specimens of *V.* × *heilbornii* having either the haplotype of their putative mother *V. stipulata* or, surprisingly, *V. weberbaueri*.

In general, the molecular studies mentioned (Aradhya et al., 1999; Kim et al., 2002; Van Droogenbroeck et al., 2002, 2004) demonstrate that AFLP fingerprinting (Vos et al., 1995) and PCR-RFLP analysis of cpDNA and mtDNA are time- and cost-efficient methods to analyze inter- and intraspecific relationships and to investigate hybridization among *Vasconcellea* species.

The objectives of this study were (1) to verify the validity of the identification key of Badillo (1993) by comparing the taxonomical grouping of the *Vasconcellea* specimens with detailed molecular and vegetative morphological data, (2) to evaluate the possible development of a vegetative identification key, (3) to investigate gaps in the current identification key by analyzing specimens that could not be identified unambiguously with this key, and (4) to evaluate possible hybridization events by comparing nuclear (AFLP) and cytoplasmic (PCR-RFLP) marker data.

MATERIALS AND METHODS

Plant material—A total of 105 *Vasconcellea* individuals and six individuals of the outgroup species *Carica papaya* were sampled (Table 1). Most specimens were collected in Ecuador by the authors. Leaf material or seeds from some individuals were kindly provided by other researchers (see Table 1 for details). For *V. sphaerocarpa*, *V. sprucei*, and *V. glandulosa* only herbarium specimens were available. Permission was given by the directors of these herbaria (HUA, BM, U) to use those specimens as sources of DNA.

Specimens were identified based on the most recent key (Badillo, 1993) and named after Badillo (2001). After identification, a taxon-specific code was given to each specimen to increase the readability of this paper (Table 1). In total, the collected individuals represent 20 described taxa of the genus *Vasconcellea*. Sixteen specimens could not be readily identified due to the absence of staminate flowers or due to lack of resemblance with any of the described species. These unidentified specimens were given a taxon code starting with 'sp.' Specimens of *V.* × *heilbornii* that could not be identified either as one of the varieties *chrysopetala* or *fructifragrans* or as the cultivar Babaco were given a taxon code starting with ''heil.'' Voucher specimens were deposited at GENT. Samples with no mature leaf material were omitted from the morphological analysis (see Table 1).

Morphological analysis—The identification key of Badillo (1993) is mainly based on staminate flowers. As most species of *Vasconcellea* have a long vegetative state, this feature is often useless. Therefore this study focused on vegetative features, although also one generative characteristic, i.e., color of petals, was studied. Because leaf dimensions within *Vasconcellea* are very variable, quantitative data were considered rather undiagnostic. Consequently, mainly qualitative features were studied. All characteristics studied are listed in Table 2.

Analysis of the morphological data was conducted using NTSYS-pc version 2.10L (Rohlf, 2000). Morphological data were converted into a similarity matrix, using the simple matching coefficient (Sneath and Sokal, 1973), with the SIMQUAL function. A dendrogram was generated from the similarity matrix by the unweighted pair-group method using arithmetic averages (UPGMA) (Sokal and Michener, 1958) with the SAHN function. The cophenetic correlation coefficient was calculated with a Mantel test (Mantel, 1967) by comparing the matrix of cophenetic values with the similarity matrix, in order to estimate how well the dendrogram represents its corresponding pairwise distance matrix. This was done with the COPH and MXCOMP modules of NTSYS-pc (Rohlf, 2000). Further, principal co-ordinate analysis (PCoA)

(Gower, 1966) was performed by extracting eigenvectors from the similarity matrix, using the EIGEN function. The data were projected onto the resulting eigenvectors using the PROJ function and two-dimensional plots of the data were achieved using MXPLOT.

Molecular analysis—*DNA extraction*—Leaf tissue was dried with silica gel and ground in liquid nitrogen. Total genomic DNA was extracted using the Qiagen Dneasy Plant Mini kit (Qiagen, Hilden, Germany). For herbarium material, an incubation in buffer AP1 (Qiagen) for 1 h at 65°C was necessary prior to extraction, and only small amounts of partially degraded DNA were obtained.

AFLP analysis—The AFLP analysis was carried out as previously described by Van Droogenbroeck et al. (2002). The following five primer combinations were used: E + GA/M + ACAA, E + GT/M + ACAA, E + GA/M + GCGT, E + GA/M + CTGT, and E + CG/M + CTGG. For each individual, DNA fingerprints were scored by visual inspection for presence (1) or absence (0) of specific AFLP fragments. Only distinct, major bands were scored. Data matrices were analyzed using Treecon 1.3b (Van de Peer and De Wachter, 1994) and NTSYS-pc version 2.10L (Rohlf, 2000). Genetic similarities were calculated using Jaccard's coefficient (Jaccard, 1908) with the SIMQUAL module of NTSYS-pc or the DISTANCE ESTIMATION option of Treecon. Similarity matrices were analyzed using the UPGMA (Sokal and Michener, 1958) clustering method in NTSYS-pc (SAHN module). Calculation of the cophenetic correlation coefficient was done as described. Reliability of clusters in each dendrogram was tested by bootstrap analysis (Felsenstein, 1985) with 1000 replications using Treecon. Additionally, a PCoA analysis was performed based on the genetic similarity matrix.

CpDNA and mtDNA haplotype determination using PCR-RFLP—The PCR-RFLP data of two cpDNA regions (*trnK*1-*trnK*2 [K1K2] and *trnM-rbcL* [ML]) and one mtDNA region (*nad*4/1-*nad*4/2 [*nad*4/1–2]) were already available for some specimens included in our sample set (Van Droogenbroeck et al., 2004). For the other specimens, additional PCR-RFLP data were generated with the eight PCR-fragment/enzyme combinations selected by Van Droogenbroeck et al. (2004): K1K2/*Eco*RV, K1K2/*Sca*I, K1K2/*Afa*I; ML/*Pst*I, ML/*Mse*I for the cpDNA regions and *nad*4/1–2/*Hinf*I, *nad*4/1–2/*Bst*OI, *nad*4/1–2/*Dde*I for the mtDNA region. Haplotypes were defined as a set of specific combinations of the observed variants for all detected mutations (for details, see Van Droogenbroeck et al., 2004).

RESULTS

Morphological analysis—The UPGMA dendrogram based on morphological similarity values (simple matching coefficient), with cophenetic value of 0.86, is presented in Fig. 1. Three main clusters can be distinguished at 50% similarity level: cluster 1 consists of all specimens with parted leaves and (palin-)actinodromous venation; cluster 2 contains the only studied species that has compound leaves, i.e., *V. palandensis*; and cluster 3 contains specimens with simple leaves and pinnate venation.

Within cluster 3, five clearly separated subclusters can be discerned. Cluster 3A contains *Vasconcellea quercifolia*, which is characterized by typical oblong leaves with many small lobes. Specimens belonging to the species *V. candicans* have heart-shaped leaves (reflected in their four basal secondary veins and a small basal extension in length) and are grouped in another discrete species-specific subcluster, 3B. Cluster 3C contains all specimens with an entire leaf margin, sometimes showing a single small lobe, i.e., *V. longiflora*, three unidentified specimens (sp256, sp259, sp260) and one specimen of *V. microcarpa* subsp. *heterophylla* (mich258); cluster 3E holds all specimens of *V. pulchra*, and one specimen of *V. microcarpa* subsp. *baccata* (micb192). One specimen of *V. microcarpa* subsp. *heterophylla* (mich190) is isolated in cluster 3D. All specimens belonging to cluster 3C, 3D, and 3E show a typical acute basal angle.

Subclusters in cluster 1 are less clearly defined. Specimens belonging to the same taxon are clustered separately and intermingled with other taxa. Only *V.* × *heilbornii* Babaco (with its typical small number of lobules, 1C), *C. papaya* (the only taxon with nine primary veins, 1N) and *V. weberbaueri* (with typical serrate leaves, 1O) are grouped in clearly separated taxon-specific clusters. Also *V. cauliflora* (bearing white flowers and showing emergences on petiole and lamina, 1B), *V. parviflora* (showing wide secondary vein spacing and lobe bases typically narrowed above attachment, 1E), and *V. monoica* (narrow lobes with a remarkably low number of lobules, 1H) are clustered in species-specific clusters, but they appear less separated from the neighboring taxa. *V. sphaerocarpa*, *V. sprucei*, and *V. glandulosa* are isolated from the other taxa (clusters 1G and 1A), but because only one specimen was included for each of them, it is difficult to define these as species-specific clusters. All specimens of *V. cundinamarcensis*, except for cund193, are clustered with sp203 (cluster 1K). Two heterogeneous and remote clusters (clusters 1F and 1L) include all specimens of *V.* × *heilbornii* var. *chrysopetala*, *V.* × *heilbornii* var. *fructifragrans*, and most of the unidentified *V.* × *heilbornii* together with their putative parent species *V. stipulata*. Both described varieties of *V.* × *heilbornii* (var. *chrysopetala* and var. *fructifragrans*) are present in cluster 1F. Furthermore, both specimens of *V. omnilingua* are grouped in cluster 1I, together with unidentified specimens, sp239, sp240, and sp241, and specimens of *V. microcarpa* subsp. *heterophylla* and subsp. *microcarpa* (mich177 and micm212). Finally, all specimens of *V. goudotiana*, some of the remaining specimens of *V. microcarpa* (subsp. *baccata*, subsp. *heterophylla*, and subsp. *microcarpa*), and unidentified specimens are interspersed between and within the afore-described (sub)clusters.

A PCoA-analysis based on the simple matching coefficient was performed (results not shown). The PcoA supports separation of the three main clusters obtained with the cluster analysis (Fig. 1). However, separation in subclusters is poor. The first three principal coordinates account for 71.1% of the variation. The second principal coordinate (10.2%) separates more or less the specimens with different leaf division, i.e., simple leaves with pinnate venation, simple leaves with (palin-)actinodromous venation, and compound leaves (clusters 1, 2, and 3 in Fig. 1). Taxa are slightly separated by principal coordinates 1 (56.9%) and 3 (4.0%), but no clear spreading is present.

AFLP analysis—Specimens (Table 1) were analyzed with AFLP using five primer combinations, selected by Van Droogenbroeck et al. (2002) for their high number of bands and polymorphism. Of a total of 254 scorable fragments, only nine (3.5%) were monomorphic in both *Vasconcellea* and *Carica*. When only *Vasconcellea* was considered, 19 (7.5%) monomorphic markers were found.

Unfortunately, the quality of the DNA obtained from herbarium material was too poor to be used in AFLP analysis, as might have been expected based on the age of the material and on similar experiences from other researchers (McLenachan et al., 2000).

The AFLP data were used to make pairwise comparisons of the genotypes on both shared and unique amplification products to generate a similarity matrix using Jaccard's coef-

TABLE 1. A list of specimens of *Vasconcellea* and *Carica* investigated in this study, their codes and origin or provider. The number of analyzed individuals per taxon is indicated between parentheses in the first column.

Taxon	Code	Origin or provider
Vasconcellea stipulata (V. Badillo) V. Badillo (4)	stip007	Loja, Loja
	stip109	Gualel, Loja
	stip169	Celica, Loja
	stip233[a]	L'Esperanza, Loja
V. × *heilbornii* (V. Badillo) V. Badillo (16)	heil011	Chantaco, Loja
	heil071	Capur, Loja
	heil218	Chuquiripamba, Loja
	heil219	Chuquiripamba, Loja
	heil235[a]	L'Esperanza, Loja
	heil245[a]	Pueblo nuevo, El Oro
	h × c 100[c]	Artificial hybrid *V.* × *heilbornii* × *V. cundinamarcensis*
var. *chrysopetala* (Heilbor)	chrys019	Chuquiripamba, Loja
	chrys076[a]	Capur, Loja
	chrys198[a]	Ayora, Pichincha
var. *fructifragrans* (Garcia. Barr. et Hern.)	fru149	San Lucas, Loja
	fru197	Ayora, Pichincha
	fru199	Ayora, Pichincha
'Babaco'	bab073	Capur, Loja
	bab120	Gonzamana, Loja
	bab155	Saraguro, Loja
V. weberbaueri (Harms) V. Badillo (5)	web005	Loja, Loja
	web006	Loja, Loja
	web009	Loja, Loja
	web148	Loja, Loja
	web267[a]	Uritusinga, Loja
V. parviflora A. DC. (4)	parv041	Catacocha, Loja
	parv045	Catacocha, Loja
	parv046	Catacocha, Loja
	parv145[a]	Zaruma, El Oro
V. goudotiana Tr. et Planch (5)	goudD4[a]	Leaf material/Dr. R. Drew
	goud276	Vilcabamba, Loja
	goud278[a]	Vilcabamba, Loja
	goud279	Vilcabamba, Loja
	goud322	Seeds/Dr. R. Drew
V. palandensis (V. Badillo et al.) V. Badillo (6)	pal062[a]	Palanda, Zamora
	pal063	Palanda, Zamora
	pal064	Palanda, Zamora
	pal068[a]	Palanda, Zamora
	pal214	Palanda, Zamora
	pal216	Palanda, Zamora
V. cundinamarcensis V. Badillo (5)	cund020	Chuquiripamba, Loja
	cund157	Saraguro, Loja
	cund193	Miraflores, Pichincha
	cund251	Molletura, Azuay
	cund252	Molletura, Azuay
V. longiflora (V. Badillo) V. Badillo (5)	long226	Maquipucuna, Pichincha
	long227	Maquipucuna, Pichincha
	long228	Maquipucuna, Pichincha
	long229	Maquipucuna, Pichincha
	long230	Maquipucuna, Pichincha
V. pulchra (V. Badillo) V. Badillo (4)	pul179	Hda San Francisco, Los Rios
	pul180	Hda San Francisco, Los Rios
	pul185	Rio Palenque, Los Rios
	pul187	Centinella, Pichincha
V. monoica (Desf.) A. DC. (3)	mon058	Valladolid, Zamora
	mon060	Valladolid, Zamora
	mon061[a]	Valladolid, Zamora
V. omnilingua (V. Badillo) V. Badillo (3)	omni236	San Antonio, El Oro
	omni237	San Antonio, El Oro
	omni238[a]	San Antonio, El Oro
V. microcarpa (Jacq.) A. DC. (12)		
subsp. *baccata* (Heilborn) V. Badillo	micb186	Rio Palenque, Los Rios
	micb192	La Abundancia, Pichincha
subsp. *heterophylla* (Poepp. et Endl.) V. Badillo	mich177	El Placer, El Oro
	mich190	La Concordia, Pichincha
	mich255[a]	Calvario, Azuay
	mich258	Calvario, Azuay
	mich266	Paute, Chimborazo

TABLE 1. Continued.

Taxon	Code	Origin or provider
subsp. *microcarpa*	micm065	Palanda, Zamora
	micm067	Palanda, Zamora
	micm212	Podocarpus, Zamora
	micm265	Paute, Chimborazo
	micm273	Palanda, Zamora
V. cauliflora (Jacq.) A. DC. (3)	caulD3[a]	Leaf material/Dr. R. Drew
	caul284	Seeds/Dr. R. Drew
	caul325	Seeds/Dr. R. Drew
V. chilensis (Planch. ex A. DC.) A. DC. (1)	chil[a]	Leaf material/Dr. T. Fichet-Lagos
V. quercifolia Saint-Hilaire (4)	querD5[a]	Leaf material/Dr. R. Drew
	quer323	Seeds/Dr. R. Drew
	quer324	Seeds/Dr. R. Drew
	querhaw8[a]	NPGS HCAR226-USDA
V. candicans (A. Gray) A. DC. (5)	cand050	Catacocha, Loja
	cand113	Catacocha, Loja
	cand126	Sozoranga, Loja
	cand171	Celica, Loja
	cand224	Pindal de Juncal, Loja
V. crassipetala (V. Badillo) V. Badillo (1)	cras282[a]	La Delicia, Imbabura
V. sphaerocarpa (García-Barr. et Hern.) V. Badillo (1)	sphaer[b]	Vargas W.G.-123771-HUA
V. glandulosa A. DC. (1)	gland[b]	Maas P.J.M. et al.-0018901-U
V. sprucei (V. Badillo) V. Badillo (1)	spruc[b]	Badillo V. et al.-000645484-BM
Unidentified *Vasconcellea* specimens (16)	sp101	Bot. Garden, Loja, Loja
	sp200	Rio Palenque, Los Rios
	sp183	Rio Palenque, Los Rios
	sp203	Cabuga, Napo
	sp205	Bot. Garden, Loja, Loja
	sp225	Mera, Pastaza
	sp239	San Antonio, El Oro
	sp240	San Antonio, El Oro
	sp241	San Antonio, El Oro
	sp256[a]	Calvario, Azuay
	sp257[a]	Calvario, Azuay
	sp259	Calvario, Azuay
	sp260	Calvario, Azuay
	sp271	Palanda, Zamora
	sp312(I)[a]	Progeny of sp205
	sp312(II)[a]	Progeny of sp205
Carica papaya L. (6)	pap026[a]	Vilcabamba, Loja
	pap048	Catacocha, Loja
	pap220	La Toma, Loja
	papD6[a]	Leaf material/Dr. R. Drew
	papPHI[a]	Philippines/Dr. T. Thuan
	papBUR[a]	Burundi/Dr. J. Bigirimana

[a] No detailed morphological data available.
[b] No molecular data available.
[c] h × c: artificial hybrid between *V.* × *heilbornii* and *V. cundinamarcensis*.

ficient. The Jaccard coefficient of band matching is recommended for the analysis of DNA fingerprint data because it only takes into account positive band matching (Weising et al., 1995).

Figure 2 shows the dendrogram with a cophenetic value of 0.85, generated using the UPGMA clustering method. On 55% Jaccard's diversity level seven clusters can be distinguished. Cluster 7 contains only *C. papaya* genotypes, clearly separated from all specimens of the genus *Vasconcellea*. Cluster 1 contains *V. parviflora* (1f), *V. weberbaueri* (1e), the hybrid *V. heilbornii* (1b, 1c, and 1d), and one of its putative progenitor species, *V. stipulata* (1a). Cluster 2 is separated into three subclusters. Cluster 2a holds *V. palandensis* (2a(IV)), *V. cundinamarcensis* (2a(I)), *V. goudotiana* (2a(V)), sp200 en sp203 (2a(III)), and subcluster 2a(II) (bootstrap value = 69%) containing sp101, sp205, sp312(I) and sp312(II). Within cluster 2b, individuals of the described species *V. pulchra* (2b(II)) and *V. longiflora* (2b(III)) are grouped together with *V. microcarpa* subsp. *heterophylla* and subsp. *baccata* and five morphologically unidentified individuals of *Vasconcellea*. Cluster 2c consists of *V. monoica* (2c(II)), *V. omnilingua* (2c(VI)), and *V. microcarpa* subsp. *microcarpa* and one specimen (mich266) of the subsp. *heterophylla*, together with five unidentified specimens. Individuals of each described species within clusters 2b and 2c are grouped together with high bootstrap values (bootstrap value = 81–100%), except for specimens belonging to *V. microcarpa* and its subspecies, which are scattered throughout these two clusters without any apparent affinity. Cluster 5 combines *V. quercifolia* (5a) and *V. candicans* (5b) with a bootstrap value of 69%. Clusters 3, 4, and 6 are species-specific, containing respectively *V. cauliflora*, *V. chilensis*, and *V. crassipetala*. Clusters 1–6 are combined in a *Vasconcellea* cluster supported by a 94% bootstrap value.

The average genetic similarity (Jaccard's coefficient) among

TABLE 2. Characteristics and their states used in the morphological analysis of *Vasconcellea* and *Carica*. For qualitative features, each descriptor is followed by a numerical code in parentheses.

Feature	States
Lamina surface	Flat (0); wavy (1)
Leaf division	Entire (0); lobed (1); parted (2); compound (3); combined compound and parted (4)
Laminar symmetry	Asymmetrical (0); symmetrical (1)
Apex angle (entire leaf or leaflet)	Acute (0); odd lobed acute (1); odd lobed obtuse (2)
Apex shape (entire leaf or leaflet)	Narrow acuminate (0); wide acuminate (1); acute (2); straight (3)
Base angle (entire leaf or leaflet)	Acute (0); obtuse (1); wide obtuse (2)
Base shape (entire leaf)	Lobate (0); cordate (1); concavo-convex (2); convex (3); rounded (4); not applicable (10)
Base shape (leaflet)	Cuneate (0); convex (1); not applicable (10)
Basal extension length (length of primary vein/length of basal extension)	Absent (0); small, i.e., $x. \geq 8$, (1); large, i.e., $x < 8$ (2)
Leaf margin folding	Flat or nearly so (0); strongly revolute (1)
Leaf margin incision	Entire (0); serrate (1)
Lobe shape	Small (0); narrow (1); moderate (2); wide (3); not applicable (10)
Lobe base	Not narrowed above attachment (0); narrowed above attachment in apical lobe (1); narrowed above attachment in all lobes (2); not applicable (10)
Basal lobes	No overlap (0); overlap (1); not applicable (10)
Lobules on central lobe	Absent (0); present (1); not applicable (10)
Lobules on upper lateral lobe	Absent (0); present (1); not applicable (10)
Lobules on central lateral lobe	Absent (0); present (1); not applicable (10)
Lobules on lower lateral lobe	Absent (0); present (1); not applicable (10)
Lobules on basal lobe	Absent (0); present (1); not applicable (10)
Vein category	Pinnate (0); actinodromous (1); palinactinodromous (2)
Primary veins	(number)
Primary veins size	Weak (0); moderate (1); stout (2); massive (3)
Prolonged midvein	Absent (0); present (1)
Leaf margin joins two basal veins	False (0); true (1); not applicable (10)
Secondary veins relative thickness (width of primary vein width of secondary vein)	Weak, i.e., $x \geq 3.50$, (0); moderate, i.e., $1.75 < x > 3.50$ (1); stout, i.e., $x \leq 1.75$ (2); not applicable (10)
Lower secondary veins	No remarkable horizontal position (0); remarkable horizontal position (1)
Basal primary veins	(number)
Basal secondary veins	(number)
Basal tertiary veins	(number)
Connective veins between primary and secondary or between primary and primary veins	Indistinct (0); distinct (1)
Angles of divergence between primary and secondary veins	Nearly uniform (0); increasing basally and/or apically (1)
Secondary vein spacing	Narrow (0); moderate (1); wide (2)
Leaf pubescense	Glabrous (0); pubescent (1)
Spiny stipules	Absent (0); weakly developed (1), strongly developed (2)
Emergences on petiole and lamina	Absent (0); small (1); obvious (2)
Dominant color of petals	Yellow (0); orange (1); green (2); white (3); purple (4)

all *Vasconcellea* taxa was 0.47. Between genus *Carica* and genus *Vasconcellea*, an average genetic similarity of 0.26 was found. All described *Vasconcellea* taxa for which more than one individual was analyzed display high (>0.80) intraspecific genetic similarity values (Table 3), except for *V.* × *heilbornii* (0.67) and *V. microcarpa* (0.57).

A PcoA analysis based on the coefficient of Jaccard was performed (results not shown). The first coordinate accounts for 12.2% of total variation and separates the group containing *V. stipulata*, *V.* × *heilbornii*, *V. parviflora*, and *V. weberbaueri* (cluster 1 in Fig. 2) from all other specimens. The second dimension (9.0%) shows the distinction between *Carica* and *Vasconcellea*. *Vasconcellea candicans* and *V. quercifolia* (cluster 5 in Fig. 2) are separated from all other specimens by the third principal coordinate (7.4%).

PCR-RFLP analysis—Eight fragment/enzyme combinations, selected by Van Droogenbroeck et al. (2004), were applied to analyze the complete sample set given in Table 1. The haplotype of each individual was determined based on a set of specific combinations of the observed variants for all detected mutations, as specified in Van Droogenbroeck et al. (2004).

Twelve different cp haplotypes (chlorotypes A–L) and four different mt haplotypes (mitotypes A–D) were found in our sample set. Specimens sharing the same chlorotype always had the same mitotype. Intraspecific haplotype diversity was only observed for individuals belonging to taxa *V.* × *heilbornii* and *V. microcarpa*.

Figure 2 gives an overview of the genetic information obtained with AFLP and PCR-RFLP. All individuals from AFLP cluster 1 share the same mitotype (C) and have three different chlorotypes (F, G, and J). Four other species also hold mitotype C, *V. cauliflora* (cluster 3), *V. crassipetala* (cluster 6), *V. candicans* (cluster 5b), and *C. papaya* (cluster 7), but have a different chlorotype (E, G, H, and L, respectively). These species are each grouped in a distinct cluster in the AFLP analysis. *Vasconcellea crassipetala* and *V. parviflora* share the same chlorotype and mitotype (G and C), although they are quite diverse according to nuclear AFLP results (mean similarity = 0.30). Cluster 2 contains the individuals with mitotypes A and B and chlorotypes A, B, C, and D. Chlorotype B

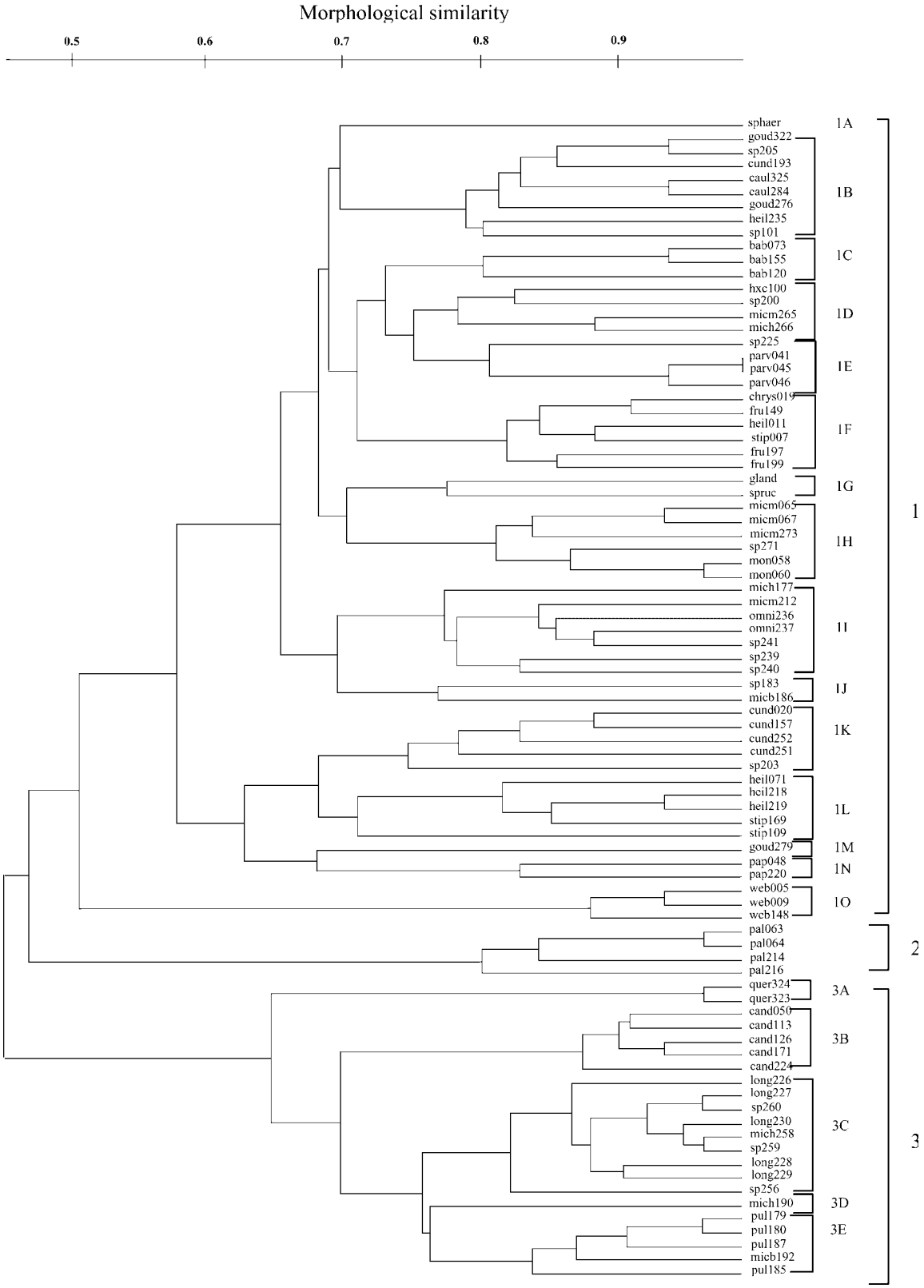

Fig. 1. The UPGMA dendrogram based on morphological data of *Vasconcellea* and *Carica*. Similarity values were calculated with the simple matching coefficient. Taxon codes are specified in Table 1.

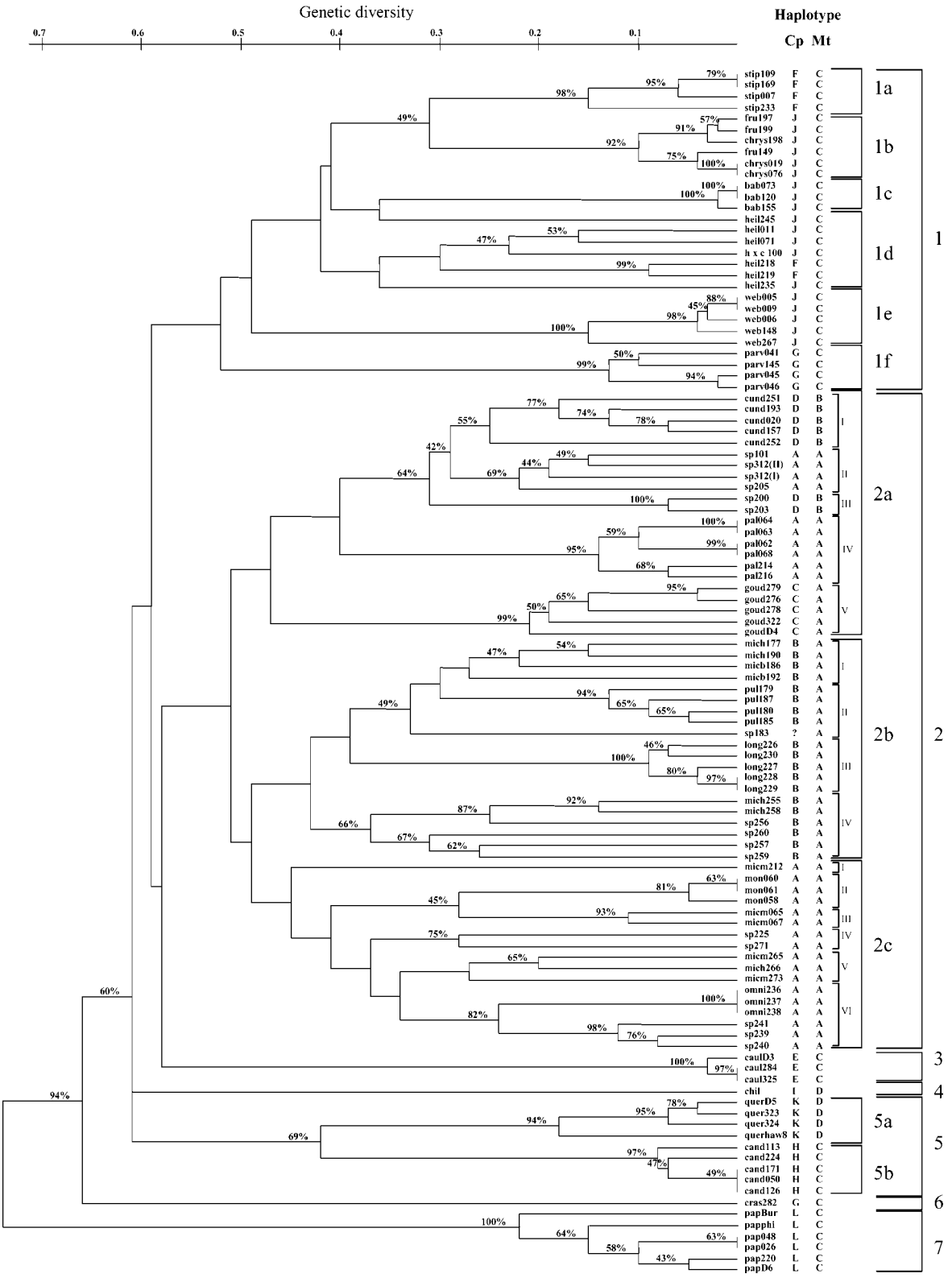

Fig. 2. The UPGMA dendrogram based on AFLP data of *Vasconcellea* and *Carica*. Genetic diversity values were calculated with the formula of Jaccard. Bootstrap values above 40 are indicated on the branches. Chloroplast (Cp) and mitochondrial (Mt) haplotype data, obtained by PCR-RFLP analysis, are given next to specimen codes.

TABLE 3. Number of individuals (*N*) and their mean intraspecific genetic similarity (Jaccard's coefficient) of all *Vasconcellea* and *Carica* species for which more than one individual was investigated, based on AFLP data.

Taxon	*N*	Mean similarity
Vasconcellea stipulata	4	0.90
V. × *heilbornii*	16	0.67
V. weberbaueri	5	0.92
V. parviflora	4	0.89
V. goudotiana	5	0.83
V. palandensis	6	0.90
V. cundinamarcensis	5	0.81
V. longiflora	5	0.93
V. pulchra	4	0.90
V. monoica	3	0.97
V. omnilingua	3	1.00
V. microcarpa	12	0.57
V. cauliflora	3	0.98
V. quercifolia	4	0.88
V. candicans	5	0.95
Carica papaya	6	0.86

is only represented in cluster 2b, while cluster 2c only includes chlorotype A. Cluster 2a comprises specimens with chlorotypes A, C, and D.

DISCUSSION

Comparing taxonomical grouping with molecular and morphological data—To verify validity of the current classification of *Vasconcellea* (Badillo, 1993), molecular and morphological UPGMA dendrograms and cytoplasmic haplotype data were compared with taxonomical grouping. If taxa are valid, they are supposed to be defined within the molecular dendrogram. In the morphological study we have focused on vegetative characters to test if it would be possible to create an identification key based on vegetative morphological data. If this is the case, molecularly validated taxa should be morphologically distinguishable based on the used data. This means that specimens belonging to the same taxon should be clustered together in the morphological dendrogram, as long as the discriminating features are included in the analysis. Taxon validity will not be evaluated for taxa represented by only one specimen.

Legitimacy of the recent generic rehabilitation of *Vasconcellea* (Badillo, 2000) is confirmed by the high AFLP-based genetic diversity (74%) between *Carica* and *Vasconcellea*. Comparable values of genetic diversity between both genera were reported before by Jobin-Decor (1997) and Van Droogenbroeck et al. (2002), who presented values of 73% and 77%, respectively. In comparison with the high genetic diversity that exists between these two genera, our geographically very diverse sample set of *Carica papaya* (collected in South America, Africa, and Asia) and the 17 studied species of *Vasconcellea* showed quite limited intrageneric genetic variation (14% and 53%, respectively). In combination with the fact that *Vasconcellea* is reported to be slightly more closely related to *Jacaratia*, another genus of the Caricaceae, than with *Carica* (Aradhya et al., 1999; Van Droogenbroeck et al., 2002), we conclude that taxonomic delimitation of the genus *Vasconcellea* is unquestionably supported by several molecular analyses, including our data. On the other hand, morphological data used in this study do not adequately support the genetic divergence between *Carica* and *Vasconcellea*. This is not surprising because only one of the discriminating features listed by Badillo (2000), particularly the number of primary veins, is a vegetative characteristic. Consequently only one of the morphologic features discriminating *Carica* and *Vasconcellea* is included in this study.

Within the genus *Vasconcellea* only two taxa exhibit intraspecific variability in the cytoplasmic fragments analyzed in this study: *V.* × *heilbornii* and *V. microcarpa* each reveal two different chlorotypes. These two taxa also show very high AFLP-based genetic diversity, 33% and 43%, respectively. The results for these taxa will be discussed further in the following section.

All other taxa for which multiple specimens were included in this study are molecularly supported by both PCR-RFLP and AFLP analysis showing no intraspecific variability with PCR-RFLP and relatively low genetic diversity values with AFLP. AFLP-based clustering shows clearly delineated clusters that are confirmed by bootstrap values between 55% (*V. cundinamarcensis*) and 100% (several taxa). The following 10 taxa are also well-defined by morphological data: *C. papaya*, *V.* × *heilbornii* 'Babaco', *V. quercifolia*, *V. candicans*, *V. weberbaueri*, *V. palandensis*, *V. parviflora*, *V. monoica*, *V. pulchra*, and *V. cauliflora*.

Although molecular relationships do corroborate the validity of *V. longiflora*, *V. cundinamarcensis*, *V. stipulata*, and *V. goudotiana*, this is not reflected in an exclusive morphological delineation of their specimens. This lack of morphological association is probably caused by the fact that none of the studied features is discriminating for these taxa or by the reported presence of high variability within taxa (Jiménez et al., 1998), possibly affected by phenotypic plasticity, introgression, or recent diversification. Rapid diversification and speciation is a typical characteristic of plant evolution on the South American continent that has been noticed since it became isolated during the late Cretaceous and early Tertiary Period, about 70 to 60 million years ago (Burnham and Graham, 1999). Genetic associations between certain groups of taxa are likewise not mirrored in the morphological clustering. For instance, the genetic relationship between *V. stipulata*, *V.* × *heilbornii*, *V. weberbaueri*, and *V. parviflora*, which was previously also reported in other AFLP and PCR-RFLP studies (Van Droogenbroeck et al., 2002, 2004), is not reflected in the morphological dendrogram. On the other hand, the clear morphological clustering of the specimens on the basis of similarity in leaf division is not confirmed by genetic associations.

In conclusion, these findings indicate that our morphological data set, containing mainly vegetative characteristics, only reveals external resemblance between the specimens that is not directly associated with genetic relationships and molecular taxon validity. Therefore we can conclude that an identification key solely based on the studied vegetative characters is problematic for this genus. Phenotypic plasticity, recent diversification, and intercompatibility between several species may confuse morphological clustering based on the vegetative data. Possible hybridization events will be discussed further in this text.

Intraspecific diversity in V.* × *heilbornii and V. microcarpa—Only two analyzed taxa reveal variation at the cytoplasmic level as well as high AFLP-based diversity and morphological separation: *V.* × *heilbornii* and *V. microcarpa*.

A 33% mean genetic diversity value and two different chlorotypes were found in *V. heilbornii*, the supposed hybrid be-

tween *V. stipulata* and *V. cundinamarcensis* (Horovitz and Jiménez, 1967; Badillo, 1971). Of the two described varieties and the cultivar within this taxon, AFLP results only support the delimitation of *V.* × *heilbornii* Babaco with a bootstrap value of 100%. On the other hand, the varieties *fructifragrans* and *chrysopetala* are intermingled in both the molecular and morphological dendrograms. According to Badillo (1993), the subdivision between both varieties is only based on the size of the spiny stipules: *chrysopetala* should bear small and weak spiny stipules, while *fructifragrans* is characterized by large and firm spiny stipules. However, our results reveal that the genetic relationship is not reflected in the size of the stipules.

The AFLP results reveal that all analyzed specimens of *V.* × *heilbornii* are genetically related with *V. stipulata* and, although more distantly, with *V. weberbaueri* and *V. parviflora*. Moreover, two of the analyzed *V.* × *heilbornii* specimens hold the chlorotype of *V. stipulata*, while all others contain the same chlorotype as *V. weberbaueri*, as reported and discussed before by Van Droogenbroeck et al. (2004). Morphologically, *V. stipulata* and *V.* × *heilbornii* are difficult to distinguish based on the features studied, because pronounced variability in leaf morphology complicates identification. However, most specimens can be classified with certainty based on fruit shape, color of petals, and general habit.

Although they are not evidenced from this study, indications of the involvement of *V. cundinamarcensis* in the hybrid formation of *V.* × *heilbornii* cannot be denied. Van Droogenbroeck et al. (2002) established that all individuals analyzed with AFLP clustered together with either *V. stipulata* or *V. cundinamarcensis*. Furthermore, the involvement of *V. cundinamarcensis* is reflected in the morphology of some specimens of *V.* × *heilbornii*. The (greenish) yellow flowers, the absence of stipules, and the slightly hairy petioles are the most pronounced features that indicate a relationship with *V. cundinamarcensis*.

A more detailed and extensive molecular and morphological analysis of *V.* × *heilbornii* and its putative parent species is being performed at this moment to clarify this problem (B. Van Droogenbroeck, E. Romeijn-Peeters, W. Van Thuyne, T. Kyndt, P. Goetghebeur, J. P. Romero-Motochi, and G. Gheysen, unpublished data). Based on all available evidence, a common maternal progenitor for *V.* × *heilbornii* and *V. weberbaueri*, or a possible triple hybridization event involving *V. stipulata*, *V. weberbaueri* and *V. cundinamarcensis*, can be hypothesized.

Intraspecific chlorotype variation in *V. microcarpa* has been revealed earlier by both Aradhya et al. (1999) and Van Droogenbroeck et al. (2004) considering relatively small sample sets (two and five specimens). It was again established in this study showing two different chlorotypes in 12 specimens belonging to three described subspecies: *microcarpa*, *baccata*, and *heterophylla*. In addition, nuclear DNA, which was never analyzed in detail before, also shows a very high within-species diversity level and accordingly very distinct grouping in the AFLP dendrogram. Subspecific classification is likewise not correlated with relationships revealed with molecular markers. Consequently, *V. microcarpa* and its subspecies do not appear to be valid taxa, as molecular data do not support their delimitation. Specimens identified as *V. microcarpa* are scattered throughout several genetically related groups, sometimes showing genetic affinities with unidentified specimens. As morphological diversity within these groups is very high, a possible hybrid origin is very plausible and will be discussed further in the following section.

Possible hybridization events and identification of unidentified specimens—Sixteen specimens involved in this study could not be identified with the key of Badillo (1993). Based on the results of this study, however, some of these specimens could be attributed to a known taxon or can be hypothesized to be of hybrid origin.

Intercompatibility between several *Vasconcellea* species has already been demonstrated by several studies (Jiménez and Horovitz, 1957; Horovitz and Jiménez, 1967; Mekako and Nakasone, 1975), and natural interspecific hybrids have been observed in areas where species are sympatric (Badillo, 1971). Of the eight species for which artificial crosses have been analyzed, six have shown to be compatible (*V. microcarpa*, *V. cundinamarcensis*, *V. stipulata*, *V. cauliflora*, *V. monoica*, and *V. horovitziana*), although not always reciprocal. *V. goudotiana* and *V. parviflora* are intercompatible but can only be crossed with a few of the abovementioned species. Based on the observed ease of hybridization, it can be hypothesized that interspecific hybrids between *Vasconcellea* species not analyzed to date might arise in nature. Taking into consideration that plant hybrid zones tend to occur more frequently in disturbed areas (Rieseberg and Ellstrand, 1993; Rieseberg, 1995), the human threat perturbing at least five species of *Vasconcellea* (IUCN, 2003) might make them more disposed to interspecific hybridization. Hybridization events can be detected molecularly by checking for incongruence between nuclear and cytoplasmic data (Rieseberg, 1995, 1997) or by non-concordance between their position in genetic and morphological analyses (Arnold, 1997). Because hybridization results in progeny with a mosaic of parental, intermediate, and extreme characters (Rieseberg, 1995) and leads to populations with a wide range of different recombinants and segregating progeny (Barton and Hewitt, 1985), morphological detection and identification of hybridization events is difficult (Rieseberg and Ellstrand, 1993). Moreover, as the morphological data presented in this study do not clearly differentiate between some genetically well-confirmed taxa, these morphological results were not always useful in the characterization of possible hybrids.

A clear evidence of incongruence is found in a group of specimens, sp101, sp205, sp312(I), and sp312(II), which are genetically closely related to *V. cundinamarcensis* based on nuclear AFLP results, although cytoplasmic PCR-RFLP results reveal that they hold the same haplotype as *V. palandensis*, *V. monoica*, and *V. omnilingua*. Preliminary results of ITS sequences (T. Kyndt, B. Van Droogenbroeck, E. Romeijn-Peeters, J. P. Romero-Motochi, X. Scheldeman, P. Goetghebeur, P. Van Damme, and G. Gheysen, unpublished data) show intra-individual sequence heterogeneity for these specimens, suggesting a hybrid origin involving *V. monoica* and *V. cundinamarcensis*. Considering that these two species are reported to be compatible (Jiménez and Hovoritz, 1957), we assume that these specimens belong to the group of superficially described (Horovitz and Jiménez, 1967; Badillo, 1971) interspecific hybrids between *V. monoica* and *V. cundinamarcensis* (m × c).

Because the haplotype of all other unidentified specimens is correlated with nuclear associations found with AFLP, no further incongruence between nuclear and chloroplast data has been observed. Nevertheless, taking into consideration that our

chloroplast data do not reveal an exclusive haplotype for every species it is not unlikely that recently divergent intercompatible species, with the same haplotype, can lead to the production of hybrids with similar nuclear and cytoplasmic characteristics and high morphological variation. Because intercompatibility has not yet been investigated in all *Vasconcellea* species, it is difficult to draw solid conclusions from our results. In the following paragraph, we point out some different genetically related groups of specimens with high morphological variation as possible hybrids or introgressed individuals between co-occuring species. Though a much more extensive survey of these specimens is necessary to understand their origin and evolution, our results suggest some preliminary conclusions that will be helpful in guiding future research.

For instance, *V. longiflora* and *V. pulchra* could be the progenitors of mich177, sp183, micb186, mich190, and micb192, all of which were sampled near the collection site of these two sympatric species. Their extremely high variability revealed in the morphological analysis is probably a result of hybrid segregation. A second group of specimens for which a hybrid origin can be hypothesized reveals a similar high range of morphological variation, while a certain molecular similarity is established: micm065, micm067, micm265, micm266, micm273, sp271, and sp225. A sympatric group of specimens, mich255, sp256, sp257, mich258, sp259, and sp260, collected in Calvario (Azuay, Ecuador), is genetically and morphologically closely associated with *V. longiflora* (mich255, sp256, and sp257 were not included in the morphological analysis due to absence of mature leaves). Our results indicate that these specimens belong to the taxon *V. longiflora* or are a divergent population or species, resulting from introgression or recent radiation. Molecular data reveal a distant genetic affinity between specimens sp200 and sp203 and (1) *V. cundinamarcensis*, sharing the same cytoplasmic haplotype, and (2) the hybrid population between *V. monoica* and *V. cundinamarcensis* (sp101, sp205, sp312(I), sp312(II): m × c). As *V. cundinamarcensis* is known to be intercompatible with at least four other *Vasconcellea* species, sp200 and sp203 might be hybrids different from *V.* × *heilbornii* and the m × c hybrid population. Finally, a group of specimens involving sp239, sp240, and sp241 has a strong molecular and morphological association with the co-occuring species *V. omnilingua*. Although these three specimens show some variation in leaf morphology, not described by Badillo (1993), genetic analysis revealed a close relationship confirmed by a high bootstrap value (82%). It is possible that the taxon description of *V. omnilingua* (Badillo, 1993) should be extended to include a higher range of morphological variation, but then again, their genetic association might also be a result of introgression or gene flow.

Based on the sufficient number of possible hybrids found among the analyzed samples, contemporary hybridization events leading to introgression between co-occuring plants are estimated to occur very frequently in the genus *Vasconcellea*. Because all plants identified as *V. microcarpa* by the key of Badillo (1993) belong to one of the proposed hybrid groups, our data suggest that this taxon is actually a combination of several hybrids from diverse origins. Investigation of faster evolving chloroplast sequences that are able to differentiate between closely related species together with an extended morphological study of these suggested hybrid specimens is needed to further investigate their origin. Moreover, more extensive intercompatibility analyses could improve our understanding of hybridization in the genus *Vasconcellea*.

In conclusion, this study suggests that evolution in *Vasconcellea* is likely to involve reticulation, introgression, and recent speciation. Our results clearly demonstrate that the taxon descriptions of Badillo (1993) are not complete and that they need a thorough revision for certain taxa. In general, we have shown that molecular marker techniques are very useful in resolving morphological identification problems in this genus.

LITERATURE CITED

ARADHYA, M. K., R. M. MANSHARDT, F. ZEE, AND C. W. MORDEN. 1999. A phylogenetic analysis of the genus *Carica* L. (Caricaceae) based on restriction fragment length variation in a cpDNA intergenic spacer region. *Genetic Resources and Crop Evolution* 46: 579–586.

ARNOLD, M. L. 1997. Natural hybridisation and evolution. Oxford University Press, New York, New York, USA.

BADILLO, V. M. 1971. Monografía de la familia Caricaceae. Publicada por la Asociación de Profesores, Universidad Central de Venezuela, Maracay, Venezuela.

BADILLO, V. M. 1993. Caricaceae, segundo esquema. *Revista de la Facultad de Agronomía de la Universidad de Venezuela* 43: 1–111.

BADILLO, V. M. 1997. Neotipificación de *Carica pubescens* Lenné et Koch y de *Carica quercifolia* (St. Hil.) Hieron. y nuevos registros de la familia para Ecuador. *Ernstia* 6: 201–205.

BADILLO, V. M. 1999. Nuevos registros para el Ecuador de especies del género *Carica*. *Ernstia* 9: 185–186.

BADILLO, V. M. 2000. *Carica* L. vs. *Vasconcella* St. Hil. (Caricaceae) con la rehabilitación de este último. *Ernstia* 10: 74–79.

BADILLO, V. M. 2001. Nota correctiva *Vasconcellea* St. Hill. y no *Vasconcella* (Caricaceae). *Ernstia* 11: 75–76.

BARTON, N. H., AND G. M. HEWITT. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* 16: 113–148.

BURNHAM, R. J., AND A. GRAHAM. 1999. The history of neotropical vegetation: new developments and status. *Annals of the Missouri Botanical Garden* 86: 546–589.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

GOWER, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53: 325–338.

HILLIS, D. M. 1987. Molecular versus morphological approaches to systematics. *Annual Review of Ecology and Systematics* 18: 23–42.

HOROVITZ, S., AND H. JIMÉNEZ. 1967. Cruziamentos interespecificos e intergenericos en Caricaceas y sus implicaciones fitotecnicas. *Agronomía Tropical* 17: 323–344.

IUCN—THE WORLD CONSERVATION UNION. 2003. The IUCN Red List of Threatened Species. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK. Available at website, http://www.iucnredlist.org/.

JACCARD, P. 1908. Nouvelles recherches sur la distribution florale. *Bulletin de la Societé Vaudoise de Sciences Naturelles* 44: 223–270.

JIMÉNEZ, H., AND S. HORIVITZ. 1957. Cruzabilidad entre especies de *Carica*. *Agronomía Tropical* 7: 207–215.

JIMÉNEZ, Y., J. ROMERO, AND X. SCHELDEMAN. 1998. Colección, caracterización y descripción de *Carica* × *heilbornii* nm. *pentagona* B.; *Carica pubescens* (A.DC.) Solms-Laub y *Carica stipulata* B. en la provincia de Loja. *Revista de Difusión Técnica y Cientifica de la Facultad de Ciencias Agricolas de la Universidad Nacional de Loja* 29: 43–54.

JOBIN-DECOR, M. P., G. C. GRAHAM, R. J. HENRY, AND R. A. DREW. 1997. RAPD and isozyme analysis of genetic relationships between *Carica papaya* and wild relatives. *Genetic Resources and Crop Evolution* 44: 471–477.

KARP, A., O. SEBERG, AND M. BUIATTI. 1996. Molecular techniques in the assessment of botanical diversity. *Annals of Botany* 78: 143–149.

KIM, M. S., P. H. MOORE, F. ZEE, M. M. M. FITCH, D. L. STEIGER, R. M. MANSHARDT, R. E. PAULL, R. A. DREW, T. SEKIOKA, AND R. MING. 2002. Genetic diversity of *Carica papaya* L. as revealed by AFLP markers. *Genome* 45: 503–512.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209–220.

MCLENACHAN, P. A., K. STÖCKLER, R. C. WINKWORTH, K. MCBREEN, S. ZAUNER, AND P. J. LOCKHART. 2000. Markers derived from amplified

fragment length polymorphism gels for plant ecology and evolution studies. *Molecular Ecology* 9: 1899–1903.

MEKAKO, H. U., AND H. Y. NAKASONE. 1975. Interspecific hybridization among 6 *Carica* species. *Journal of the American Society of Horticultural Science* 100: 237–242.

NATIONAL RESEARCH COUNCIL. 1989. Highland papayas. *In* F. R. Ruskin [ed.], Lost crops of the Incas: little-known plants of the Andes with promise for worldwide cultivation, 252–261. National Academy Press, Washington, D.C., USA.

RIESEBERG, L. H. 1995. The role of hybridization in evolution: old wine in new skins. *American Journal of Botany* 82: 944–953.

RIESEBERG, L. H. 1997. Hybrid origins of plant species. *Annual Reviews in Ecology and Systematics* 28: 359–389.

RIESEBERG, L. H., AND N. C. ELLSTRAND. 1993. What can molecular and morphological markers tell us about plant hybridisation? *Critical Reviews in Plant Science* 12: 213–241.

ROHLF, F. J. 2000. NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, Setauket, New York, USA.

ROMEIJN-PEETERS, E. 2004. Biodiversity of the genus *Vasconcellea* (Caricaceae) in Ecuador: a morphological approach. Ph.D. dissertation, Faculty of Sciences, Ghent University (UGent), Ghent, Belgium.

SCHELDEMAN, X. 2002. Distribution and potential of cherimoya (*Annona cherimola* Mill.) and highland papayas (*Vasconcellea* spp.) in Ecuador. Ph.D. dissertation, Faculty of Agricultural and Applied Biological Sciences, Ghent University (UGent), Ghent, Belgium.

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy: the principles and practice of numerical classification. W.H. Freeman, San Francisco, California, USA.

SOKAL, R. R., AND C. D. MICHENER. 1958. A statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin* 38: 1409–1438.

VAN DE PEER, Y., AND R. DE WACHTER. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Computational Applications in Bioscience* 10: 569–570.

VAN DROOGENBROECK, B., P. BREYNE, P. GOETGHEBEUR, E. ROMEIJN-PEETERS, T. KYNDT, AND G. GHEYSEN. 2002. AFLP analysis of genetic relationships among papaya and its wild relatives (Caricaceae) from Ecuador. *Theoretical and Applied Genetics* 105: 289–297.

VAN DROOGENBROECK, B., T. KYNDT, I. MAERTENS, E. ROMEIJN-PEETERS, X. SCHELDEMAN, J. P. ROMERO-MOTOCHI, P. VAN DAMME, P. GOETGHEBEUR, AND G. GHEYSEN. 2004. Phylogenetic analysis of the highland papayas (*Vasconcellea*) and allied genera (Caricaceae) using PCR-RFLP. *Theoretical and Applied Genetics* 108: 1473–1486.

VOS, P., R. HOGERS, M. BLEEKER, M. REIJANS, T. VAN DE LEE, M. HORNES, A. FRIJTERS, J. POT, J. PELEMAN, M. KUIPER, AND M. ZABEAU. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407–4414.

WEISING, K., H. NYBOM, K. WOLFF, AND W. MEYER. 1995. DNA fingerprinting in plants and fungi. CRC Press, Boca Raton, Florida, USA.