Plant Systematics and Evolution (2020) 306:63
https://doi.org/10.1007/s00606-020-01693-5

ORIGINAL ARTICLE

Phylogeny and taxonomy of the Antillean endemic genus *Leptocereus* (Cactaceae) inferred from chloroplast markers and morphological evidence

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Received: 16 February 2020 / Accepted: 17 May 2020 / Published online: 4 June 2020

Abstract

Leptocereus is an Antillean genus of thin-stemmed cacti with 17 described species. We carried out a phylogenetic reconstruction with plastid DNA sequence data and a combined analysis with a set of 39 morphological characters using Maximum Likelihood and Bayesian inference criteria to explore the monophyly of the genus. We further analyzed the evolution of eight morphological characters to interpret the circumscription of *Leptocereus* and test for putative synapomorphies for the clade. Five plastid markers (*trn*L-F, *trn*Q-*rps*16, *psb*A-*trn*H, *pet*L-*psb*E, and *rpl*16) were sequenced for fifteen species of *Leptocereus*, seven species of the related genera (*Armatocereus, Dendrocereus, Strophocactus*) and ten species from Hylocereeae, Pachycereinae, Stenocereinae were used as outgroup taxa. Our phylogenetic analyses suggest that *Leptocereus* is paraphyletic with a clade of the two *Dendrocereus* species nested within it. All Cuban species constitute a monophyletic group, as do the species of Hispaniola and Puerto Rico, which are sister to the Cuban clade + *Dendrocereus* clade. No morphological character analyzed here was synapomorphic for the genus, but sunken areoles in the depressions of the ribs were a character present in all subclades of *Leptocereus*. Based on our molecular data and extensive fieldwork, a new circumscription of *Leptocereus* is proposed, which includes three new combinations (*Leptocereus albellus* comb. et stat. nov., *L. nudiflorus* comb. nov., *L. undulosus* comb. nov.).

Keywords *Armatocereus* · Caribbean · Cuba · *Dendrocereus* · Hispaniola · Morphology · Plastid DNA

Introduction

Leptocereus (A.Berger) Britton & Rose is a genus of cacti that includes erect, scandent or prostrate shrubs, or which are sometimes arborescent, with segmented and often thin branches, 17 described species (Anderson 2001; Areces-Mallea 2003, 2018), and is endemic to the Antilles. There are 12 recognized species on Cuba, three on Hispaniola and two on Puerto Rico, one of which is shared with Culebra Island and the other with Anegada (British Virgin Islands), representing the easternmost distribution of the genus (Areces-Mallea 1992, 1997, 2017, 2018; Hunt et al. 2006). Species of *Leptocereus* mostly inhabit tropical dry forests or coastal shrublands on limestone, from sea level to 685 m in elevation (see: *Majure* et al. *6438*, DES) in the Sierra Martín García, Dominican Republic, although populations of *Leptocereus santamarinae* Areces have been observed on serpentine soils on Cuba (see: *Barrios* et al. *HFC88505,* HAJB).

Handling Editor: Livia Wanntorp.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00606-020-01693-5) contains supplementary material, which is available to authorized users.

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Berger (1905) described *Leptocereus* as a subgenus of *Cereus* distinguished by its elongated climbing stems and short flowers. The subgenus initially included three species: the type *Cereus assurgens* C.Wright ex Griseb. from Cuba and two other Costa Rican species (*Cereus gonzalezii* F.A.C.Weber and *Cereus tonduzii* F.A.C.Weber) currently circumscribed under *Weberocereus* (*W. tunilla* and *W. tonduzii*, respectively; Korotkova et al. 2017). Soon after, Britton and Rose (1909) elevated *Leptocereus* to the generic level by circumscribing it to thin ribbed species with diffuse ramification patterns, small flowers and prickly fruits—this was restricted to just two species, *L. assurgens* (C.Wright ex Griseb.) Britton & Rose from Cuba and *L. quadricostatus* (Bello) Britton & Rose from Puerto Rico. A decade later in their monograph on Cactaceae, Britton and Rose (1920) recognized eight Antillean species (six on Cuba, one on Puerto Rico and the last on Hispaniola). By the end of the twentieth century, six new species of *Leptocereus* had been described, and *Cactus paniculatus* Lam. (also known as *Neoabbottia paniculata* (Lam.) Britton & Rose) was transferred by Hunt and Taylor (1991) to *Leptocereus*, so at that time it was considered a genus with 15 species. Subsequently, Hunt et al. (2006) considered four Cuban species as synonyms, reducing the genus to 11 species. However, the major taxonomic contribution in *Leptocereus* was carried out by Areces-Mallea (1992, 1993, 2003, 2017, 2018), who recognized 17 species and one variety. In addition, while Hunt et al. (2006) reduced *L. assurgens* and *L. ekmanii* (Werderm.) F.M.Knuth as synonyms, based on their taxonomic assessment, Barrios and González-Torres (2015) performed a morphological analysis (including vegetative and reproductive attributes) and found differences that support the recognition of both as separate species.

There are several approximations about the phylogenetic relationships of *Leptocereus* in the subfamily Cactoideae. Buxbaum (1958) hypothesized that this genus was part of the first evolutionary line in Cactoideae (tribe Leptocereeae) taking into account the morphology of the flower. Gibson and Horak (1978) reduced the number of genera belonging to the Leptocereeae to three based on their anatomical observations and the compilation of morphological and biochemical characters (*Leptocereus*, *Armatocereus* and *Samaipaticereus*). In later classifications, the Leptocereeae were included in several tribes (Echinocereeae, Barthlott and Hunt 1993; Pachycereeae, Anderson 2001; Phyllocacteae, Nyffeler and Eggli 2010), although Wallace (2002) redefined it with three genera (*Leptocereus*, *Acanthocereus* and *Dendrocereus*), two of them different from those of Gibson and Horak (1978) and sister to the Pachycereeae. In the most recent decades, several phylogenetic analyses in Cactaceae have incorporated samples of some species of *Leptocereus* and of putative closely related species; analyses by Wallace (2002) suggested that *Armatocereus* and *Leptocereus* represent sister genera, while Nyffeler (2002) found that *Leptocereus leonii* Britton & Rose together with species of *Armatocereus*, *Castellanosia*, *Neoraimondia*, *Acanthocereus*, and *Disocactus* form a clade. On the other hand, Areces-Mallea (2003) found a greater relationship between *Dendrocereus* and *Harrisia* than between *Leptocereus* and *Dendrocereus* based on the intron *trn*L (this relationship has not been supported in any subsequent phylogenetic study). Using three plastid markers (*trn*L intron, *trn*L-F spacers and *rpl*16 intron), Arias et al. (2005) obtained, with high support, a clade with *Leptocereus* and *Dendrocereus* and two species of *Armatocereus* as a sister group of the previous species, rejecting the Wallace hypothesis (2002) of *Acanthocereus* as a sister group of *Leptocereus*. Results from Hernández-Hernández et al. (2011) agree with the results of Arias et al. (2005), in recovering *Leptocereus* in a clade (although with low support) together with *Dendrocereus*, *Armatocereus*, *Neoraimondia*, *Castellanosia* (Browningieae) and *Pseudoacanthocereus*. Interestingly, Hernández-Hernández et al. (2011) resolved *Dendrocereus nudiflorus* (Engelm. ex C.Wright) Britton & Rose nested inside the clade of two species of *Leptocereus* but could draw no conclusions of relationships in the clade because of the lack of sufficient taxon sampling.

The phylogenetic relationships among *Leptocereus* species have only been explored comprehensively by Areces-Mallea (2003). This author used both molecular DNA sequences (intron *trn*L) and morphological data (100 characters), as well as their combination. In his DNA sequence analyses, 10 species of *Leptocereus* were included, while in the morphological analyses 100 characters and 20 taxa of *Leptocereus* were used (including infraspecific taxa). The Areces-Mallea study (2003) based on the intron *trn*L shows *Leptocereus* to be monophyletic; however, a polytomy is formed among three clades: (1) the species of Cuba, (2) the species of Hispaniola-Puerto Rico and 3) the Hispaniola taxon *L. weingartianus* (E.Hartmann) Britton & Rose subsp. *weingartianus*. The main objectives of this study were to carry out a phylogenetic reconstruction of *Leptocereus* and close relatives of Phyllocacteae to assess the monophyly of the genus and species relationships, based on five plastid markers and the inclusion of morphological characters. The evolution of eight morphological characters were analyzed to test their potential for aiding in species circumscription in *Leptocereus* and to test for putative synapomorphies for the clade.

Materials and methods

Taxon sampling

In this study, 15 of the 17 species of *Leptocereus* recognized by Areces-Mallea (2003) were selected (one species from Cuba, *L. chrysotyrius* Areces, and one from Hispaniola, *L.*

demissus Areces, were not sampled in this study). To represent the greatest amount of variation and determine species relationships, we included more than one terminal in seven species with some conflict in their taxonomic circumscription based on morphology: *L. assurgens* (3), *L. paniculatus* (Lam.) D.R.Hunt (2), *L. quadricostatus* (2), *L. wrightii* León (2), *L. scopulophilus* Areces (3), *L. santamarinae* (3) and *L. maxonii* Britton & Rose (4). To determine the sister clade to *Leptocereus,* seven species from three genera (*Dendrocereus*, *Armatocereus* and *Strophocactus*) were included, based on the results of Hernández-Hernández et al. (2011). The functional outgroup consisted of five species of the tribe Hylocereeae sensu Korotkova et al. (2017), two species of the subtribe Pachycereinae, one species of Stenocereinae sensu Hernández-Hernández et al. (2011), as well as *Corryocactus melanotrichus* (K.Schum.) Britton & Rose and *Calymmanthium substerile* F.Ritter (Online Resource 3).

DNA extraction and amplification

DNA was extracted from desiccated stem tissue in silica gel and subsequently frozen at – 20 °C. The day prior to extraction the samples were frozen at – 70 °C, the tissue size was approximately 1 cm^3. DNA from *Leptocereus quadricostatus* (*Sustache* and *Figuerola 1952*, SJ) was extracted from three dehydrated and frozen seedlings in the same way as the rest of the samples. Extractions were carried out according to the procedures of the DNeasy Plant Mini kit (Qiagen, Inc., Valencia, California), except that the incubation time in tissue lysis buffer was extended to 120 min, or through a modified CTAB extraction (Doyle and Doyle 1987) followed by chloroform/isoamyl alcohol and silica column-based purification steps, from silica-dried epidermal tissue (Neubig et al. 2014; Majure et al. 2019). The presence of DNA was confirmed by agarose gel electrophoresis (1%) with GelRed™ (Biotium, USA), and the samples were stored in collecting tubes at – 20 °C until used. For the phylogenetic analyses, five plastid markers were selected based on previous work in Cactaceae (Hernández-Hernández et al. 2011; Sánchez et al. 2018; Tapia et al. 2017); four intergenic spacers (*pet*L-*psb*E, *psb*A-*trn*H, *trn*L-*trn*F, *trn*Q-*rps*16) and one intron (*rpl*16). The amplification of the different regions was carried out by means of the polymerase chain reaction (PCR) in a volume of 25 µl. Each amplification reaction contained 19.025 µl of H_2O, 2.5 µl of 10× buffer, 0.5 µl of dNTPs at a concentration of 200 µM, 1 µl of BSA, 0.75 µl of MgCl 2, 0.3 µl first Forward, 0.3 µl of first Reverse, 0.125 µl of Platinum Taq DNA Polymerase (Invitrogen™) at 5 U/µl, 0.6 µl of total DNA. The *pet*L-*psb*E intergenic spacer was amplified using primers F (*pet*L) and R (*psb*E) (Shaw et al. 2007) and the following reaction sequence: 2 min at 94 °C initial denaturation, followed by 1 min at 94 °C, 30 s at 52 °C and 1 min at 72 °C for 30 cycles and a final extension of 5 min at 72 °C. For *psb*A-*trn*H primers F (*psb*A) (Sang et al. 1997) and R (*trn*H) (Tate and Simpson 2003), and the following reaction sequence were used: 2 min at 94 °C initial denaturation, followed by 30 s at 94 °C, 30 s at 52 °C and 1 min at 72 °C for 29 cycles and a final extension of 7 min at 72 °C. For *trn*L-*trn*F the primers F (c), R (f), F-int (e) and R-int (d) (Taberlet et al. 1991) and the following reaction sequence were used: 2 min at 94 °C initial denaturation, followed by 1 min at 94 °C, 1 min at 54 °C and 1.30 min at 72 °C for 30 cycles and a final extension of 7 min at 72 °C. For *trn*Q-*rps*16 the first F (*trn*QUUG) and R (*rps*16X1) (Shaw et al. 2007) and the following reaction sequence were used: 2 min at 95 °C initial denaturation, followed by 1 min at 95 °C, 50 s at 55 °C and 1 min at 72 °C for 35 cycles and a final extension of 10 min at 72 °C. For *rpl*16 the first F (1F) and R (3R) (Hernández-Hernández et al. 2011) and the following reaction sequence were used: 5 min at 94 °C initial denaturation, followed by 1 min at 94 °C, 50 s at 55 °C and 2 min at 72 °C for 30 cycles and a final extension of 4 min at 72 °C. The samples obtained by PCR were sequenced at the University of Washington (High-throughput Genomics Center: http://www.htseq.org) and at the Universidad Nacional Autónoma de México (Instituto de Biología, Molecular Systematics Laboratory). Several of the samples were sequenced using a "genome skimming" approach (see: Ripma et al. 2014; Majure et al. 2019) on the Illumina HiSeq X platform. Raw, whole genomic DNAs were sent to Rapid Genomics LLC (http://rapid-genomics.com/home/; Gainesville, FL) for library preparation and paired-end read sequencing (yielding 150 bp reads). Raw reads were then referenced-mapped in Geneious (v. 11.1.5, Biomatters Ltd.) to the plastid loci outlined above for their incorporation into the dataset. Majority consensus sequences were created for each locus from the mapped reads for their incorporation into the larger dataset.

Alignment of the sequences

Chromatograms were assembled and edited in PhyDE v. 0.9971 (Müller et al. 2005; http://www.phyde.de). The resulting sequences were manually aligned using the same program with the Best match option and examined by eye—non-alignable poly A/T sites were excluded from the analyses. Before concatenating the DNA sequences, the 5′ and 3′ ends of each sequence were removed because of ambiguities. The insertions and deletions (Indels) were coded according to the simple indel coding method (Simmons and Ochoterena 2000).

Morphological data

A total of 39 morphological characters was compiled (Online Resource 4) from field data, herbarium materials [DES, HAC, HAJB, JBSD, MEXU, NY; acronyms follow

Thiers (2018)] and living collections (National Botanical Garden, University of Havana). In addition, a majority of the information of genera outside of *Leptocereus* was compiled through bibliographic review (Britton and Rose 1920; Bravo-Hollis 1978; Barthlott and Hunt 2000; Anderson 2001; Hunt et al. 2006; Sánchez-Salas et al. 2009; Ostolaza 2011; Cruz et al. 2016; Franco 2017; Rosas 2017; Tapia et al. 2017; Areces-Mallea 2017). The matrix of morphological characters (Online Resource 5) was coded with binary and multistate characters and edited in Mesquite 3.02 (Maddison and Maddison 2015).

Phylogenetic analyses

Two matrices were built; the first included only molecular data (DNA sequences and indels) and the second one comprised DNA sequences, indels and morphological data (combined matrix). Matrices have been deposited as an online resource (Online Resource 1–2) associated with this manuscript. Both matrices were analyzed using Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analyses was performed using the program RAxML 8.2.10, in the CIPRES Science Gateway portal (2018; https://www.phylo.org/portal2/login!input.action), and each region was partitioned under 25 rate categories using GTR as the model of molecular evolution and carrying out 10,000 nonparametric bootstraps for the separated and combined partitions. For BI analyses, the model of molecular evolution for each marker was determined by means of the Akaike information criterion (AIC), using the program PartitionFinderV1.0.1, and the partitions corresponding to the morphological and indel data were analyzed by means of the Mkv model (Lewis 2001). All analyses of BI were implemented in MrBayes-v3.2 allowing the probability rates of substitution models to vary between partitions, four chains were run simultaneously for 10,000,000 generations sampling a tree every 1000 generations. The first 25% of trees were excluded as burn-in, with the remaining trees used to construct the majority rule consensus tree and its subsequent probabilities as support of the nodes. All trees were edited in TreeGraph 2.4.0–456 beta (Stöver and Müller 2010).

Ancestral state reconstruction in *Leptocereus*

The majority rule consensus tree from the BI analysis based on our DNA sequence only dataset was selected to evaluate the evolution of morphological characters. This tree was pruned and only included the three species of *Armatocereus*, two species of *Dendrocereus* and one terminal of each species of *Leptocereus*. From the 38 characters initially included, two vegetative (habit and level of areoles in the rib margin) and six reproductive (anthesis initiation, anthesis length, color of inner tepals, fruit length, mucilage in fruit, and seed color) characters were selected, which we considered important, because they are diagnostic characters for some species (see: Areces-Mallea 1992, 2003, 2018), or because the characters were phylogenetically informative in the combined analysis for the *Leptocereus–Dendrocereus* clade. The reconstruction of ancestral states was performed in Mesquite v.3.04, using the maximum likelihood (ML) method and implementing the Mk1 model of evolution (Maddison and Maddison 2015).

Results

Sequence characteristics

The concatenated DNA matrix with five markers included 3628 characters, of which 9.6% were variable. We excluded 9 hotspots (highly variable regions) and coded 35 indels from the sequences of the four DNA regions (Table 1), most of which represented deletions (Online Resource 6). The *rpl*16 marker in most Cuban *Leptocereus* species presented difficulties for amplification from 890 to 1050 bp, and thus those 159 bp were removed from the analyses for all samples. For *Leptocereus* and *Dendrocereus*, *rpl*16 had the highest number of variable sites (37) followed by *trn*L-F (21) and *psb*A (14). Missing data represented 1.17% of the molecular matrix (Table 1), which came mainly from *rpl*16 (represents 87.4% of missing data); the terminals with the highest amount of missing data were *Leptocereus maxonii* (*HFC88784*), *Dendrocereus nudiflorus* (*LCM7048*) and *Calymmanthium substerile*.

Phylogenetic analyses

The ML and BI analyses of the DNA dataset showed high support (93% bs, 0.99 pps) for *Armatocereus* as the sister to *Leptocereus*, but with low support (63% bs; 0.73 pps) with respect to species of the Echinocereeae (*Cephalocereus, Peniocereus, Stenocereus*), and of the genus *Strophocactus* sensu Korotkova et al. (2017), and there was no resolution between the *Strophocactus* and Echinocereeae clades. ML and BI analyses resulted in mostly congruent topologies (other than support values), with high support (100% bs, 1.0 pps) for a clade containing all *Leptocereus* and *Dendrocereus* species (Fig. 1). Two clades of *Leptocereus* were resolved and well supported (100% bs, 1.0 pp), the Cuban clade (CU) and the Hispaniolan–Puerto Rican (EPR) clade, which was sister to the CU + *Dendrocereus* (D) clade (91% bs, 0.76 pps). The CU clade is supported by six substitutions (molecular synapomorphies), while the EPR clade is supported by 13 substitutions; six substitutions support *Dendrocereus* nested within *Leptocereus* (Table 2). Although there was low resolution among the species in the CU clade

Table 1 Informative data of the alignment of the sequences

Region	*pet*L-*psb*E	*psb*A-*trn*H	*rpl*16	*trn*L-F	*trn*Q-*rps*16	DNA matrix
Sequence length	547	443	1066	1043	529	3628
Variables sites	35	58	104	101	51	349
% missed data	0.60	0.22	3.49	0.10	0	1.17
No. of parsimony informative characters	15	31	68	45	26	185
% informative characters	2.74	6.99	6.37	4.31	4.91	5.09
No. of hotspots	1	1	2	3	2	9
Informative coded indels	0	7	7	14	7	35
Model of nucl. subs.	GTR + I + G	GTR + G	GTR + I + G	F81 + I + G	GTR + I + G	

(Fig. 1), four clades were recovered, the *Arboreus* (AR), *Assurgens* (AS), *Leonii* (LE) and *Carinatus* clades, with *L. carinatus* resolved as sister to the rest. Species level relationships were resolved in the AS clade, however, and showed that *L. assurgens* was not monophyletic. Species level relationships also were resolved for both the *Dendrocereus* and EPR clades. *Leptocereus weingartianus* from Hispaniola was sister to the rest of the EPR clade species, and *Dendrocereus undulosus* was recovered as sister to *D. nudiflorus*.

The ML and BI analyses of the combined DNA and morphological matrices resulted in congruent topologies and showed a similar topology to that of just DNA sequence data, although with better resolution among the terminals (Fig. 2). *Armatocereus* was resolved as sister to the *Leptocereus* and *Dendrocereus* clades, and furthermore, *Strophocactus* was resolved as sister to an *Armatocereus, Leptocereus, Dendrocereus* clade; Echinocereeae was resolved as sister to that clade. *Dendrocereus* remained nested within the *Leptocereus* clade (100% bs; 1.0 pps), but with low support (40% bs; 0.60 pps) still as sister to the CU clade. Furthermore, the CU clade formed four highly supported clades with *L. carinatus* Areces as sister to the rest of the species, *L. assurgens* var. *albellus* Areces as sister to the rest of the AS clade (97% bs; 1.0 pps), again showing non-monophyly of *L. assurgens*; *L. scopulophilus* (*HFC88329*) was sister to the rest of the LE clade (76% bs; 1.0 pps) and *L. arboreus* Britton & Rose was sister to the rest of the AR clade (94% bs; 0.99 pps) (Fig. 2). The EPR clade again was resolved, as in the DNA sequence only analyses, with the Hispaniolan species, *L. weingartianus*, sister to the rest of the EPR clade (Fig. 2).

Ancestral state reconstruction in *Leptocereus*

Optimization through likelihood on the BI majority rule consensus tree from the DNA sequence only dataset shows that the ancestral habit in *Leptocereus* s.l. (including *Dendrocereus*) is ambiguous, with equal probability ($p = 0.50$) for tree or shrub (Fig. 3a). Sunken areoles in the depressions of the ribs are likely pleisiomorphic ($p = 0.96$), and there was a shift to marginal areoles in the AS clade (Fig. 3b). Flowers with nocturnal anthesis ($p = 0.96$) and short life span ($p = 0.99$) are likely symplesiomorphies for *Leptocereus* s.l. and white inner tepals ($p = 0.88$) are also most likely ancestral (Fig. 3c–e; outgroups not given here are also plesiomorphic for nocturnal anthesis and short life span). The medium fruits ($p = 0.92$) with mucilage ($p = 0.76$) also represent ancestral states of the genus (Fig. 3f–g), as well as the brown-black color of the seeds ($p = 0.96$), although, there was a switch to black seeds in the Cuban clade ($p = 0.84$) (Fig. 3h), a putative synapomorphy of that clade that was reversed in the AR clade back to brown-black seeds.

Discussion

Paraphyly in *Leptocereus*

Areces-Mallea (2003) was the first to conduct a phylogenetic analysis including several species of *Leptocereus* and the two *Dendrocereus* species. However, in his significant study based on the *trn*L. intron, he finds that both genera are not close relatives, but rather that *Dendrocereus* and *Harrisia* are closely related, as was suggested by Gibson and Nobel (1986) who placed them in the tribe Hylocereeae. Almost a decade later Hernández-Hernández et al. (2011) recovered a close relationship between *Dendrocereus* and two species of *Leptocereus* (one from Cuba and one more from Puerto Rico) with high support values for ML analyses (99% bs) and with *Dendrocereus* nested with *Leptocereus.* Although these authors only commented on this relationship as intriguing, our study corroborates the relationship with high support values for both analysis (ML and BI) and likewise recovers a paraphyletic *Leptocereus* s.s. with *Dendrocereus* nested within.

Dendrocereus shares several morphological characters with *Leptocereus* such as, a diffuse and sympodial branching pattern, sunken areoles in the depressions of the ribs, same range of rib numbers (3–5), nocturnally opening flowers, fruits with mucilage, green, indehiscent fruits

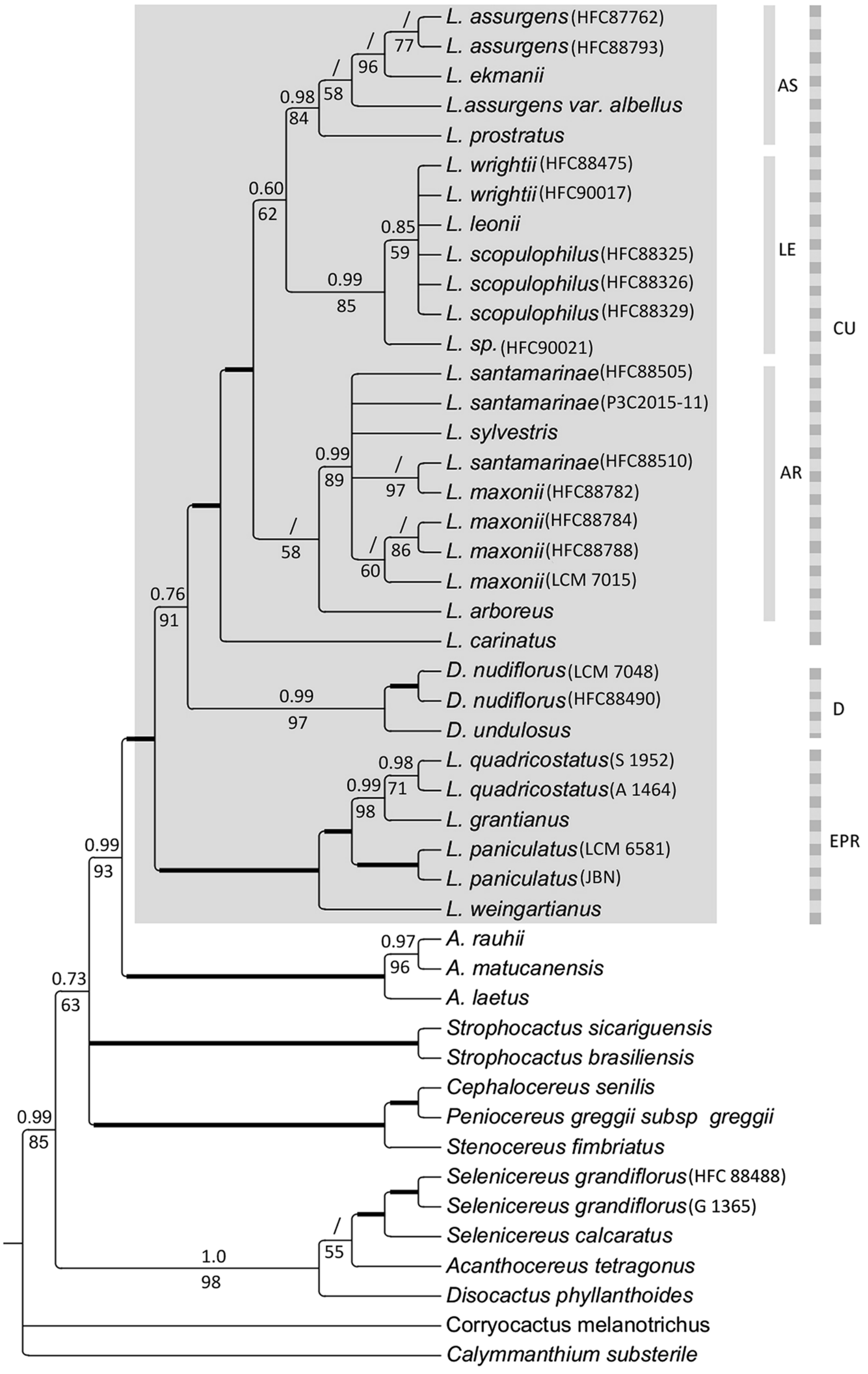

Fig. 1 Majority-rule consensus BI tree from our DNA sequence only matrix. Values above branches are posterior probabilities and values below branches are bootstrap support values from Maximum Likelihood (10,000 pseudoreplicates). Thickened branches denote maximum support. The EPR (Hispaniola + Puerto Rico) clade is sister to the D (*Dendrocereus*) + CU (Cuban) clade. Within the CU clade, *L. carinatus* is sister to the rest of the species, and the AR (*Arboreus*) clade is sister to the LE (*Leonii*) + AS (*Assurgens*) clade

when mature, and the shape and color of the seeds (Fig. 4). Several species of *Leptocereus* form trees (Fig. 4b), and although they do not reach the giant dimensions of some specimens of *Dendrocereus nudiflorus*, two species share the same habit: *L. paniculatus* (endemic to Hispaniola) and *L. arboreus* (endemic to Cuba). In general, *Dendrocereus* appears to be a *Leptocereus* with structures of larger dimensions (e.g., ribs more than 4 cm wide, flower up to 20 cm long, fruit more than 10 cm long, seed more than 3.1 mm long) and therefore could be considered as gigantism within *Leptocereus*. While gigantism in animals on islands is well documented (see: Foster 1964; Jaffe et al. 2011; Raia and Meiri 2011), including in the Antilles (Silva et al. 2007; Arredondo 2011), this phenomenon has rarely been studied in plants (Manihera and Burns 2017), especially in the Antilles, and the reason for the evolution

Table 2 Molecular characteristics that support clades within the genus *Leptocereus* s.l. EPR: Hispaniola and Puerto Rico

Clades	No. of characters	Character type, marker and position in alignment
Cuban *Leptocereus*	6	substitutions: *trn*L-F (86), *trn*Q-*rps*16 (73), *psb*A-*trn*H (303), *rpl*16 (29; 397; 793)
Cuban *Leptocereus* and *Dendrocereus*	2	*Indel*: *trn*Q-*rps*16 (420), substitution: *pet*L-*psb*E (235)
EPR *Leptocereus*	13	substitutions: *trn*L-F (75; 890; 1571*), trn*Q-*rps*16 (67; 156; 209), *pet*L-*psb*E (235; 273), *rpl*16 (220; 238; 320; 516; 920)
Leptocereus with *Dendrocereus*	6	substitutions: *trn*L-F (961), *trn*Q-*rps*16 (92), *psb*A-*trn*H (96; 213; 394), *rpl*16 (881)

of size has not yet been clarified (Vermeij 2016; Manihera and Burns 2017).

The main differences between *Leptocereus* s.s. and *Dendrocereus* are floral features; in the latter the tepals are narrowly oblong to narrowly triangular with an acute apex with respect to those that occur in *Leptocereus*, which are usually short and rounded or ligulate (Fig. 4i–p). Floral morphology can be modified in response to adaptations to different groups of pollinators (Fenster et al. 2004; Schlumpberger 2012; Armbruster 2014). For example, the nocturnal habit of *Dendrocereus* flowers restricts the group of pollinators to nocturnal species, probably moths with long proboscises, although two species of bats that consume their pollen have also been reported (Silva 1979). However, in *Leptocereus* Areces-Mallea (2003) noted the visits of five functional groups (butterflies, moths, bees, hummingbirds and bats) both day and night with 38 species, although not all of them may act as pollinators (Barrios et al. 2012). We hypothesize that the floral diversity in *Leptocereus* and morphological divergence in the *Dendrocereus* clade may be the result of selection by different pollinators.

Regarding the DNA sequence data matrix, *Leptocereus* s.s. and *Dendrocereus* present several molecular synapomorphies represented by different base changes (Table 2). In addition, both taxa share the same habitats and geographic range, being endemic to the Antilles. Therefore, we here recircumscribe *Leptocereus* to include *Dendrocereus*, and thus render *Leptocereus* s.l. monophyletic, given the high support of the ML and BI analyses, as well as the characters previously discussed.

Relationships among *Leptocereus* s.l. and other clades

The close relationship obtained in our study between *Leptocereus* and *Armatocereus* was first recognized by Buxbaum (1958), who considered both genera within the Leptocereeae based on characteristics of the flowers (relatively small pericarpel and receptacle short with numerous spiny, bristly or at least hairy areoles). Anderson (2001) incorporated *Leptocereus* in Echinocereeae (previously Pachycereeae), but included *Armatocereus* in Browningieae; Wallace (2002) proposed a new circumscription of Leptocereeae (with *Leptocereus*, *Dendrocereus* and *Acanthocereus*), while *Armatocereus* was maintained in Browningieae. In fact, Wallace (2002) published a phylogenetic tree based on the *rbc*L gene where *Leptocereus* and *Armatocereus* appear as sister taxa, but he did not consider this result significant. Our results agree with the results obtained by Korotkova et al. (2017); a polytomy with low support we found (63% bs; 0.73 pps; Fig. 1), which includes three clades: (1) *Leptocereus* (CU)-*Dendrocereus* (D)-*Leptocereus* (EPR)-*Armatocereus*, (2) *Strophocactus,* and (3) Pachycereinae-Stenocereinae. More comprehensive future studies could help elucidate relationships among these three clades.

Relationships within *Leptocereus* s.l

All three subclades of *Leptocereus* s.l. (CU, EPR, and D subclades) were well supported and are geographically cohesive, with CU restricted to Cuba, EPR to Hispaniola and Puerto Rico (and adjacent islands) and *Dendrocereus* to Hispaniola and Cuba. In the EPR and CU clades, the sister species to those clades (*L. weingartianus* and *L. carinatus* Areces, respectively) exhibit plesiomorphic characters such as tubular and nocturnal flowers, and white perianth segments with less than 25 tepals (in *L. maxonii* they can reach 60). However, while *L. weingartianus* is a widely distributed species (Areces-Mallea 2003), *L. carinatus* is restricted to a location near the eastern region of Cuba (Areces-Mallea 1993). Intriguingly, *L. carinatus* inhabits an intermediate zone between the species of the subclade AR (Fig. 5) in an area generally of low floristic relevance with similar vegetation and climatic conditions to the species that inhabit the karstic elevations in western Cuba (Areces-Mallea 2003).

The three Cuban subclades (AR, LE and AS) are congruent with the geographic distribution of their species (Fig. 5). The subclade AR is composed of four species that inhabit mainly coasts of eastern Cuba, although some populations can be found in elevations close to them (Fig. 5). The species of this subclade present the spiniest fruits (Fig. 4t) within the genus, with yellow spines, generally > 30 per areole when immature and > 40 per areole in fruit. Another common characteristic of the AR subclade is the presence of

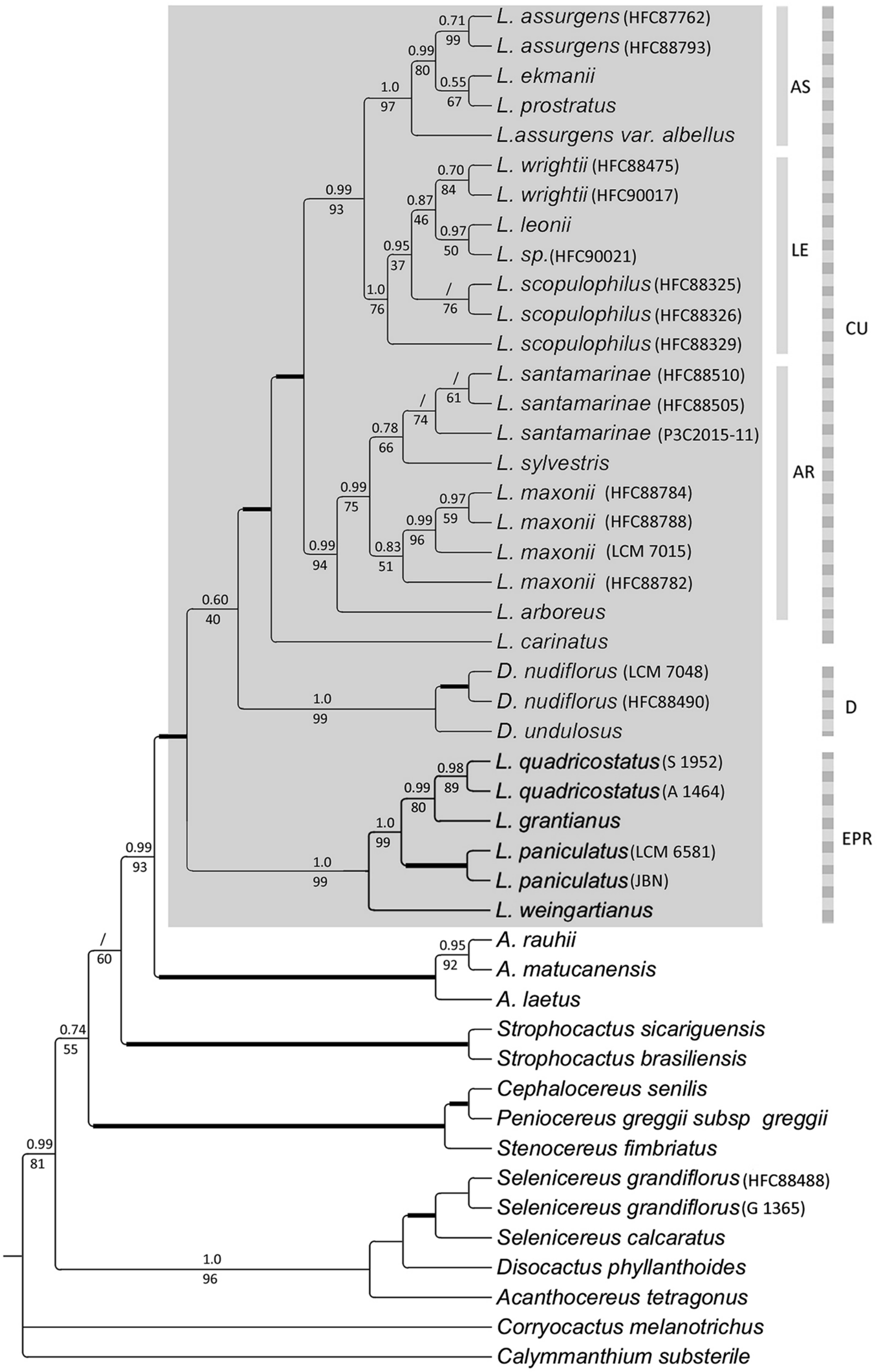

Fig. 2 Majority-rule consensus BI tree from combined DNA sequence and morphological matrix. Values above branches are posterior probabilities and values below branches are bootstrap support values from Maximum Likelihood (10,000 pseudoreplicates). Thickened branches denote maximum support. All major clade relationships remain the same, however, species relationships are in many cases better resolved, especially in the CU subclades LE and AR

nocturnal flowers with white tepals with > 10 stigma lobes (Fig. 4l, p). Subclade LE comprises a taxon (*Leptocereus* sp.) of unknown natural origin (from the JBN collection), and three species that inhabit the low karst hills of four provinces of western Cuba (Artemisa, Havana, Mayabeque and Matanzas) and some coastal areas. Orange to pale pink flowers are a synapomorphy for these species (Figs. 3e and 4m–o). In addition, black seeds appear to be synapomorphic for the Cuban clade among the species that inhabit the karstic hills throughout the island (although brown seeds have re-evolved in the AR subclade; Fig. 3h). In this group, *L. leonii* stands out due to the presence of several autapomorphies, such as red fruits less than 4.5 cm in length (Fig. 4s) without mucilage or acidity in the pulp; fruit size and the lack of mucilage are homoplasious in species of the AS subclade (Fig. 3f–g). The AS subclade is represented by

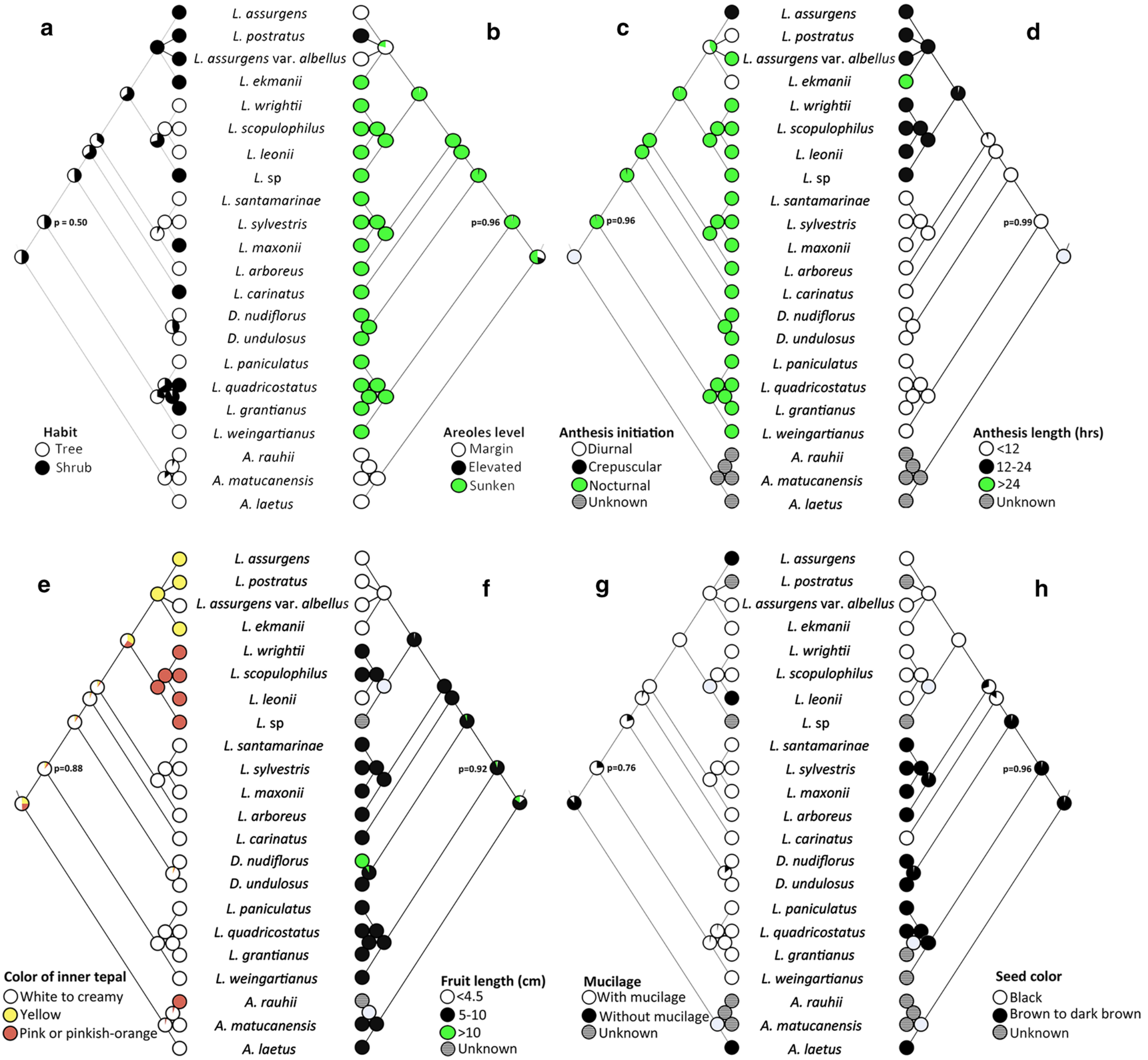

Fig. 3 Maximum likelihood reconstructions of ancestral character states in *Leptocereus*: **a** habit; **b** level of areoles in the rib margin; **c** anthesis initiation; **d** anthesis length; **e** color of inner tepals; **f** fruit length; **g** presence of mucilage in fruit pulp and seeds; **h** seed color

allopatric species distributed in several mountain ranges of the Coordillera de Guaniguanico, in the westernmost region of Cuba. The representatives of this subclade are prostrate to scandent shrubs, and are composed of species with yellow tepals (Fig. 4j, k) and small, yellow fruits (except *L. assurgens* var *albellus*; Fig. 3e). Adaptations presented in this group (sometimes autapomorphies and other times synapomorphies) are unique in the genus such as: raised areoles (*L. prostratus* Britton & Rose); small flowers with diurnal initiation of anthesis (*L. ekmanii*, *L. prostratus*, and *L. chrysotyrius*), with > 24 h duration and less than 200 ovules in *L. ekmanii* (in *L. maxonii* we have counted more than 4000 ovules in some flowers) (Fig. 3b-d). Of all the *Leptocereus*, the AS subclade is the group with clear adaptations to diurnal pollination, this being an ancestral characteristic in cacti (Schlumpberger 2012), but apparently derived in the genus (Fig. 3c).

The large trees of the *Dendrocereus* clade (two species) are found throughout lowland areas across both Cuba and Hispaniola. Our phylogenetic results suggest that the two species, *D. undulosus*, endemic to Hispaniola, and *D. nudiflorus*, endemic to Cuba, are genetically divergent from one another and likely represent true species, although Anderson (2001) suggested that they could be the same. Future

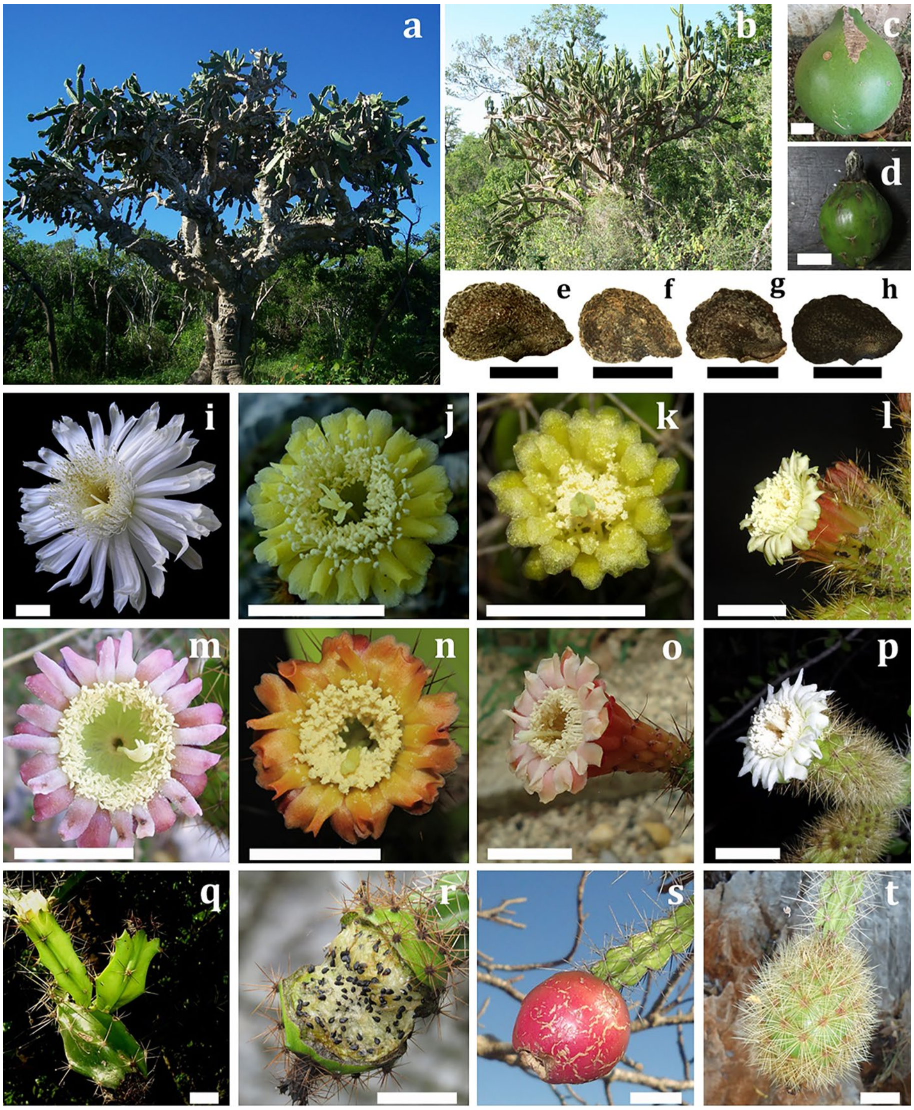

Fig. 4 Morphological features of some species from the redefined *Leptocereus* s.l. (see taxonomic treatment). Tree growth form of **a** *L. nudiflorus* and **b** *L. arboreus* protruding above surrounding vegetation; smooth fruit of **c** *L. nudiflorus* and multi-areoled fruit of **d** *L. paniculatus*; seeds of **e** *L. nudiflorus*, **f** *L. sylvestris*, **g** *L. santamarinae* and **h** *L. wrightii*, showing the transition from brown to black seeds; floral variation in *Leptocereus*, **i** *L. nudiflorus*, **j** *L. assurgens*, **k** *L. ekmanii*, **l** *L. sylvestris*, **m** *L. scopulophilus*, **n** *L. wrightii*, **o** *L. leonii*, **p** *L. maxonii*, flower bud and fruit of **q** *L. carinatus* showing little-modified pericarpel; dehiscing fruit of **r** *L. albellus* exposing pulp and seeds, smooth fruit of **s** *L. leonii*, and **t** densely spiny fruit of *L. maxonii*. The seed scale is equivalent to 2 mm, and the flowers and fruits to 2 cm except in **k** where equivalent to 1 cm

phylogenetic and morphological work should incorporate more populations of both species.

The EPR clade consists of the widespread *L. weingartianus* and *L. paniculatus* on Hispaniola, which are subsequent sisters to the more restricted *L. quadricostatus* and *L. grantianus* from Puerto Rico and parts of the Lesser Antilles. *Leptocereus weingartianus* occurs in a diverse array of elevations over limestone, ranging from near sea level to nearly 700 m, while *L. paniculatus* is more restricted mostly to lower elevations over heavier, silty soils (Majure, pers. obsv.). Thus, ecological niche specialization may have played a role in speciation in this clade.

Ancestral state reconstruction in *Leptocereus* s.l

Hernández-Hernández et al. (2011) considered that the shrub habit is the most probable ancestral state in the clade to which *Leptocereus* belongs. Our results are ambiguous for tree and shrub, so if we consider the tree state as derived, then it evolved at least four times in the genus (Fig. 3a). The sunken position of the areoles in the margin of the ribs is a plesiomorphic character in most species of the genus, however, in the AS subclade both marginal and elevated areoles are seen (Fig. 3b). Regarding the color, anthesis and duration of the flower, our results show white flowers and nocturnal anthesis to be the plesiomorphic condition, as is the short duration of the flower (Fig. 3c–e). These results agree with those obtained by Franco (2017) for *Peniocereus*. However, Schlumpberger (2012) reports that in cacti, pollination by bees is ancestral, and pollination by bats, moths and hummingbirds is the derived condition. In *Leptocereus*, a change from bat-moth pollination syndrome to pollination by bees or diurnal butterflies is observed in some species of the AS subclade (Fig. 3c).

Additionally, medium-sized fruits with brown seeds and mucilage correspond to plesiomorphic characters in

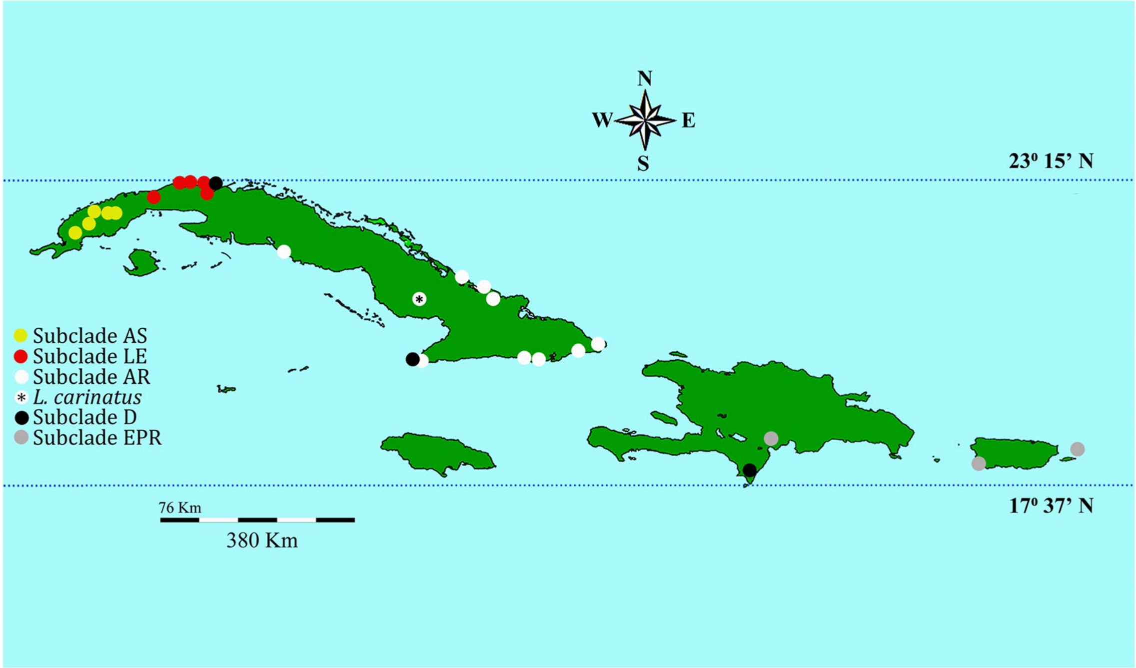

Fig. 5 Distribution of collection sites given for subclades of *Leptocereus*

Leptocereus (Fig. 3f–h). The presence of mucilage is a state that has evolved in several lineages in Cactaceae (see: Barrios et al. 2015), even being present in *Leuenbergeria* species (Leuenberger 1986); and it is also found in the relatives of cacti (Portulacaceae and Anacampserotaceae) (Ogburn and Edwards 2009). Its presence in fruits is associated with different functions mainly related to dispersion and germination of seeds (Yang et al. 2012; Western 2012). However, in the Cuban clade, at least two species (*L. leonii* and *L. assurgens*) present fruit without mucilage (Fig. 3g). Finally, the black color of the seeds in the CU subclade could have evolved in response to wetter habitats, as our results show (Figs. 3h and 5) that this feature is present almost exclusively in the species that inhabit inland hills on the island of Cuba, where the current and past rainfall (Ortega 1983; Ortega et al. 2011) are more abundant than in coastal habitats.

Taxonomic considerations for some entities of the Cuban clade

The limits between close species in Cuban subclades are not clearly defined, due to the low variability of the markers used (Fig. 1). This fact may be related to the CU subclade being a rapid radiation, which corresponds to the results suggested by Hernández-Hernández et al. (2014) for the group, who found that the clade was of a very young age (8.16–7.37 Ma), although based on limited sampling. However, based on the extensive field work carried out on Cuban *Leptocereus* species, it is necessary to underscore some taxonomic considerations. *Leptocereus maxonii* can be clearly differentiated from *L. santamarinae* and *L. sylvestris* Britton & Rose, as it is a generally decumbent species, with thinner stems (usually thinner than 2.5 cm wide, whereas in *L. santamarinae* and *L. sylvestris* they are between 2.5 and 4.6 cm wide), densely spiny pericarpel and fruits, more than 30 spines per areole, and is the only species of *Leptocereus* where the pericarpel is not visible due to the density of spines (Fig. 4p). Meanwhile, the phylogenetic and morphological data obtained in this study do not support the separation of *L. santamarinae* and *L. sylvestris* as different species. An eventual phylogeographic study may be necessary to determine whether these taxa should be considered one or two species.

On the other hand, in the AS subclade, *L. assurgens* var. *albellus*, recently described by Areces-Mallea (2018), should be raised to species according to our phylogenetic results (Figs. 1 and 2), where it was recovered as distantly related to other accessions of *L. assurgens*. Although Areces-Mallea (2018) considered the color of the tepals only as a variation in *L. assurgens*, and that there were no other floral characters that distinguish both taxa, there are several aspects to consider. As far as we have observed, the only species in the genus with polymorphism in the color of the tepals in the same population is *L. scopulophilus* (González-Torres et al. 2012). All populations of *L. assurgens* in the Viñales range (their type locality) have yellow tepals and stems that are thinner than the specimens of Sierra de San Carlos, which Areces-Mallea (2018) himself recognizes; another notable difference is that *Leptocereus assurgens* var. *albellus* of Sierra de San Carlos is the only taxon that has dehiscent fruits (Fig. 4r), and unlike *L. assurgens* var. *assurgens* the pulp contains mucilage (Online Resource 5). Finally, the westernmost populations of *L. assurgens* in Chichones del Indio are 21 km away from the Sierra de San Carlos, and between both locations *L. prostratus* is distributed in the Sierra de Sumidero. Thus, *L. assurgens* var. *albellus* is phylogenetically, morphologically, and geographically distinct from *L. assurgens* var. *assurgens*, and we therefore recognize the taxon at the species level here.

Conclusions

One of two endemic genera of Cactaceae in the Antilles that form a major in situ radiation, *Leptocereus,* was shown here to be non-monophyletic as currently circumscribed, with the insular giant *Dendrocereus* embedded within it. Three major clades make up *Leptocereus* s.l., the CU (Cuban) clade, the D (*Dendrocereus*) clade, and the EPR (Hispaniola/Puerto Rican) clade. Species relationships among the EPR and D clades are resolved in our phylogeny, however, species relationships among the members of the Cuban clade will need to be further clarified in future work. Although, no clear morphological synapomorphies were discovered for the clade, suites of characters can be used to recognize the group, including sunken areoles in the rib margins, white tepals, nocturnal flowers, fruit with brown seeds and the production of mucilage in most species (Fig. 3). However, these mostly plesiomorphic characters have been lost in some subclades that show apomorphic conditions to these character states (e.g., elevated areoles, black seeds, lack of mucilage). The re-circumscription of *Leptocereus* (see Taxonomic Treatment below), including *Dendrocereus*, encompasses 19 species of trees and shrubs, these all endemic to the diverse, seasonally dry tropical forest of the Antilles and with the major center of diversity for the clade on Cuba.

Taxonomic treatment

Based on our phylogenetic results to reflect monophyly and the comparison of morphological characters, three new combinations in *Leptocereus* are presented here.

Leptocereus albellus (Areces) D.Barrios & S.Arias, **comb. et stat. nov.** ≡ *Leptocereus assurgens* (C.Wright ex Griseb.) Britton & Rose var. *albellus* Areces, Cact. Suc. J. (Los Angeles) 90(4): 260. 2018.—TYPE (not found): Cuba, Pinar del Río province: approximately 20 km NNE from Guane, in Sierra de San Carlos mountain range, on cliffs surrounding the "Hoyo de los Helechos," a deep solution hole within the Majagua-Cantera cavernous system, 28 Mar 1992 (fl.), *A.E. Areces 6343*.—LECTOTYPE (**designed here**): illustration of "flower of the type specimen of *Leptocereus assurgens* var. *albellus* (*Areces 6343*)", in Areces, Cact. Succ. J. (Los Angeles) 90(4): 260, Fig. 5. 2018.—EPITYPE (**designed here**): Cuba, Pinar del Río, Minas de Matahambre, mogote de la cueva de Cando, en ladera sur, Sierra de San Carlos, 15 May 2018, *D. Barrios* and *J.L. Guerra HFC90015* (HAJB 001213!; isoepitypes: HAJB 001208!, 001209!, 001210!, 001211!, 001212!).
Note: According to Areces (2018), type specimens were deposited in HAJB (holotype) and NY (isotype), however, these specimens were not located, thus, we have designated a lectotype and epitype of the species here.

Leptocereus nudiflorus (Engelm. ex C.Wright) D.Barrios & S.Arias, **comb. nov.** ≡ *Cereus nudiflorus* Engelm. ex C.Wright, Anales Acad. Ci. Méd. Fís. Nat. Habana Revista Ci. 6: 98. 1869. ≡ *Dendrocereus nudiflorus* (Engelm.) Britton & Rose, The Cactaceae 2: 113. 1920. —TYPE: Cuba, en las playas cerca de la Habana y de Guantánamo, 1866–1867, *C. Wright 3570* (Holotype MO?; GH isotypes 00303982! 00061820!; NY isotype 00120653!).

Leptocereus undulosus (DC.) D.Barrios & Majure, **comb. nov.** ≡ *Cereus undulosus* DC., Prod. Syst. Nat. 3: 467. 1828. ≡ *Dendrocereus undulosus* (DC.) Britton & Rose, J. New York Bot. Gard. 26(310): 220. 1925. ≡ *Acanthocereus undulosus* (DC.) Croizat, Caldasia 2: 137. 1943.—LECTOTYPE (**designed here**): illustration in Plumier, Plantarum americanarum fasc. 8. 187. t. 194. 1758.— EPITYPE (**designed here**): Haiti, 12–18 Jan 1929, *E.C. Leonard* and *G.M. Leonard 12085* (NY 03305544!).
Note: De Candolle (1828) refers to an illustration in Burman's work (1758, t. 194), based on a drawing by Charles Plumier. Therefore, this figure represents the lectotype (art. 8.1, 9.4). Hunt (1984) included this name in a review of cacti in the work of Plumier, referring to the illustration as an "iconotype".

Acknowledgments The present study is part of the PhD thesis of the first author. We appreciate the collaboration between the Jardín Botánico Nacional, the Jardín Botánico of the Instituto de Biología, Universidad Nacional Autónoma de México, and the Desert Botanical Garden that made possible a great part of the collection expeditions and the laboratory work. The study was funded by the UNAM (DGAP PAPIIT IN208619 to S. Arias) and a National Geographic Society Explorer's Grant (Grant 9939-16 to L.C. Majure). The results obtained are part of the project ¨Taxonomy of the Cactaceae family in Cuba: A contribution to its conservation¨. We want to thank the Empresa Flora and Fauna, BIOECO, ECOVIDA, a Planta! and the Cuban Botany Society for their support before and during the expeditions. The help in the field of J.A. García-Beltrán, E. Palacio, A. Legra, C. Legra, A. Hernández, Y. Revilla, M. Deloncelet, A. Morales, S. Toledo, G. Begué, W. Carmenate, J.L. Gómez, J.M. Acuña, E. Martínez, R. Chaves, J. Serrano, Y. Lorenzo, Y. Hernández, M. Torres, Y. Martínez and R.M. Verdecia were of inestimable value for carrying out the expeditions, as well as the support of the workers and managers of the protected areas and herbarium visited. Field work in the Dominican Republic was aided by T. Clase (JBSD) and Y. Encarnación (JBSD, UF) and made possible through the collaboration of the Jardín Botánico Nacional “Dr. Rafael Ma. Moscoso” (Dept. de Botánica) and funded by the Florida Museum of Natural History Biodiversity Institute (to D.W. Steadman) and the National Science Foundation (grant GSS-1461496 to D.W. Steadman and J. Franklin). Seeds of *L. quadricostatus* were kindly provided by J.A. Sustache and C.E. Figuerola. The first author wants to thank the support provided in the laboratory by L. Cabrera, Y. Morales, D. Franco, H. Tapia, I. Rosas, D. Aquino, M. Cruz, D. Sánchez, and L.M. Márquez (Instituto de Biología); and the support provided by The University of British Columbia and J. Whitton to the Faculty of Science. The expeditions were made according to the permits LH175 AN (09), LH AN (110) 2015, AN (63) 2017, AN (64) 2017, AN (65) 2017 and 76/2017.

Authors' contributions DB, SA and LCM carried out fieldwork, labwork, analyzed the data and wrote the manuscript. LRG-T helped write the manuscript.

Funding The study was funded by the UNAM (DGAP PAPIIT IN208619 to S. Arias), the Florida Museum of Natural History Biodiversity Institute (Grant to D.W. Steadman), the National Science Foundation (Grant GSS-1461496 to D.W. Steadman and J. Franklin), and a National Geographic Society Explorer's Grant (Grant 9939-16 to L.C. Majure).

Availability of Data and Material All sequence data are available in GenBank, and our DNA sequence and morphological matrices are available as Online Resource 1 and 2 associated with this manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All authors have carried out fieldwork and data generation ethically, including obtaining appropriate permitting.

Consent for publication All authors have consented to publishing this work.

Information on Electronic Supplementary Material

Online Resource 1. Nexus file of our DNA sequence matrix.
Online Resource 2. Nexus file of DNA sequence + morphological data matrix.
Online Resource 3. A list of taxa sampled with the herbarium number and location.
Online Resource 5. Our matrix of morphological characters used in the phylogeny of *Leptocereus*.
Online Resource 4. A table of our 39 morphological characters and their character states.
Online Resource 6. A table of our coded indels used in our phylogenetic analysis.

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