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Phylogenetic Relationships of the Cultivated Neotropical Palm *Bactris gasipaes* (Arecaceae) with its Wild Relatives Inferred from Chloroplast and Nuclear DNA Polymorphisms

T. L. P. COUVREUR,[1,6] W. J. HAHN,[2] J.-J. DE GRANVILLE,[3] J.-L. PHAM,[1] B. LUDEÑA,[4] and J.-C. PINTAUD[1,4,5]

[1]Institut de Recherche pour le Développement (IRD), UMR DGPC/DYNADIV, 911 Avenue Agropolis BP 64501, 34394 Montpellier cedex 5, France;

[2]Office of the Dean, Georgetown College and Dept. of Biology, Georgetown University, 37th & O Sts., NW, Washington, DC 20057-1003, U.S.A.;

[3]Herbarium CAY, Institut de Recherche pour le Développement (IRD), B.P. 165, 97323 Cayenne Cedex, France;

[4]Pontificia Universidad Católica del Ecuador, Laboratorio de Biología Molecular de Eucariotes, Av. 12 de Octubre y Roca, Quito, Ecuador

[5]Present address: Institut de Recherche pour le Développement, Whimper 442 y Coruña, A.P. 17-12-857, Quito, Ecuador

[6]Author for correspondence, present address: Nationaal Herbarium Nederland (NHN), Wageningen University, Generaal Foulkesweg 37, 6703 BL Wageningen, The Netherlands (Thomas.Couvreur@wur.nl)

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ABSTRACT. Peach palm (*Bactris gasipaes* Kunth.) is the only Neotropical palm domesticated since pre-Columbian times. It plays an important role not only at the local level due to its very nutritious fruits, but also in the international market for its gourmet palm heart. Phylogenetic relationships of the peach palm with wild *Bactris* taxa are still in doubt, and have never been addressed using molecular sequence data. We generated a chloroplast DNA phylogeny using intergenic spacers from a sampling of cultivars of *Bactris gasipaes* as well as putative wild relatives and other members of the genus *Bactris*. We estimated phylogenetic relationships using maximum parsimony (MP), maximum likelihood (ML), and Bayesian analysis. Our results indicated a close affinity between three taxa: *Bactris gasipaes* var. *gasipaes*, *B. gasipaes* var. *chichagui*, and *B. riparia*. There was no clear differentiation between these three taxa at the level of chloroplast sequences, and they shared a unique inversion that we characterized in this paper. *Bactris setulosa*, a species potentially related to the *Bactris gasipaes* complex, appeared highly divergent, and seemed to be a composite taxon with affinities outside the complex. We also investigated nuclear microsatellite polymorphisms at 8 loci within *Bactris gasipaes*, *B. riparia*, and *B. setulosa*, finding a pattern of relationships in agreement with the cpDNA data. The results presented here are important for future studies on domestication and crop improvement of *Bactris gasipaes*.

KEYWORDS: Arecaceae, *Bactris gasipaes*, crop-wild relatives, DNA inversions, domestication, microsatellite polymorphisms.

Bactris gasipaes Kunth (peach palm or Pijebaye) has been cultivated by Neotropical Amerindian tribes since pre-Colombian times (Clement 1988; Patiño 1958). It was widely distributed throughout the Amazon basin, the lower Andes, the Pacific Coast of Northern South America, and throughout Central America well before the arrival of the Europeans (Mora Urpi 1983). Its cultivation extends from Bolivia to southern Mexico, from sea level to 1000 m elevation (Mora Urpi 1983). Peach palm represents an important crop for Amerindian tribes because of a multitude of uses of the whole palm (Clement and Mora Urpi 1987). The fruit is the major traditional product, with high nutritive content, surpassing maize and carrots in energetic values (Metzler et al. 1992). Peach palm has been intensively cultivated for palm heart production in Costa Rica, Brazil, Ecuador, and other Latin American countries for more than two decades (Clement 1995). More recently, plantations have been established in other tropical countries such as Indonesia, Hawaii and the French island of La Reunion, making it a truly important crop worldwide.

Over the past three decades, the value of peach palm has been rediscovered, with promising nutritional and commercial benefits for resource-poor families in Latin America, and the exportation of the fine palm heart to northern countries. However, the peach palm is still neglected and largely understudied (Hernández Bermejo and León 1994; Hunter 1969; Popenoe and Jimenez 1921) in comparison to other native Neotropical crops, such as maize, cocoa, papaya, tomato, or potato.

Taxonomically, the diverse and widely distributed genus *Bactris* is still problematic, and the status of the cultigen *B. gasipaes* has been widely

TABLE 1. Circumscription of *Guilielma* in two recent classifications, and the new circumscription proposed here.

Sanders (1991)		Henderson (2000)	This study
	Non-ocreate clade	***Guilielma* group**	**Antillean clade**
Antillean clade	*Bactris cubensis*	*Bactris cubensis*	*Bactris cubensis*
	Bactris jamaicana	*Bactris jamaicana*	*Bactris jamaicana*
	Bactris plumeriana	*Bactris plumeriana*	*Bactris plumeriana*
			***Bactris gasipaes-riparia* clade**
Guilielma clade	*Bactris macana*	*Bactris gasipaes* var. *chichagui*	*Bactris gasipaes* var. *chichagui*
	Bactris dahlgreniana	(syn. *B. macana, B. dahlgreniana)*	(syn. *B. macana, B. dahlgreniana)*
	Bactris gasipaes	*Bactris gasipaes* var. *gasipaes*	*Bactris gasipaes* var. *gasipaes*
	Tuberculate clade		*Bactris riparia*
Setulosa clade	*Bactris setulosa*	*Bactris setulosa*	
Corosilla clade	*Bactris riparia*	*Bactris riparia*	

debated (e.g. Sanders 1991). *Bactris* (Arecoideae, Cocoseae, Bactridinae Dransfield et al. 2005) is the first or second most diverse palm genus in tropical America, comprising as few as 73 or as many as 239 species, according to Henderson (2000) and Uhl and Dransfield (1987), respectively. During the 19th and early 20th centuries, many specimens of peach palm and closely related wild palms were described as distinct species. These wild and cultivated species were at first described mostly in the genus *Guilielma* (Martius 1824) and later transferred to the genus *Bactris* (MacBride 1960; Wessels Boer 1988). Gradually, names typified on cultivated plants were reduced into synonymy of the widely accepted *B. gasipaes* (Clement 1988). However, the status of the wild relatives of the peach palm remained unclear. Clement (1988, 1999) considered two species as putative wild relatives of the peach palm: the predominantly Andean *B. macana* (Martius) Pittier and the Amazonian *B. dahlgreniana* Glassman, which occurs in Peruvian Amazonia and western Brazil.

The first cladistic study of *Bactris*, based on morphological characters, was published by Sanders (1991) confirming the inclusion of *Guilielma* within *Bactris*. Sanders recognized a "*Guilielma* clade" including the peach palm and its putative wild relatives *B. dahlgreniana* and *B. macana*. Sanders also grouped the *Guilielma* clade with the "Antillean clade" which comprised the three Greater Antillean species of *Bactris*, into the "non-ocreate clade". The Antillean species were also recovered as a monophyletic group by Salzman and Judd (1995) based on morphological and anatomical data. Henderson (1995) then suggested that *B. riparia* Mart. and *B. setulosa* Karst. might also be closely related to the non-ocreate clade. Sanders (1991) did not recognize a close affinity between the non-ocreate clade and *B. setulosa*, placing the latter in a separated group, the "Tuberculate clade" (Table 1). He did not include *B. riparia* in his cladistic analysis but nevertheless assigned it to the Tuberculate clade as well. Ferreira (1999) performed a cladistic analysis based on morphological and anatomical characters on the Antillean and *Guilielma* clades, including *B. setulosa* and *B. riparia*. *Bactris dahlgreniana* and *B. macana* were also included but under the names "macana S" (from the south) and "macana N" (from the north) respectively. This analysis recovered a clade comprising *B. macana* S and N and *B. gasipaes*, with *B. riparia* being sister to the former three. *Bactris setulosa* was sister to this clade, and included two morphologically differentiated forms. Finally, in a recent monograph of *Bactris*, Henderson (2000) considered an informal *Guilielma* group to contain the non-ocreate clade of Sanders plus *B. riparia* and *B. setulosa*. Moreover, Henderson (2000) distinguished two varieties within *B. gasipaes*: the cultivated variety *Bactris gasipaes* var. *gasipaes* and the wild variety *B. gasipaes* var. *chichagui* (Karsten) Henderson, the latter including the two previously recognized wild species *B. macana* and *B. dahlgreniana*, in synonymy (Table 1). We will adopt Henderson's nomenclature throughout the article, and shall refer to the *Bactris gasipaes* complex as the group that contains wild and cultivated forms of *B. gasipaes*.

Recently, several studies have been undertaken on the molecular phylogeny of the Cocoseae tribe (Gunn 2004; Hahn 2002). These studies addressed only intergeneric relationships within Cocoseae and included a single species of *Bactris*, leaving broader relationships within the genus uncertain.

The purpose of the present work is to conduct a molecular phylogeny of *B. gasipaes* and related species to answer two questions. First, can we consider all wild and cultivated forms of *B. gasipaes* sensu Henderson as part of the same species? Second, is the *Guilielma* group of Henderson monophyletic and how are the species included in this group related to *Bactris gasipaes*? Answering

TABLE 2. Summary of microsatellite genotyping data in *B. gasipaes* (Bg), *B. riparia* (Br), and *B. setulosa* (Bs).

Locus name	Repeat motif cloned	Allelic range (amplicon size in bp)	No. of alleles in the whole sampling	% of amplification success in each species
mBgCIR010	$(GA)_8$	159–180	6	Bg: 100%; Br: 100%; Bs: 0%
mBgCIR57	$(GA)_7$	263–280	5	Bg: 100%; Br: 100%; Bs: 0%
mBgCIR58	$(GA)_{17}$	265–299	13	Bg: 100%; Br: 100%; Bs: 75%
mBgCIR62	$(GA)_{16}$	199–239	8	Bg: 100%; Br: 100%; Bs: 75%
mBgCIR71	$(GA)_{17}$	122–146	8	Bg: 80%; Br: 100%; Bs: 75%
mBgCIR87	$(GA)_{19}$	174–214	7	Bg: 100%; Br: 0%; Bs: 0%
mBgCIR94	$(GA)_{15}$	203–225	4	Bg: 100%; Br: 20%; Bs: 0%
mBgCIR204	$(GA)_{14}(GAA)_2$	212–251	10	Bg: 100%; Br: 100%; Bs: 100%

these questions will help our understanding of the evolutionary relationships between the *B. gasipaes* complex and related species, and in turn allow us to address important questions on the origin and domestication process of the peach palm. Aside from these aspects, as we have included a substantial sample of *Bactris* species (29 of the 73 species recognized by Henderson 2000) and four out of five genera in the tribe Bactridinae, we will also test the monophyly of *Bactris* and explore other internal relationships.

MATERIALS AND METHODS

Plant Materials. A total of 38 samples representing 29 species of *Bactris* from all major groups recognized by Sanders (1991) and Henderson (2000) were gathered from wild or cultivated plants (Appendix 1). Species nomenclature follows Henderson (2000), except for *B. trichophylla* which we maintain as distinct from *B. mexicana* and *B. hondurensis*. Henderson (2000) considered *B. trichophylla*, as a variety of *B. mexicana*. The species that are the main focus of this study (*B. gasipaes*, *B. setulosa*, *B. riparia*) were sampled more than once in order to represent the genetic diversity across their geographic range. In addition, eight species in five genera of the Bactridinae-Elaeidinae clade (Gunn 2004; Hahn 2002) were also included in order to assess the monophyly of *Bactris*. Two species, *Cocos nucifera* and *Attalea crassispatha*, were chosen as outgroups because they belong to the clade sister of Bactridinae-Elaeidinae (Hahn 2002).

DNA Extraction, Amplification and Sequencing. Total genomic DNA was extracted from leaf fragments dried in silica gel, using DNeasy® Plant maxi Kit (Qiagen, Valencia California) and then stored at −20°C. Sequence data were obtained for all taxa from two cpDNA intergenic spacers, *trnD-trnT* (DT) and *trnQ-rps16* (Q16), using primers reported in Hahn (2002b) and reviewed in Shaw et al. (2005). An additional sequence (CfM) spanning parts of the *psbC-trnS* and *trnS-trnfM* adjacent spacers, was also obtained for *Bactris gasipaes*, *B. riparia* and *B. setulosa* samples. These regions were first amplified and sequenced using universal primers (Grivet et al. 2001). Because most of the *psbC-trnS* spacer proved invariable and the *trnS-trnfM* universal primers proved difficult to amplify in *Bactris*, a new primer pair was designed in the most variable region (850 bp) for subsequent sequencing (*psbC*fshort: 5′ ATT TGT GGC ATG CGG GAA GG 3′ and *trnfM*rshort: 5′ GGA TCG GGG AAA TAC CAA ATA AGT 5′). Non-specific amplification pattern with universal *trnS-trnfM* primers could be due to multiple annealing sites of the *trnS* (UGA) primer because *trnS* (UGA), *trnS* (GCU), *trnG* (UUC) and *trnG* (GCC) genes show very little sequence difference across taxa (Shaw et al. 2005). All spacers are located in the large single copy region of the chloroplast which is slightly less conserved than the rest of the chloroplast genome (Clegg et al. 1994) and is thus appropriate to address questions at lower taxonomic levels, especially using non-coding sequences. PCR amplifications were conducted using the FailSafe kit with Premix E (Epicentre, Madison, Wisconsin), according to manufacturer's instructions. The PCR program included 35 cycles at 95°C for 30 sec, 50°C (Q16) or 54°C (DT and CfM) for 45 sec, 72°C for 2 min and a final extension at 72°C for 7 min. The PCR products were sequenced on ABI automated sequencers using Big Dye chemistry (PE Biosystems, Foster City California).

Additionally, seven microsatellite markers isolated from *B. gasipaes* var. *gasipaes* (mBgCIR 10, 57, 58, 62, 71, 87, 94) described in Billotte et al. (2004) and one isolated from *Bactris gasipaes* var. *chichagui* (mBgCIR 204) described in Couvreur et al. (2006) were amplified in five individuals of *B. gasipaes*, one of *B. riparia* and four of *B. setulosa* (Table 2) using the protocol of Billotte et al. (2004).

Alignment of Sequence Data and Character Coding. Manual alignment was undertaken because the cpDNA sequences recovered were relatively invariant, and because this allows for the identification of motifs involved in secondary structures, such as inversions, that are not always identifiable with multiple alignment software (Kelchner 2000). Microsatellites were excluded from the analysis, as these structures originate through slipped-strand misparing (Levinson and Gutman 1987) and are highly homoplastic. Inversions and indels corresponding to direct repeat polymorphisms were coded separately as binary characters, while the gaps introduced in the sequences alignment to account for these structures were coded as missing characters. The matrices used in this study are available in TreeBASE (study number S1686).

Phylogenetic Analysis. Phylogenetic analyses were conducted with both maximum parsimony (MP) and maximum likelihood (ML) methods using PAUP* 4.0b10 (Swofford 2002). All analyses were conducted with the DT and Q16 datasets, separately or combined. To test incongruence between the two datasets, a Partition Homogeneity test (ILD, Farris et al. 1994) as implemented in PAUP* was used, with 1,000 iterations with a full heuristic search and random taxon addition. The test provides a reasonable indication of the phylogenetic signal of both data sets, and a nonsignificant *P* value suggests that the data sets can be combined.

For the MP analysis, the data matrices for each of the two cpDNA regions (Q16 and DT), and a combined data matrix were analyzed using 1,000 replicates of random taxon-addition to find multiple islands of equally most parsimonious trees (Maddison 1991), tree bisection-reconnection (TBR) branch swapping, retaining all equally most parsimonious trees (MULPARS) on, and unordered characters (Fitch Parsimony, Fitch 1971). A limit of ten trees was set for each

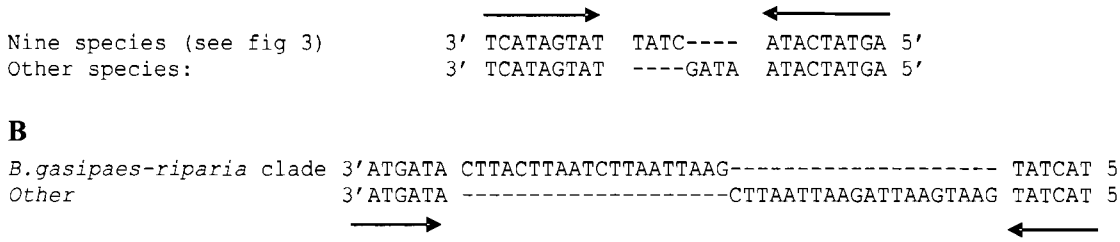

FIG. 1. Inversions detected within the *trnD-trnT* chloroplast spacer. A. Minute inversion. B. Middle-sized inversion. Arrows indicate the conserved inverted repeats flanking the inversion. The two forms of the sequence corresponding to the inversion are separated to indicate non-homology of base positions in the aligned sequences.

replicate. After completing the replicates, all trees found were then used as starting trees for another round of swapping with a tree limit of 5,000. Relative support for each branch was assessed by 1,000 bootstrap replications (Felsenstein 1985; Salamin et al. 2003) and TBR branch swapping (10 random addition and saving 10 trees per replicate).

Maximum Likelihood analyses were conducted with the combined data set. Modeltest 3.06 (Posada and Crandell 1998) was used to identify the substitution model that best fits our data. Modeltest scores indicated that the best model to fit our data under the Akaike information criterion (AIC, Akaike 1973) is the HKY85 + G model (Hasegawa et al. 1985) which accounts for both transition/transversion bias and nucleotide frequency biases. The likelihood parameters of the HKY85 + G model were estimated with PAUP* using the maximum parsimony topology (first tree in memory). These parameters were then included in a heuristic search with ten replicates of random taxon addition sequence and TBR branch swapping. The whole procedure was reiterated on the basis of the newly generated ML tree until all parameters and tree topologies stabilized. One hundred bootstrap replicates were completed to test branch support.

Bayesian phylogenetic reconstructions were conducted in MrBayes v2.01 (Huelsenbeck and Ronquist 2001) with the program's default priors and model parameters estimated as part of the analyses. The data was partitioned into three datasets: DT, Q16 and indels + inversions. Three heated chains and a single cold chain were used in all Markov Chain Monte Carlo (MCMC) analyses and runs were initiated with random trees. Trees were sampled every 500 generations and majority rule consensus trees and posterior probabilities for nodes were assembled from all post-burn-in sampled trees. Each of these data-partition runs was conducted with a total of 10 million generations, the first million of which were discarded as burn-in.

Additionally we undertook two distance analyses using the NJ algorithm (Saitou and Nei 1987) on a subset of the taxa sampling including the *B. gasipaes* complex, *B. setulosa*, and *B. riparia* (Table 1), to compare the cpDNA and microsatellite data. The first analysis included the three spacers regions (DT, Q16 and CfM) and was computed using the Jukes-Cantor distance implemented in PAUP*. The microsatellite data were analyzed using the shared allele distance DAS (Chakraborty and Jin 1993) and NJ reconstruction method implemented using the software package Populations 1.2.28 (Olivier Langella, CNRS, available at http://bioinformatics.org/project/?group_id=84). For both data sets, 10,000 bootstrap replications were completed.

RESULTS

trnD-trnT *Analysis (MP).* Of the 983 base positions included in this analysis, 36 (3.6%) were variable and of these 22 (2.2%) were potentially parsimony informative. After the initial search, 6,810 most parsimonious trees were found. Using those trees as starting trees, 5,000 equally most parsimonious trees were saved, each of 70 steps with a consistency index (CI) of 0.914 and a retention index (RI) of 0.921. The strict consensus tree was poorly resolved (not shown) and weakly supported, but a few clades did appear. The genus *Bactris* formed a monophyletic group supported by several synapomorphic substitutions with bootstrap support (BS) of 70%. Wild and cultivated *B. gasipaes* clustered together with *B. riparia* in a weakly supported clade (BS 67%) without internal resolution. This clade was supported both by substitutions and by the intermediate sized inversion reported below. Samples of *B. setulosa* from the western and eastern sides of the Andes did not cluster together. Finally, the three Caribbean species clustered together (Antillean clade) but with little support (provided by one synapomorphic substitution; BS 59%).

Two inversions were found in the DT spacer. The first one, commonly called a minute inversion (Kelchner and Wendel 1996), was 4 bp long and flanked by a perfect inverted repeat (IR) of 9 bp, located 33 bp from the 5'extreme end of *trnE* gene (Fig. 1A). It was present in nine species, including one outgroup (Fig. 3, species with * indicated). The second was an intermediate-sized inversion of 22 bp and was synapomorphic for the *Bactris gasipaes-riparia* clade. This inversion was also bordered by a pair of perfect inverted repeat sequences of 6 bp, 179 bp from 3' extreme of the *trnY* gene (Fig. 1B). Both IR's were highly conserved throughout the ingroup and the outgroup.

Analysis of* trnQ-rps16 *(MP). Of the 1,040 positions included in this analysis, 47 (4.5%) were variable and of these 38 (3.6%) were potentially parsimony informative. In addition, we coded 5 indels corresponding to direct repeats. Finally, three microsatellites were identified within the Q16 spacer. One has a repeat motif of 4 bp (GATA),

repeated 1 to 6 times depending on accessions, the two other were stretches of "Ts" and "As" of variable length (6–15 bp; 7–11 bp respectively). They were thus excluded from the phylogenetic analysis although they could be useful for future population genetic studies on these species. The analysis yielded 24 equally parsimonious trees of 99 steps. The Q16 spacer data had a CI of 0.889 and an RI of 0.933. The strict consensus tree (not shown) was slightly more informative and with higher branch support than the DT tree. Monophyly of *Bactris* was strongly supported (BS 100%). Support for the *B. gasipaes-riparia* clade was also higher (BS 80%), and *B. riparia* was recovered as a monophyletic group (BS 63% for the three specimens analyzed). *Bactris setulosa* was split in two groups, as with the DT data. Finally, the Antillean clade is not recovered with the Q16 spacer data.

Combined Analysis (MP). The ILD test (Farris et al. 1994) for the two datasets indicated good congruence between DT and Q16 (p = 0.23). The Fitch parsimony analysis was run on 2,023 characters (90 were excluded), of them 60 were parsimony informative (3%), and 9,680 trees were recovered. Using these trees as starting points for a second parsimony analysis, 5,000 trees were saved with a length of 173 steps (CI = 0.879, RI = 0.912). Resolution was not much improved, but branch supports increased significantly in the strict consensus tree of the combined analysis compared to the separate ones. This also indicated that the two datasets are combinable. In this combined analysis, the genus *Bactris* was strongly supported as a monophyletic group (Fig 2, BS 100%). The *B. gasipaes-riparia* clade had good support (Fig 2, BS 92%). The weakly supported western group of *B. setulosa* (Fig 2, BS 65%) formed a well supported clade with *B. coloradonis* and *B. concinna* (Fig 2, BS 86%), while the eastern sample fell in a large unresolved basal polytomy that included the majority of *Bactris* species together with the Caribbean ones. Finally four species (*B. major, B. militaris, B. brongniartii,* and *B. bifida*) form a clade with no bootstrap support.

ML and Bayesian Analysis. Two identical trees were obtained using the maximum likelihood method (Fig. 3) with a transition/transversion ratio of 0.9287 and a log likelihood of − 4,126.98. These maximum likelihood trees were more informative than the combined MP strict consensus tree. Clades highly supported in the MP analysis were also supported in the ML analysis (100% BS for the genus *Bactris*, 93% for the *B. gasipaes-riparia* clade, 89% for *B. riparia*, 83% for western Andean *B. setulosa* with *B. concinna* and *B. coloradonis*). The Antillean clade was recovered but with minimal support (BS 53%). The Bayesian analysis tree (not shown) was topologically identical to the ML tree. Clades with posterior probability values of 95% or higher were generally congruent with clades having bootstrap support of 70% or greater found in the ML analysis, which took 25 times longer (24 hours for the Bayesian analysis, 5 weeks for 100 replicates of the ML bootstrap analysis). Posterior probabilities of the Bayesian analysis were always higher when compared to the ML bootstraps except for the Bactridineae clade (Fig. 3).

NJ Analyses. Both NJ analyses, using two different types of markers, showed the same groupings and similar relationships. The unrooted three-spacers chloroplast distance tree (Fig. 4) was in agreement with the previous two-spacers analyses. *Bactris setulosa* was highly divergent from the *B. gasipaes-riparia* clade. Divergence between the eastern and western Ecuadorian origins of *B. setulosa* was very marked also suggesting polyphyly of this species as well. The NJ tree based on microsatellite markers showed the same groupings but with much less marked divergence between the *B. gasipaes-riparia* clade and *B. setulosa*. Incomplete transferability of the microsatellites markers to *B. setulosa* may have biased the result, but this is an indication that this species is distantly related to *B. gasipaes*. Transferability was best with *B. riparia*, which exhibited little allelic differentiation (Table 2).

Discussion

The* Bactris gasipaes-riparia *Clade. Prior to the present analysis, only morphological and anatomical characters were used to infer the phylogenetic structure of *Bactris* and especially the putative sister taxa of the peach palm (Ferreira 1999; Salzman and Judd 1995; Sanders 1991). All three phylogenic analyses of the cpDNA sequences recovered the same topology, grouping *B. gasipaes* and *B. riparia*. This grouping was supported by various substitutions in both DT and Q16 spacers and also by the 22-bp inversion (Fig. 1B). However, both cpDNA sequences and nuclear microsatellites failed to separate the wild *B. gasipaes* var. *chichagui* from the cultivated *B. gasipaes* var. *gasipaes*. Similar results were obtained with RAPD markers (Rodrigues et al. 2004) and might be explained in various ways. It is possible that the domestication process had little impact on mostly neutral markers like SSR, RAPD, and non-coding cpDNA, which therefore do not show significant differentiation between the wild and cultivated varieties. Another possible explanation would be polyphyly of *B. gasipaes* var. *gasipaes* resulting from independent

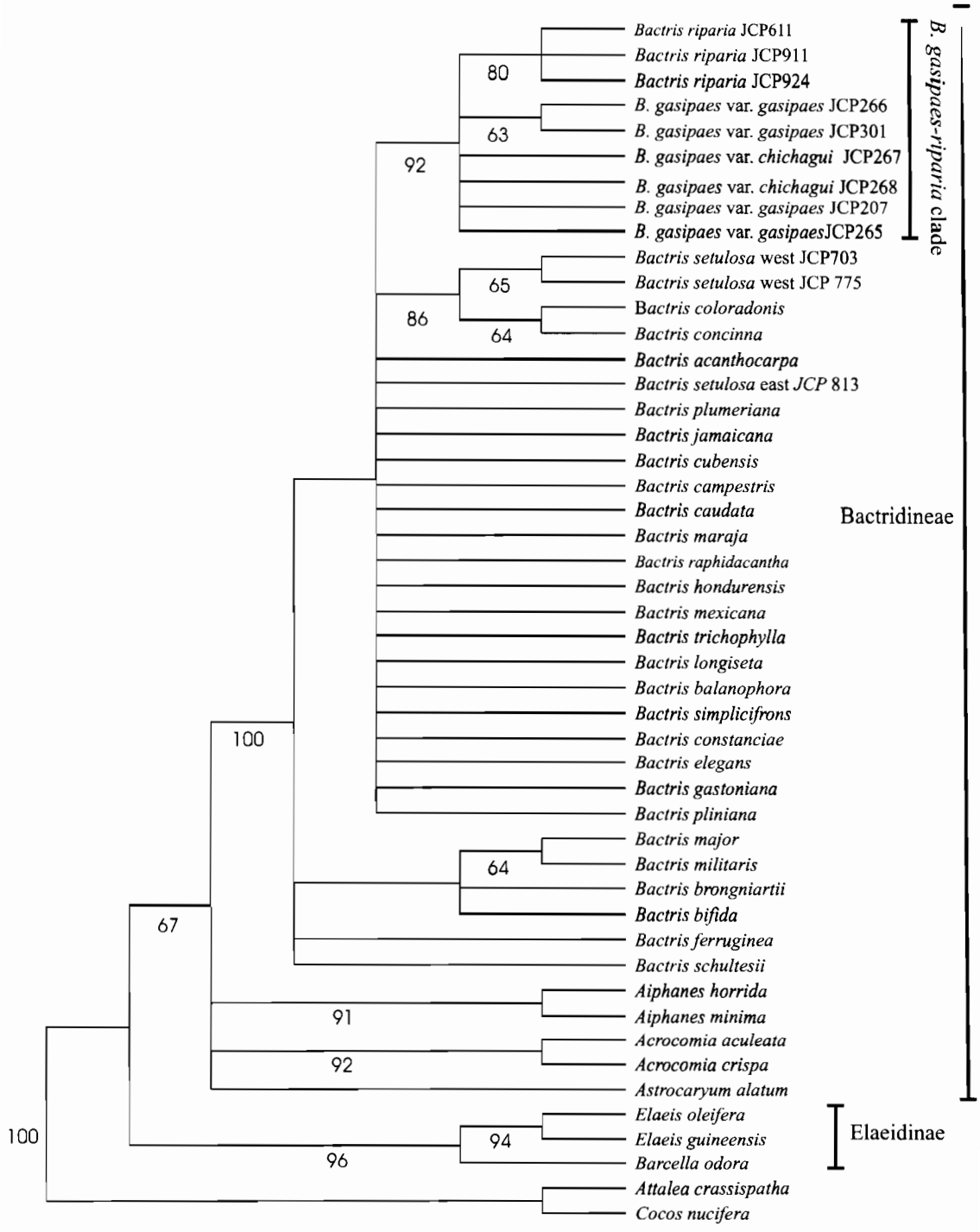

FIG. 2. Maximum parsimony strict consensus tree of 5,000 trees from the combined *trnD-trnT* and *trnQ-rps16* sequence data. Tree length = 173 steps; CI = 0.879 and RI = 0.912. Bootstraps replicate values are indicated under the branches.

events of domestication of distinct wild populations. Henderson (2000) reported three different fruit types in *Bactris gasipaes* var. *chichagui*, which were subsequently referred to as types I, II, and III (Da Silva and Clement 2005). Type I corresponds to the former *B. dahlgreniana*, type III to the former *B. macana pro parte* and both were included in the cpDNA dataset. The two types could have been involved in the domestication process. Finally, introgression between wild and cultivated populations in sympatry may further complicate their relationships. Rodrigues et al. (2004) and Clement

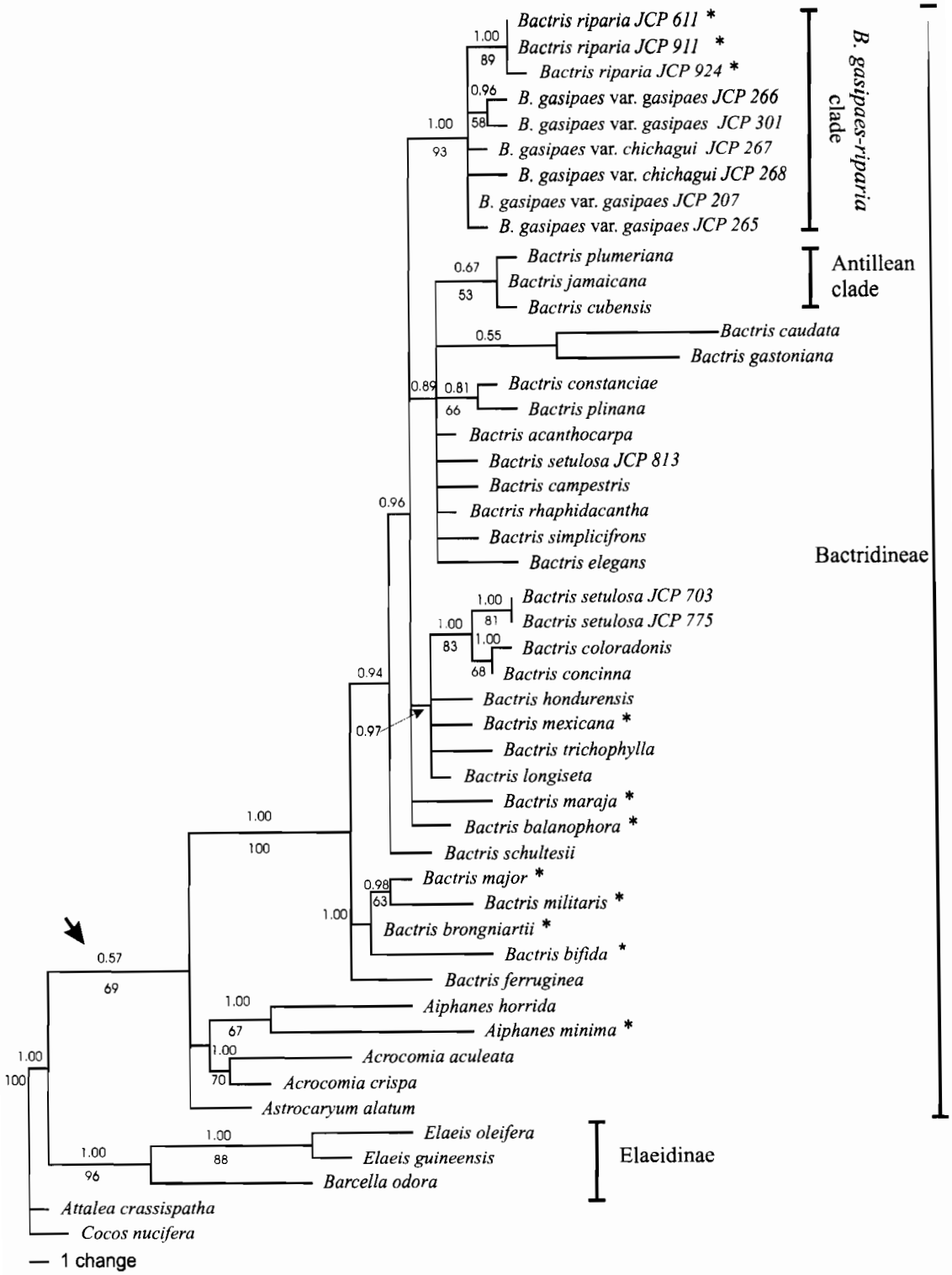

FIG. 3. Maximum likelihood tree (*trnD-trnT* and *trnQ-rps16* spacers data combined). Model used = HKY85 + G; base frequencies: A = 0.34; C = 0.15; G = 0.16; T = 0.34; Substitution model: Ti/tv ratio = 0.93; gamma distribution shape = 0.0044; 100 bootstrap replicates values are indicated under branches. Bayesian analysis: posterior probability values (PP) are indicated above branches. Arrow indicates where the PP value is smaller than the bootstrap values. * indicates the distribution of the minute inversion.

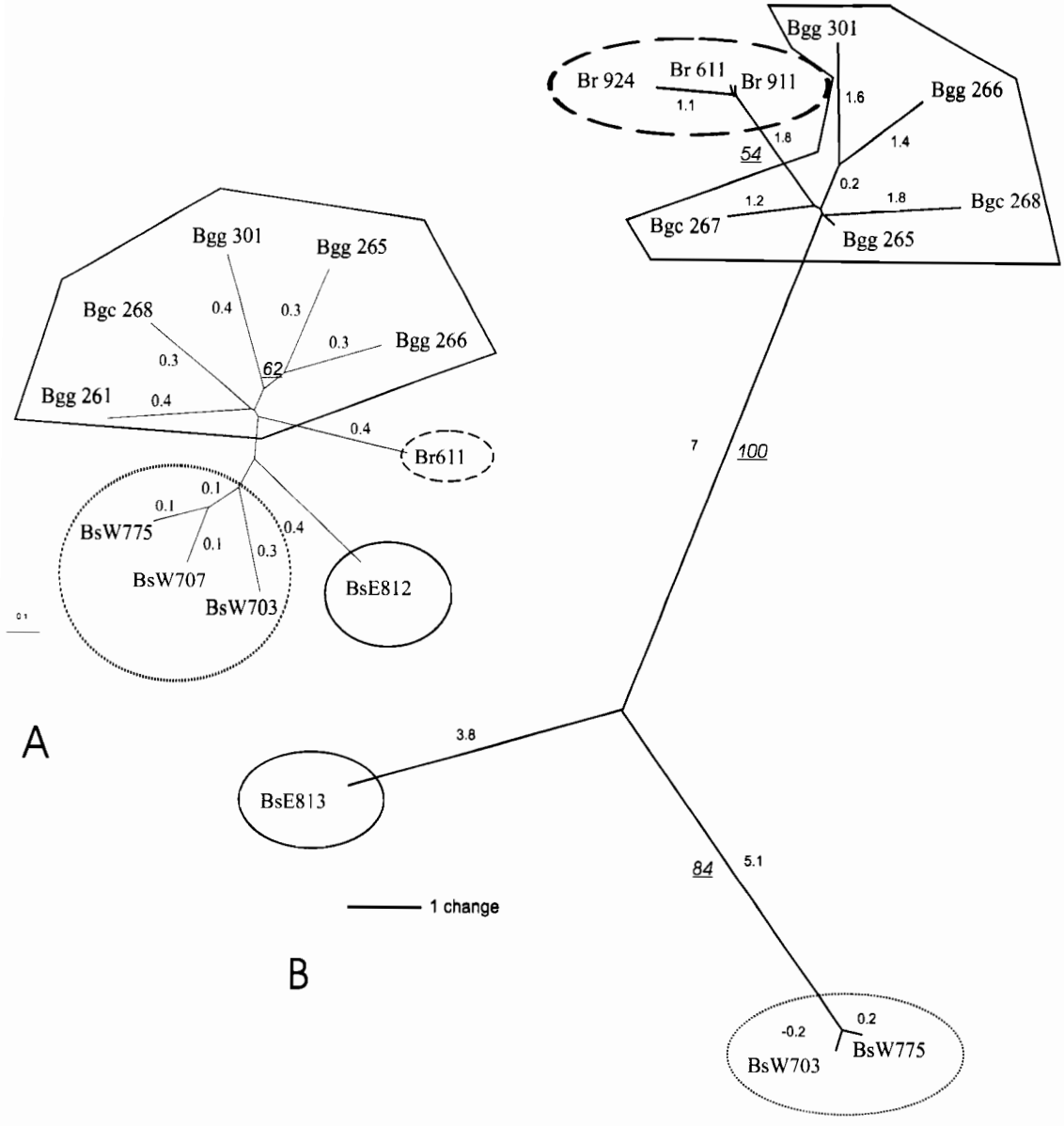

FIG. 4. Neighbor Joining (NJ) analyses of *B. gasipaes*, *B. riparia* and *B. setulosa* with two different molecular markers. A. NJ tree of 8 nuclear microsatellite markers, computed with the D_{AS} distance. B. NJ tree from nucleotide substitution data of three cpDNA spacers (*trnD-trnT*, *trnQ-rps16* and *psbC-trnfM* p.p.), computed with the Jukes-Cantor distance. Bgg: *B. gasipaes* var. *gasipaes*; Bgc: *B.gasipaes* var. *chichagui*; Br: *B. riparia*; BsE: *B. setulosa* of eastern Ecuador; BsW: *Bactris setulosa* of western Ecuador. Values in italic and underlined indicate bootstrap values > 50% after 10,000 iterations. Numbers on branches indicate the NJ distance values.

et al. (1999) suggested that introgression between wild and cultivated populations was very limited in Brazil, while Couvreur et al. (2006) showed evidence of extensive gene flow between wild and cultivated plants in western Ecuador.

At a higher level, the taxonomic treatment of *B. gasipaes* by Henderson (2000), which included *B. macana* and *B. dahlgreniana* with *B. gasipaes*, was consistent with all available molecular data. In addition, our study indicated that *B. riparia* is very closely related to the *B. gasipaes* complex which confirms Ferreira's study (1999), where *B. riparia* appeared sister to both *B. macana* and *B. gasipaes*. *Bactris riparia* was also considered by Henderson (1995) as possibly related to *B. gasipaes* based on various vegetative and reproductive characteristics, but was not included in the cladistic analysis of Sanders (1991). *Bactris riparia* has red-orange starchy fruits similar to those of both wild and cultivated *B. gasipaes* varieties (Henderson 2000). The cpDNA data grouped together all individuals of *B. riparia* on the basis of point substitutions and

the minute inversion, but failed to recover *B. gasipaes* as sister to *B. riparia* due to a lack of synapomorphies for *B. gasipaes* alone (although there were synapomorphies for *B. riparia* alone and for *B. gasipaes* + *B. riparia*). More variable markers may distinguish the two species, which are very distinctive. In addition, *B. riparia* has a highly specialized ecology, being restricted to the vicinity of inundated sites, especially in black water areas.

Henderson (2000) suspected that *B. setulosa* was related to *B. gasipaes* and therefore placed the former within the Guilielma group. *Bactris setulosa* did not cluster within the *B. gasipaes-riparia* clade in our analyses. Low transferability of the microsatellite markers from *B. gasipaes* to *B. setulosa* also indicated that these two taxa are not as closely related as previously thought. Moreover, different samples of *B. setulosa* are always found in two groups, corroborating the findings of Ferreira (1999), who identified a marked morphological difference between two groups of specimens. *Bactris setulosa* is, therefore, much in need of taxonomic revision. In our analysis, *B. setulosa* sampled west of the Andes grouped with *B. coloradonis* and *B. concinna* with some support. More markers are needed to confirm this relationship. The eastern Andean specimens clustered weakly with other species, including the Antillean specimens. The Antillean clade, considered close to the *Guilielma* clade by Sanders (1991), appeared monophyletic, but its relationship to other groups remained poorly supported in the present analysis. Monophyly of the Antillean clade as shown here agrees with previous morphological studies (Ferreira 1999; Salzman and Judd 1995). From these results we propose a new circumscription for the Guilielma clade comprising *B. riparia* and the *B. gasipaes* complex (Table 1).

Monophyly of* Bactris *and Other Relationships Within this Genus. Our results supported the monophyly of *Bactris* with the maximum value of branch support. This result was interesting taking into consideration the great morphological diversity of this large and widespread genus, and close affinity with other genera of Bactridinae like *Aiphanes* and *Astrocaryum* (Gunn 2004; Hahn 2002), also included in this analysis.

From the ML and Bayesian analyses, there were a few other weakly to moderately supported clades that might be of phylogenetic significance. A clade including *B. major*, *B. brongnartii*, *B. bifida*, and *B. militaris* grouped together species that shared the small inversion and, except for *B. militaris*, belong to the *Pyrenoglyphis* group. The two other species of the *Pyrenoglyphis* group included in this analysis, *B. concinna* and *B. gastoniana*, have the alternative and more common condition of the small-inversion locus. These two species did not form a clade with each other, nor with the other *Pyreneglyphis* species. These results suggest polyphyly for the *Pyrenoglyphis* group as delimited by Henderson (2000), who did not attempt to define monophyletic entities within *Bactris*, but rather artificial groups to facilitate species identification. Thus it is not surprising to find various groups recognized by Henderson to be polyphyletic, but the grouping of *B. constanciae* with *B. pliniana* (Fig. 3) is hard to explain. These two species are highly distinct morphologically and do not show any obvious affinity. At the molecular level, they share a 38 bp deletion in the *trnD-trnT* spacer, which was not been found elsewhere in the sampling. Further study is needed to clarify this relationship. *Bactris mexicana*, *B. trichophylla*, and *B. hondurensis* showed several differences in cpDNA sequences, including substitutions, inversions, and indels. They did not form a clade in any of our analyses and therefore are best considered distinct species.

Inversions. We report the presence of two inversions flanked by perfect inverted repeats in the DT spacer (Fig. 1). The discovery of such inversions in angiosperms has increased over the past ten years (Graham et al. 2000; Ohsako and Ohnishi 2000; Sang et al. 1997) as predicted by Graham and Olmstead (2000). Asmussen and Chase (2001) documented inversions in non-coding chloroplastic sequences of palms but did not described them. A few putative mechanisms for the origin of such inversions have been proposed and are reviewed in Kelchner (2000). These minute inversions are thought to be highly susceptible to reversal and parallelism within a studied group (Kelchner 2000). For example, Graham et al. (2000) characterized a minute inversion (4 bp) detected in four distantly related genera. This indicated that minute inversions may be highly homoplastic. However, inversion polymorphism could be phylogenetically informative at lower taxonomic levels. Indeed, the minute inversion (4 bp) detected in our dataset occurred convergently within *Bactris* and also in *Aiphanes* but distinguished *B. gasipaes* from *B. riparia* and also *B. trichophylla* from *B. mexicana* (Fig. 3). Nevertheless, the overall high homoplasy (CI=0.25; RI=0.70) of this character in the dataset weakened the support of the *B. gasipaes – riparia* clade. On the contrary, the medium-sized inversion (22 bp) was highly informative phylogenetically (CI=RI=1.00), representing a synapomorphy for the *B. gasipaes-riparia* clade. Sang et al. (1997) also detected a midsized inversion (21 or 6 bp) that was specific to the genus *Paeonia* (Paenoiaceae). These results indicate that midsize

inversions convey valuable phylogenetic information, and therefore should be carefully examined during alignment (Kelchner 2000).

The present molecular analysis clarified several important points. Our results confirmed that the wild and cultivated varieties of *B. gasipaes* are part of the same species, and revealed that *B. riparia* is closely related to this complex. This has important implications regarding the geographic origin of *Bactris gasipaes*, as the wild populations of the *Bactris gasipaes-riparia* clade are restricted to the western Amazon and Andes. Moreover, *Bactris riparia* may be regarded as a potential genetic resource for the improvement of the peach palm.

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APPENDIX 1. Species name; extraction number (only for *B. setulosa, B. gasipaes*, and *B. riparia*); GeneBank number (*trnQ-rps16; trnD-trnT; psbC-trnfM*) '-' = missing; number of SSR loci genotyped (only for *B. setulosa, B. gasipaes, and B. riparia*); origin; collector information; location of voucher specimen; group as defined by Henderson 2000. * = sequence from genebank (Hahn 2002).

Bactris riparia Mart.; JCP611; DQ159262; DQ159230; DQ645576; 6 loci; Sucumbios, Ecuador; AAU 44381 (AAU); ***Guilielma.*** *Bactris riparia* Mart.; JCP91; DQ159263; DQ159231; DQ645577; Orellana, Ecuador; Balslev 4345 (AAU); ***Guilielma.*** *Bactris riparia* Mart.; JCP924; DQ159264; DQ159232; DQ645578; Loreto, Peru, Millán 717 (USM); ***Guilielma.*** *Bactris gasipaes* Kunth var. *chichagui* Henderson; JCP267; DQ159256; DQ159224; DQ645571; Rôndonia, Brazil; Clement 501 (FTG); ***Guilielma.*** *Bactris gasipaes* Kunth var. *chichagui* Henderson; JCP268; DQ159257; DQ159225; DQ645572; 8 loci; Puertovelo, Western Ecuador; Balslev 62013 (AAU); ***Guilielma.*** *Bactris gasipaes* Kunth var. *gasipaes;* JCP207; DQ159258; DQ159226; -; French Guyana; Granville & Perthuis 17288 (CAY); ***Guilielma.*** *Bactris gasipaes* Kunth var. *gasipaes;* JCP261; - ; - ;- ; 8 loci; Brazil; no voucher; ***Guilielma.*** *Bactris gasipaes* Kunth var. *gasipaes;* JCP265; DQ159261; DQ159229; DQ645575; 8 loci; Nicaragua Granville & Perthuis 17289 (CAY); ***Guilielma.*** *Bactris gasipaes* Kunth var. *gasipaes;* JCP266; DQ159259; DQ159227; DQ645573; 8 loci; Brazil; Granville & Perthuis 17290 (CAY); ***Guilielma.*** *Bactris gasipaes* Kunth var. *gasipaes;* JCP301; DQ159260; DQ159228; DQ645574; 7 loci; Loreto, Peru; no voucher; ***Guilielma.*** *Bactris setulosa* H. Karst.; JCP703; DQ159266; DQ159234; DQ645580; 5 loci; Western Ecuador; AAU 97637 (AAU); ***Guilielma.*** *Bactris setulosa* H. Karst.; JCP707; DQ159265; DQ159233; -; 3 loci; Western Ecuador, Balslev 4298 (AAU); ***Guilielma.*** *Bactris setulosa* H. Karst.; JCP775; DQ159267; DQ159235; DQ645581; 4 loci; Western Ecuador; ***Guilielma.*** *Bactris setulosa* H. Karst.; JCP812;-;-;-; 2 loci; Eastern Ecuador; Balslev 62117 (AAU); ***Guilielma.*** *Bactris setulosa* H. Karst.; JCP813; DQ159233; DQ159265; DQ645579; Eastern Ecuador; Balslev 4288 (AAU); ***Guilielma.*** *Bactris plumeriana* Mart.; DQ372521; DQ372509; -; Hispaniola; Hahn 7758 (MBC, WIS); ***Guilielma.*** *Bactris jamaicana* L.H. Bailey; -; DQ372510;-; Jamaica; Hahn 7400 (WIS); ***Guilielma.*** *Bactris cubensis* Burret; DQ372522; DQ372511; -; Cuba; Hahn 7519 (WIS); ***Guilielma.*** *Bactris acanthocarpa* Mart. (as *B. humilis* in Hahn 2002); AY044604*; AY044509*; - ; **Piranga**. *Bactris rhaphidacantha* Wess.Boer; DQ159270; DQ159238; - ; French Guyana; Perez 878 (CAY); **Piranga.** *Bactris pliniana* Granv. & A.J. Hend.; DQ159284; DQ159252; - ; French Guyana; Perez 938 (CAY); **Piranga.** *Bactris schultesii* (L.H. Bailey) Glassman; DQ159279; DQ159247; - ; Ecuador; AAU 64761 (AAU); **Amylocarpus.** *Bactris simplicifrons* Mart.; DQ159280; DQ159248; - ; French Guyana; Perez 874 (CAY); **Amylocarpus.** *Bactris balanophora* Spruce; DQ372527; DQ372514; - ; Amazonas, Brasil; Hahn 7620 (WIS); **Orange-fruited.** *Bactris campestris* Poepp.; DQ159268; DQ159236; - ; French Guyana; Perez 825 (CAY); **Orange-fruited.** *Bactris caudata* H. Wendl. ex Burret; DQ372523; DQ372512; - ; Costa Rica; Hahn 7935 (WIS); **Orange-fruited.** *Bactris coloradonis* L.H. Bailey; DQ159271; DQ159239; -; Costa Rica; Janovec 1302 (FTG); **Orange-fruited.** *Bactris hondurensis* Standl.; DQ372525; - ; - ; Costa Rica; Hahn 7517 (WIS); **Orange-fruited.** *Bactris mexicana* Mart.; DQ372525; - ; - ; Mexico; Hahn 7401 (WIS); **Orange-fruited.** *Bactris militaris* H.E. Moore; DQ372526; DQ372513; - ; Costa Rica; Hahn 7382 (WIS); **Orange-fruited.** *Bactris trichophylla* Burret; DQ159272; DQ159240; - ; Belize; Zona 1097 (FTG); **Orange-fruited.** *Bactris constanciae* Barb. Rodr.; DQ15928; DQ159249; - ; French Guyana; Perez 881 (CAY); **Purple-fruited.** *Bactris elegans* Barb.Rodr. & Trail; DQ159282; DQ159250; - ; French Guyana; Perez 854 (CAY); **Purple-fruited.** *Bactris ferruginea* Burret; DQ159273; DQ159241; - ;

Brazil; Noblick 4836 (FTG); **Purple-fruited.** *Bactris longiseta* H. Wendl. ex Burret; DQ159274; DQ159242; - ; Costa Rica; Noblick 5457 (FTG); **Purple-fruited.** *Bactris maraja* Mart.; DQ159269; DQ159237; - ; Peru; Millán 562 (USM); **Purple-fruited.** *Bactris bifida* Mart. ; DQ159278 ; DQ159246 ; - ; Peru; Millán 719 (USM); **Pyrenoglyphis.** *Bactris brongniartii* Mart.; DQ159275; DQ159243; - ; Peru; FTG 99 2111A; no voucher; **Pyrenoglyphis.** *Bactris concinna* Mart.; DQ159277; DQ159245; -; Peru; Moore 8411 (BH); **Pyrenoglyphis.** *Bactris gastoniana* Barb.Rodr.; DQ159283; DQ159251; -; French Guyana; Perez 864 (CAY); **Pyrenoglyphis.** *Bactris major* Jacq.; DQ159276 ; DQ159244; - ; Guyana ; Zona 1094 (FTG); **Pyrenoglyphis.** *Aiphanes horrida* (Jacq.) Burret; AY044603*; AY044507 (DT)*; - ; ?; Zona 1095 (FTG). *Aiphanes minima* (Gaertn.) Burret; DQ159285; DQ159253; - ; Miami, USA (from Lesser Antilles); Zona 873 (FTG). *Acrocomia aculeata* (Jacq.) Lodd ex Mart. ; AY044602*; AY044506 (DT)*; - ; Brasil; Noblick 5019 (MBC). *Acrocomia crispa* (Kunth) C.F. Baker ex Becc.; AY044607*; AY044511*; - ; Cuba; Perry, s.n. (MBC). *Astrocaryum alatum* Loomis; AY044604*; AY044508*; - ; Costa Rica; Hubbuch & Nemenyi 54 (FTG). *Barcella odora* (Trail) Drude; AY044608* ; AY044512* ; - ; Brazil; Kahn 3608 (CEN). *Elaeis guineensis* Jacq.; DQ159255; DQ159223; - ; Democratic Republic of Congo; Lame PH 562; no voucher. *Elaeis oleifera* (Kunth) Cortés; AY044609*; AY044513*; - ; Panama; FTG 87 117, no voucher. *Cocos nucifera* L.; AY044613*; AY044517*; - ; Miami Div. of Forestry, USA; Zona 1098 (FTG). *Attalea crassispatha* (Mart.) Burret; DQ159286; DQ159254; -; Haiti; Zona 1099 (FTG).