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PHYLOGENETICS OF *PUYA* (BROMELIACEAE): PLACEMENT, MAJOR LINEAGES, AND EVOLUTION OF CHILEAN SPECIES[1]

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Puya (Bromeliaceae), a large genus of terrestrial bromeliads found throughout a range of elevations in the Andes and central Chile, is of great systematic, evolutionary, and biogeographical interest. This first molecular phylogenetic study of *Puya* and related bromeliads employs *matK*, *trnS-trnG*, *rps16*, and *PHYC* sequences. Chloroplast DNA, nuclear DNA, and combined DNA data all place *Puya* closest to subfamily Bromelioideae. Nuclear and combined data support *Puya* as monophyletic, and the two subgenera are nonmonophyletic. All data indicate that the Chilean species of *Puya* are early diverging within the genus, consistent with Chilean genera as the first-diverging members of subfamily Bromelioideae. Central Chile is identified as a key region for understanding the biogeographical history of Bromeliaceae, as is true with other South American plant groups. A complicated history involving early chloroplast capture and later secondary hybridization and/or introgression is seen in Chilean lineages. These events help explain the occurrence of sterile inflorescence tips, floral color and shape, and leaf indument. The ecological radiation of *Puya* appears coincident with the final, recent rise of the Andes and subsequent high-elevation habitat diversification. Additionally, geographical distribution, rather than moisture or elevational adaptations, correlates to species relationships. Evolution of CAM photosynthesis has occurred multiple times.

Key words: Bromeliaceae; Andes; Chile; chloroplast capture; CIPRES; *matK*, *PHYC*; phytochrome C; *Puya*; *rps16*; *trnS-trnG*.

Puya, a large genus (ca. 200 spp.; Luther, 2004) of terrestrial, rosette-leaved bromeliads, is of considerable ecologic, biogeographic, systematic, and evolutionary interest. As one of the largest radiations in the Bromeliaceae, *Puya* extends from the mountains of Central America, through the mid- to high-elevations of the Andes, and south to lower elevations of central Chile. In a diversity of both wet and dry habitats, its species exhibit considerable morphological variation in growth form, semelparity and iteroparity, inflorescence structure, and floral color (Fig. 1). Surprisingly, considering that *Puya* is one of the most characteristic and recognized elements of the páramo and subpáramo floras (Luteyn, 1999), little is known about phylogenetic relationships of species and evolutionary transitions in biogeography, habitat, habit, and morphological features. Moreover, *Puya* occupies an important position in the phylogenetic tree of Bromeliaceae as the putative sister to the large and diverse subf. Bromelioideae (Givnish et al., 2007; Schulte and Zizka, 2008). However, sampling of *Puya*, subfamily Bromelioideae and other close relatives has been limited; the exact placement of *Puya* and composition of its major lineages are unknown.

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The genus *Puya* was first described by the Chilean priest G. I. Molina in 1782 and, starting with the treatment of Wittmack (1888), has always been placed with other bromeliad genera with capsular fruits lacking villous seeds. *Puya* was monographed by Lyman Smith as the first group revised in his comprehensive treatment of Bromeliaceae in *Flora Neotropica* (Smith and Downs, 1974). In this definitive treatment, *Puya* was placed in the subf. Pitcairnioideae with other genera including primarily mesic *Pitcairnia* and primarily xeric *Deuterocohnia*, *Dyckia*, and *Hechtia*. Most other morphology-based classification schemes placed *Puya* with *Hechtia/Deuterocohnia/Dyckia* (among others) apart from *Pitcairnia* (subtribe Puyinae: Mez, 1896; tribe Puyeae: Mez, 1934–1935; tribe Puyeae: Varadarajan and Gilmartin, 1988). Thus, phylogenetic relationships among genera comprising subf. Pitcairnioideae have long troubled bromeliad systematists, and no classification scheme proposed solely on morphological characters has been supported by modern molecular systematic data. The first molecular phylogenies of Bromeliaceae, all using various single chloroplast DNA (cpDNA) regions, revealed a broadly paraphyletic subf. Pitcairnioideae (Terry et al., 1997; Horres et al., 2000; Crayn et al., 2004) and placed *Puya* as sister to subf. Bromelioideae with fleshy fruits. The recent molecular phylogenetic analysis using *ndhF* by Givnish et al. (2007) placed the two sampled *Puya* as sister to subf. Bromelioideae and this clade as sister to another clade comprising *Pitcairnia* + (*Deuterocohnia*, *Dyckia*, *Fosterella*, and others), hereafter referred to as subf. Pitcairnioideae. The many morphological similarities of *Puya* with these other xeric genera were argued to be due to convergence (Givnish et al., 2007). *Puya* was thus placed in the newly described and monogeneric subf. Puyoideae (Givnish et al., 2007). These results have been corroborated by the five cpDNA region Bromelioideae phylogeny of Schulte and Zizka (2008) and the cpDNA plus low-copy nuclear PRK study of Schulte et al. (2009). Recent polyploid events are quite rare in Bromeliaceae and $N = 25$ has been found for all *Puya* surveyed (Smith and Downs, 1974; Brown and Gilmartin, 1989; Gitaí et al., 2005).

Fig. 1. Representative vegetative and floral diversity in both subgenera of *Puya* and in the Chilean species. Species in left gray box are members of subg. *Puya*. The remaining photos show species from subg. *Puyopsis*. Species united by the black line are Chilean *Puya* species.

Puya is found primarily above 1500 m a.s.l. in both moist and dry habitats from the Cordillera Talamanca of Costa Rica to the dry chaco of Tucumán, Argentina (Fig. 2A). A noticeably disjunct group of seven species are found at just above sea level in central Chile (Fig. 2B). This region with a Mediterranean climate represents the southernmost and lowest elevational range of any species of *Puya* and is separate from the distribution of all other *Puya* by the highest and oldest parts of the Andes to the east and the desolate Atacama desert to the north. This region of Chile is also home to all species of *Ochagavia* and *Fascicularia* and several species of *Greigia*, three putative early-diverging genera of subf. Bromelioideae (Horres et al., 2007; Schulte and Zizka, 2008). Little systematic effort is evident at the infrageneric level within *Puya* besides the erection of subg. *Puya* and *Puyopsis* (Smith and Downs, 1974). The paucity of systematic studies within *Puya* may be due to two issues. First, many species are known only from types, and second, obvious characters for morphological comparisons are elusive or are not preserved on most herbarium specimens. Smith and Downs (1974) placed eight species into subg. *Puya* based on the presence of elongate sterile tips of the compound inflorescence, apparently an adaptation to pollination by perching birds (Johow, 1898; Anderson et al., 2005). *Puya chilensis*, *P. gilmartiniae*, *P. alpestris*, *and P. berteroniana* are generally restricted to central Chile and *P. boliviensis* is found in a disjunct area further north in Chile. *Puya weddelliana* is found in xeric southern Bolivia, *P. castellanosii* occurs in xeric northwest Argentina, and the more widespread *P. raimondii* grows in the high puna of Bolivia and Peru. No formal classification has been proposed within subg. *Puyopsis* that contains the remaining 190+ species. Additionally, no molecular phylogenetic approach has tested the monophyly of the two subgenera or even examined relationships of more than a few species.

Of special evolutionary, phylogenetic, and biogeographic interest are species of *Puya* restricted to Chile. The Chilean group includes members from both subg. *Puya* and *Puyopsis* (Figs. 1, 2B). Five species have elongate, sterile inflorescence tips and are placed in subg. *Puya. Puya chilensis*, *P. gilmartiniae*, and *P. boliviensis* possess yellow flowers and leaves becoming glabrous on the abaxial surface. *Puya berteroniana* and *P. alpestris* have leaves with dense, white, appressed scales on the abaxial surface, but are often difficult to tell apart because the former has blue-green flowers and the latter has blue flowers. *Puya alpestris* is generally a smaller plant. The flowers in each of these species are shallower and have a wider diameter than the narrower, longer flowers typical of *Puya* (R. S. Jabaily, personal observation). The two species of subg. *Puyopsis* in Chile, *P. coerulea* and *P. venusta*, are generally shorter plants. They have dense, white scales on both the upper and lower leaf surfaces, wholly fertile inflorescences, and dark blue-purple flowers that are tubular and narrowly open. Many of these Chilean species have overlapping ranges and flowering times and appear to share pollinators (R. S. Jabaily, personal observation). Because of the unique nature of the central coastal Chilean ecosystem compared to the remainder of the Andes and the occurrence of both subgenera and individual speciesin close proximity, placing the Chilean *Puya* within the context of a broader phylogeny is a major goal of this study. Thus, two broad and important phylogenetic issues concerning *Puya* warrant more detailed molecular phylogenetic surveys with additional genes and broader sampling of taxa of *Puya* and related genera. First, the emerging phylogenetic evidence places the large genus *Puya* with dehiscent fruits as sister to an even larger radiation of Bromelioideae with baccate fruits. Second, *Puya* exhibits a remarkable species radiation, involving morphological and ecological variation, along a biogeographical distribution from southern Chile to Costa Rica.

Our aims here are to (1) solidify the placement of *Puya* in relation to related subfamilies and genera using greater taxa sampling and additional chloroplast and nuclear gene regions, (2) test the monophyly of subg. *Puya* and *Puyopsis*, and (3) examine the phylogenetic relationships and morphological evolution of Chilean species in the context of a broader phylogeny of *Puya*.

MATERIALS AND METHODS

Collections—Fieldwork was conducted from 2006 to 2008 in all major biogeographical regions where *Puya* occurs (northern Andes wet páramo, northern Andean valleys, central Andean puna, central Andean valleys, central coastal Chile). Multiple accessions of several species were taken to test for species monophyly, particularly of Chilean species and widespread taxa (e.g., *P. raimondii* and *P. ferruginea*). Some samples were taken from the living plant collections at the Huntington Botanical Garden (San Marino, California, USA) and Marie Selby Botanical Garden (Sarasota, Florida, USA) (Appendix 1). All vouchers are deposited at WIS and in home-country herbaria. Forty-three accessions of *Puya* are analyzed in this paper, including seven of eight species from subgenus *Puya*, all species from Chile and a representative sampling of subgenus *Puyopsis* encompassing the geographical range of the genus and major morphological types (Smith and Downs, 1974) (Table 1). Based the recent molecular phylogenetic results in Bromeliaceae (Givnish et al., 2007), we also included taxa (for a total of 75 taxa) representing major groups of subf. Bromelioideae and the allied genera *Pitcairnia, Deuterocohnia, Fosterella*, and *Dyckia* and the ultimate outgroup *Hechtia*.

Selection of gene regions—A subset of *Puya* representing the breadth of geographic and morphological diversity seen in the genus, as well as a robust sampling of outgroups, was included in a pilot survey to select appropriate gene regions for more extensive sampling (Tables 2, 3). Eight cpDNA regions from Shaw et al. (2005, 2007), Barfuss et al. (2005), and others developed by Dr. Linda Prince (Rancho Santa Ana Botanical Garden, California, USA) were initially screened for variation within *Puya.* Similarly, four nuclear gene regions were screened including the ITS region of nuclear ribosomal DNA (nrITS) and three low copy nuclear regions: RNA polymerase II (*RPB2*) (Denton et al., 1998), alcohol dehydrogenase (*Adh*) (Roalson and Friar, 2004), and phytochrome C (*PHYC*) (Samuel et al., 2005; C. Pires, University of Missouri-Columbia, personal communication).

Three cpDNA regions *(trnS-trnG*, *matK*, *rps16*) were then selected for more extensive taxon sampling based on the pilot survey (Table 3). These three cpDNA regions with complete taxon sampling were chosen in part because of compatibility with existing DNA data sets of Bromeliaceae (Barfuss et al., 2005) and the higher number of informative indels. Of the nuclear regions surveyed in the pilot analysis, *PHYC* was chosen because it was the easiest to amplify and sequence and had the most variation.

Gene sequencing—Total genomic DNA was extracted from silica-dried leaves following the manufacturer's protocol for the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). Polymerase chain reaction (PCR) primers for all regions were based on previous studies as indicated in Table 2. Primers for the four more extensively sampled cpDNA regions *trnS-trnG* spacer, *matK*, and *rps16* intron, and intron 1 of nuclear region *PHYC* included primers trnS GCU and "3′trnG UUC" following Shaw et al. (2005), *matK-trnK* primers 390F and 1326R (Cuénoud et al., 2002), universal *rps16* primers rpsF and rpsR2 (Oxelman et al., 1997), and *PHYC* primers Poales PHYC P2F and P2R (C. Pires, University of Missouri-Columbia, personal communication). PCR reaction mixes for all regions were 13.75 μL ddH_2O, 1 μL template DNA, 3.5 μL TaKaRa *Taq* polymerase (Otsu, Shiga, Japan), 10× ExTaq buffer, 0.5 μL BSA, 1.25 μL dimethyl sulfoxide (DMSO), 0.5 μL Q-Soln (Quiagen), 3.5 μL dNTP, 0.25 μL Tween, 0.5 μL of each 10 mM primer, and 0.25 μL of TaKaRa ExTaq.

All regions were PCR amplified in 25 μL with an MJ Research PTC-200 thermal cycler using an initial 5 min denaturation at 94°C followed by 32 cycles of 94°C denaturation for 30 s, 1 min annealing at 52°C, and 2 min extension at 72°C; followed by a 7 min final extension at 72°C. PCR products were then

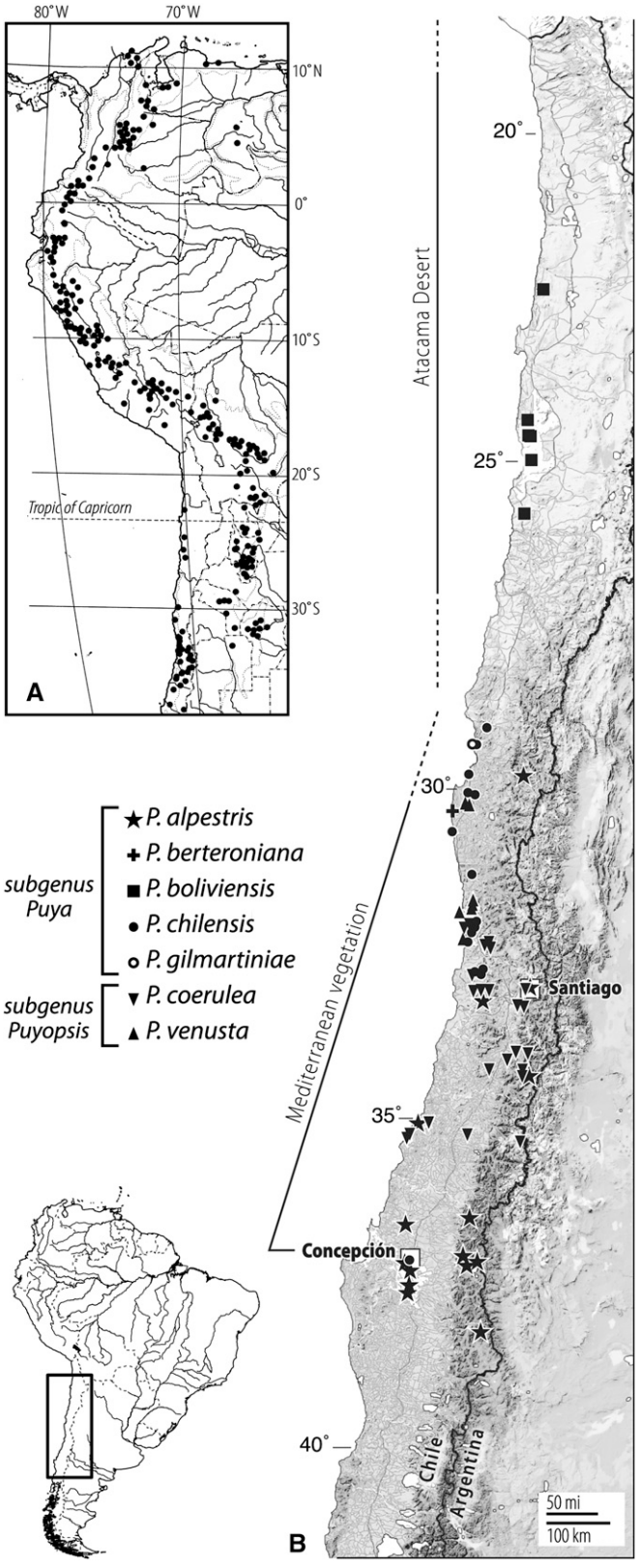

Fig. 2. Distribution of *Puya* species based on herbarium specimens. Locations are from the collections of R. Jabaily and from CONC, Rundel and Dillon (1998), and Smith and Downs (1974). (A) Entire range of *Puya*, updated from Smith and Downs (1974). (B) Localities of Chilean *Puya*.

purified and cleaned using the AMPure PCR purification protocol (Agencourt, Beverly, Massachusetts, USA). Gel electrophoresis of PCR products was used to determine product size and amount. Cycle sequencing reactions used the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA) using the thermocycler parameters 94°C for 5 min, 50 cycles of 94°C for 1 min; and final elongation at 60°C for 10 min. Samples were electrophoresed on an Applied Biosystems 3730xl automated DNA sequencing instrument, using 50 cm capillary arrays and POP-7 polymer. Data were analyzed using PE-Biosystems version 3.7 of the program Sequencing Analysis at the University Wisconsin-Madison Biotechnology center. Sequences were manually edited in the program Sequencher 3.0 (Gene Codes, Ann Arbor, Michigan, USA) and the resulting sequences were manually aligned in the program Se-Al v2.0a7b (Rambaut, 2003). All sequences could be aligned without difficulty except for nrITS. For nrITS, we employed the procedure of Wheeler (1996) in selecting the alignment that minimized steps in the most parsimonious trees. Sequences from the four genes were submitted to NCBI-GenBank (FJ968163–FJ968437; Appendix 1), and gene alignments were submitted to TreeBASE (http://treebase.org, SN4576-23204 [5, 6, 7]).

Phylogenetic analyses—Phylogenetic analysis of the three chosen cpDNA regions included nonoverlapping indels that were coded as separate characters scored as present or absent and appended to nucleotide data as additional characters following the procedure of Baum et al. (1994). Analysis of the one nuclear gene chosen warranted additional steps in formation of the final aligned data set. In all cases, gel electrophoresis revealed a single, clear band for *PHYC*. *PHYC* is shown to be a single copy locus in all monocots thus far surveyed (M. Kinney, University of Missouri-Columbia, personal communication) and was treated as such by Samuel et al. (2005). Direct sequencing of *PHYC* produced clean sequences, and thus cloning was not performed. Sporadic occurrences of double peaks, inferred as allelic variation, were carefully evaluated to see if their patterns suggested the presence of alleles either restricted to a taxon or shared with two or more taxa. The polymorphic sites were then treated in two ways in separate analyses and the phylogenetic results compared. First, base pairs showing double peaks were coded as polymorphic and included in one analysis. Issues with scoring taxa as polymorphic are known (e.g., Nixon and Davis, 1991), and thus in the second approach, we randomly divided polymorphic taxa into subunits that are monomorphic for each character used in the analysis. We generated duplicate sequences for the subset of taxa exhibiting double peaks and then randomly assigned each of the two possible states at each polymorphic site to one or the other duplicated sequences. This expanded data set with additional "taxa" was included in a second analysis. If the majority of polymorphic sites actually represent allelic variation restricted to a given species, we would expect the duplicate taxa to be sisters. However, if many of these sites represent more ancient alleles shared in common by species, we would expect increased homoplasy and/or occurrences of duplicate taxa not forming monophyletic clades.

The cpDNA data (concatenated as one data set) and nuclear *PHYC* data were analyzed both separately and combined using maximum parsimony (MP), maximum likelihood (ML; Felsenstein, 1973), and Bayesian MCMC inference (BI; Yang and Rannala, 1997). MP analyses were implemented in the program PAUP* 4.0b10 (Swofford, 2003). Heuristic searches were performed using 100 stepwise random addition sequences replicates, holding one tree at each step, with tree-bisection-reconnection (TBR) branch swapping and MULTREES and steepest descent in effect. Because analyses were not able to complete under the above search parameters due to excessive numbers of most parsimonious trees, an alternative search strategy was employed. The resulting consensus tree of all trees was then used as a backbone constraint to search for trees not consistent with the initial trees. If the initial heuristic search was sufficient, this additional search strategy should detect that there are no shorter trees and that the strict consensus tree reflects all most parsimonious trees, even though all equal length trees have not been found (Catalán et al., 1997; Davis et al., 2001; Hall et al., 2002). Ensemble consistency indices (CI) and retention indices (RI) (Farris, 1989) were calculated to evaluate the amount of homoplasy in the data. Bootstrap replicates (Felsenstein, 1985) were performed to assess character support. One thousand bootstrap replicates were performed with stepwise random addition sequences, holding three trees at each step, with MULTREES and steepest descent in effect. To decrease the amount of time necessary to run large bootstrap replicates, we held a limit of 10 trees at each step. MP bootstrap analyses are available (SSAppendices S1–S3: see Supplemental Data with the online version of this article).

ML and BI analyses employed the program MODELTEST v. 3.6 (Posada and Crandall, 1998) and the likelihood ratio test (Felsenstein, 1988) to select models of nucleotide evolution for each of the different gene regions. The ML

TABLE 1. Accessions of *Puya* included in this study with general geographic locality, subgeneric classification, and position in the phylogenetic analyses. HBG = accessions from the Huntington Botanical Garden (San Marino, California).

Accession	Geographic location	Subgenus	Clade in		
			cpDNA phylogeny (Fig. 3)	*PHYC* phylogeny (Fig. 4)	Combined data phylogeny (Fig. 5)
Puya alpestris 1	central Chile	*Puya*	Chilean Puya	Blue Puya	Chilean Puya
Puya alpestris 2	central Chile	*Puya*	Chilean Puya	Blue Puya	Chilean Puya
Puya angusta 1	central Peru	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya angusta 2	central Peru	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya berteroniana	central Chile	*Puya*	Chilean Puya	—	Chilean Puya
Puya bicolor	central Colombia	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya boliviensis 1	northcentral Chile	*Puya*	Core Puya: Central Andes	Yellow Puya	Core Puya: Central Andes
Puya boliviensis 2	northcentral Chile	*Puya*	Core Puya: Central Andes	Yellow Puya	Core Puya: Central Andes
Puya castellanosii	northwest Argentina	*Puya*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya chilensis 1	central Chile	*Puya*	Chilean Puya	Yellow Puya	Chilean Puya
Puya chilensis 2	central Chile	*Puya*	Chilean Puya	Yellow Puya	Chilean Puya
Puya coerulea 1	central Chile	*Puyopsis*	Chilean Puya	Blue Puya	Chilean Puya
Puya coerulea 2	central Chile	*Puyopsis*	Chilean Puya	Blue Puya	Chilean Puya
Puya compacta	central Ecuador	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya dasylirioides	Costa Rica	*Puyopsis*	Core Puya: Central Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya dyckioides	northwest Argentina	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya ferreyrae	northern Peru	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya ferruginea 1	HBG-central Bolivia	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya ferruginea 2	southern Peru	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya ferruginea 3	northern Peru	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya gilmartiniae	central Chile	*Puya*	Chilean Puya	Yellow Puya	Chilean Puya
Puya goudotiana	central Colombia	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya hamata	northern Ecuador	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya harmsii	northwest Argentina	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya herrerae	central Peru	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya lanata	southern Ecuador	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya lineata	central Colombia	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya macrura	northern Peru	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya mima	northern Peru	*Puyopsis*	Core Puya	—	Core Puya: Central Andes
Puya mirabilis	northwest Argentina	*Puyopsis*	Core Puya: Central Andes	—	Core Puya: Central Andes
Puya nana	central Bolivia	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya nitida 1	southern Ecuador	*Puyopsis*	Core Puya: Northern Andes	—	Core Puya: Northern Andes
Puya nitida 2	central Colombia	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya nutans	central Ecuador	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya obconica	central Ecuador	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya pygmaea	central Ecuador	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya raimondii 1	HBG-southern Peru	*Puya*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya raimondii 2	central Peru	*Puya*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya santosii	central Colombia	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Central Andes	Core Puya: Northern Andes
Puya trianae	central Colombia	*Puyopsis*	Core Puya: Northern Andes	Core Puya: Northern Andes	Core Puya: Northern Andes
Puya ultima	HBG-central Bolivia	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya venusta 1	central Chile	*Puyopsis*	Chilean Puya	Blue Puya	Chilean Puya
Puya venusta 2	central Chile	*Puyopsis*	Chilean Puya	Blue Puya	Chilean Puya
Puya yakespala	northwest Argentina	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes
Puya wrightii	HBG-northern Peru	*Puyopsis*	Core Puya: Central Andes	Core Puya: Central Andes	Core Puya: Central Andes

and BI phylogenies produced in this study were generated on the Cyberinfrastructure for Phylogenetic Research (CIPRES) portal 2 (http://www.phylo.org/sub_sections/portal/; National Science Foundation collaborative project). The most likely phylogeny for cpDNA, nuclear DNA, and combined data sets were produced in the program GARLI v. 0.96 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl, 2006) using GTR+I+γ as the model for chloroplast and combined data and GTR+γ for nuclear data. Multiple models for each gene partition are not allowed in GARLI, so the more complex model for a given set of genes was chosen. ML bootstrapping (MLB), with different models allowed for each gene partition, was completed using the program RAxML 7.0.4 (Randomized Accelerated Maximum Likelihood; Stamatakis, 2006; Stamatakis et al., 2008). The program automatically determines the number of bootstrap runs necessary to reach completion. BI analyses were done using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) allowing different models for each region. Four independent runs of 5 000 000 generations were completed with four chains each (three heated, one cold), using a chain temp of 0.2 and uniform priors. Trees were sampled every 1000 generations. Likelihood-by-generation plots were created, and the first 25% of runs were discarded as burn-in. A majority rule consensus of the remaining trees from the four runs was produced in PAUP* 4.0b10 and used as the Bayesian inference tree with posterior probabilities (PP).

Congruence between the cpDNA and nuclear DNA data sets was assessed using the incongruence length difference (ILD) test (Farris et al., 1994) with a heuristic search and simple taxon addition for 100 random partitions of data in PAUP* 4.0b10. When incongruence was detected with the ILD test, we explored likely explanations for incongruence by removal of taxa strongly placed in different clades with the two data sets.

Support for several taxonomic and biogeographic hypotheses were tested using the cpDNA, *PHYC*, and combined data sets (Table 4). We examined the support for (1) monophyletic *Puya*, (2) monophyletic subf. Bromelioideae, (3) monophyletic subg. *Puya*, (4) monophyly of northern Andean *Puya*, (5) monophyly of all Chilean *Puya*, and (6) monophyly of Chilean *Puya* minus *P. boliviensis*. These clades were written as topological constraints into the PAUP* block, and differences in length between the optimal and constrained most parsimonious trees were given as Bremer support values (Bremer, 1994). The constrained topologies were evaluated for significance using the Templeton test (Templeton, 1983) at $P = 0.05$ (Table 4).

Morphological analysis—Although a morphological phylogenetic analysis is beyond the scope and intent of this paper, a number of key morphological characters that are critical in understanding the evolution of Chilean species

TABLE 2. Information and new primers for cpDNA and nuclear gene regions surveyed but not chosen for full sampling in pilot study. Regions were surveyed for phylogenetic utility across a subset of *Puya* and outgroup taxa. Regions underlined are nuclear, nonunderlined are chloroplast regions. PIC = parsimony informative characters. New primer sequences for *atpF* intron, *trnS-trnQ*, *ndhJ-trnF*, and nrITS were graciously provided and created by Dr. Linda Prince of Rancho Santa Ana Botanic Garden. *atpF* intron: atpF-E1F, AGCAACAAATCCAATAAATCT; atpF-E2R, CTCTGTTTTCGATTATCTAATAAAT; *trnS-trnQ*: psbK-IGSR, CCAATCGTAGATGTTATGCC; psbK-IGSF, ATCGAAAACTTGCAGCAGCTTG; trnQ-IGSR, ACCCGTTGCCTTACCGCTTGG; trnS-IGSF, GGAGAGATGGCTGAGTGGA; *ndhJ-trnF*: ndhJ-IGSF, RCCCCTAATTTYTATGAAATACA; trnF-IGSF, ATCCTCGTGTCACCAGTTCAAA; nrITS: 5A, CCTTATCATTTAAGAGGAAGGAG; 5.8SR, ACGGGATTCTGCAATTCACAC; 5.8SF, TCACGGCAACGGATATCTCGG; 241R, CAGTGCCTCGTGGTGCGACA.

Region	Primers	Size (bp)	With outgroup				No. indels	No. *Puya* species	Only *Puya*			
			No. variable	% Variable	No. PIC	% PIC			No. variable	% Variable	No. PIC	% PIC
atpF intron	Present study	864	54	6	13	1.5	1	10	22	2.5	7	0.8
trnS-trnQ	Present study	1307	113	8.6	34	2.6	1	13	25	1.9	14	1
ndhJ-trnF	Present study	1230	94	7.6	19	1.5	5	9	13	1	8	0.65
psbD-trnT	Shaw et al., 2007	1149	109	9.5	26	2.3	4	8	44	3.8	14	1.2
trnL-F	Taberlet et al., 1991	991	75	7.6	37	3.7	0	9	10	1	1	0.1
Adh copy1	Roalson and Friar, 2004	800	65	8	15	1.8	0	9	27	3.3	9	1.1
Adh copy2	Roalson and Friar, 2004	1000	N/A	N/A	N/A	N/A	0	6	27	2.7	4	0.4
RPB2	Denton et al., 1998	1500	N/A	N/A	N/A	N/A	0	10	49	3.2	14	0.9
nrITS	Present study	755	86	11.4	32	4.2	0	47	63	8.3	17	2.2

were examined in more detail. Characters examined of the Chilean species included: flower color, flower shape, presence of dense appressed leaf scales on abaxial surface, and inflorescence tip sterility (Fig. 6). Initial observations were made in central Chile while on a collecting trip in 2006. Further observations of inflorescence and flower development for *P. alpestris, P. venusta*, and *P. coerulea* were made at the living collection of the Desert Garden of the Huntington Botanical Garden in April 2007. Young flowers and inflorescences were dissected and photographed to examine similarities in morphology at earlier stages of development between species.

RESULTS

Chloroplast regions trnS-trnG, matK, rps16—The combined matrix of cpDNA data comprised 2580 bp of characters, of which 124 characters were parsimony informative. Four indels (one each from *trnS-trnG* and *rps16* and two from *matK*) were coded and included in MP analyses. MP analyses recovered over one million trees of length 399, with a CI of 0.769 and RI of 0.889 with parsimony uninformative characters included. Reverse constraint analyses using the strict consensus of these trees as a topological constraint were not able to find alternative more parsimonious topologies, indicating that treespace was amply surveyed and that this consensus tree adequately estimates the main relationships within *Puya* and among other genera. Because heuristic MP searches could not be completed, MP bootstrap analyses used constraints to speed up analysis time. These MP boostrap values (online SSAppendix S1) were highly congruent with other ML and BI support analyses. MODELTEST generated the following nucleotide evolution models for the cpDNA regions: *trnS-trnG* fits to k81uf+γ, *matK* to GTR+I+γ, *rps16* to TIM+γ. The ML GARLI analysis using GTR+γ+I model (the most complex model chosen in MODELTEST for any of the gene partitions) yielded a tree with –ln 6343.6827. The BI consensus tree from MrBayes runs with different models allowed for each partition had a best score of –ln 6704.95. Support values from MLB were sometimes markedly lower than BI posterior probability values for the same clade, generally supported by relatively few characters (Figs. 3–5).

The MP, ML, and BI analyses based on three cpDNA regions yielded nearly nonconflicting topologies, with ML and BI resolving and better supporting clades that were in a polytomy or poorly supported in MP analysis. A summary of the cpDNA phylogenetic results is shown in Fig. 3 with ML and BI support values (MLB and PP, respectively). In all these analyses, *Puya* and subf. Bromelioideae are sister to a clade of (*Deuterocohnia*, *Dyckia*, *Fosterella*) + *Pitcairnia*, representatives of the subfamily Pitcairnioideae. Subfamily Bromelioideae is monophyletic (99 PP, 65 MLB), but the earliest-diverging lineages of subf. Bromelioideae are weakly supported (Fig. 3). Two strongly supported clades of *Puya* ("Core *Puya*," "Chilean *Puya*") form an unresolved relationship with subf. Bromelioideae. All species of *Puya* from Chile except *Puya boliviensis* comprise the clade "Chilean *Puya*," a clade further supported by two unique indels. The majority of the most parsimonious trees (86%) place most members of subf. Bromelioideae as sister to "Core *Puya*," though majority rule consensus trees should not be interepreted as indicative of support of relationships (Sharkey and Leathers, 2001). Subgenus *Puya* clearly is not monophyletic as *P. boliviensis*, *P. castellanosii*, and *P. raimondii* are placed in "Core *Puya*," whereas *P. chilensis*, *P. alpestris*, and *P. berteroniana* are placed in "Chilean *Puya*." Species

TABLE 3. Information on cpDNA and nuclear gene region chosen for complete study. Region underlined is nuclear, nonunderlined are cpDNA regions. PIC = parsimony informative characters.

Region	Primers	Size (bp)	With outgroup				No. indels	No. *Puya* species	Only *Puya*			
			No. variable	% Variable	No. PIC	% PIC			No. variable	% Variable	No. PIC	% PIC
trnS-trnG	Shaw et al., 2005	1048	104	11.2	49	5	4	45	25	2.7	17	1.8
matK	Barfuss et al., 2005	819	77	9.3	32	3.9	2	45	21	2.6	11	1.3
rps16	Barfuss et al., 2005	827	108	13	43	5.2	1	45	21	2.5	8	1
PHYC	Kinney et al., unpubl.[a]	1048	178	17	97	9.3	0	45	59	5.6	32	3

[a] Mike Kinney and J. Chris Pires, unpublished data.

TABLE 4. Results of hypothesis testing for cpDNA, *PHYC*, and combined data sets. Ranges of Templeton *P*-values are given where appropriate. ** indicates highly significant result ($P = 0.05$).

Hypotheses	Bremer decay cpDNA/*PHYC*/Combined data sets	Templeton cpDNA/*PHYC*/Combined data sets
Monophyletic *Puya*	0/0/0	1/1/1
Monophyletic Bromelioideae	0/1/0	1/1/1
Monophyletic subgenus *Puya*	22/21/32	0.0001**/0.0001**/0.0001**
Monophyletic N. Andean taxa	4/1/00	0.3173–0.2059/1/1
Monophyletic Chilean *Puyas*	19/6/6	0.0001**/0.0833–0.0339**/0.3657–0.2008
Monophyletic Chilean *Puyas-P. boliviensis*	3/12/01	0.3173–0.2568/0.0047–0.0013**/0.8084–0.8811

monophyly is not recovered within "Chilean *Puya*" although branch lengths are very short. Very low resolution is also found within "Core *Puya*," but two moderately supported clades (96 and 66% PP; less than 50% MLB) include most taxa from the northern Andes and many taxa from the central Andes, respectively. An unusual zygomorphic-flowered species, *P. mima*, from the central Andes does not group with any other species within "Core *Puya*."

Nuclear DNA region phytochrome C—Analyses of the effects of polymorphisms in the *PHYC* data indicated that very similar results were seen with either method of coding polymorphisms. Evidence of allelic variation in *PHYC* was found in 24 of sampled taxa as indicated by obvious double peaks at a few base pairs. The number of polymorphic sites in a given taxon ranged from 1 to 15 (the latter seen in *P. macrura*) with a mean of 3.5 (SD ±3.01) However, polymorphic sites were evident in only 80 bp of the entire 1060-bp matrix across all taxa. Additionally, 65 of these 80 polymorphic sites were polymorphic only within a single taxon. Thus, the two parsimony approaches to evaluate the effects of polymorphisms in the *PHYC* data indicated that the allelic variation encountered appears not to have an adverse effect on phylogenetic reconstruction. The two types of analyses (the first with sites scored as polymorphic, and the second with duplicate taxa randomly assigned alternative states at each polymorphic) generated identical topologies and duplicate taxa in the second analysis were always recovered as sister lineages, generally with moderate to high support (BS > 70). Although these analyses only indirectly assess the issue of polymorphic taxa as discussed by Nixon and Davis (1991), the results suggest that there is a not a strong phylogenetic bias in not having allelic data. We use the polymorphism-coded *PHYC* data set for the remainder of this paper.

Because of concerns about the appropriateness of using a low-copy nuclear gene across greater phylogenetic distances without resorting to cloning (Samuel et al., 2005), we restricted *PHYC* sequencing to *Puya* and subf. Bromelioideae, and two species of *Pitcairnia* serve as outgroups. Several species of subf. Bromelioideae and *Puya* could not be sequenced for *PHYC*, despite multiple attempts. The final matrix of 58 taxa is 1047 characters in length, of which 171 were variable and 97 (9.3%) were parsimony informative. MP analysis revealed over 900 000 trees of length 223, CI = 0.789 and RI = 0.906 before the search was terminated. Reverse constraints found no conflicting topologies of similar or shorter lengths. The maximum likelihood analysis of GARLI using GTR+γ model selected in MODELTEST yielded a tree –ln 2915.8267. RAxML performed 400 ML bootstrapping replicates. The BI consensus tree from MrBayes had a best score of –ln 3182.621.

As with the cpDNA regions, *PHYC* was not able to fully resolve relationships between *Puya* and subf. Bromelioideae (Fig. 4). Monophyly of subf. Bromelioideae is not recovered. A clade comprising three genera (*Ochagavia*, *Greigia*, and *Deinacanthon*) is weakly supported (87% PP, <50% MLB) as sister to all *Puya*. The remainder of subf. Bromelioideae is strongly monophyletic (99% PP, 83% MLB). *Puya* is moderately supported as monophyletic (99% PP, <50% MLB) with *PHYC*, unlike that seen with cpDNA (see Fig. 3). Also, neither subgenus is monophyletic as members of subg. *Puya* are included in all major subclades of *Puya*. Three major subclades of *Puya* are recovered with strong support: "Core *Puya*" (98% PP, 80% MLB, (which differs slightly in taxon composition from the cpDNA "Core *Puya*" clade), "Yellow *Puya*" (100% PP, 92% MLB) (taxa with yellow flowers from Chile), and "Blue *Puya*" (100% PP, 99% MLB) (taxa with some shade of blue flowers from Chile). "Core *Puya*" and "Yellow *Puya*" are strongly monophyletic (100% PP, 95% MLB), with "Blue *Puya*" sister to this clade (99% PP, <50% MLB). Resolution within "Core *Puya*" remains low as seen also with cpDNA, but the "Northern Andes" clade (with a few differences) is recovered as with cpDNA (see Fig. 3).

Analysis of combined data set—ILD results show significant incongruence between the cpDNA and *PHYC* data sets. We explored the effects of removing individual taxa and sets of taxa (e.g., outgroups and Chilean *Puya* taxa), but significant incongruence persisted between data sets. Incongruence may be exacerbated by the relatively large number of taxa and often very short branch lengths seen in this study. Concatenation of the data sets was still carried out, as ILD tests should not be interpreted as a clear indication that concatenation is inappropriate (Yoder et al., 2001; Hipp et al., 2004).

The combined data set of 75 taxa was 3631 characters in length, of which 462 were variable and 221 (6% of total) were parsimony informative. MP analysis revealed over 1 000 000 trees of length 667, CI = 0.730 RI = 0.864 before runs were terminated. Reverse constraints found no conflicting topologies of similar or shorter lengths. ML analysis using GARLI and the GTR+γ+I model (the most complex model chosen in MODELTEST for any of the gene partitions, in this case, *trnS-G*) yielded a tree with –ln 10526.13. RAxML performed 350 ML bootstrapping replicates using the specified models for each gene partition. The BI tree from MrBayes runs with different models allowed for each partition had a best score of –ln 10129.713.

The tree obtained from combined cpDNA and *PHYC* data (Fig. 5) contains aspects of both the cpDNA tree (see Fig. 3) and the *PHYC* tree (see Fig. 4). Subfamily Pitcairnioideae is sister to *Puya* and subf. Bromelioideae, and the latter subfamily is strongly well supported (99% PP, 85% MLB) as seen with cpDNA but not *PHYC*. *Puya*, however, is monophyletic (99% PP, 68% MLB) as seen with *PHYC* but not cpDNA. The sister

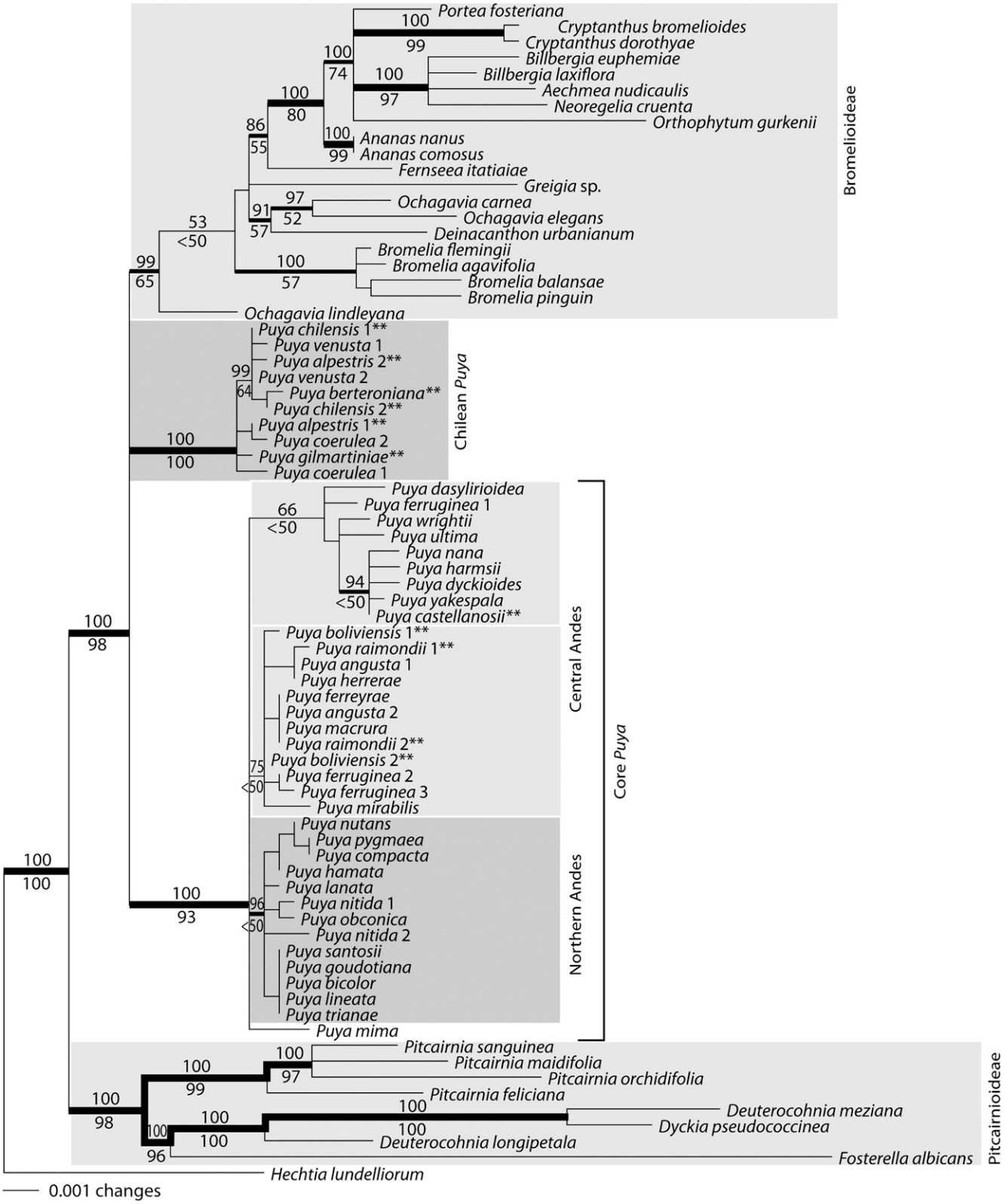

Fig. 3. Maximum likelihood cpDNA phylogeny for *Puya* and all outgroups based on *trnS-trnG*, *matK*, and *rps16*. *Hechtia* is used as the outgroup. Numbers above branches correspond to Bayesian MCMC inference posterior probabilities (PP) and numbers below correspond to maximum likelihood bootstrap MLB) values calculated using RAxML. Support values are shown for only clades of interest. Line thickness corresponds to relative support: thickest lines indicate strong support (both PP and MLB above 80), medium thick lines indicate medium support (either PP or MLB above 80), and thin lines indicate weak support (both PP and MLB below 80). If both PP and MLB are below 50, the clade is collapsed. Two asterisks (**): subgenus *Puya*.

relationship between *Puya* and subf. Bromelioideae is more strongly supported with combined data than with either of the two data sets individually (100% PP, 84% MLB). The early divergence of *Ochagavia*, *Deinacanthon*, and *Greigia* from within subf. Bromelioideae—a clade sister to all *Puya* with *PHYC* data—is also more strongly supported with combined data. The combined topology more strongly points to the early divergence of *Bromelia* within the remainder of subf. Bromelioideae. Within the monophyletic *Puya*, "Chilean *Puya*" is recovered with the same species composition as seen in the cpDNA analysis. However, taxa within the clade "Chilean *Puya*" segregate based on flower color as seen with *PHYC* alone. *Puya mima* and *Puya boliviensis* are strongly supported as the earliest diverging lineages within "Core *Puya*." Combined data do little to increase resolution within the remainder of *Puya*, though the "Northern Andes" clade is still weakly supported.

Hypothesis testing—Assessments of Bremer support indices and Templeton tests for all three data partitions reject the hypotheses of a monophyletic subg. *Puya* (Table 4). The cpDNA data reject monophyly of all *Puya* from Chile and *PHYC* data reject the hypothesis of monophyletic clade of Chilean *Puya* minus *P. boliviensis* (the topology seen with cpDNA data). The three data sets are equivocal about monophyly of subf. Bromelioideae, the genus *Puya* itself, and the "Northern Andes" clade—all clades with relatively short branches.

Morphological analysis of Chilean species—For the Chilean species of *Puya*, herbarium records and plants in botanical gardens and in the field of central Chile were observed for select key characters. Character-state presence or absence was generalized across species and was typically consistent with given species descriptions if the characters were initially noted in the original species description (Fig. 6). Abaxial, appressed leaf scales unite Chilean species with bluish flowers. Yellow pollen and abaxially glabrous leaves unite Chilean species with yellow flowers. Inflorescence development was studied in three Chilean species at the Huntington Botanical Garden to see whether there was any similarity at early developmental stages between *P. alpestris* (with sterile inflorescence tips) and *P. coerulea* and *P. venusta* (with nonsterile tips). Abortion of the terminal flowers began very early in development of the compound inflorescence in *P. alpestris*. Flowers were fully fertile from the base of the inflorescence branch. The abortive whorls of flowers had underdeveloped stamens and petals, were greatly reduced in size, and died very early in development. In contrast, the entire axis of the inflorescences of both *P. coerulea* and *P. venusta* were fertile throughout, and no evidence was seen of the abrupt developmental timing change typical in *P. alpestris*. There is thus no similarity in inflorescence fertility between the two groups of species at any developmental point.

DISCUSSION

This study demonstrates the utility of considering both chloroplast and nuclear data to illuminate complex relationships in rapidly evolving groups. Key questions about the evolutionary history of this group of bromeliads are answered, and a working hypothesis is presented for the evolution of Chilean *Puya*.

Utility of chloroplast and nuclear data—The weak support for monophyly of *Puya* and subf. Bromelioideae and for relationships between taxa in "Core *Puya*" and "Chilean *Puya*" can be attributed to very short branches leading to these three major radiations (Figs. 3, 4). For example, only one character unites *Puya* in the combined data MP analysis. These results support prior observations of low rates of nucleotide substitution in Bromeliaceae (Gaut et al., 1992; Givnish et al., 2007; Smith and Donoghue, 2008), which may be especially problematic for *Puya* and subf. Bromelioideae. Barfuss et al. (2005) used *rps16,* in addition to many other cpDNA regions, across the subf. Tillandsioideae, another major bromeliad radiation. They reported 14.1% parsimony informative characters (PIC) for *rps16* across subf. Tillandsioideae and outgroups, whereas only 5.2% (43 total) PIC are seen across *Puya*, subf. Bromelioideae, and all outgroups, and just 1% (8 total) PIC within *Puya* for *rps16*. At this rate, concatenation of thousands of nucleotides from a great many loci would be necessary to get significant resolution in "Core *Puya*." Low-copy nuclear region *PHYC* was significantly more variable (9.3% [97 total] PIC across *Puya* and outgroups and 3% [32 total] PIC within *Puya*), but resolution within "Core *Puya*" was still low. Other low-copy nuclear DNA regions screened in this study showed low variability, suggesting that commonly used low-copy nuclear gene regions may not hold the ultimate solution for resolution of recently evolved groups of bromeliads. Restriction-based methods surveying the nuclear genome (e.g., AFLPs) may be of more utility and appropriate at these taxonomic levels in Bromeliaceae (Jabaily et al., 2008).

Combining cpDNA and nuclear DNA data increases support for monophyly of *Puya* and subf. Bromelioideae and resolves relationships among *Puya*, subf. Bromelioideae, and outgroups. However, by viewing results of the chloroplast and nuclear stories separately, we are also able to hypothesize a specific and more complicated scenario of evolution for the Chilean *Puya* (see below).

Placements and monophyly of Puya and subf. Bromelioideae—The placement of major lineages in the combined data set topology agrees with previous phylogenetic studies across Bromeliaceae (Terry et al., 1997; Crayn et al., 2004; Barfuss et al., 2005; Givnish et al., 2007; Schulte and Zizka, 2008) *Puya* and subf. Bromelioideae are sister clades, and they in turn are sister to the subf. Pitcairnioideae. Given the weak support for monophyly of *Puya* in this molecular analysis, it is worth asking if we should still consider *Puya* monophyletic. If we were to consider the two major clades of "Chilean *Puya*" and "Core *Puya*" produced by the combined data set as distinctive enough for generic status, "Chilean *Puya*" would retain the name *Puya*, as the type specimen is *Puya chilensis* Molina, and "Core *Puya*" would be then elevated to the genus *Puyopsis*. The monophyly of *Puya* has never been questioned by specialists in Bromeliaceae and has been supported in most familywide revisions and molecular phylogenies to date, although few representatives of *Puya* typically have been included. The paraphyly of *Puya* in the analysis of Rex et al., (2009) is weakly supported and is likely an artifact of poor taxon sampling of Bromelioideae and *Puya*. A suite of characters including leaf-base constriction and leaf-sheath texture has traditionally been used to separate *Puya* from other genera. Smith (1968, p. 461) argued that for *Puya* "…the really significant characters appear to be the twisting together of the petals after anthesis and the winged seed." The winged seeds of *Puya* can sometimes be confused with seeds of *Pitcairnia* subg. *Pepinia* (Varadarajan and Gilmartin, 1988; Robinson and Taylor, 1999). Additionally, seed diversity

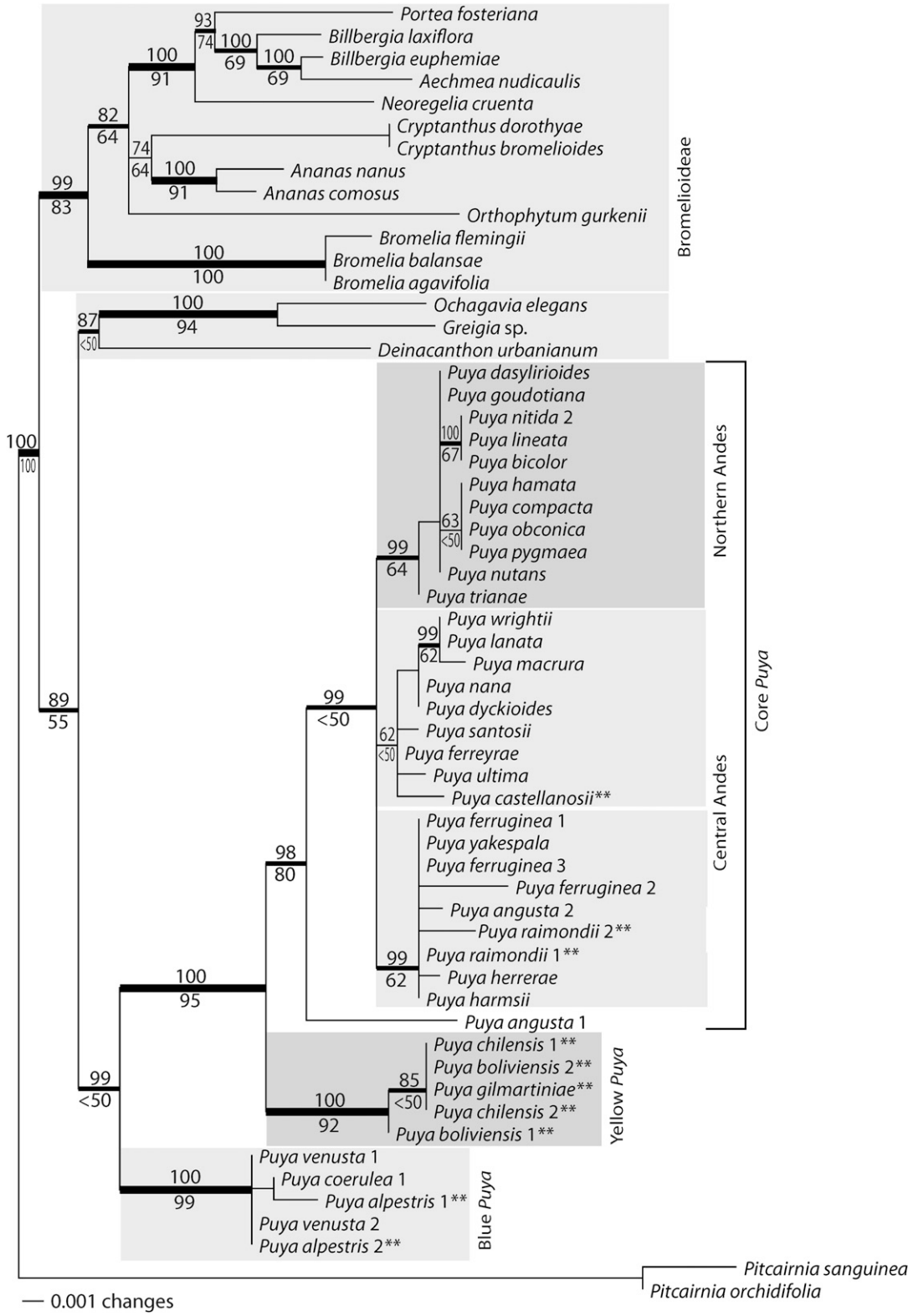

Fig. 4. Maximum likelihood nuclear DNA (*PHYC*) phylogeny for *Puya* and subf. Bromelioideae with *Pitcairnia* spp. used as a monophyletic outgroup. Numbers above branches correspond to Bayesian inference posterior probabilities (PP), and numbers below correspond to maximum likelihood bootstrap MLB) values calculated using RAxML. Support values are shown for only clades of interest. Line thickness corresponds to relative support: thickest lines indicate strong support (both PP and MLB above 80), medium thick lines indicate medium support (either PP or MLB above 80), and thin lines indicate weak support (both PP and MLB below 80). If both PP and MLB are below 50, the clade is collapsed. Two asterisks (**): subgenus *Puya*.

within *Puya* may preclude a simple, synapomorphic definition of *Puya* based on seeds (Varadarajan and Gilmartin, 1988). Therefore, the most readily diagnosable character for all species placed in *Puya* is the twisting of petals into a tight spiral after anthesis. While some species of other genera may have slight twisting of petals after anthesis, none form the regular tight spiral seen in *Puya*. This character is also very useful for identifying *Puya* from old inflorescences because the twisted petals generally persist. On the basis of this key morphological synapomorphy and the results of our molecular phylogenies, although not well supported at this node, we argue that *Puya* should still be considered a cohesive, monophyletic lineage. The less likely alternative is that the feature of twisting petals was lost in subf. Bromelioideae if the latter is derived from within a paraphyletic *Puya* (Fig. 3).

The subf. Bromelioideae has long been recognized as a natural group based on possession of distinctive fleshy fruits and inferior ovaries, though current generic circumscriptions are strongly in flux. Molecular systematics studies with a variety of sampling densities have all recovered a monophyletic subf. Bromelioideae, albeit with low support (e.g., 64 PP in Schulte and Zizka, 2008; 89% bootstrap in Givnish et al., 2007; 63% of trees on majority rule consensus in Horres et al., 2000). The sampling for our study included several putatively early-diverging genera of subf. Bromelioideae (*Ochagavia* and *Greigia,* following Horres et al., 2007), as well as representatives of many other genera. The cpDNA data were able to resolve a monophyletic subf. Bromelioideae with moderate support, placed in a polytomy with "Chilean *Puya*" and "Core *Puya*." *PHYC* data identified a clade comprising *Ochagavia Greigia* and *Deinacanthon* as sister to *Puya*, and this larger clade sister to the remainder of subf. Bromelioideae. However, only one synapomorphic nucleotide character unites *Ochagavia*, *Greigia*, and *Deinacanthon* with *Puya* and apart from the remainder of subf. Bromelioideae. Combined data strongly support a monophyletic subf. Bromelioideae as sister to a monophyletic *Puya,* in agreement with all other familywide molecular phylogenies. Within subf. Bromelioideae, primarily terrestrial or saxicolous genera (e.g., *Ochagavia*, *Deinacanthon*, *Greigia*, *Bromelia*, *Ananas*, *Cryptanthus*, *Orthophytum*) form a basal grade to the more derived, primarily epiphytic Bromelioideae (e.g., *Billbergia*, *Portea*, *Aechmea*), in agreement with the topologies of Schulte and Zizka (2008) and Schulte et al., (2009). The relative position of these basalmost lineages differs slightly from Schulte et al., (2009), which recovered a single species of *Bromelia* as sister to the remainder of Bromelioideae. Our analysis used several additional species of *Bromelia* and some different species from other basal genera but also was unable to resolve the basalmost lineage of Bromelioideae with strong support.

Monophyly of Puya subgenera and major interspecific clades—In no analysis was subg. *Puya* monophyletic, as subgenus members *P. raimondii* and *P. castellanosii* were clearly embedded in "Core *Puya*" not as sister species, *P. boliviensis* was in "Core *Puya*" in the cpDNA and combined analysis, and *P. berteroniana*, *P. alpestris*, and *P. chilensis* were in separate clades of entirely Chilean taxa. Hypothesis testing clearly rejected a monophyletic subg. *Puya* with all data sets. The two subgenera of *Puya* should thus be considered nonmonophyletic, given this evidence. The character of sterile inflorescence tips, previously used to define subg. *Puya*, has either evolved several times independently in *Puya*, perhaps in response to pressure from similar perching pollinators (Johow, 1898) or evolved once and subsequently lost multiple times in the remainder of *Puya*. Careful analysis of different sterile tip inflorescences during development is needed to test the now likely hypothesis that sterile inflorescence tips evolved in a convergent fashion.

Morphological variation among the 190+ species comprising subg. *Puyopsis* is not well characterized, and no interspecific classification has been presented for subg. *Puyopsis.* Hornung-Leoni and Sosa (2008) constructed a morphological matrix of 93 discrete and continuous characters, including many characters emphasized in the monograph of *Puya* (Smith and Downs, 1974), for 28 species of *Puya* from both subgenera. Only a few clades within *Puya* had any support in the parsimony analysis with the character of sterile inflorescence branch apex not surprisingly supporting the unity of all members of subg. *Puya*. The DNA-based phylogenies of this study will allow for independent assessment of morphological characters as either synapomorphic or homoplasious. We use several morphological characters to support our hypothesis of the evolution of Chilean taxa below (see section *Evolution of Chilean Puya*).

Besides testing the monophyly of subgenera in this study, we were able to delineate the Chilean *Puya* as a unique clade(s) separate from "Core *Puya*." Other clades in "Core *Puya*" are apparent in these analyses, though clade support is minimal. A clade of species from the wetter northern Andes is supported by all three data sets, although its species composition deviates slightly among the three. This mesic northern Andean clade appears to be derived from a group of taxa from the more xeric central Andes. Relationships among species in the central Andes are more weakly supported and these species do not form consistent clades. Several taxa within the northern Andean clade are from more xeric valleys (e.g., *P. obconica*, *P. bicolor*) geographically near the high elevation páramo taxa (e.g., *P. goudotiana*, *P. hamata*, *P. pygmaea*). These northern Andean xeric species do not group with other species from xeric valleys in the central Andean valleys. Likewise, *Puya* from high elevation central Andean areas (e.g., *P. raimondii*, *P. angusta*) do not group with high elevation species from the northern Andes. *Puya* appears to have colonized mid and high elevations multiple times. Thus, broad geographic distributional patterns rather than ecological parameters of moisture and elevational adaptations might be the most accurate predictor of phylogenetic relationships within "Core *Puya*." The low levels of phylogenetic resolution found with our chosen regions were not able to resolve sister relationships between the multiple accessions of widespread taxa in Core "*Puya*" (e.g., *P. ferruginea*). AFLP analyses appear to provide finer-resolved relationships among species in this group (Jabaily et al., 2008). *Puya mima* from central Peru deserves further investigation as it appears to hold a pivotal placement in the phylogenetic reconstructions, often as sister to the remaining "Core *Puya*." This species is morphologically very distinct, having a simple, few-flowered inflorescence that is nearly glabrous. The flowers are pinkish, quite large, and zygomorphic, all quite rare character states in *Puya*.

Biogeographic implications—Our results not only show that early-diverging species of subf. Bromelioideae (all *Ochagavia*, many *Greigia*) occur in central Chile as previously suggested (Horres et al., 2007), but importantly that one of the first-diverging clades in *Puya* is from central Chile as well. If a molecular clock is assumed, the initial radiation of major clades of subf. Bromelioideae and *Puya* in central Chile occurred very quickly, as indicated by very short branch lengths. A subsequent radiation created the majority of extant "Core *Puya*" species in the Andes.

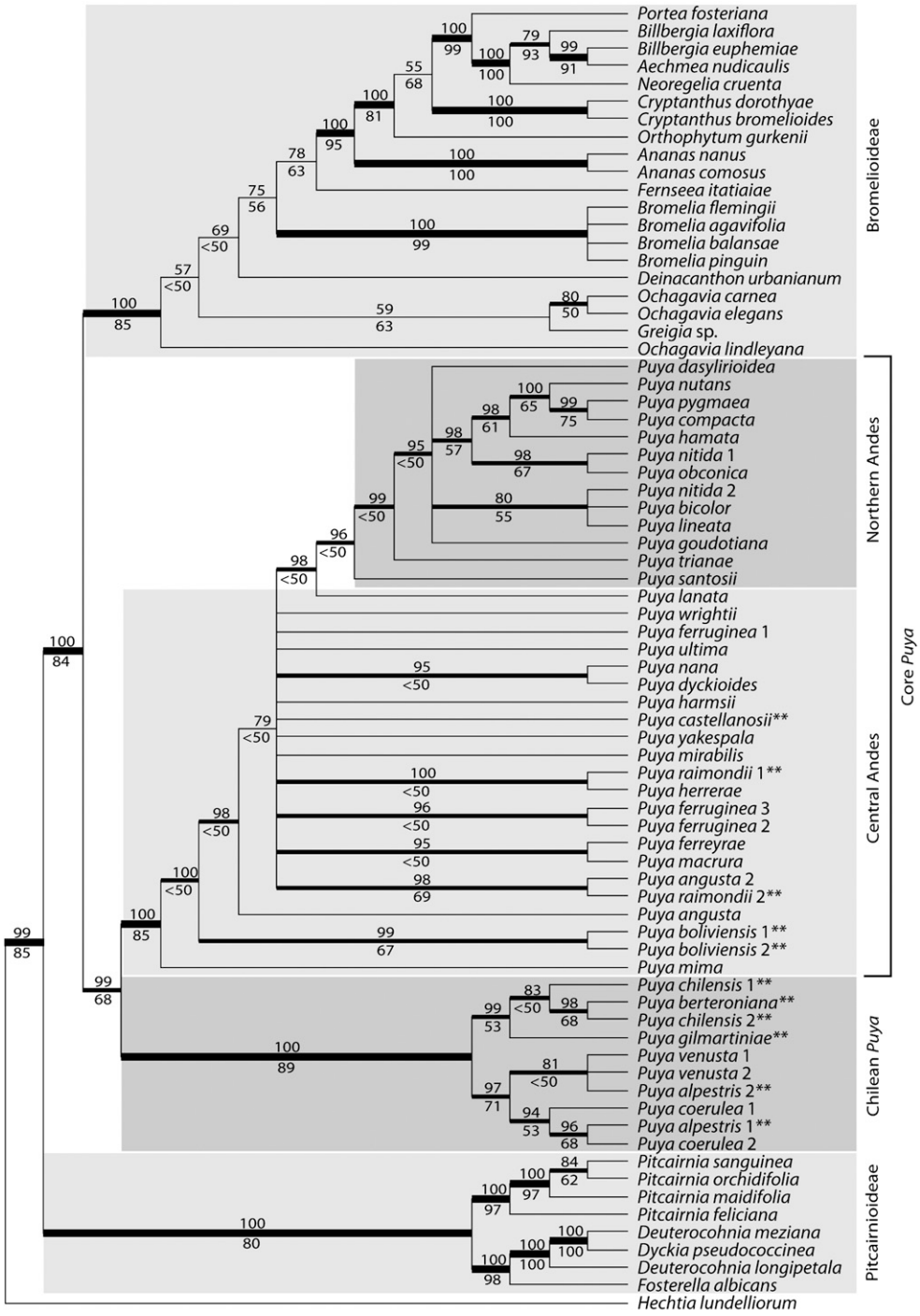

Fig. 5. Bayesian posterior probability phylogeny of combined cpDNA (*trnS-trnG*, *matK*, *rps16)* and nuclear DNA (*PHYC*) data for *Puya* and all outgroups. *Hechtia* is used as the outgroup. Numbers above branches correspond to Bayesian inference posterior probabilities (PP) and numbers below correspond to maximum likelihood bootstrap MLB) values calculated using RAxML. Support values are shown for only clades of interest. Line thickness corresponds to relative support: thickest lines indicate strong support (both PP and MLB above 80), medium thick lines indicate medium support (either PP or MLB above 80), and thin lines indicate weak support (both PP and MLB below 80). If both PP and MLB are below 50, the clade is collapsed. Two asterisks (**): subgenus *Puya*.

In the context of biogeography and habitats seen in *Puya*, the pattern of C_3 and CAM photosynthesis is notable. The evolution of CAM photosynthesis has occurred multiple times in Bromeliaceae (Martin, 1994; Benzing, 2000; Crayn et al., 2004; Givnish et al., 2007) and is associated with an invasion of arid habitats or the evolution of epiphytism. The majority of subf. Bromelioideae are CAM, but members of *Greigia*, *Fascicularia*, and *Ochagavia* are C_3, suggesting that C_3 is the ancestral condition in Bromelioideae (Horres et al., 2007) and that CAM did not arise in response to aridity in central Chile, at least for

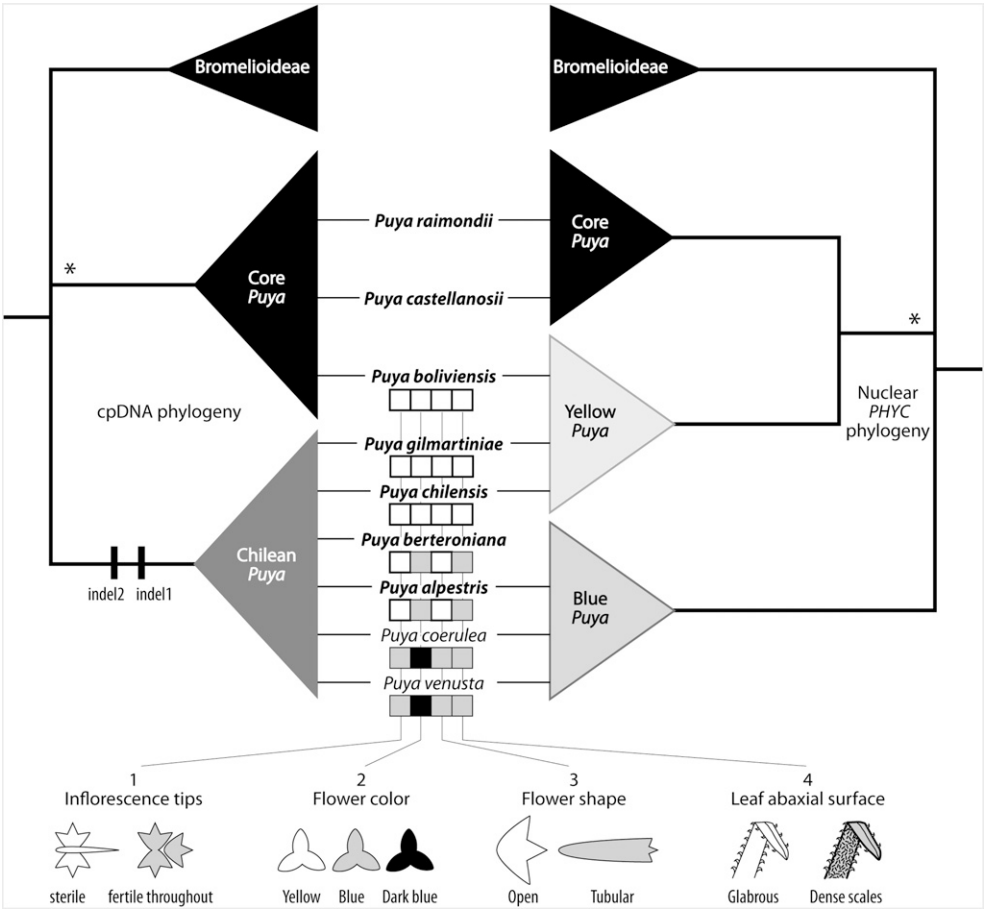

Fig. 6. Comparison of cpDNA and nuclear *PHYC* phylogenies illustrating relationships of "Chilean *Puya*" (or its smaller clades), "Core *Puya*", and subf. Bromelioideae. States of select morphological characters discussed in the text are shown for "Chilean *Puya*" only. Asterisk indicates the weakly supported relationships between Bromelioideae and all *Puya*. Two *matK* indels are placed as synapomorphies for "Chilean *Puya*".

subf. Bromelioideae. Members of subf. Pitcairnioideae and subf. Tillandsioideae native to central and northern Chile are either obligate CAM plants or have a mixed C_3/CAM system (Rundel and Dillon, 1998). Most species of *Puya* surveyed by Martin (1994) had carbon isotope values indicative of a mixed C_3/CAM system, the given value being proportional to the frequency the plant switched to CAM photosynthesis, presumably because of water stress. Carbon isotope values for a given species varied between individuals surveyed by Martin (1994) and Crayn et al. (2004), the latter who surveyed many more species and indicated that *Puya* is the only genus of Bromeliaceae with both C_3 and CAM photosynthesis. Species of *Puya* with CAM are in the minority (24% of species surveyed, Crayn et al., 2000) and include the Chilean species *P. chilensis* and *P. gilmartiniae*, as well as many species from lower elevations and generally dry valleys in the central Andes. Species of *Puya* with mixed C_3/CAM included *P. venusta* and *P. berteroniana.* C3 species include *P. alpestris* and *P. coerulea* from Chile, as well as most surveyed species from higher elevations and the northern Andes. Interestingly, putative closely related species pairs *P. berteroniana/P. alpestris* and *P. venusta/P. coerulea* show different forms of photosynthesis, but this may be impacted by the physiological history of the individuals surveyed. Clearly, CAM photosynthesis has arisen at least twice within the *Puya*/Bromelioideae clade and merits further study within *Puya*.

Bromeliaceae are particularly depauperate in Chile compared to the rest of its primary range in South America (Rundel and Dillon, 1998). Nine species of subf. Bromelioideae are found in Chile, whereas the remaining 700+ species are primarily in southeastern Brazil. Seven species of *Puya* are endemic to Chile (though there are a few reports of *P. alpestris* from far western Argentina), whereas the remaining 190+ species are found throughout the Andes. The Mediterranean region of central Chile is recognized as a biodiversity hotspot (Myers et al., 2000), but the phylogeographic affinities of taxa endemic to central Chile with those from the remainder of the Andes are complex and varied (Katinas et al., 1999). A number of taxa with primarily Andean distributions have disjunct species in central Chile. Phylogenetic and systematic studies of these various groups do not necessarily exhibit the same pattern seen in *Puya* of basal, depauperate lineages in Chile with a subsequent radiation in the Andes and elsewhere. Berry et al. (2004) showed that the Chilean *Fuchsia* sect. *Kierschlegeria* (Onagraceae) are sister to a larger radiation of southern South American and Brazilian taxa in sect. *Quelusia*, not sister to the main Andean *Fuchsia* radiation. This pattern may be more similar to that seen in subf. Bromelioideae. *Tristerix* (Loranthaceae) is equally diverse in central/southern Chile and central/northern Andes and species from these two geographical regions form sister clades (Amico et al., 2007). Similarly, extant *Chuquiraga* (Asteraceae)

have equally large radiations in southern South America, including central Chile, and in the rest of the Andes (Ezcurra, 2002). The species endemic to central Chile were derived from a more widespread species. *Loasa* s.s. (Loasaceae) and allied genera form a major radiation in central Chile, but relationships with northern Andean *Nasa* remain unresolved (Weigend et al., 2004). Multiple clades of the tribe Mutisieae (Asteraceae) have members in central Chile and in other regions of the primarily southern Andes. However, the clade of *Moscharia/Polyachyrus/Leucheria* has its center of origin and diversity in central Chile, and the arid climate may have prompted evolution of secondary capitula (Katinas et al., 2008).

More phylogenetic work needs to be done on other species rich genera from central Chile and the Andes to determine whether there is a general phytogeographic relationship between the two regions. In the above examples illustrating links between central Chile, the Andes, and the rest of South America, determining the ages of lineage diversifications will be important to more fully understand these phytogeographic relationships. The Andean uplift greatly altered the weather patterns and vegetation of Chile, and subsequent Pleistocene glacial cycles may have served to isolate and subsequently diversify lineages (Simpson, 1975). Groups that were forming before or during the uplift of the southern Andes in the mid Pliocene-Pleistocene may have a very different distribution and diversification pattern than those that formed after the major uplift was completed (Arroyo et al., 1988).

Molecular calibration of the diversification of Bromeliaceae using monocot fossils and an *ndhF* phylogeny of monocots (Givnish et al., 2007) placed the stem node of the clade comprising subf. Bromelioideae and *Puya* to around 10 Mya and their subsequent crown radiation to about 7.8 Mya. These time periods of origination and diversification of *Puya* and subf. Bromelioideae are within the Miocene/Pliocene when the southern Andes were building, although not yet at full height, and affecting climate changes. Extant taxa of *Puya* may have arisen considerably more recently (even as late as 370 000 yr; Givnish et al., 2007), when the northern Andes were actively rising and the older southern Andes of Chile were at full height. Recent glaciation cycles in the Andes and their impact on Andean flora via successive rounds of allopatry and sympatry (Simpson, 1975) undoubtedly played a critical role in the radiation of extant taxa of *Puya* (Varadarajan, 1990). An expanded and updated phylogeny of Bromeliaceae calibrated with molecular dating methods, including sampling from the here defined "Chilean *Puya"* lineage and denser taxon sampling of early diverging lineages within subf. Bromelioideae, will do much to improve our understanding of the evolutionary history of Bromeliaceae and of South American phytogeography more generally. Bromeliaceae, and *Puya* in particular, may prove to be some of the most rapid examples of plant speciation, with rates similar to or exceeding those currently found in other rapid radiation examples (Richardson et al., 2001; Klak et al., 2004; Hughes and Eastwood, 2006; Scherson et al., 2008).

Evolution of the Chilean Puya—The results of these molecular phylogenetic analyses further enhance the phylogenetic, evolutionary, and biogeographic significance of the species of *Puya* restricted to Chile. As demonstrated, Central Chile is implicated as an ancestral area for the radiation of extant *Puya* and subf. Bromelioideae. Moreover, the fundamental phylogenetic conflict between the cpDNA and nuclear *PHYC* data resides in the placement of the Chilean species. The cpDNA data strongly support a monophyletic Chilean clade, whereas *PHYC* data strongly support two clades of Chilean *Puya* with one sister to the remainder of *Puya* ("Core *Puya*"). This discordance is best explained by ancient chloroplast capture in Chile (see below). Additionally, at least two other more recent episodes of hybridization and/or chloroplast capture involve Chilean species (see below).

Ancient reticulation in the Chilean Puya—A reticulate evolutionary history is invoked to explain the conflicting results from individually strongly supported cpDNA and nuclear DNA clades (Fig. 7). Chloroplast capture and hybridization are well-established explanations for points of conflict between cpDNA and nuclear DNA data, though assigning either as causation of the conflict should proceed with caution as other stochastic processes can create similar patterns (Smith and Sytsma, 1990; Baum et al., 1998; Wendel and Doyle, 1998; Linder and Rieseberg, 2004; Friar et al., 2008). Incomplete lineage sorting may be a pertinent explanation for such data set conflict, but generally it is invoked for groups that exhibit complex patterns of organellar DNA relationships within and among species generally not in sympatry (Wendel and Doyle, 1998; Comes and Abbott, 2001). The weight of the evidence in *Puya*, with good sampling in Chile and broad sampling elsewhere, does not point toward incomplete lineage sorting or other stochastic population-level processes as causative for this discordance. Rather, chloroplast capture following hybridization and unidirectional introgression explains the evidence best because the incongruence between the data sets is confined to the Chilean *Puya* and is readily explained by biogeography, morphology, and ecology in sympatry (Kim and Donoghue, 2008). The possibility that *PHYC* is providing a discordant phylogeny relative to other portions of the nuclear genome (Ané et al., 2007) also may be discounted as phylogenetic analysis of AFLP data, primarily nuclear in origin, produces highly congruent results relative to *PHYC* (Jabaily et al., 2008). On the basis of the extensive sampling of *Puya* and phylogenetic patterns of both cpDNA and nuclear DNA, we propose the following scenario to explain the discordance in phylogeny and morphology for the Chilean species of *Puya* (Fig. 7).

(1) Prior to any episode of chloroplast capture, ancestral lineages of subf. Bromelioideae and three clades of *Puya* had already diversified in Central Chile. Strong support is seen with *PHYC* (Fig. 4) for three clades within *Puya*, with the Chilean "Yellow *Puya*" sister to the "Core *Puya*" and these two in turn sister to the Chilean "Blue *Puya*."

(2) In Central Chile, an ancestor of the nuclear DNA defined clade "Yellow *Puya*" crossed as the pollen donor to an ancestor of the nuclear DNA defined clade "Blue *Puya*." This hybrid, possessing the cytoplasm of "Blue *Puya*," backcrossed repeatedly as the egg donor to the paternal "Yellow *Puya*" ancestor. The final introgressant product, the ancestor to all extant "Yellow *Puya*," thus swamped out most, if not all, nuclear contributions from the "Blue *Puya*" ancestor, but captured the latter's chloroplast. This early chloroplast capture event followed by subsequent diversification of both "Yellow *Puya*" and "Blue *Puya*" clades thus united all Chilean species into a single cytoplasm clade distinct from both "Core *Puya*" and subf. Bromelioideae. The sole exception in Chile is *P. boliviensis,* which more recently captured a distinctive chloroplast from within "Core *Puya*," as discussed below.

(3) In theory, the directionality of the chloroplast capture could be from the yellow-flowered species to the blue-flow-

ered species, or vice versa. Assuming that cpDNA and *PHYC* sequence evolution have proceeded at a relatively constant rate (although each different) through time, the relative amount of change and branching pattern evident between cpDNA and *PHYC* of Chilean species relative to that evident in both the "Core *Puya*" and subf. Bromelioideae should provide a means of identifying the directionality of chloroplast capture. The *PHYC* phylogeny (Fig. 4) depicts a trichotomy at the base of *Puya* with three clades, subf. Bromelioideae, "Blue *Puya*," and "Yellow *Puya* + Core *Puya*," indicating all three are essentially of equal age. However, the stem lineage leading to subsequent splitting of the "Yellow *Puya* + Core *Puya*" is approximately half in ML branch length relative to the entire length of the clade. If the "Yellow *Puya*" cytoplasm had been captured by the "Blue *Puya*" lineage early on in Central Chile, the cpDNA phylogeny should retain a clade of "Chilean *Puya*" with "Core *Puya*" and that this stem branch would be roughly half that in length relative to the entire branch. Instead, the cpDNA phylogeny (Fig. 3) also depicts a trichotomy at the base of *Puya* with each of the stem lineages roughly equal in length (0.003 ML branch length). This topology and similarity in divergence among the three clades (subf. Bromelioideae, "Core *Puya*," and "Chilean *Puya*") strongly argue that the "Blue *Puya*" cytoplasm is found in the extant Chilean species.

(4) Following this chloroplast capture event, subsequent diversification of this "mosaic" "Yellow *Puya*" ancestor and of the "Blue *Puya*" ancestor created all extant species in Chile. Prior to or subsequently after the chloroplast capture event, several morphological features evolved in their respective clades although it is not possible to ascertain when or what state was apomorphic (Fig. 6). The "Yellow *Puya*" clade is now defined by yellow, bowl-shaped flowers, sterile-tipped inflorescences, and glabrous leaves; the "Blue *Puya*" clade is defined by some shade of blue flowers, and densely scaly leaves below. Flower shape and inflorescence sterility vary within "Blue *Puya*." All morphological traits discussed here vary in "Core *Puya*," although sterile-tipped inflorescences of unknown homology occur in at least two of these species (*P. raimondii* and *P. castellanosii*).

Secondary chloroplast capture in P. boliviensis—An additional hybridization and possibly introgression event was involved in the formation of *P. boliviensis*. The maternally inherited cpDNA unites all *Puya* taxa from Chile minus *P. boliviensis* and instead places this species within "Core *Puya.*" Nu-

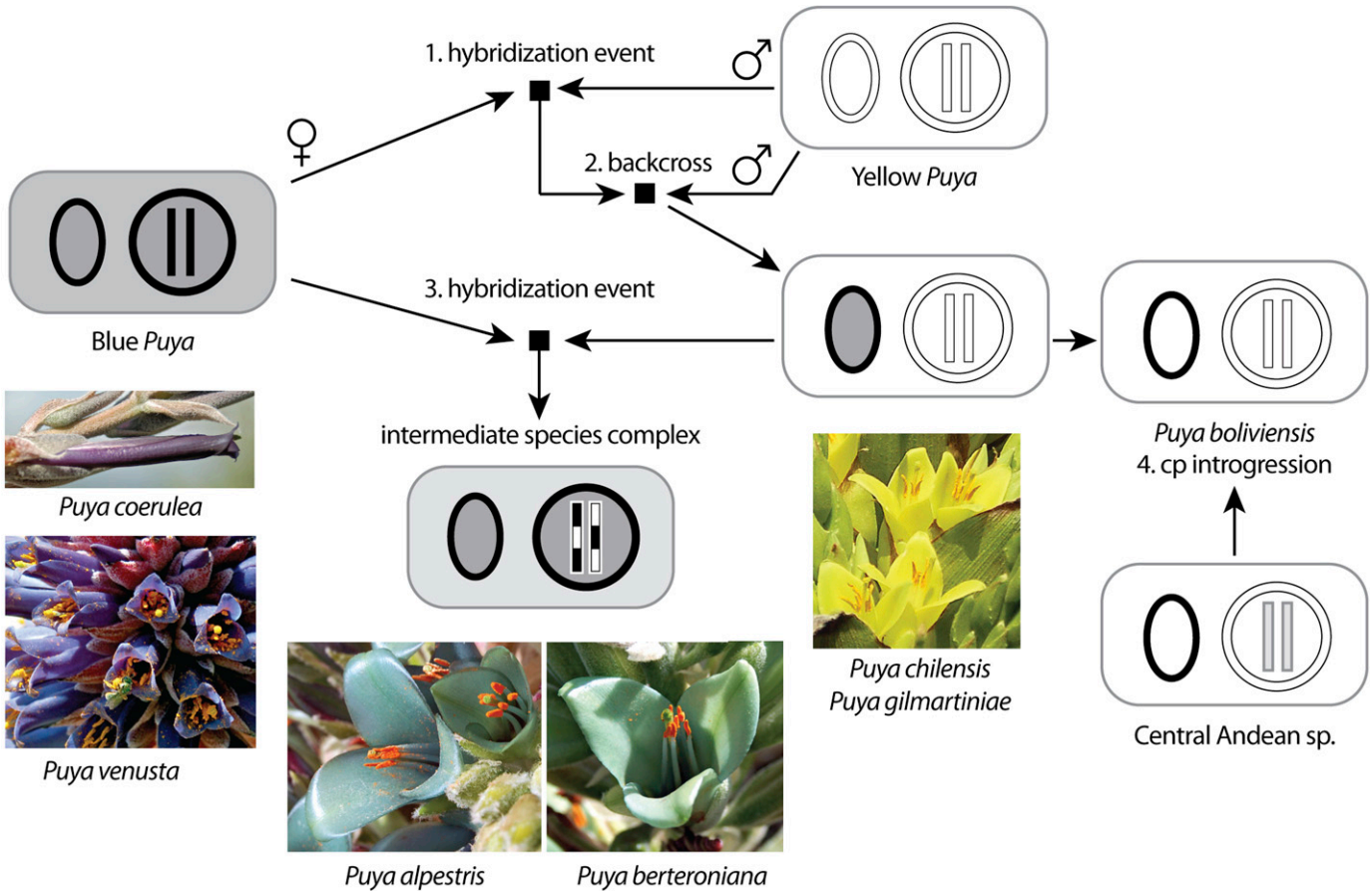

Fig. 7. Scheme depicting hypothesized genome evolution in the "Chilean *Puya*". Rounded box and circle diagrams depict cells: the smaller circle is the maternally inherited chloroplast genome and the larger circle with two internal bars represents the biparentally inherited nuclear genome. (1) Hybridization event between maternal "Blue *Puya*" ancestral lineage and paternal "Yellow *Puya*" ancestral lineage. (2) Repeated hybrid (cytoplasm donor) backcrossing to paternal "Yellow *Puya*" giving rise to modern "Yellow *Puya*" lineage with "Blue *Puya*" cytoplasm. (3) Subsequent, recent hybridization event between modern "Yellow *Puya*" and "Blue *Puya*" with no subsequent backcrossing generating the *P. alpestris/P. berteroniana* intermediate species complex. (4) Secondary chloroplast introgression event in *P. boliviensis* involving a central Andean species of "Core *Puya*".

clear *PHYC*, however, places *P. boliviensis* as expected based on floral and vegetative features squarely within the "Yellow *Puya*" clade from Chile. *Puya boliviensis* thus shares a similar nuclear history with other "Yellow *Puya"* but experienced a later, secondary chloroplast capture event from a maternal lineage elsewhere in the Andes. *Puya boliviensis* occurs at an isolated location in northern Chile beyond the boundary of the Mediterranean zone and several hundred kilometers north of the other Chilean species. It is one of only two *Puya* species found in the fog-fed lomas formations of arid coastal Chile and Peru, the most xeric areas in which *Puya* are currently found (Rundel and Dillon, 1998). If we interpret the *PHYC* history as the closest approximation available to the species history for *Puya*, *P. boliviensis* is derived from a central Chilean clade ("Yellow *Puya*"), and not from elsewhere in the Andes. This relationship is similar to that seen in other Atacaman endemic clades: *Oxyphyllum* (Luebert et al., 2009), *Polyachyrus* (Katinas and Crisci, 2000; Katinas et al., 2008), and *Tropaeolum* sect. *Chilensis* (Hershkowitz, 2006a). Hyperaridity of these coastal areas may be a recent phenomenon and Arroyo et al. (1988) suggested that the western slope of the Andes may have been wetter than the eastern side during the Miocene, which would have most likely supported many more species of *Puya*. The maternal cytoplasm in *P. boliviensis* may have come from a more widespread *Puya* species group that is now just represented as "Core *Puya*" in the higher Andes. Alternatively, *P. boliviensis* could have captured a chloroplast from an extant taxon. The cpDNA phylogeny is not sufficiently resolved within "Core *Puya*" to clearly identify the cytoplasm source for *P. boliviensis*, but it may be *P. ferruginea*, a widespread species found in the lomas of southern Peru and also at higher elevations in several countries. The chloroplast donor to *P. boliviensis* could also have been from other taxa restricted to the higher elevation central Andean Altiplano. Valleys connecting the Altiplano with the arid coast may have been a corridor of gene exchange between *P. boliviensis* and central Andean "Core *Puya*," which have been proposed as important for the dispersal and subsequent isolation of groups that originated in the high central Andes (Palma et al., 2005; Luebert et al., 2009). The other members of subg. *Puya* not from Chile are all found in the vicinity of the Altiplano.

Hybridization and recent origin of P. alpestris/P. berteroniana—More recently, a "Yellow *Puya*" taxon with sterile inflorescence tips (most likely extant *P. chilensis*) and a blue-flowered taxon (perhaps *P. coerulea* or *P. venusta*) hybridized to create the intermediary species complex *P. alpestris / P. berteroniana*. *Puya berteroniana* may be a lineage from within *P. alpestris* or derived in a similar fashion. *Puya berteroniana* is reported from a few locations in the northern part of the Mediterranean district near Fray Jorge NP, a refugium for much more temperate, southern floristic elements (Simpson Vuilleumier, 1971). In the field, *P. alpestris* and *P. berteroniana* are very difficult to tell apart, grading together in overall body size and general floral color. This gradation has generated discrepancies between major collectors in Chile as to which name they apply to a given collection. If morphological characters can behave as single nuclear loci (Baum et al., 1998), the traits of sterile inflorescence tips and shallow, open flowers in these two species would trace the contribution of the "Yellow *Puya*" clade. However, the trait of abaxial leaf indument would trace the contribution of the "Blue *Puya*" clade. In addition, the only *PHYC* copy in *P. alpestris* accessions is also contributed by the "Blue *Puya*" clade. Despite numerous attempts, we were unable to sequence *PHYC* from *P. berteroniana*. Both the "Yellow *Puya*" and "Blue *Puya*" clades would have provided parental copies for the expression of the intermediate floral coloration seen in the two species. The lighter blue flowers of *P. alpestris/P. berteroniana* are intermediate between the yellow flowers of *P. chilensis*, *P. gilmartiniae* and *P. boliviensis* and the dark blue flowers of *P. coerulea* and *P. venusta*. We cannot rule out that such a hybridization event and possible subsequent limited introgression may have happened multiple times to create the complex seen in *P. alpestris/P. berteroniana*. Much finer-scale population genetic markers would be necessary to address this question further.

The persistence of hybrid species *P. alpestris* raises questions of how speciation in sympatry can occur and how maintenance of species boundaries is achieved. The Mediterranean vegetation region of Chile extends from roughly 31°S–37°S and is flanked on the north by the Atacama Desert and to the south by cooler and wetter climates. The majority of Chilean *Puya* are restricted to this zone. Putative hybrid *P. alpestris* is common throughout this region (32°S–38°S for the majority of collections) and grows in relative close proximity to both putative extant parent taxa: *P. chilensis* (possible "Yellow *Puya*" parent*)* and *P. coerulea/P. venusta* (possible "Blue *Puya*" parent) (Fig. 2B). In some sites (e.g., the beaches of Zapallar), species were found growing in true sympatry. However, most Chilean species of *Puya* live in general close proximity. Most species also overlap, at least partially, in flowering times and appear to share pollinators (R. S. Jabaily, personal observation). During our field season from November to December 2006, all observed populations of *P. chilensis* had recently finished flowering, though a few individuals were still flowering. Populations of *P. venusta*, *P. coerulea*, *P. alpestris*, and *P. berteroniana* were all in full flower. *Patagonia gigas*, the largest species of hummingbird on earth, was observed visiting the flowers of both *P. alpestris* and *P. venusta*, although the inflorescence and flower shapes of species in subg. *Puya* suggest pollination by perching birds.

As no prezygotic barriers to successful reproduction may exist between *P. alpestris* and its putative parents, postzygotic mechanisms such as chromosomal rearrangement and genic sterility should be investigated to determine why *P. alpestris* persists. Similar questions could perhaps be asked about all *Puya* species, because interspecies fertility has not been systematically studied. Interspecific hybrids in *Puya* have naturally arisen in the Huntington Botanical Garden (R. S. Jabaily, personal observation). The Chilean endemics of *Tropaeolum* sect. *Chilensia* (Tropaeoleaceae) have a similar history of reticulate evolution based on conflict between nrITS paralogs (Hershkovitz et al., 2006a). Chile has been suggested to be an ideal habitat for sympatric speciation, with varied habitats and climate throughout many latitudes (Hershkovitz et al., 2006b). The Chilean *Puya* may well be an ideal group in which to further explore these questions of biogeography, hybridization, introgression, and sympatric speciation.

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APPENDIX 1. Voucher information and GenBank accession numbers for species of *Puya* and outgroup genera. Subfamilies follow Givnish et al. (2007). Herbaria abbreviations: WIS, = University of Wisconsin-Madison, Wisconsin, USA, HNT = Huntington Botanical Garden, San Marino, California, USA, SEL = Marie Selby Botanical Garden, Sarasota, Florida, USA, MO = Missouri Botanical Garden, St. Louis, Missouri, USA, USZ = Universidad Autónoma Gabriel René Moreno, Santa Cruz, Bolivia, RFA = Universidad Federal do Rio de Janeiro, Rio de Janeiro, Brazil, HB = Herbarium Bradeanum, Rio de Janeiro, Brazil, MBML = Museo de Biologia Mello Leitão, Santa Teresa, Brazil, CONC = Universidad de Concepción, Concepción, Chile; LP = Museo de La Plata, La Plata, Argentina, COL = Universidad Nacional de Colombia, Bogotá, Colombia, QCNE = Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador, USM = Universidad Nacional Mayor de San Marcos, Lima, Peru.

Subfamily	Genus	species	Author	Voucher (herbaria)	*trnS- trnG*	*matK*	*rps16*	*PHYC*
Hechtioideae	***Hechtia***	***lundelliorum***	L.B.Smith	RSJ 024 (WIS)	FJ968380	FJ968178	FJ968308	—
Pitcairnioideae	***Deuterocohnia***	***longipetala***	(Baker) Mez	RSJ 235 (HNT)	FJ968413	FJ968210	FJ968340	—
Pitcairnioideae	***Deuterocohnia***	***meziana***	Kuntze ex Mez	RSJ 071 (WIS)	FJ968390	FJ968187	FJ968318	—
Pitcairnioideae	***Dyckia***	***pseudococcinea***	L.B.Smith	RSJ 233 (HNT)	FJ968411	FJ968208	FJ968338	—
Pitcairnioideae	***Fosterella***	***albicans***	(Grisebach) L.B.Smith	C. Nowiche 2359 (USZ)	FJ968420	FJ968217	FJ968347	—
Pitcairnioideae	***Pitcairnia***	***feliciana***	(A. Chevalier) Harms & Moldboard	SEL 1998-0116	FJ968386	FJ968183	FJ968314	—
Pitcairnioideae	***Pitcairnia***	***maidifolia***	(C.Morren) Decaisne	SEL1986-0313A	FJ968387	FJ968184	FJ968315	—
Pitcairnioideae	***Pitcairnia***	***orchidifolia***	Mez	SEL1994-0086A	FJ968388	FJ968185	FJ968316	FJ968253
Pitcairnioideae	***Pitcairnia***	***sanguinea***	H. Luther	SEL 1992-0015	FJ968385	—	FJ968313	FJ968252
Bromelioideae	***Aechmea***	***nudicaulis***	(L) Grisebach	Wendt 332 (RFA)	FJ968392	FJ968189	FJ968320	FJ968256
Bromelioideae	***Ananas***	***comosus***	(Linnaeus) Merrill	RSJ 018 (WIS)	FJ968379	FJ968177	FJ968307	FJ968247
Bromelioideae	***Ananas***	***nanus***	(L. B. Smith) L. B. Smith	A. Genrty (MO)	FJ968374	FJ968172	FJ968303	FJ968242
Bromelioideae	***Billbergia***	***euphemiae***	E. Morren	RSJ 017 (WIS, SEL)	FJ968378	FJ968176	FJ968306	FJ968246
Bromelioideae	***Billbergia***	***laxiflora***	L.B. Smith	Wendt 379 & G. K. Brown, L. Kolmann (MBML, RFA)	FJ968366	FJ968164	FJ968295	FJ968236
Bromelioideae	***Bromelia***	***balansae***	Mez	RSJ 031 (HNT)	FJ968381	FJ968179	FJ968309	FJ968248
Bromelioideae	***Bromelia***	***pinguin***	L.	BAB sn (WIS)	FJ968436	FJ968233	FJ968363	—
Bromelioideae	***Bromelia***	***agavifolia***	Brongniart ex Houllet	J. Kress 88-2529 & Stone (SEL)	FJ968373	FJ968171	FJ968302	FJ968241
Bromelioideae	***Bromelia***	***flemingii***	I. Ramirez & Carnevali	F. Oliva-Esteva s.n. (SEL)	FJ968372	FJ968170	FJ968301	FJ968240
Bromelioideae	***Cryptanthus***	***bromelioides***	Otto & Dietrich	RSJ 016 (WIS,SEL)	FJ968377	FJ968175	FJ968305	FJ968245
Bromelioideae	***Cryptanthus***	***dorothyae***	Leme	L. C. Araujo s.n. (SEL)	FJ968370	FJ968168	FJ968299	FJ968238
Bromelioideae	***Deinacanthon***	***urbanianum***	(Mez) Mez	RSJ 004 (SEL)	FJ968376	FJ968174	—	FJ968244
Bromelioideae	***Fernseea***	***itatiaiae***	(Wawra) Baker	Katia Ribeiro 291 (RFA)	FJ968367	FJ968165	FJ968296	—
Bromelioideae	***Greigia***	**sp.**		RSJ 184 (CONC, WIS)	—	FJ968224	FJ968354	FJ968284
Bromelioideae	***Neoregelia***	***cruenta***	(Graham) L.B.Smith	B. Whitman s.n. (SEL)	FJ968371	FJ968169	FJ968300	FJ968239
Bromelioideae	***Ochagavia***	***carnea***	(Beer) L.B.Smith & Looser	Leme 2418 (HB)	FJ968368	FJ968166	FJ968297	—
Bromelioideae	***Ochagavia***	***elegans***	Philippi	Tod Stuessey s.n. (SEL)	FJ968369	FJ968167	FJ968298	FJ968237
Bromelioideae	***Ochagavia***	***lindleyana***	Mez	RSJ 058 (HNT, WIS)	FJ968437	FJ968234	FJ968364	—
Bromelioideae	***Orthophytum***	***gurkenii***	Hutchison	RSJ 234 (SEL)	FJ968412	FJ968209	FJ968339	FJ968272
Bromelioideae	***Portea***	***fosteriana***	L.B. Smith	Wendt 397 & G. K. Brown, L. Kolmann (MBML, RFA)	FJ968365	FJ968163	FJ968294	FJ968235
Puyoideae	***Puya***	***alpestris* 1**	(Poeppig) Gay	RSJ 177 (WIS,CONC)	FJ968401	FJ968198	FJ968329	FJ968264
Puyoideae	***Puya***	***alpestris* 2**	(Poeppig) Gay	RSJ 174 (WIS,CONC)	FJ968407	FJ968204	FJ968335	FJ968269
Puyoideae	***Puya***	***angusta* 1**	L.B.Smith	RSJ 226 (WIS,USM)	FJ968431	FJ968228	FJ968358	FJ968289
Puyoideae	***Puya***	***angusta* 2**	L.B.Smith	RSJ 230 (WIS,USM)	FJ968435	FJ968232	FJ968362	FJ968293
Puyoideae	***Puya***	***berteroniana***	Mez	RSJ 168 (WIS,CONC)	FJ968398	FJ968195	FJ968326	—
Puyoideae	***Puya***	***bicolor***	Mez	RSJ 202 (COL)	FJ968425	FJ968222	FJ968352	FJ968282
Puyoideae	***Puya***	***boliviensis* 1**	Baker	M. Rosas 3351 (WIS)	FJ968402	FJ968199	FJ968330	FJ968265
Puyoideae	***Puya***	***boliviensis* 2**	Baker	D. Stanton and J. Villagra sn (WIS)	FJ968409	FJ968206	—	FJ968270
Puyoideae	***Puya***	***castellanosii***	L.B.Smith	RSJ 149 (LP, WIS)	FJ968393	FJ968190	FJ968321	FJ968257
Puyoideae	***Puya***	***chilensis* 1**	Molina	RSJ 164 (WIS,CONC)	FJ968396	FJ968193	FJ968324	FJ968260
Puyoideae	***Puya***	***chilensis* 2**	Molina	RSJ 172 (WIS,CONC)	FJ968406	FJ968203	FJ968334	FJ968268
Puyoideae	***Puya***	***coerulea* 1**	Lindley	RSJ 175 (WIS,CONC)	FJ968400	FJ968197	FJ968328	FJ968263
Puyoideae	***Puya***	***coerulea* 2**	Lindley	RSJ 176 (WIS,CONC)	FJ968408	FJ968205	FJ968336	—
Puyoideae	***Puya***	***compacta***	L.B.Smith	RSJ 129 (QCNE)	FJ968417	FJ968214	FJ968344	FJ968275
Puyoideae	***Puya***	***dasylirioides***	Standley	Grant 92-01895 (MO)	FJ968375	FJ968173	FJ968304	FJ968243
Puyoideae	***Puya***	***dyckioides***	(Baker) Mez	RSJ 150 (LP, WIS)	FJ968394	FJ968191	FJ968322	FJ968258
Puyoideae	***Puya***	***ferreyrae***	L.B.Smith	RSJ 222 (USM, WIS)	FJ968430	FJ968227	FJ968357	FJ968288
Puyoideae	***Puya***	***ferruginea* 1**	(Ruiz & Pavón) L.B.Smith	RSJ 059 (USM, WIS)	FJ968383	FJ968181	FJ968311	FJ968250
Puyoideae	***Puya***	***ferruginea* 2**	(Ruiz & Pavón) L.B.Smith	RSJ 210 (USM, WIS)	FJ968428	FJ968225	FJ968355	FJ968286
Puyoideae	***Puya***	***ferruginea* 3**	(Ruiz & Pavón) L.B.Smith	RSJ 209 (USM, WIS)	FJ968427	—	—	FJ968285
Puyoideae	***Puya***	***gilmartiniae***	G.S.Varadarajan & Flores	RSJ 169 (CONC, WIS)	FJ968399	FJ968196	FJ968327	FJ968262
Puyoideae	***Puya***	***goudotiana***	Mez	RSJ 182 (COL)	FJ968424	FJ968221	FJ968351	FJ968281
Puyoideae	***Puya***	***hamata***	L.B.Smith	RSJ 122 (QCNE)	FJ968414	FJ968211	FJ968341	FJ968273
Puyoideae	***Puya***	***harmsii***	(Castellanos) Castellanos	RSJ 145 (LP, WIS)	FJ968391	FJ968188	FJ968319	FJ968255
Puyoideae	***Puya***	***herrerae***	Harms	RSJ 212 (USM, WIS)	FJ968429	FJ968226	FJ968356	FJ968287
Puyoideae	***Puya***	***lanata***	Kunth	RSJ 105 (QCNE)	FJ968418	FJ968215	FJ968345	FJ968276

APPENDIX 1. Continued.

Subfamily	Genus	species	Author	Voucher (herbaria)	*trnS- trnG*	*matK*	*rps16*	*PHYC*
Puyoideae	***Puya***	***lineata***	Mez	RSJ 180 (COL)	FJ968426	FJ968223	FJ968353	FJ968283
Puyoideae	***Puya***	***macrura***	Mez	RSJ 227 (USM, WIS)	FJ968432	FJ968229	FJ968359	FJ968290
Puyoideae	***Puya***	***mima***	L.B.Smith&Read	RSJ 228 (USM, WIS)	FJ968433	FJ968230	FJ968360	—
Puyoideae	***Puya***	***mirabilis***	(Mez) L.B.Smith	RSJ 161 (LP, WIS)	FJ968404	FJ968201	FJ968332	—
Puyoideae	***Puya***	***nana***	Wittmack	RSJ 062 (WIS)	FJ968389	FJ968186	FJ968317	FJ968254
Puyoideae	***Puya***	***nitida* 1**	Mez	RSJ 112 (QCNE)	FJ968416	FJ968213	FJ968343	—
Puyoideae	***Puya***	***nitida* 2**	Mez	RSJ 206 (COL)	FJ968421	FJ968218	FJ968348	FJ968278
Puyoideae	***Puya***	***nutans***	L.B.Smith	RSJ 117 (QCNE)	FJ968403	FJ968200	FJ968331	FJ968266
Puyoideae	***Puya***	***obconica***	L.B.Smith	RSJ 106 (QCNE)	FJ968419	FJ968216	FJ968346	FJ968277
Puyoideae	***Puya***	***pygmaea***	L.B.Smith	RSJ 135 (QCNE)	FJ968415	FJ968212	FJ968342	FJ968274
Puyoideae	***Puya***	***raimondii* 1**	Harms	RSJ 229 (USM, WIS)	FJ968434	FJ968231	FJ968361	FJ968292
Puyoideae	***Puya***	***raimondii* 2**	Harms	RSJ 232 (USM, WIS)	FJ968410	FJ968207	FJ968337	FJ968271
Puyoideae	***Puya***	***santosii***	Cuatrecasas	RSJ 194 (COL)	FJ968422	FJ968219	FJ968349	FJ968279
Puyoideae	***Puya***	***trianae***	Baker	RSJ 192 (COL)	FJ968423	FJ968220	FJ968350	FJ968280
Puyoideae	***Puya***	***ultima***	L.B.Smith	RSJ 051 (HNT, WIS)	FJ968384	FJ968182	FJ968312	FJ968251
Puyoideae	***Puya***	***venusta* 1**	Philippi	RSJ 166 (CONC, WIS)	FJ968397	FJ968194	FJ968325	FJ968261
Puyoideae	***Puya***	***venusta* 2**	Philippi	RSJ 165 (CONC, WIS)	FJ968405	FJ968202	FJ968333	FJ968267
Puyoideae	***Puya***	***wrightii***	L.B.Smith	RSJ 039 (HNT, WIS)	FJ968382	FJ968180	FJ968310	FJ968249
Puyoideae	***Puya***	***yakespala***	Castellanos	RSJ 157 (LP, WIS)	FJ968395	FJ968192	FJ968323	FJ968259