Apparent similarity, underlying homoplasy: Morphology and molecular phylogeny of the North American clade of *Manihot*[1]

María-Angélica Cervantes-Alcayde[2,5], Mark E. Olson[2], Kenneth M. Olsen[3], and Luis E. Eguiarte[4]

[2]Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, 3er Circuito de Ciudad Universitaria s/n, Del. Coyoacán, México D.F. 04510 Mexico; [3]Department of Biology, Washington University, St. Louis, Missouri 63130 USA; and [4]Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, 3er Circuito de Ciudad Universitaria s/n, Del. Coyoacán, México D.F. 04510 Mexico

- *Premise of the study:* Morphologically diverse clades are useful for detecting adaptive morphological evolution. Each of their variants may have evolved once or several times, suggesting that their repeated appearance may be due to environmental pressures. The North American *Manihot* species are an excellent system to detect possible adaptations and to assess the effect of mono- or polyphyly on classification. With 20 species, this group includes growth forms from tuberous herbs to trees. The monophyly of this group and its relationship with the economically important *M. esculenta* were tested for the first time with complete sampling of North American species.
- *Methods:* We carried out maximum likelihood and Bayesian phylogenetic analyses on a matrix of 3662 bp from chloroplast (*psbA-trnH*, *trnL-trnF*) and nuclear loci (*PEPC* and two paralogous copies of *G3pdh*). We included all North American *Manihot* species, *Manihotoides pauciflora*, and published sequences from 34 South American species.
- *Key results:* Our results support monophyly of the North American *Manihot* group. Its taxonomic sections are paraphyletic, and three to four growth forms evolved repeatedly. *Manihotoides pauciflora* is nested within North American *Manihot* species. Some *PEPC* and *G3pdh* clones grouped with clones of other species and not with clones from their own species.
- *Conclusions:* North and South American *Manihot* species are sister clades. Paraphyly of North American sections suggests that taxonomic revision is warranted. The position of *Manihotoides pauciflora* confirms that *Manihotoides* should remain subsumed within *Manihot*. Most growth forms likely evolved repeatedly in this group. The behavior of *PEPC* and $G3pdh_{NA}$ clones is probably due to incomplete lineage sorting.

Key words: Bayesian analyses; Euphorbiaceae; growth form evolution; incomplete lineage sorting; maximum likelihood; *Manihotoides pauciflora*; morphological homoplasy; North American *Manihot* clade.

Detecting morphological convergence allows the identification of possible adaptations and environmental factors that promote morphological diversity. The study of morphologically diverse clades is particularly useful because the species share a common ancestor and evolved from the same morphological starting point. Within morphologically diverse clades, each of the different variants might have evolved once, with their occurrence in multiple species reflecting shared ancestry, or they might have evolved convergently in separate species (e.g., Baker et al., 2000; Möller and Cronk, 2001; Perret et al., 2003; Edwards et al., 2005; Hearn, 2006). To identify one of these scenarios as the most plausible requires knowing the phylogenetic relationships among the members of these clades. One of the best-known examples of convergent morphological evolution comes from the lizard genus *Anolis* (Losos, 2001). On each of the largest islands of the Caribbean, there are similar assemblages of lizards, each made up of species with distinct body forms and divergent habitat uses. Molecular phylogenies indicate repeated and independent evolution of each body form on each island as the most likely scenario (see also Reinthal and Meyer, 1997, Turgeon and Bernatchez, 2003; Østbye et al., 2005; Østbye et al., 2006). Distinguishing between single and multiple origins of morphological features not only affects inference of the processes involved in species diversification, but

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[5]Author for correspondence (e-mail: angelicacervantesalcayde@yahoo.com.mx), phone: +52 55 5622-9124

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also is vital for identifying the features that can diagnose natural supraspecific classifications. We detail a case in the morphologically diverse and economically important plant genus *Manihot* Mill. Our molecular phylogenetic data suggest that morphologically similar species, which have traditionally been grouped together in supraspecific classifications, are actually instances of homoplasious evolution of growth form.

Manihot occurs exclusively in the New World and belongs, with the genera *Cnidoscolus* Pohl, *Elateriospermum* Blume, *Glycydendron* Ducke, *Hevea* Aubl., and *Micrandra* Benn. & R. Br., to the lineage of articulated crotonoids within Euphorbiaceae Juss. (Wurdack et al., 2005). *Manihot* and *Cnidoscolus* have been considered sister genera based on morphological and molecular evidence (Miller and Webster, 1962; Webster, 1994; Wurdack et al., 2005). With approximately 100 species, *Manihot* is a phenotypically diverse genus that includes tuberous herbs with annual shoots, shrubs, vines, and trees over 10 m tall (Figs. 1, 2). Its geographical range spans southern Arizona to Argentina, but there are two main concentrations of taxa. The largest is in eastern and central Brazil, where almost 80 species occur in a region including the states of Goias, Minas Gerais, and Bahia (Rogers and Appan, 1973). Mexico is the center of diversity in North America, with 15 of the 20 North American species occurring exclusively there, and the remaining five species having the bulk of their ranges in Mexico. Only one species, *M. brachyloba* Müll. Arg., has a distribution that overlaps with those of both South and North American species, occurring in the northwestern Amazon basin, in a very small area of Costa Rica, and on Hispaniola (Duputié et al., 2011). Most species of *Manihot* are found in tropical dry habitats, and even those that occur in wet forest tend to grow in sunny clearings. The best-known species is the economically important starch crop cassava (also known as manioc or yuca), *M. esculenta* subsp. *esculenta* Crantz, which was domesticated from the South American *Manihot esculenta* subsp. *flabellifolia* (Pohl.) Cif. (Olsen and Schaal, 1999, 2001).

Phylogenetic relationships among North American *Manihot* species have been little studied. Although most previous phylogenetic hypotheses for the genus have suggested that the North American species make up a monophyletic group (Schaal et al., 2006; Chacón et al., 2008), an alternative hypothesis proposes that the North American species form a grade basal to the South American clade (Duputié et al., 2011). Ascertaining whether the North American species form a clade requires extensive sampling of these species. A phylogenetic analysis of the North American species would help clarify the relationships within this group, because less than half of the species have been included in previous phylogenetic studies. In addition, a robust phylogenetic hypothesis for the North American species of *Manihot* would help settle the long debate regarding the possible involvement of three North American species in the domestication of the most economically important species in the genus, *M. esculenta* subsp. *esculenta* (e.g., Rogers, 1965; Renvoize, 1972; Bertram, 1993; Fregene et al., 1994; Roa et al., 1997; Olsen and Schaal, 1999, 2001; Léotard et al., 2009). Showing that the species are only distantly related to the cultivated manioc would further support *M. esculenta* subsp. *flabellifolia* as the sole wild progenitor (Olsen and Schaal, 1999, 2001).

A phylogenetic hypothesis for North American species would also allow us to address whether the proposed supraspecific taxonomy (Rogers and Appan, 1973) represents monophyletic groups. Rogers and Appan (1973) split the North American species into two sections, *Foetidae* Rogers and Appan and *Parvibracteatae* Pax emend. Rogers and Appan, based on the growth form and the size of fruits and seeds. There is evidence that these sections might not reflect monophyletic groups, with South and North American species from different sections being intermingled in molecular phylogenetic hypotheses (Bertram, 1993; Chacón et al., 2008; Duputié et al., 2011). Moreover, since the monograph of Rogers and Appan (1973), two new

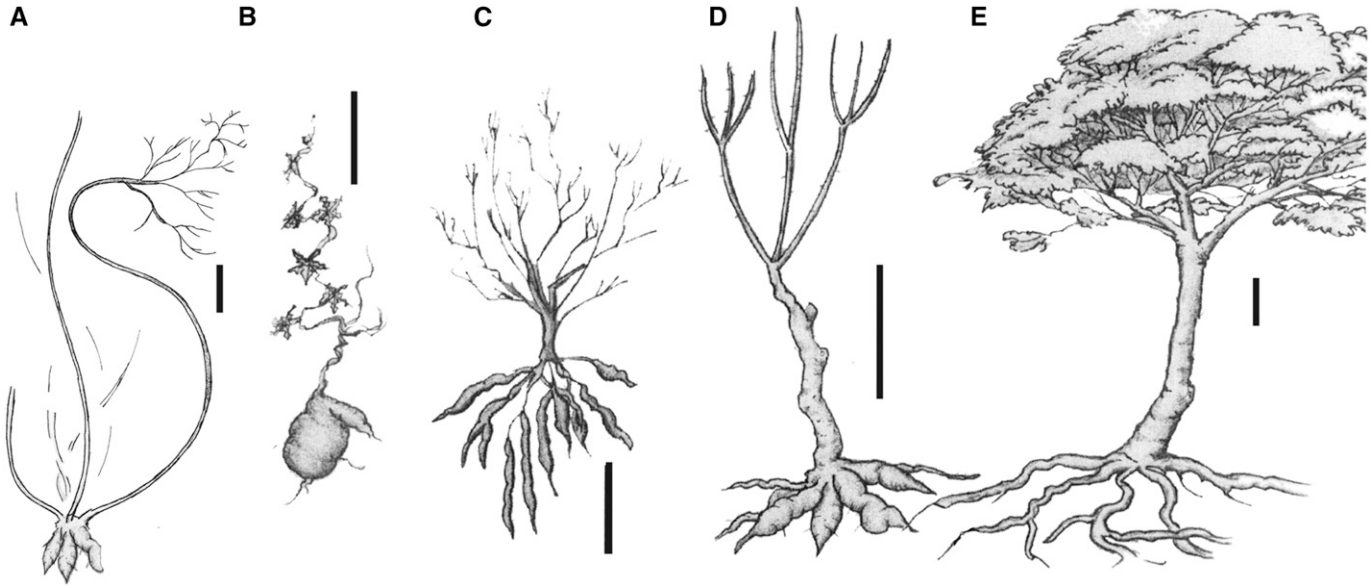

Fig. 1. Growth form diversity in North American *Manihot* species. (A) Lianas (plants with non self-supporting stems, commonly exceeding 10 m in length, but usually less than 5 cm in diameter). (B) Herbs (short plants with annual stems and massive tuberous roots). (C) Shrubs (plants with tuberous roots, at most 3 m in height, lacking distinct trunks and having numerous branches rarely exceeding 5 cm in diameter). (D) Treelets (plants with tuberous roots normally not exceeding 3 m in height, with distinct trunks sparingly branched well above the base and branches rarely exceeding 5 cm in basal diameter). (E) Trees (tall plants with fibrous roots, reaching 5 m or more, with distinct trunks commonly exceeding 10 cm in basal diameter branched well above the base). Scale bars: A, C–E = 1 m; B = 10 cm.

Fig. 2. Eight North American *Manihot* species representing the different growth forms in this genus and *Manihotoides*. (A, B) Trees (A, *M. foetida*; B, *M. websteri*). (C, D) Treelets (C, *M. pringlei*; D, *M. aesculifolia*). (E, F) Shrubs (E, *M. obovata*; F, *Manihotoides pauciflora*). (G, H) Herbs (G, *M. rhomboidea*; H, *M. subspicata*).

Mexican species, *M. obovata* (Jiménez-Ramírez, 1990) and *M. mcvaughii* (Steinmann, 2005) have been described. Jiménez-Ramírez (1990) described the section *Obovatae* to include *M. obovata*, while Steinmann (2005) did not designate a sectional affinity for *M. mcvaughii*. For a stronger test of the monophyly of the North American sections and to examine the placement of newly described taxa, we included in our analyses all known North American species.

In addition to evaluating the monophyly of North American species and sections of *Manihot*, a phylogenetic hypothesis would make it possible to address the taxonomic status of two additional enigmatic taxa. One is the genus *Manihotoides*, the only species of which, *M. pauciflora* (Brandegee) D.J. Rogers & Appan, was previously considered a species of *Manihot* (Brandegee, 1910). *Manihotoides* was erected for it (Rogers and Appan, 1973) because it has condensed stems and uniflorous inflorescences, conditions that made it unique in *Manihot*. The subsequent description of *M. obovata* (Jiménez-Ramírez, 1990) and *M. mcvaughii* (Steinmann, 2005), which share with *Manihotoides* these features, suggested that *M. pauciflora* should again be subsumed within *Manihot* (Webster, 1994, 2014; Radcliffe-Smith, 2001; Martínez-Gordillo et al., 2002). We test its position here.

Another elusive relationship that a phylogenetic hypothesis would help clarify is that between *M. crassisepala* Pax & K. Hoffm. and *M. foetida* (Kunth) Pohl. *Manihot crassisepala* is known from only a few wild populations, whereas *M. foetida* lacks known wild populations but is a part of traditional horticulture. These species are extremely similar morphologically, differing only in the glabrous ovary of *M. crassisepala* and the hairy ovary of *M. foetida*. However, many morphological characters are likely markedly homoplasious in *Manihot* (Chacón et al., 2008), so this similarity might not reflect ancestry. Our phylogenetic reconstruction will allow us to evaluate whether the morphological similarity between these two species reflects common ancestry or whether it might represent an example of repeated evolution of similar morphologies.

In addition to its economic importance, the North American *Manihot* species are of interest because they offer a useful

system for the study of growth form evolution. The distribution of North American species of *Manihot* seems congruent with a single or with multiple origins of each growth form in this genus. Several species of *Manihot*, together often representing two to five growth habits, can be found in the same biogeographical units (Fig. 3; following Morrone, 2005). Within these general units, most *Manihot* species have restricted distributions. This situation, with localized species assemblages, often isolated from one another, and each with similar arrays of growth forms, represents an archipelago-like distribution, analogous to the distribution of *Anolis* ecomorphotypes on the Greater Antillean islands (Losos, 2001). Each growth habit in *Manihot* might have arisen in each of the geographical areas in which it is found, or it might have arisen only once and subsequently spread to different areas prior to speciation. Identifying which of the two options is the more likely is a primary goal of this study.

Finally, a phylogenetic hypothesis would allow us to evaluate whether the phenomenon of incomplete lineage sorting is as common in North American species as in those of South America. Incomplete lineage sorting may occur in recently radiated lineages, where there has not been sufficient time for sequences to diverge to the point of reciprocal monophyly between species. It has been suggested that the relatively recent appearance of *Manihot* (approximately 6.6 million years ago [Ma]; Chacón et al., 2008) would give insufficient time for the evolution of reproductive barriers (Chacón et al., 2008) and for coalescence leading to reciprocal monophyly (Duputié et al., 2011). Potentially congruent with these observations is that the South American species of *Manihot* intergrade morphologically with one another. In contrast, North American species are readily distinguished from one another, having clear morphological discontinuities between them (M. E. Olson and K. M. Olsen, personal observations). This discontinuity might suggest that there has been more time since divergence in the North American species and consequently more frequent reciprocal monophyly.

In summary, this study has four main goals. The first is to evaluate whether the North American *Manihot* species are a monophyletic group. The second is to explore whether the supraspecific classification of Rogers and Appan (1973), which is based on growth form, reflects monophyletic groups, as well as to assess the generic status of *Manihotoides* and the phylogenetic relationships of two species described in the last two decades. The third is to analyze whether each growth habit arose one or more times. The final goal is to assess whether incomplete lineage sorting, which is prevalent in the South American species, is also widespread in the North American species.

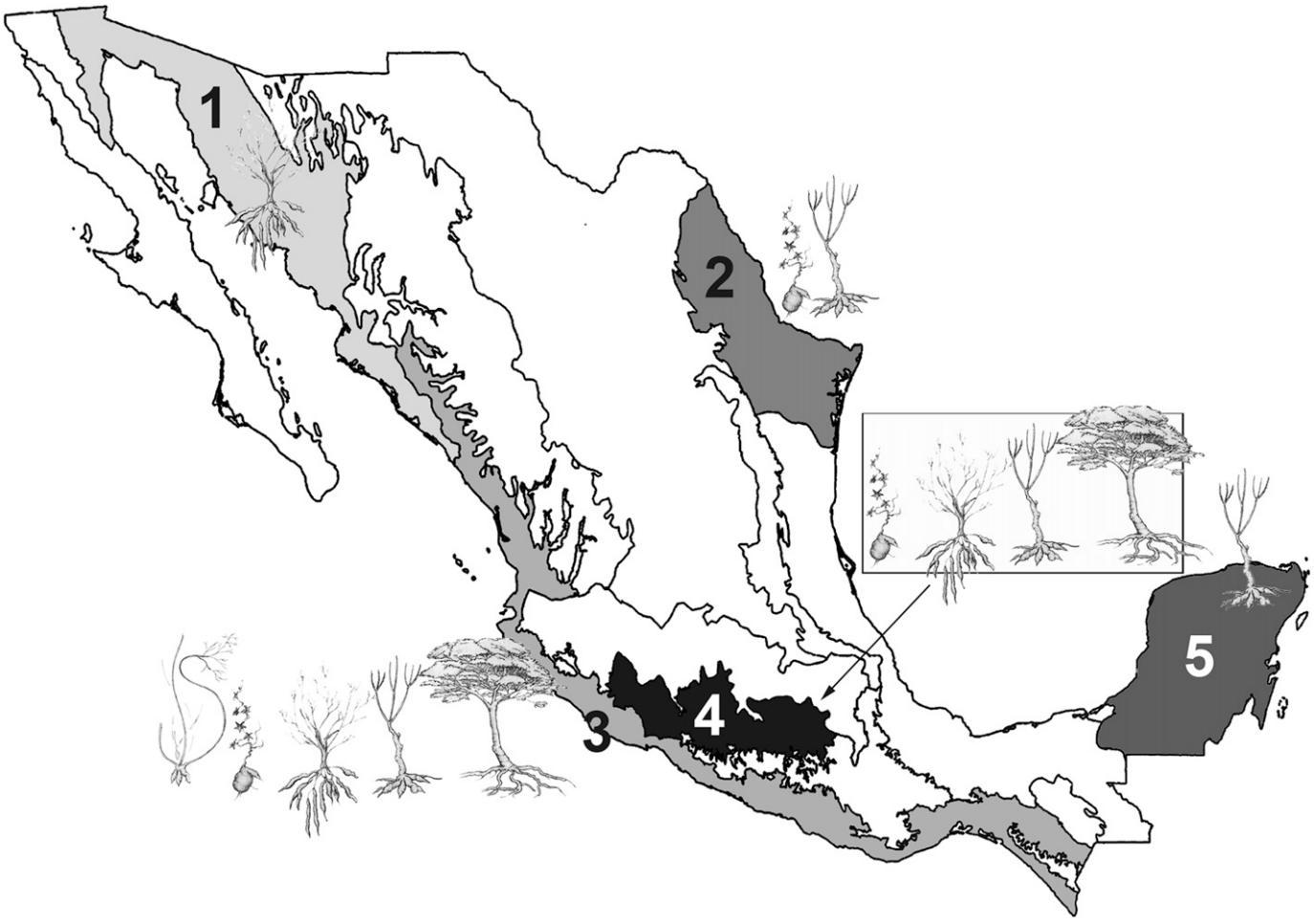

Fig. 3. Map of North American *Manihot* growth forms. Different growth forms often occur together in the same biogeographic unit. This distribution creates an archipelago-like situation analogous to that described by Losos (2001). Biogeographic units: 1 = Sonora, 2 = Tamaulipas, 3 = Mexican Pacific Coast, 4 = Balsas Basin, 5 = Yucatan Peninsula (Morrone, 2005).

MATERIALS AND METHODS

***Taxon sampling*—**We sampled one individual for 17 North American species that have restricted distributions and little intraspecific morphological variability and two individuals for one morphologically variable species (*M. caudata*, some of whose populations have individuals with flat canopies and exfoliating bark, whereas other populations have individuals with rounded canopies and smooth bark) and two widespread species (*M. aesculifolia* and *M. rhomboidea*). For these taxa, we obtained sequences for all markers from one individual, and sequences for two to four markers for the second. In the phylogenetic analyses, we only included the sequences of one individual for each North American species because the sequences were invariant within species. In addition to this sampling of all 20 North American species of *Manihot*, we included as outgroups *M. brachyloba* (the northernmost South American species), *M. esculenta*, and *Cnidoscolus egregius* Breckon ex Fern. Casas. We generally extracted DNA from material collected during the rainy season from young leaves. For species collected during the dry season (*M. angustiloba*, *M. auriculata* McVaugh, *M. davisiae*, *M. obovata*, *M. rubricaulis* I.M. Johnst., *M. websteri* D.J. Rogers & Appan and *M. pauciflora*), we extracted DNA from bark.

Our phylogenetic analyses included 126 original sequences from *Manihot* species and *C. egregius*. These sequences were from three plastid and three nuclear markers. The plastid markers were the intergenic spacer *psbA-trnH*, the intron *trnL*, and the intergenic spacer *trnL-trnF* (primers from Taberlet et al. [1991] and Sang et al. [1997]). The sequences from the intron *trnL* and the intergenic spacer *trnL-trnF* were analyzed as a single marker (*trnL-trnF*) following Chacón et al. (2008). The nuclear markers were the gene for phosphoenol pyruvate carboxylase (*PEPC*) (primers from Olson [2002]), a version of the low-copy nuclear gene encoding glyceraldehyde 3-phosphate dehydrogenase that was not sequenced in previous phylogenetic studies of *Manihot* ($G3pdh_{NA}$) (primers from Strand et al. [1997]), as well as a possible paralog of $G3pdh_{NA}$ sequenced by previous authors for several North and South American species (hereafter $G3pdh_{NASA}$). In addition to our original sequences, we also used 74 sequences of one plastid and one nuclear marker from Chacón et al. (2008) and Duputié et al. (2011) for 45 species of *Manihot* and two of *Cnidoscolus* (see Appendix 1 for vouchers and GenBank accession numbers). We included the sequences of *trnL-trnF* and $G3pdh_{NASA}$ of Chacón et al. (2008) for all the species of *Manihot* for which these authors amplified both markers. Additionally, we used all the sequences of $G3pdh_{NASA}$ of Duputié et al. (2011) for North American species, and the sequences of $G3pdh_{NASA}$ from these authors for 11 South American species representing all the major clades they recovered, the six major South American biogeographic units inhabited by *Manihot* (Duputié et al., 2011, following Morrone, 2006), most growth forms found in the South American species, and the entire South American range of *Manihot* (Appendix S1, see Supplemental Data with the online version of this article).

***DNA extraction, amplification, cloning, and sequencing*—**We ground leaves or bark in liquid nitrogen or in a TissueLyser LT (Qiagen, Valencia, California, USA) with one or two cycles of 50 shakings per second for 3 min. We extracted total genomic DNA using DNeasy Plant Mini kits (Qiagen) following the manufacturer's protocols, eluting the samples in deionized water.

Details on the amplification protocols, reaction contents, and kits used are in Appendices S2 and S3 (see online Supplemental Data). We obtained all sequences of *psbA-trnH*, *trnL-trnF*, and $G3pdh_{NASA,}$ as well as most *PEPC* sequences and some $G3pdh_{NA}$ sequences, by direct sequencing, whereas the remaining *PEPC* and $G3pdh_{NA}$ sequences were obtained after cloning. Before direct sequencing, we purified amplification products using the QIAquick PCR Purification Kit (Qiagen). We followed the manufacturer instructions, eluting the amplifications in 30 μL of deionized water rather than in dilution buffer. For sequencing, we used BigDye Terminator v3.1 Cycle Sequencing Kits (Invitrogen, Carlsbad, California, USA). For purifying sequencing reactions, we used Centri-Sep spin columns (Princeton Separations, Freehold, New Jersey, USA) (for *PEPC* and *psbA-trnH*), or Centri-Sep 96-well plates (Applied Biosystems, Foster City, California, USA) (for *trnL-trnF* and $G3pdh_{NA}$) with Sephadex G-50 Fine (GE Healthcare BioSciences, Piscataway, New Jersey, USA).

We carried out cloning to obtain sequences of *PEPC* for seven species and of $G3pdh_{NA}$ for 13 species with more than one haplotype of these markers. Although we made several modifications to the amplification protocols and/or reactions, we could not increase specificity of amplification reactions for direct sequencing. Therefore, we made four 50 μL amplification reactions per species with nonproofreading polymerases, separated the fragments using electrophoresis, and purified the desired fragments using the QIAquick Gel Extraction Kit (Qiagen) and the manufacturer instructions. We cloned the purified fragments into plasmids using standard protocols. For ligations, we used the Pgem-T Easy Vector System I kit (Promega, Madison, Wisconsin, USA) or the pJET1.2/blunt Cloning Vector (Fisher Scientific, Pittsburgh, Pennsylvania, USA). We cloned the ligations into JM109 (Promega), Z (Zymo Research, Orange, California, USA), or NEB 5-alpha competent cells (New England Biolabs, Ipswich, Massachussets, USA). We digested bacterial plasmids with EcoRI (Invitrogen; New England Biolabs). We purified the digested plasmids with Montage Plasmid Miniprep 96 well kits (Millipore Corp., Billerica, Massachussets, USA,) or QIAprep Spin Miniprep Kits (Qiagen). After plasmid purification, we followed the same sequencing procedure described above for the plastid markers and $G3pdh_{NASA}$. We obtained three to four clone sequences for all species, except for *M. obovata*, for which we only found and sequenced two clones.

We sequenced *psbA-trnH*, *trnL-trnF*, *PEPC*, and $G3pdh_{NA}$ using the ABI PRISM 3100 Genetic Analyzer platform (Applied Biosystems). We sequenced *PEPC* and *psbA-trnH* sequences at the facilities of the Instituto de Biología and the Laboratorio de Evolución Molecular y Experimental of the Instituto de Ecología, both of the Universidad Nacional Autónoma de México. We sequenced *trnL-trnF* and $G3pdh_{NA}$ at the facilities of Washington University in Saint Louis. We sent our amplifications of North American $G3pdh_{NASA}$ to be sequenced at the High Throughput Genomics Center, Seattle, Washington (http://www.htseq.org/).

***Phylogenetic analyses*—**We edited sequences using the program Sequencher v4.8 (Gene Codes, Ann Arbor, Michigan, USA). After editing, we made preliminary alignments in Clustal W (Larkin et al., 2007) as implemented in the program BioEdit v. 5.0.6 (Hall, 1999) using the default parameters. Later we corrected these alignments using the program Se-Al v.2.0a11 Carbon (Rambaut, 2002), seeking to maximize similarity and simultaneously minimize substitutions, eliminating ambiguous zones from further analysis. We carried out all our phylogenetic analyses with the CIPRES Science Gateway (http://www.phylo.org/portal2; Miller et al., 2010).

Bayesian analyses—We carried out Bayesian MCMC analyses (Yang and Rannala, 1997) of each marker separately using the program MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001). The first purpose of these analyses was to assess qualitatively whether markers yielded contradictory topologies or not. The second was to detect putatively paralogous clones in the nuclear markers *PEPC* and $G3pdh_{NA}$, which we cloned. We excluded *PEPC* and $G3pdh_{NA}$ clones with singleton mutations that were likely produced by sequencing errors. Therefore, we only included in phylogenetic analyses *PEPC* and $G3pdh_{NA}$ clones that differed by at least two bases from the other clones of the same species or that differed only by one, provided that this distinctive character state was shared with at least another clone or sequence from another species.

We carried out separate analyses for each of four markers to observe which clades were recovered, as well as the behavior of the clones of nuclear markers. For $G3pdh_{NA}$, we carried out two separate analyses. The first included our sequences plus the sequences of Chacón et al. (2008) and Duputié et al. (2011). In this analysis, our *G3pdh* sequences made up a clade distinct from the sequences of Chacón et al. (2008) and Duputié et al. (2011) (online Appendix S4; alignment available at http://purl.org/phylo/treebase/phylows/study/TB2:S16916). This result and the great difference between our sequences and the sequences of Chacón et al. (2008) and Duputié et al. (2011) (Fig. 4) suggested that the two versions were not orthologous. Therefore, we designated our sequences as $G3pdh_{NA}$ and the previously published sequences as $G3pdh_{NASA}$. Using GenBank sequences of $G3pdh_{NASA}$ from *Manihot*, we designed two specific primers using the program Primer3 v. 0.4.0 (Koressaar and Remm, 2007; Untergasser et al., 2012). These primers (*GPDX*7F*Spe*: 5′-AAGCTTAGAAGCACAAGT-3′, *GPDX*9R*Spe*: 5′-CATTCGTCGTGTTGATCCC-3′) allowed us to obtain sequences of $G3pdh_{NASA}$ for most North American samples. For the second separate analysis of $G3pdh_{NA}$, the $G3pdh_{NASA}$ sequences were analyzed separately.

In addition to allowing us to observe which clades were recovered by each of the five markers, separate analyses of *PEPC* and $G3pdh_{NA}$ allowed us to determine how to treat the clones from species of the ingroup in further analyses. On the basis of these analyses, we constructed a consensus sequence for each species whenever the clones grouped with the others from their own species or did not group with any other clone. Therefore, we included in subsequent analyses consensus sequences of all the clones of the nuclear markers, with the exception of three clones of *PEPC* from two species of the ingroup (online Appendix S5; alignment available at http://purl.org/phylo/treebase/phylows/study/TB2:S16916). We deleted these clones because they grouped with clones from a member of the outgroup and they were so different from the other clones from their own species that it would

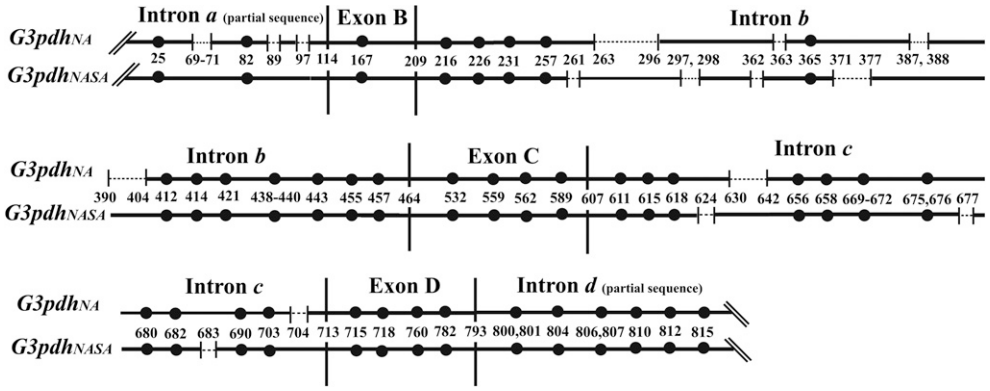

Fig. 4. Map depicting the differences between a version of *G3pdh* exclusively sequenced in North American species of *Manihot* ($G3pdh_{NA}$) and a version of this gene sequenced in North and South American species ($G3pdh_{NASA}$). Black circles represent positions with only two character states, one shared by all sequences of $G3pdh_{NA}$, and the second by all the sequences of $G3pdh_{NASA}$. Dashed lines represent bases or fragments absent in one of the versions of *G3pdh.* Names of exons and introns follow Olsen and Schaal (1999).

have been impossible to construct a consensus without having many uncertain character states. For South American clones, we constructed consensus sequences for each species, provided they were similar enough not to generate consensus sequences with many ambiguous characters. Summarizing the variation in South American species in this way would not affect our conclusions because the South American species were used only to test North American species monophyly, not to infer South American species phylogeny.

Before each Bayesian analysis, we identified the models that best fit the data for each of the five markers using the Akaike information criterion (Akaike, 1974) in the program jModeltest 0.1.1 (Posada, 2008). Preliminary trials indicated that a heating parameter of 0.05 and runs of 20 million generations allowed optimal exchange among chains and acceptably low standard deviations.

For concatenated analyses, we joined our sequences with those of Chacón et al. (2008) and Duputié et al. (2011). We carried out several preliminary trials to identify the parameters that would produce acceptably low standard deviations and proper exchange levels among chains. To find these values, as well as the number of generations to include in the burn-in during the final analyses, we examined the results using the program Tracer v1.6 (Rambaut et al., 2014). Based on these preliminary tests, we carried out a final analysis of 240 million generations using six chains, 100 swaps among chains per generation, a heating parameter of 0.03, and a burn-in of 60 million generations. After discarding the topologies included in the burn-in, we constructed a consensus for each search with the remaining trees.

Maximum likelihood analyses—We carried out maximum likelihood analyses (Felsenstein, 1973) of the concatenated matrix of all markers using the program RAxML BlackBox 8.0.24 (Stamatakis et al., 2008; Stamatakis, 2014) as implemented in the CIPRES Science Gateway. We specified one partition for each marker and used GTR + Γ, as suggested by Stamatakis et al. (2008). We recovered slightly differing topologies depending on which species of *Cnidoscolus* was used as outgroup. Therefore, we ran three trials using three different sequences of addition of the species of *Cnidoscolus* and a randomly selected seed number. To estimate clade support, we ran rapid bootstrap replicates allowing RAxML to stop bootstrapping automatically (Pattengale et al., 2010). We compared the topologies with highest scores produced by the three different sequences of addition of *Cnidoscolus*.

***Growth habit evolution*—**As compared with the often plastic growth habits exhibited by South American species, North American *Manihot* species are readily distinguished from one another morphologically (Rogers and Appan, 1973), and they can be clearly divided into five growth habit categories (Figs. 1, 2). The single liana species has nonself-supporting stems, commonly exceeding 10 m in length, but usually less than 5 cm in diameter. Herbs are very short plants, less than a meter tall, with annual stems and massive tuberous roots. Shrubs are short plants, rarely over 3 m, lacking distinct trunks and branched from the base. Branches rarely exceed 5 cm in diameter. Adult shrubs have tuberous roots. Treelets are short plants, rarely exceeding 3 m, with distinct trunks sparingly branched well above the base and basal diameters seldom over 5 cm. Adult treelets have tuberous roots. Finally, trees are tall plants, reaching 5 m or more, with distinct trunks that branch well above the base and commonly exceed 10 cm in basal diameter. Adult trees have fibrous roots. To infer the number of times these growth habits likely evolved in the North American species of *Manihot*, we mapped this character onto the topology obtained with the Bayesian and maximum likelihood analyses of the concatenated matrix. For mapping growth habit, we carried out a maximum likelihood optimization using a Markov model with one parameter (Mk1) (Lewis, 2001) in the program Mesquite v. 2.75 (Maddison and Maddison, 2011).

RESULTS

We sequenced *psbA-trnH*, *trnL-trnF*, *PEPC*, and $G3pdn_{NA}$ for all North American species, and $G3pdh_{NASA}$ sequences for 16 (Appendix S1). For a molecular sampling as complete as possible, we added to the alignment of three of the four species for which we were not able to obtain $G3pdh_{NASA}$ (*M. angustiloba*, *M. michaelis*, and *M. oaxacana*) the sequences of Duputié et al. (2011). The final matrix included the sequences of five markers for 19 terminals, four markers for four terminals, two markers for 23 terminals, and one marker for 12 (alignment available at http://purl.org/phylo/treebase/phylows/study/TB2:S16916). There were great differences in sequence length between the outgroup and the North American *Manihot* species. However, the segments shared by all the sequences were very similar, so it was possible to align them unambiguously. The only exceptions were *trnL-trnF* and *PEPC*, which had segments ambiguously aligned of 19 and 30 bp, respectively, which were not included in the analyses.

As reported for other taxa (Wolfe et al., 1987; Clegg et al., 1994), individual plastid markers had fewer point mutations per unit length than did the individual nuclear markers. The lengths of the five individual matrices and the single concatenated matrix, the number of species included within each of them, their numbers of variable sites, and the descriptive statistics for each phylogenetic method are shown in Table 1. Recovery of clades in separate gene analyses helped to identify the few clades that were recovered by more than one marker (Appendix S6).

Two of the three nuclear markers, *PEPC* and $G3pdh_{NA}$, had more than one copy per species.

***Possible incomplete lineage sorting or hybridization in North American species*—**Separate analyses of these loci provided evidence consistent with incomplete lineage sorting or hybridization in North American *Manihot* species. We found that some clones from several species did not coalesce with one

TABLE 1. Features of separate and concatenated matrices, as well as statistics of each phylogenetic method. I: First separate analysis. II: Second separate analysis.

	Chloroplast		Nucleus			
Feature	*psbA-trnH*	*trnL-trnF*	*PEPC*	*G3pdh*$_{NA}$	*G3pdh*$_{NASA}$	Concatenated matrix
Matrix						
Length (bp)	863	1034	I: 455 II: 451	I: 816 II: 646	668	3662
Number of taxa	23	44	I: 33 II: 24	I: 81 II: 20	54	58
Informative characters	17 (1.96%)	16 (1.55%)	I: 36 (7.91%) II: 22 (4.88%)	I: 154 (18.87%) II: 14 (2.17%)	53 (7.93%)	123
Outgroup	*Cnidoscolus egregius*	*C. tubulosus*	*C. egregius*	*M. aesculifolia*	*C. urens*	*C. urens*
Bayesian analyses						
Model selected	TPM1uf+Γ	TrN+ Γ	I: TIM2 + Γ II: TIM2 + I	I: HKY+I II: HKY	TIM1+I	One model for each partition
Average SD	0.001820	0.002090	I: 0.001392 II: 0.001268	I: 0.001482 II: 0.000765	0.001458	0.000817
ML analyses						
ML scores	—	—	—	—	—	−8407.987831

Note: ML = maximum likelihood.

another, grouping instead with clones from other species. *PEPC* clones that made up clades with *PEPC* clones from other species were found in 20% of the North American taxa, whereas *G3pdh*$_{NA}$ clones that made up clades with clones from other species were found in 30% of them. Some of the clades recovered by the second analyses of *PEPC* and *G3pdh*$_{NA}$ were identical to those in the concatenated analyses (e.g., both markers recovered the clade *M. angustiloba-M. davisiae* and clade A) (Figs. 5, 6; Appendices S6, S8) or very similar to them (e.g., a clade similar to B was recovered both by *PEPC* and *G3pdh*$_{NA}$; *PEPC* recovered a polytomy made up of *M. mcvaughii*, *M. michaelis*, and *M. tomatophylla*; and *G3pdh*$_{NA}$ recovered a clade including four species from clade C). These findings suggest that these markers are evolving congruently with the species phylogeny. Other clades recovered by the second analyses of *PEPC* and *G3pdh*$_{NA}$ were not recovered in any other analysis, suggesting that the species making up such clades have gone through interspecific hybridization or, alternatively, through duplication events of this marker followed by subsequent episodes of lineage sorting. The results of the *G3pdh*$_{NA}$ separate analysis of all North and South American sequences suggested that *G3pdh*$_{NA}$ and *G3pdh* $_{NASA}$ are not orthologous (see Materials and Methods and Appendix S4). Our 34 *G3pdh*$_{NA}$ sequences made up a clade (PP = 1.00) separated by a long branch from a clade made up of the South and North American *G3pdh*$_{NASA}$ sequences of Chacón et al. (2008) and Duputié et al. (2011).

Final concatenated analyses and evolutionary questions— The concatenated analyses carried out with the two phylogenetic methods recovered very similar topologies (Figs. 5, 6; Appendices S7, S8). However, the support values of clades recovered using maximum likelihood were low. This seems to imply that most clades were supported by relatively few characters. Additionally, maximum likelihood analyses (hereafter MLA) failed to recover the genus *Manihot* as monophyletic with respect to *Cnidoscolus*, with two species of *Cnidoscolus* mingling with South American species of *Manihot*. This result was probably due to the fact that we were able to sequence only two markers for *C. egregius* and had only one marker for each of the remaining species of *Cnidoscolus*. The incomplete molecular sampling for these three species could have led to their unstable positions.

Both Bayesian analysis (hereafter BA) and MLA suggested that North American *Manihot* species together make up a monophyletic group (BSML = 36%, PP = 0.81). Results from the two phylogenetic methods agreed on the nonmonophyly of North American *Manihot* sections. Members of section *Foetidae*, section *Parvibracteatae*, and *Manihotoides pauciflora* intermingled in all analyses. Both BA and MLA indicated convergent evolution in habit in North American *Manihot* species (Fig. 6; Appendix S8). There are herbs in two clades, treelets in three clades, and trees and shrubs in two (BA) to four and five clades (MLA), respectively. Although the growth forms of many ancestral nodes were ambiguous, the growth forms with the highest likelihoods at most of them were the shrubby (BA) or tree habits (BA and MLA). This suggests that herbs likely arose two (BA), three (MLA), or even four (BA) times, treelets between three (BA, MLA) and four (BA) times, trees between one (BA, MLA) and three (BA) times, and shrubs two (BA), five (BA), or six (MLA) times, with the lianescent growth having arisen in *M chlorosticta* (BA and MLA). Thus, despite evidence of lineage sorting and the low support values of several clades, the resolution recovered in our analyses allowed us to address the main objectives of our study.

DISCUSSION

North American Manihot monophyly— Our study addressed the issue of whether North American *Manihot* species make up a distinct clade within this large neotropical genus. Although most authors have suggested that North American species form a clade (Bertram, 1993; Schaal et al., 2006; Chacón et al., 2008), others have suggested that North American species make up a grade basal to the South American ones (Duputié et al., 2011). However, all previous studies were based on incomplete sampling of North American *Manihot* species and on three loci at most (Chacón et al., 2008; Duputié et al., 2011). The current study was based on four to five molecular regions for all the North American *Manihot* species, as well as including an extensive representation of potentially closely related species. Our results suggested that North American species make up a single clade (Bertram, 1993; Schaal et al., 2006; Chacón et al., 2008).

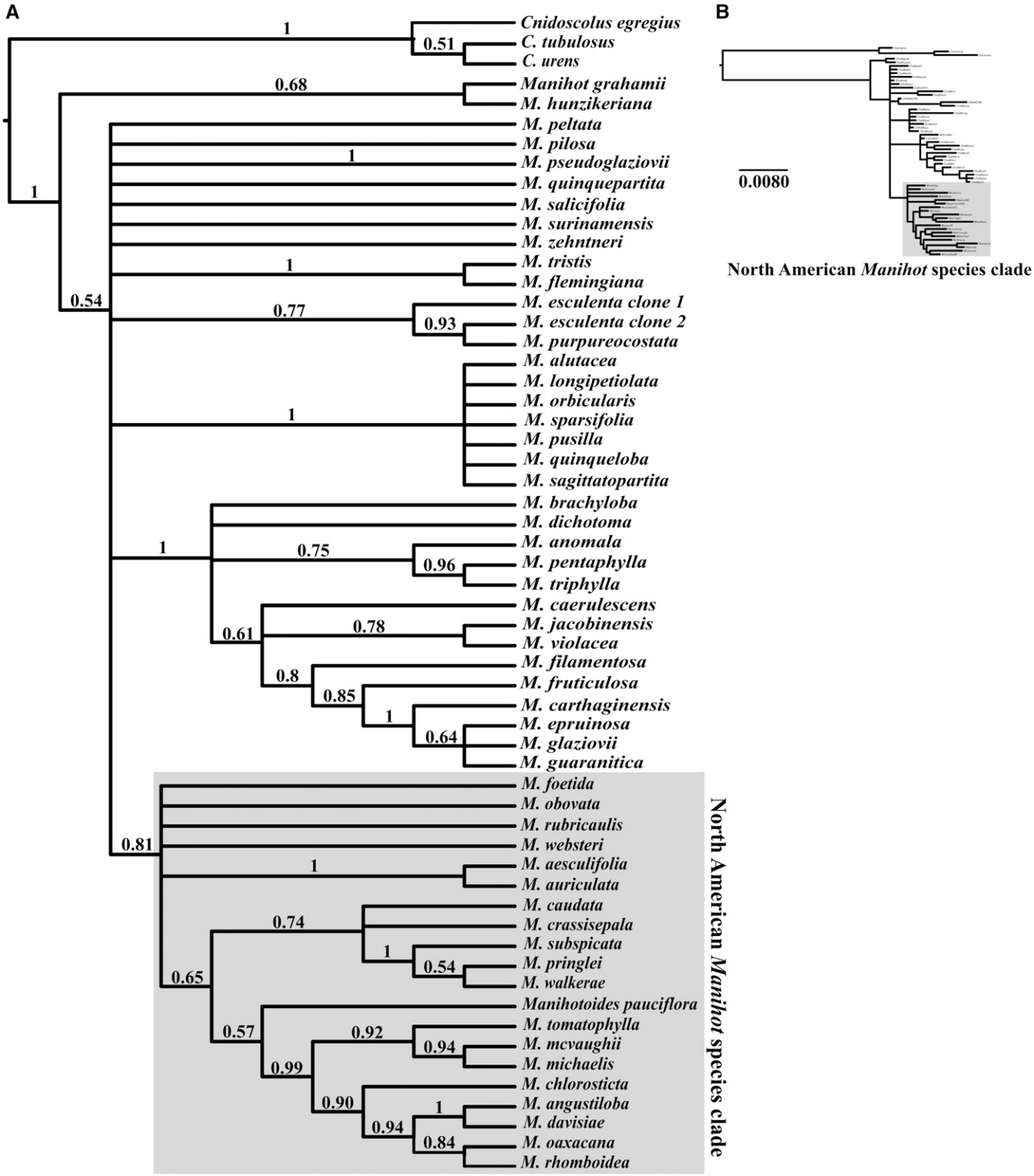

Fig. 5. Bayesian 50% majority rule consensus tree showing the relationships among all North American *Manihot* species and a selected group of South American species encompassing all growth forms and the entire geographical range of the South American species. The phylogeny is based on sequences of two plastid markers (*psbA-trnH* and *trnL-trnF*), and three nuclear markers (*PEPC*, $G3pdh_{NA}$, and $G3pdh_{NASA}$). Posterior probabilities are shown above the branches. (A) Consensus tree showing the relationships among species. (B) Reduced consensus tree showing the branch lengths.

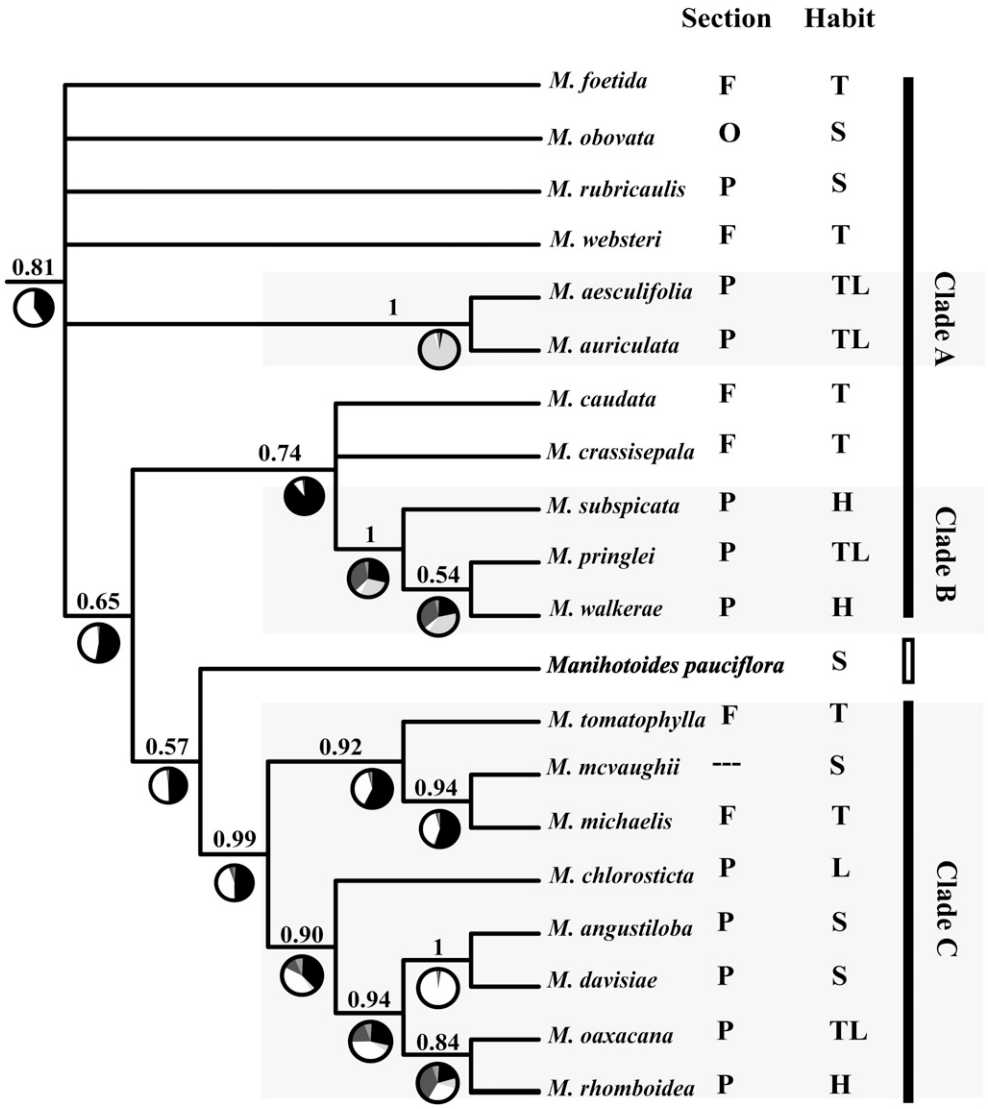

Fig. 6. Habit and sectional affinity of the North American *Manihot* clade mapped onto the topology recovered using Bayesian inference, showing that sectional classification is not based on monophyletic groups and that the habit categories that they are in part based on are highly homoplasious. The tree summarizes the phylogeny in Fig. 5. Clades A, B, and C are those recovered both by Bayesian and maximum likelihood analyses. Posterior probabilities are shown above the branches. Maximum likelihood growth form mapping for the North American species is shown below the branches. Liana: medium gray, herb: dark gray, shrub: white, treelet: light gray, tree: black. P = section *Parvibracteatae*, F = section *Foetidae*, O = section *Obovatae*. T = tree, TL = treelet, S = shrub, H = herb, L = liana. *Manihotoides pauciflora* falls within the North American *Manihot* species clade.

Our results also seem to cast some light on the debated relationship between North American species and *M. esculenta*. None of the concatenated phylogenetic analyses we carried out suggested a close relationship between *M. esculenta* subsp. *esculenta* and any North American species (Fig. 5; Appendix S7). Therefore, our results agree with previously published results that suggested that North American and Mesoamerican *Manihot* are only distantly related to the cultivated manioc and were not involved in its domestication (Olsen and Schaal, 1999, 2001; Chacón et al., 2008; Léotard et al., 2009).

***Supraspecific classification of North American* Manihot *species*—** Our phylogenetic analyses, based on a complete sampling of North American species, suggested that sections *Foetidae* and *Parvibracteatae* are not monophyletic, and, therefore that the morphological characters they are based on (the size of individual plants, fruits, and seeds) are likely homoplasious. Even without perfectly resolved phylogenetic hypotheses, we can identify numerous instances of members of the two sections making up clades. For example, clade C recovered by BA and MLA (Fig. 6; Appendix S8) was made up of members from sections *Foetidae* and *Parvibracteatae*. These sections are delimited based on features that are positively related with plant stature. Our results would make it seem that the sections of Rogers and Appan (1973) simply reflect plant size, with section *Foetidae* including the largest species, and section *Parvibracteatae* including medium to small ones. These apparently frequent changes in size in North American *Manihot* species also seem to be accompanied by frequent shifts in growth form.

***Growth form evolution*—** Although not completely congruent regarding the phylogenetic relationships recovered, our

results helped to assess whether each growth form in *Manihot* originated only a single time before speciation or originated multiple times, as in *Anolis* (Losos, 2001). If each growth form evolved only once, we would have obtained topologies with clades made up of species sharing the same growth form. Given that all our analyses recovered clades made up of species with different growth forms, our results provide evidence that each growth form evolved more than once in the North American clade.

Of these likely independent origins of growth forms, the similarity between *Manihot crassisepala* and *M. foetida* is particularly striking. *M. crassisepala* is known from only a few populations in a small, remote area of the western Balsas Depression, whereas *M. foetida* lacks known wild populations but is a part of traditional horticulture in the northern central Balsas, where it is used in living fences. This species pair is apparently morphologically identical except for ovary pubescence. That *M. foetida* grows exclusively in human settlements and *M. crassisepala* in the wild and that both species are so similar morphologically made us think that *M. foetida* was probably domesticated from *M. crassisepala*. Given this scenario, we expected to recover these two species as closely related. However, these taxa were not recovered as sister species in any of our analyses, implying that these species evolved their similar morphologies independently. There is no information regarding the original wild range of *M. foetida*. Therefore, not only the relationship between these two species demands further clarification, but also the history of domestication of *M. foetida*.

Growth form is intimately associated with environmental conditions. One of the most important adversities that plants of the dry tropics have to face is the scarcity of water during much of the year. In the North American clade of *Manihot*, larger species seem to live in areas with greater moisture availability, but in general the selective factors that favor different life forms are not clear. Tuberous roots in *Manihot* have been interpreted not only as water storage adaptations, but also as adaptations to disturbance, allowing to the plants to resprout after fire (Duputié et al., 2011). However, fire is absent from most Mexican *Manihot* habitats, in contrast to the Brazilian cerrado, where many species occur. Therefore, the environmental conditions favoring the development of tuberous roots in the North American species of *Manihot* remain to be investigated.

***Positions of new North American* Manihot *species and taxonomic status of* Manihotoides pauciflora—**Our results help place the recently described species *M. mcvaughii*, while the position of *M. obovata* remains ambiguous. *Manihot mcvaughii* was recovered as a member of clade C, both by BA and MLA. In the case of *M. obovata*, while BA did not associate this species with any other, in MLA this species was recovered as sister to *M. foetida* with low support (BSML = 32%; Appendix S8).

Our phylogenetic analyses suggested, even if they were not congruent on the exact position of *Manihotoides*, that the genus should not be recognized and *Manihotoides pauciflora* should remain synonymized with *Manihot* (i.e., Webster, 1994, 2014; Radcliffe-Smith, 2001; Martínez-Gordillo et al., 2002). In all our separate and concatenated analyses, *M. pauciflora* was nested within the North American clade. Our results also suggested that the unusual features of *Manihotoides* (Rogers and Appan, 1973) are examples of the morphological lability in *Manihot*. *Manihotoides pauciflora* shares with *Manihot mcvaughii* and *M. obovata* reduced inflorescences (Martínez-Gordillo et al., 2002; Steinmann, 2005), and with *M. obovata* the presence of leaves clustered on short shoots (Martínez-Gordillo et al., 2002; Webster, 1994). None of our concatenated analyses indicated that *M. mcvaughii*, *M. obovata*, or *Manihotoides pauciflora* are closely related, implying that condensed stems and uniflorous inflorescences might have evolved at least twice. Furthermore, these results add support to the hypothesis that these features have evolved in response to arid environments (Steinmann, 2005) given that these three unrelated species are located in some of the driest areas of occurrence of *Manihot* in Mexico, which also have the highest minimum temperatures. This association might imply that the evolution of stature is influenced by water availability, with larger plants being favored in areas where drought is less severe (Moles et al., 2009). Additionally, while *M. mcvaughii*, *M. obovata*, or *Manihotoides pauciflora* have short shoots, the shrub species that reach into the northern desert areas (*Manihot angustiloba*, *M. davisiae*, and *M. rubricaulis*, all of them occurring in northwestern Mexico) do not have condensed stems. This suggests that the capacity to leaf out from existing stems is favored only in areas that are not subject to frost, whereas in arid areas where stems are subject to freezing, leaf area is increased only from apical and not axial meristems.

Incomplete lineage sorting and molecular polymorphisms in North American* Manihot *species—As the distribution of *PEPC* and $G3pdh_{NA}$ haplotypes seemed to suggest, North American *Manihot* species have either undergone lineage sorting or frequent hybridization. Sequences often showed patterns of coalescence that were incongruent with species boundaries, rather than consistent patterns of reciprocal monophyly between species. Given that incomplete lineage sorting has also been detected for South American species (Olsen and Schaal, 2001; Duputié et al., 2011), this phenomenon, rather than hybridization, would seem to be the more likely alternative to explain the distribution of haplotypic diversity across the clade. However, in the absence of enough information on the reproductive biology of *Manihot*, the possibility that the hybridization between species has caused this pattern, at least partially, cannot be ruled out.

Conclusions—Based on a taxonomic sampling including all North American *Manihot* species and *Manihotoides pauciflora*, and a molecular sampling of two chloroplast and three nuclear marker sequences, we have assembled the most complete phylogenetic hypothesis of North American *Manihot* to date, using Bayesian and maximum likelihood analyses. Our concatenated analyses suggested that the group of North American species of *Manihot* is monophyletic.

Our results consistently suggested that accepting the current supraspecific classification of *Manihot* would require recognizing paraphyletic groups. Our concatenated analyses recovered clades made up of members of both sections *Foetidae* and *Parvibracteatae*, so these sections are best eliminated. In the case of section *Obovatae*, our analyses did not provide any conclusive result regarding its position. In contrast, our analyses consistently recovered *Manihotoides pauciflora* as nested within the North American *Manihot* clade. Therefore, *Manihotoides* should remain synonymized with *Manihot* to avoid a paraphyletic *Manihot*.

Illustrating the morphological lability of North American *Manihot* species, most of our results suggested that trees, treelets, shrubs, and herbs have evolved repeatedly in this genus. Rather than grouping by growth form, North American *Manihot* species form species assemblages mainly based on their geographical distribution, with several of the most strongly supported clades including species whose ranges are close or overlapping (e.g., the *M. angustiloba-M. davisiae* and *M. aesculifolia-M. auriculata* clades) and that often differ in growth form (e.g., members of clade B and some members of clade C) (Fig. 6; Appendix S8).

Finally, even though North American species of *Manihot* have clearer morphological boundaries than South American ones, the interspecific distribution of nuclear haplotypes in the North American clade suggested that they have undergone widespread incomplete lineage sorting, just as has been reported for the South American species (Olsen and Schaal, 2001; Duputié et al., 2011).

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APPENDIX 1. Voucher and sequence information for the species collected and sequenced by the authors of this paper, as well as accession numbers of the sequences from Chacón et al. (2008) and Duputié et al. (2011) employed in this study. All vouchers collected by the authors of this paper and *M. auriculata* are deposited at the Herbario Nacional de México (MEXU). ACA: María-Angélica Cervantes-Alcayde.

Taxon, voucher, Locality, locality, GenBank accession numbers: [a] *psbA-trnH*, [b] *trnL-trnF*, [c] *PEPC*, [d] $G3pdh_{NA}$, [e] $G3pdh_{NASA}$.

Ingroup: ***Manihot aesculifolia***, ACA 31, México: Michoacán, KP692091 [a], KP691988 [b], KP692008 [c], KP692041 [d], KP692075 [e], EU518807 [e], FN551748 [e], FN551744 [e]. ***Manihot angustiloba***, ACA 49, México: Sinaloa, KP692092 [a], KP691989 [b], KP692009 [c], KP692042 [d], KP692043 [d], KP692044 [d], FN551745 [c], FN551751 [c]. ***Manihot auriculata***, MELR 3408, México: Nayarit, KP692093 [a], KP691990 [b], KP692010 [c], KP692045 [d], KP692076 [e]. ***Manihot caudata***, ACA 23, México: Michoacán, KP692094 [a], KP691991 [b], KP692011 [c], KP692046 [d], KP692047 [d], KP692048 [d], KP692077 [e], FN551746 [e]. ***Manihot chlorosticta***, ACA 29, México: Colima, KP692095 [a], KP691992 [b], KP692012 [c], KP692049 [d], KP692078 [e], EU518814 [e], FN551743 [e]. ***Manihot crassisepala***, ACA 37, México: Michoacán, KP692096 [a], KP691993 [b], KP692013 [c], KP692050 [d], KP692051 [d], KP692052 [d], KP692079 [e]. ***Manihot davisiae***, ACA 50, México: Sinaloa, KP692097 [a], KP691994 [b], KP692014 [c], KP692053 [d], KP692054 [d], KP692055 [d]. ***Manihot foetida***, ACA 1, México: Morelos, KP692098 [a], KP691995 [b], KP692015 [c], KP692016 [c], KP692056 [d], KP692057 [d], KP692058 [d], KP692080 [e]. ***Manihot mcvaughii***, ACA 35, México: Michoacán, KP692099 [a], KP691996 [b], KP692017 [c], KP692059 [d], KP692060 [d], KP692081 [e]. ***Manihot michaelis***, ACA 25, México: Colima, KP692100 [a], KP691997 [b], KP692018 [c], KP692019 [c], KP692061 [d], FN551742 [e]. ***Manihot oaxacana***, Olson 1116, México: Oaxaca, KP692101 [a], KP691998 [b], KP692020 [c], KP692062 [d], FN551739 [e]. ***Manihot obovata***, ACA 8, México: Guerrero, KP692102 [a], KP691999 [b], KP692021 [c], KP692063 [d], KP692082 [e]. ***Manihotoides pauciflora***, ACA 20, México: Puebla, KP692103 [a], KP692000 [b], KP692022 [c], KP692064 [d], KP692065 [d], KP692083 [e]. ***Manihot pringlei***, ACA 42, México: Tamaulipas, KP692104 [a], KP692001 [b], KP692023 [c], KP692024 [c], KP692025 [c], KP692066 [d], KP692084 [e]. ***Manihot rhomboidea***, ACA 26, México: Colima, KP692105 [a], KP692002 [b], KP692026 [c], KP692027 [c], KP692028 [c], KP692067 [d], KP692068 [d], KP692069 [d], KP692085 [e]. ***Manihot rubricaulis***, ACA 46, México: Durango, KP692106 [a], KP692003 [b], KP692029 [c], KP692069 [d], KP692070 [d], KP692086 [e], EU518831 [e]. ***Manihot subspicata***, ACA 40, México: Nuevo León, KP692107 [a], KP692004 [b], KP692030 [c], KP692031 [c], KP692071 [d], KP692087 [e], FN551871 [e]. ***Manihot tomatophylla***, ACA 34, México: Michoacán, KP692108 [a], KP692005 [b], KP692032 [c], KP692033 [c], KP692072 [d], KP692088 [e], FN551747 [e]. ***Manihot walkerae***, KP692109 [a], KP692006 [b], KP692034 [c], KP692073 [d], KP692089 [e]. ***Manihot websteri***, ACA 17, México: Puebla, KP692110 [a], KP692007 [b], KP692035 [c], KP692074 [d], KP692090 [e], FN551740 [e]. Outgroups- ***Cnidoscolus egregius***, ACA 19, México: Oaxaca, KP692113 [a], KP692040 [c]. ***Cnidoscolus tubulosus***, EU518895 [b]. ***Cnidoscolus urens***, FN551736 [e]. ***Manihot alutacea***, EU518897 [b], EU518808 [e]. ***Manihot anomala***, EU518898 [b], EU518809 [e]. ***Manihot brachyloba***, KP692111 [a], EU518899 [b], KP692036 [c], EU518810 [e]. ***Manihot caerulescens***, EU518900 [b], EU518811 [e]. ***Manihot carthaginensis***, EU518901 [b], EU518812 [e]. ***Manihot dichotoma***, FN551741 [e], FN551773 [e]. ***Manihot epruinosa***, EU518904 [b], EU518815 [e]. ***Manihot esculenta* subsp. *esculenta***, KP692112 [a], KP692037 [c], KP692038 [c], KP692039 [c]. ***Manihot esculenta* subsp. *flabellifolia***, EU518906 [b], EU518816 [e]. ***Manihot filamentosa***, EU518908 [b], EU518818 [e]. ***Manihot flemingiana***, FN551821 [e]. ***Manihot fruticulosa***, EU518909 [b], EU518819 [e]. ***Manihot glaziovii***, EU518910 [b], EU518820 [e]. ***Manihot grahamii***, FN551792 [e]. ***Manihot guaranitica***, EU518911 [b], EU518821 [e]. ***Manihot hunzikeriana***, FN551791 [e]. ***Manihot jacobinensis***, EU518914 [b], EU518823 [e]. ***Manihot longipetiolata***, EU518915 [b], EU518824 [e]. ***Manihot orbicularis***, EU518916 [b], EU518825 [e]. ***Manihot peltata***, EU518917 [b], EU518826 [e]. ***Manihot pentaphylla***, EU518918 [b], EU518827 [e]. ***Manihot pilosa***, EU518919 [b], EU518828 [e]. ***Manihot pseudoglaziovii***, EU518920 [b], EU518829 [e]. ***Manihot purpureocostata***, EU518921 [b], EU518830 [e]. ***Manihot pusilla***, FN551809 [e]. ***Manihot quinqueloba***, FN551805 [e], FN551807 [e]. ***Manihot quinquepartita***, FN551783 [e]. ***Manihot sagittatopartita***, FN551811 [e]. ***Manihot salicifolia***, FN551837 [e]. ***Manihot sparsifolia***, EU518923 [b], EU518832 [e]. ***Manihot surinamensis***, FN551822 [e]. ***Manihot triphylla***, EU518924 [b], EU518833 [e]. ***Manihot tristis***, EU518925 [b], EU518834 [e]. ***Manihot violacea***, EU518926 [b], EU518835 [e]. ***Manihot zehntneri***, FN551825 [e].