Historical biogeography and life-history evolution of Andean *Puya* (Bromeliaceae)

RACHEL S. JABAILY[1,2]* and KENNETH J. SYTSMA[1]

[1]*Department of Botany, University of Wisconsin, Madison, WI, USA*
[2]*Department of Biology, Rhodes College, Memphis, TN, USA*

Puya (Bromeliaceae), with > 200 species, is a classic example of a recent, rapid species-level radiation in the Andes. To assess the biogeographical history of this primarily Andean species group and the evolution of different life histories, amplified fragment length polymorphism (AFLP) data were generated for 75 species from throughout the geographical range of the genus. Distribution data for latitudinal and elevational ranges were compiled for almost all species. The greatest number of species is found at mid-elevations and mid-latitudes south of the equator. The genus originated in central Chile and first moved into the Cordillera Oriental of the central Andes via inter-Andean valleys. Cladogenesis progressed in a general south to north direction tracking the final uplift of the Andes. All taxa north of the Western Andean Portal form a monophyletic group implying a single colonization of the northern Andes, with no subsequent transitions back south from the Northern Andes. Repeated evolutionary transitions of lineages up and down in elevation are suggestive of allopatric speciation driven by Pleistocene glaciation cycles. True semelparity evolved once in *P. raimondii*, with similar semi-semelparity evolving repeatedly in páramos of the northern Andes. Fieldwork and phylogenetic characterization of high-elevation *Puya* are priorities for future efforts. © 2012 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2013, **171**, 201–224.

ADDITIONAL KEYWORDS: AFLP – BayesTraits – elevation – latitude – monocarpic – semelparity – Western Andean Portal.

INTRODUCTION

Understanding evolution in the Andes, the most extensive mountain system on earth, is both fascinating and challenging due to the impact of extensive recent geological upheavals on the biota and the difficulties involved in elucidating relationships between the large number of species found within this complex geographical space, which are often of recent origin. The tropical latitudes of the Andes are the most important biodiversity hotspot on the planet, in terms of both number of species and levels of endemism (Luteyn, 1999; Myers *et al*., 2000; Young *et al*., 2002). The Andes are home to rapid and extensive plant and animal species radiations (Smith & Sytsma, 1994; Cardillo, 1999; Berry *et al*., 2004; Bell & Donoghue, 2005; Kay *et al*., 2005; Fjeldsa & Rahbek, 2006; Hughes & Eastwood, 2006; Drummond, 2008; Scher-son, Vidal & Sanderson, 2008; Jabaily & Sytsma, 2010; Chaves, Weir & Smith, 2011; Givnish *et al*., 2011; Sklenář, Dušková & Balslev, 2011). The extent, recent evolution and high rates of species diversification found for several species-rich Andean plant groups [e.g. *Lupinus* L. (Hughes & Eastwood, 2006; Drummond, 2008; Drummond *et al*., 2012), *Valeriana* L. (Bell & Donoghue, 2005; Moore & Donoghue, 2007), core Tillandsioideae (Givnish *et al*., 2011)] and the uniquely steep and extended latitudinal and altitudinal gradients found in the Andes highlight this region as an ideal study system for investigating plant diversification (e.g. Särkinen *et al*., 2011). Understanding why the Andes are rich in species remains a key challenge in Neotropical biology (Rull, 2011). A more thorough understanding of the processes involved in making the Andes so rich in species can also help inform more broadly the mechanisms behind the genesis and maintenance of extensive species-level radiations.

*Corresponding author. E-mail: jabailyr@gmail.com

Geologically driven allopatric speciation with subsequent differentiation and/or parapatric ecological speciation along habitat gradients are the main evolutionary scenarios postulated for many Andean animals and plants (Gentry, 1982; Young *et al.*, 2002; Brumfield & Edwards, 2007). However, detailed studies involving these cladogenic processes in the Andes and the Neotropics are more common in vertebrates than in plants to date (Young *et al.*, 2002; Hall, 2005; Weir, 2006; Brumfield & Edwards, 2007; Ribas *et al.*, 2007; Torres-Carvajal, 2007; Elias *et al.*, 2009; Santos *et al.*, 2009; Chaves *et al.*, 2011), in part because of greater baseline field knowledge and collecting efforts in some animal groups compared with plant groups. Historical biogeographical explanations for Andean radiations are increasingly based on time-calibrated phylogenies and use of explicit biogeographical models (Richardson *et al.*, 2001; Berry *et al.*, 2004; Kay *et al.*, 2005; Moore & Donoghue, 2007; Alzate, Mort & Ramirez, 2008; Drummond, 2008; Antonelli *et al.*, 2009; Cosacov *et al.*, 2009; Givnish *et al.*, 2011; Luebert, Hilger & Weigend, 2011; Särkinen *et al.*, 2011; Drummond *et al.*, 2012).

The charismatic genus *Puya* Molina (Bromeliaceae), with > 200 described species of terrestrial rosette-forming bromeliads (Fig. 1), is a striking example of recent rapid species diversification in the Andes, providing an ideal study group to investigate Andean species radiations (Jabaily & Sytsma, 2010; Schulte *et al.*, 2010; Givnish *et al.*, 2011). The genus comprises two major clades, one endemic to lowland and coastal habitats in central Chile and the other almost exclusively Andean and spanning all tropical and significant portions of temperate Andean latitudes. Species are found from sea level to > 4500 m elevation (Fig. 2) in habitats including high elevation páramo and puna, mesic and xeric inter-Andean valleys, portions of the lowland chaco and the coastal Chilean matorral (Fig. 1). Two species are found in Panama and Costa Rica, one endemic and one widespread into the northern Andean lowlands. *Puya* flowers provide nectar for hummingbirds, the main pollinators, and are utilized as a food source by the spectacled bear. *Puya* fruits produce copious seed with a small, papery wing appendage but are generally thought to be poor dispersers. Perhaps as a consequence, many species are narrow endemics, often found in a single valley. Bromeliaceae are rosette-forming monocots typically with terminal inflorescences that do not continue to grow after flowering (Benzing, 2000) (Fig. 1). Many bromeliads reproduce asexually via production of clonal offshoot ramets ('pup' rosettes), which either remain attached to the mother plant, or sever to produce physiologically independent individuals. *Puya* is one of several genera of Bromeliaceae that vary in the ability of

different species to produce pup rosettes (Barbará *et al.*, 2009). Most species of *Puya* are iteroparous (R. S. Jabaily, pers. observ.) forming large colonies of attached clonal rosettes, especially in marginal habitats such as rocky cliff faces. A small number of high-elevation species show reduced ability or even inability to produce pup rosettes before or after inflorescence production and thus are effectively reproducing only sexually. *Puya* is also one of the relatively few lineages of long-lived plants with taxa that are semelparous (or monocarpic: Young & Augspurger, 1991). The repeated evolution of semelparity particularly in tropical montane ecosystems is a fascinating case of convergent evolution (Hedberg & Hedberg, 1979; Smith & Young, 1987), and the evolution of this risky life-history strategy raises many evolutionary questions that can best be framed in the light of established phylogenetic relationships between taxa.

An initial phylogenetic analysis of *Puya* uncovered two major clades with robust support using a combination of plastid DNA and nuclear single-copy PHYC gene sequences (Jabaily & Sytsma, 2010), but levels of informative sequence variation for these loci in the primarily Andean 'core *Puya*' clade were too low to resolve species relationships. The family-wide phylogeny of Givnish *et al.* (2011) used nearly 10 000 bp of plastid sequence, but phylogenetic resolution between the eight sampled species of *Puya* (beyond retrieval of the two major clades) was minimal, in line with known low rates of sequence evolution in the family as a whole (Gaut *et al.*, 1992; Givnish *et al.*, 2007; Smith & Donoghue, 2008; Sass & Specht, 2010). However, Andean bromeliads are by no means unique, as most published DNA sequence-derived phylogenetic trees for high-elevation Andean clades are poorly resolved (e.g. Emshwiller, 2002; Andersson, 2006; Hershkovitz *et al.*, 2006; Alzate *et al.*, 2008; Soejima *et al.*, 2008; Cosacov *et al.*, 2009).

The use of amplified fragment length polymorphism (AFLP) provides an alternative to direct sequencing for species-level phylogenetics particularly for recent and rapidly radiating groups (Albertson *et al.*, 1999; Després *et al.*, 2003; Richardson *et al.*, 2003; Koopman, 2005; Spooner, Peralta & Knapp, 2005; Pellmyr *et al.*, 2007; McKinnon *et al.*, 2008; Dasmahapatra, Hoffman & Amos, 2009; Kropf, Comes & Kadereit, 2009; Arrigo *et al.*, 2011; Bacon *et al.*, 2011; Gaudeul *et al.*, 2012). Despite the often mentioned advantages and limitations of using AFLP in phylogenetic analyses (see recent review by Gaudeul *et al.*, 2012), few studies have explicitly assessed congruence between phylogenetic analyses based on AFLP and DNA sequence data or provide a theoretical basis for using or not using AFLP. Importantly, recent theoretical studies indicate that the major drawback of this technique is the low information content of AFLP
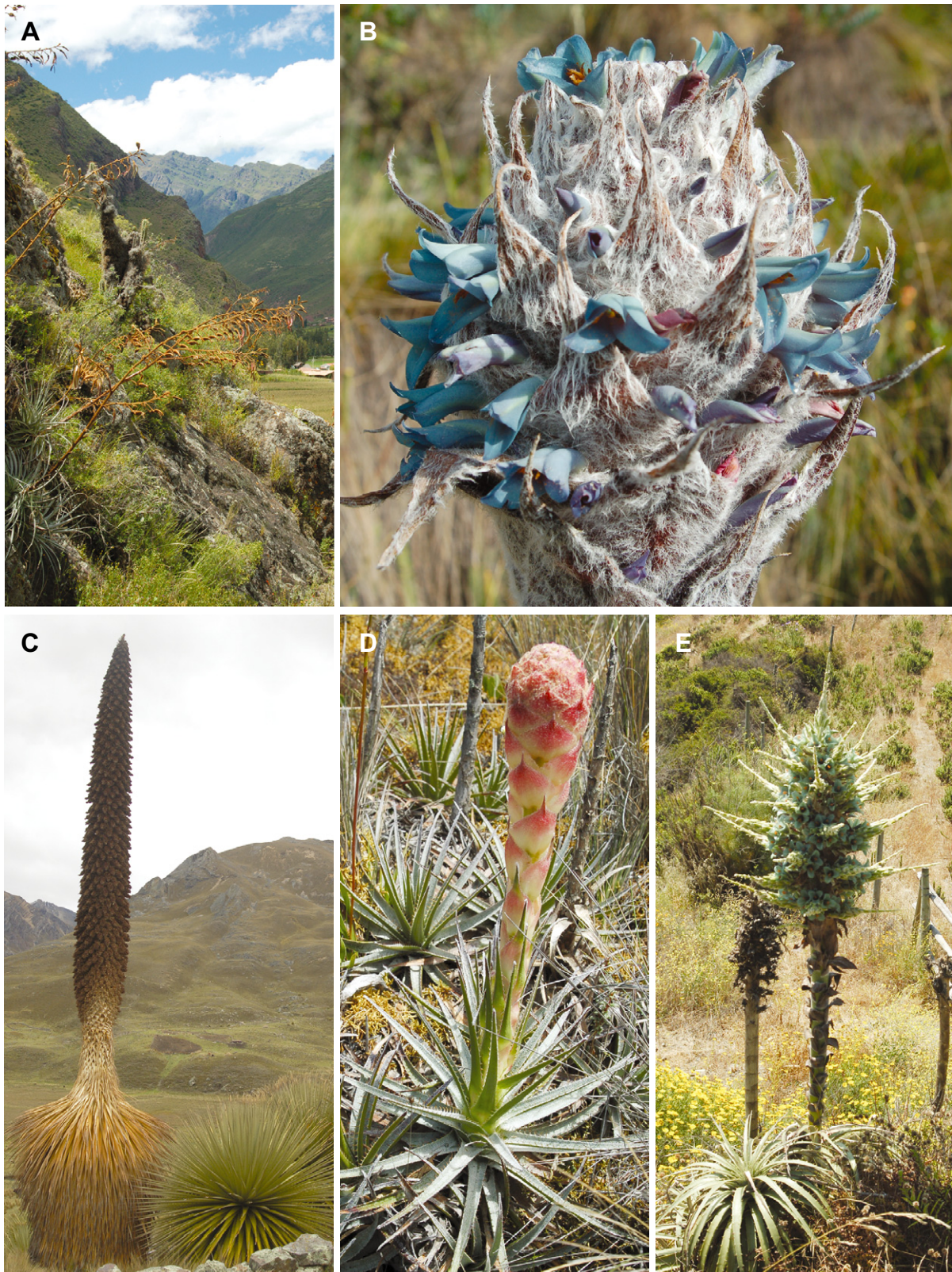
Figure 1. Habitats and life forms of *Puya* species. A, *P. ferruginea* (Ruiz & Pav.) L.B.Sm., Cusco, Peru. B, *P. compacta* L.B.Sm., Azuay, Ecuador. C, *P. raimondii* Harms, Ancash, Peru. D, *P. exigua* Mez, Azuay, Ecuador. *P. alpestris* Poepp., Coquimbo, Chile. Photos: A–D, R. S. Jabaily; E, M. J. Jabaily.
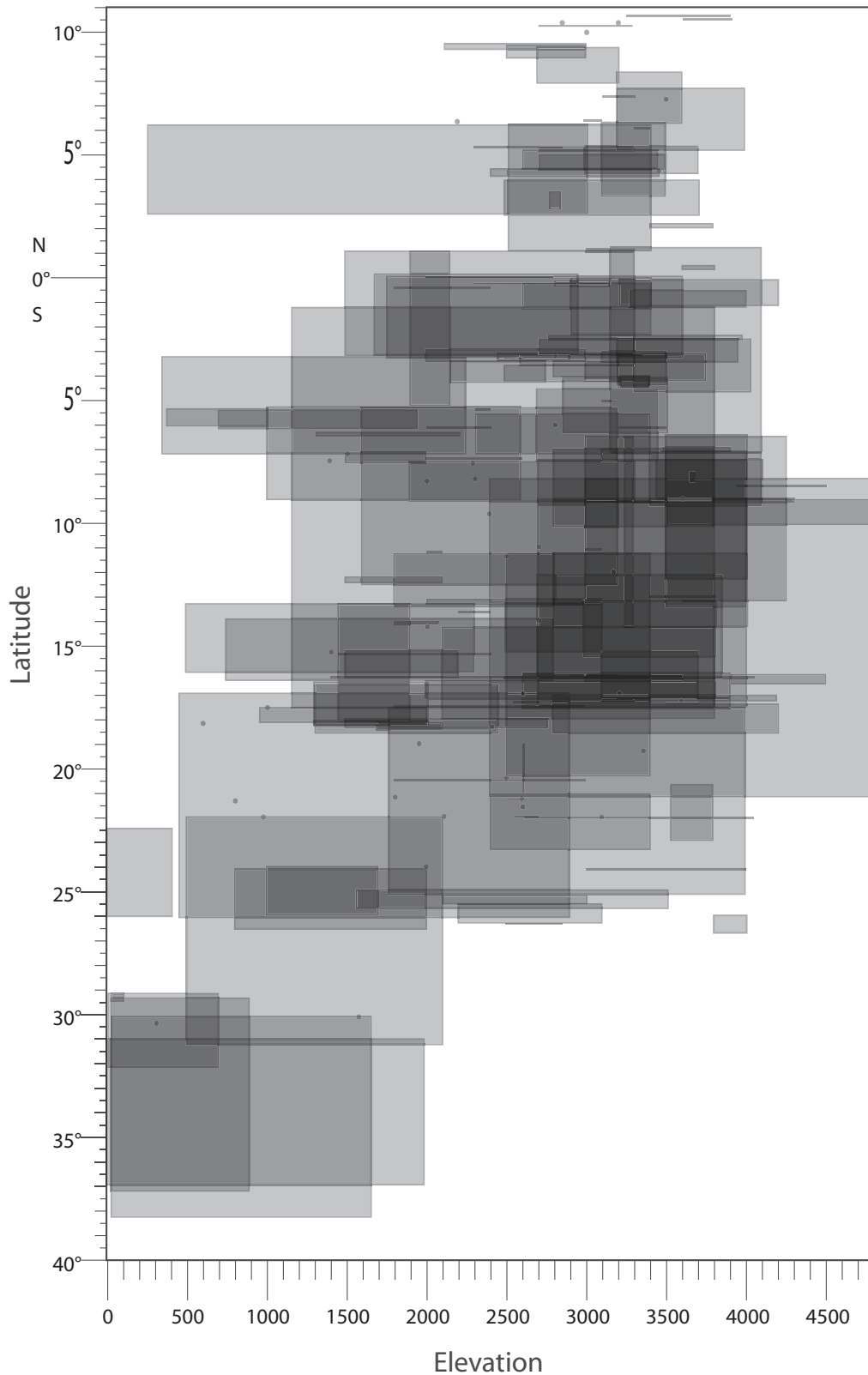
Figure 2. Latitude and elevation ranges for individual species of *Puya* (Bromeliaceae). Species ranges known only from type specimens are represented as a dot or a line; species with multiple collections are depicted as boxes with the dimensions corresponding to known latitude and elevational limits.

markers (Simmons *et al*., 2007; García-Pereira, Caballero & Quesada, 2010, 2011) and not the other commonly invoked limitations such as the lack of homology of co-migrating fragments (García-Pereira *et al*., 2010), the dominant nature of AFLP characters (Simmons *et al*., 2007) and correlation with genome size (Fay, Cowan & Leitch, 2005; Althoff, Gitzendanner & Segraves, 2007; Caballero & Quesada, 2010). Recent studies on the effectiveness of AFLP markers indicate that they are appropriate for phylogenetic inference as long as sequence divergence is low, the topology of the underlying evolutionary tree is not strongly asymmetric and basal nodes do not have short branch lengths (García-Pereira *et al*., 2011). Under certain circumstances, AFLP may be suitable to reconstruct even deeper phylogenies than usually accepted (García-Pereira *et al*., 2010). Current evidence also suggests that AFLPs largely behave as neutral characters (Bonin, Ehrich & Manel, 2007) and that an AFLP-based clock may be appropriate with shallow divergences (Kropf *et al*., 2009).

AFLPs have been used at both the population and the species level in Bromeliaceae (Sgorbati *et al*., 2004; Rex *et al*., 2007) and in other Andean plant groups for which lack of DNA sequence variation is also an issue (Tremetsberger *et al*., 2006; Schmidt-Lebuhn, Seltmann & Kessler, 2007; Emshwiller *et al*., 2009; Nakazato & Housworth, 2011). Schulte *et al*. (2010) explored the utility of AFLP at both the interspecific and the intrapopulation level in the 'Chilean *Puya*' clade to determine relationships between species and detect putative hybrid individuals. In this study the AFLP-based analyses supported species monophyly (except for noted hybrid individuals) and were congruent with the main phylogenetic divisions in *Puya* based on nuclear and plastid DNA sequence data (Jabaily & Sytsma, 2010). Thus there is evidence to suggest that AFLP data can be used to provide a reasonable estimate of species relationships, at least in *Puya*.

The goals of this study were to (1) reconstruct a preliminary phylogenetic framework for *Puya* with emphasis on the Andean species of *Puya*, (2) use this framework to investigate historical biogeographical patterns in the Andes and (3) analyse patterns in life-history (reproductive strategy) variability and evolution. To that end, a baseline phylogenetic tree employing representative sampling of species from all major Andean regions was generated using a large AFLP data set. This new framework is combined with distribution data to investigate the historical biogeography of *Puya* in the Andes. Specifically, the frequencies of evolutionary transition across latitudinal and elevational space are quantified under different analytical models to gain insight into the potential role of Andean uplift versus glaciation cycles in driving species diversification.

MATERIAL AND METHODS

Plant material was collected throughout the Andes from 2006 to 2008 and from the extensive living plant collection at the Huntington Botanical Garden (San Marino, CA, USA). Herbarium material was not used because of the necessity of high-quality DNA for AFLP analysis. As many *Puya* spp. as possible were observed and collected at localities spanning the geographical range of the genus and including all major habitats where *Puya* occurs. In addition, latitudinal and elevational ranges for 193 of the 214 recognized species were generated from field data, information from Smith & Downs (1974) and Manzanares (2005) and specimen data from herbaria in the USA and South America (NY, US, F, MO, SEL, WIS, HNT, USZ, LPB, QCNE, LP, MCNS, CONC, COL, USM, CUZ, HUT).

AFLP ANALYSIS

Ninety-eight accessions representing 75 *Puya* spp., all identified by the first author, were included in the AFLP analysis (Appendix 1). These accessions span the taxonomic, morphological and geographical range of the genus and included 40 species not sampled in the previous analysis of Jabaily & Sytsma (2010). Multiple individuals from multiple populations of 18 species were included to test species monophyly. Several putative new species from Apurimac, Peru, were also included. For a subset of accessions and primer pairs multiple independent AFLP analyses were performed to test for repeatability of the fragments generated.

AFLP fragment generation and isolation protocols follow Emshwiller *et al*. (2009). Total genomic DNA was extracted with the DNeasy Plant Kit (Qiagen, Valencia, California) following the manufacturer's protocol. All enzymes and buffers used for the entire AFLP fragment process were from New England Biolabs (Ipswich, MA, USA). For the initial digestion step, 3.7 μL DNA was digested with 0.5 μL *Mse*I, 0.25 μL *Eco*RI (enzyme concentrations were 50 U/μL), 0.5 μL *Eco*RI buffer and 0.05 μL bovine serum albumin at 37 °C for 2 h. Immediately after completion of the digestion step, double stranded adapters were ligated to each digestion product in reactions with 5 μL digestion product, 1 μL ligase buffer, 0.19 μL each of *Eco*RI and *Mse*I adaptors, 0.10 μL T4 DNA ligase and 3.52 μL water for a total reaction volume of 10 μL, held at 16 °C for 14 h.

Before the first round of amplification, 7 μL of product was diluted with 29 μL of water. The first round of amplification used primers *Eco*RI+A and *Mse*I+C. Reaction mixes used 5 μL of the diluted digested DNA with attached adaptors, 2.5 μL 10×

buffer, 2 μL dNTPs, 0.38 μL each primer, 0.25 μL *Taq* polymerase and 14.5 μL of water for a total reaction volume of 25 μL. The cycling regime was 1 min denaturation at 72 °C, followed by 20 cycles of 94 °C for 1 min, 56 °C for 1 min and 72 °C for 2 min, and a final extension of 72 °C for 2 min. Before the final amplification step, 20 μL of the product was diluted with 360 μL water. A second, more selective round of amplifications followed with the following primer combinations (M+CGA/E+ATG, E+AGC, E+AC; M+CGG/E+ATT; M+CTC/E+AGC, E+AC, E+ATG; M+CAT/E+AC; M+CCC/E+AC). Reaction mixes for the second round of amplifications were 5 μL of the diluted product from the first round of amplifications, 2.5 μL of 10× buffer, 3 μL dNTPs, 0.5 μL deionized Hi-Di formamide (Applied Biosystems, Carlsbad, CA, USA), 2.5 μL of the Mse*I* primer, 0.5 μL of Eco*RI* primer fluorescently labelled with 6-FAM, 0.25 μL *Taq* and 10.75 μL water for a total reaction volume of 25 μL. The cycling regime was nine cycles of 94 °C for 50 s, 65 °C for 1 min (decrease by 1 °C per cycle) and 72 °C for 2 min, followed by 20 cycles of 95 °C for 50 s, 56 °C for 1 min, 72 °C for 2 min and a final extension of 72 °C for 10 min.

PCR products were electrophoresed on an ABI 3700 automated sequencer (Applied Biosystems, Foster City, CA, USA) with a 500ROX-labelled internal lane standard at the University of Wisconsin-Madison Biotechnology Center. Output profiles were visualized and analysed using GeneMarker (SoftGenetics, State College, PA, USA) using the settings of Holland, Clarke & Meudt (2008). After visual inspection of every profile generated for each accession from each primer pair, some manual adjustments were made to the determinations of peaks by the program.

ROOTING OF THE PHYLOGENY

A positive relationship between phylogenetic distance and AFLP homoplasy has been well documented (Fay *et al.*, 2005; Koopman, 2005; Althoff *et al.*, 2007). Initially, *Ananas* Mill. from the sister subfamily Bromelioideae and the more distantly related *Deuterocohnia* Mez were included for potential rooting purposes, but were ultimately discarded because of suspected non-homology of fragments. For both outgroup accessions, the number of bands scored as present was much lower than in *Puya* (166 for *Ananas*, 168 for *Deuterocohnia*, average 245 for *Puya* taxa) and distance analyses found the outgroups to be more similar to each other than either was to *Puya*, which does not coincide with the current understanding of relationships in Bromeliaceae (Givnish *et al.*, 2011). Rooting of the AFLP phylogeny using non-*Puya* outgroups was also deemed inappropriate based on

comparison of sequence similarity at various phylogenetic levels in a nuclear ribosomal internal transcribed spacer (ITS) dataset (data not shown). The level of ITS sequence divergence between the outgroup genera and *Puya* was three times higher than that recommended by Koopman (2005) for application of AFLP and was in the appropriate range of 10–30 variable nucleotide positions in *Puya*. Thus, based on these data and outgroup rooting of the AFLP dataset indicating relationships not seen with other plastid or nuclear genes in previous studies, we only included *Puya* for AFLP analysis in this study.

Rooting the core Andean *Puya* phylogenetic tree using the Chilean clade is also complicated by ancient plastid introgression that means that almost all taxa from Chile group with the 'Chilean *Puya*' clade in the plastid gene tree, generating incongruence between the plastid DNA and PHYC nuclear gene trees (Jabaily & Sytsma, 2010). The AFLP topology places taxa from Chile in two clades, corresponding in composition to the 'Yellow *Puya*' and 'Blue *Puya*' clades identified through analysis of the low-copy nuclear region PHYC (Jabaily & Sytsma, 2010). The 'Blue *Puya*' clade was identified as the sister to all other *Puya* in this former analysis based on nuclear DNA. Issues of gene tree/species tree discordance are potentially avoided by analysing hundreds of AFLP fragments (Giannasi, Thorpe & Malhotra, 2001), which effectively span the nuclear genome (Althoff *et al.*, 2007). Given the primary focus of the AFLP analyses presented here on non-Chilean Andean *Puya*, the 'Blue *Puya*' clade was used as the functional outgroup (the sister group to all other *Puya*) for all subsequent analyses.

PHYLOGENETIC ANALYSES

The resultant AFLP presence/absence matrix was analysed using distance and Bayesian inference. Neighbour-joining (NJ) trees were calculated in PAUP*4.0b10 (Swofford, 2002) using the Nei–Li distance matrix, minimum evolution, NJ start tree and TBR branch swapping. A consensus network was constructed using NeighborNet (Bryant & Moulton, 2004) to visually assess non-bifurcating events and conflicting phylogenetic signal. Nei–Li distances (Nei & Li, 1979) were generated and analysed in the program SplitsTree4, version 4.12.4 (Huson & Bryant, 2006).

Genetic distance methods are often employed for analysis of AFLP and other restriction-site data, but converting all the data into pairwise distance measurements may cause a loss of information and uncertainty in the topology is not conveyed. We thus implement model-based methods that provide topologies with branch lengths that can be used

in BayesTraits (Pagel, Meade & Barker, 2004) for subsequent character analyses. Likelihood-based methods for analysing AFLP data include a simple binary data/restriction site model implemented in MrBayes 3.1 (Ronquist & Huelsenbeck, 2003) and the more sophisticated model specifically designed for AFLP data of Luo, Hipp & Larget (2007). This newer model takes into account the length of restriction sites and the possibility of substitutions or indels in the interior of fragments. Unfortunately, the method is currently computationally costly and analysis of a dataset this size was computationally intractable (B. Larget, pers. comm.). The model of restriction site evolution implemented in MrBayes, although perhaps overly simplistic in its assumptions of restriction site gain and loss, approximates the gain and loss of fragments. The dataset was analysed with two independent runs in MrBayes using the prior settings Dirichlet (2.65, 1.00) (lset coding = noabsencesites) and an MCMC run of 120 000 000 generations, samplefreq = 5000 and the default setting to discard the first 25% of runs as burn-in. Convergence and stationarity of the MrBayes analysis were determined by evaluating the standard deviation of split frequency values at the end of the run, a plot of the generation vs. log likelihood values and the potential scale reduction factor convergence diagnostic.

For subsequent biogeographicaql analyses in which bifurcating trees were required, the program Summary Tree Explorer (Derthick, 2008) was used to generate a priority-rule consensus tree from 2000 randomly chosen trees from the post burn-in MrBayes output. The priority-rule consensus tree allows for clades with < 50 posterior probability if not in conflict with other clades.

HISTORICAL BIOGEOGRAPHY

The geographical distributions of taxa included in the AFLP dataset were scored as present or absent in three different categories of geographical space: (1) discrete Andean cordilleras *sensu* Simpson (1975) – Coastal, Principal, Pampean, Oriental, Occidental, Western Colombian Andes, Central Colombian Andes, Eastern Colombian Andes; (2) broad latitudinal belts; and (3) broad elevational belts. The latter two categories were analysed as three- and two-state area codings. For scoring of broad latitudinal belts, the break between northern and central/southern Andes was placed at the Western Andean Portal/Rio Marañon Valley/Amotape-Huancabamba deflection zone (hereafter WAP, following Antonelli *et al.*, 2009), located between 3 and 5°S. For the two-state latitude analysis, species were coded as present north or south of the WAP. For the three-state latitude analysis, species were coded as north of the WAP, between the

WAP and the Tropic of Capricorn or south of the Tropic of Capricorn. For the two-state elevational analysis, taxa were scored as low-elevation if found below 3000 m and high elevation if found above 3000 m. For the three-state coding, taxa found at 0–1500 m were coded as low elevation, taxa at 1500–3000 m as mid elevation and taxa at $\geq$ 3000 m as high elevation.

The three sets of discrete biogeographical characters were mapped onto the priority-rule Bayesian inference phylogeny using several methods. First, ancestral state was reconstructed under the maximum parsimony (MP) criterion using the program MESQUITE (Maddison & Maddison, 2008). All possible ancestral reconstructions were examined. Character homoplasy was assessed with the consistency and retention indexes (CI, RI; Felsenstein, 1978).

Second, we employed ancestral state reconstruction using the MultiState program implemented in BayesTraits ver. 1.0 (Pagel *et al.*, 2004). Although phylogenetic analyses based on AFLP data typically only involve assessment of topology and support, AFLP branch length information was used by Whittall & Hodges (2007) to model character evolution in *Aquilegia* L. More recently, Kropf *et al.* (2009) documented a linear relationship between the degree of AFLP divergence and time of isolation in three unrelated species of alpine plants and advocated the use of an AFLP-based clock for absolute dating. We explored the AFLP trees using more explicit model-based approaches offered in BayesTraits. For these analyses 2000 rooted phylogenies chosen at random from the post-burnin MrBayes analyses were used and the data were first optimized under a maximum-likelihood (ML) framework to find the parameters and likelihood scores in order to inform the subsequent Bayesian inference analysis. This analysis was run in a reverse-jump MCMC framework with rate coefficients drawn from an exponential (0–10) hyperprior distribution. The transition rate parameter (ratedev) was adjusted until the acceptance values averaged 20–40%, as recommended (Pagel & Meade, 2006). The number of generations and priors were adjusted to minimize differences between the average log likelihood and the log likelihood from the initial ML run, minimize change across runs in the harmonic mean and have an appropriate average ratedev. Each analysis was run twice with 10 000 000 iterations per run, the first 2500 000 discarded as burn-in and trees were sampled every 1000 iterations. Outputs from the two runs were combined and the average probabilities of the character states for each character were determined for each of the analysed ancestral nodes. Likelihood ratio tests were performed to assess the significance of transition rate values and test various

hypotheses of character evolution (Pagel & Meade, 2006). Analyses were conducted in a likelihood framework and the average log-likelihood value was compared between analyses using the likelihood ratio test statistic.

Third, we utilized the program S-DIVA (Yu, Harris & He, 2010) using just the Andean cordilleran dataset to reconstruct past dispersal and vicariance events in *Puya* while incorporating uncertainty in the underlying phylogeny. The Andean cordillera dataset was optimized across the same 2000 randomly chosen phylogenies analysed in BayesTraits. Constraining ancestral areas to two, three or four cordilleras was explored, as well as the impact of restricting ancestral areas to cordilleras that are currently adjacent.

In addition to these three approaches to reconstruct the biogeographical history of species diversification in *Puya*, we also assessed the degree of correlated elevational and latitudinal transitions across the phylogeny of *Puya* using binary characters for both elevation and latitude. The degree of potential covariance of changes in the two-state elevation and latitude datasets was assessed in a phylogenetic framework using the Discrete program in BayesTraits (Pagel & Meade, 2006). This program evaluates two models, the first in which elevation and latitude evolve independently on the tree. This creates two rate coefficients per trait or four rate coefficients that must be estimated. The second model allows the traits to evolve in a correlated fashion such that the rate of change in one trait depends on the background state of the other. The dependent model has four states, one for each combination of the two binary traits or eight rate coefficients that must be estimated. A likelihood ratio test with four degrees of freedom was used to determine if a dependent or independent model of character evolution was favoured by the data.

LIFE-HISTORY EVOLUTION

We also examined life-history transitions across the *Puya* phylogeny. Three life-history types (Table 3) were scored for all accessions of *Puya*: 0 = iteroparous, 1 = semi-semelparous and 2 = semelparous. Transitions between any pair of states were allowed. This character was analysed in BayesTraits MultiState using the same subset of phylogenies and parameters as for biogeographical data.

RESULTS

PATTERNS OF DISTRIBUTION IN LATITUDE AND ELEVATION

Puya spp. are found throughout the Andes from sea level to > 4500 m, at latitudes from 40°S to 10°N, but species diversity is not evenly distributed across these geographical axes (Figs 2, 3). The greatest numbers of species are found at mid elevations and mid latitudes, and the majority of these have relatively restricted latitudinal and elevational ranges. Low-elevation species (1500 m and below) are most common at the southernmost latitudes, but are found to a limited extent scattered across all latitudes, and these species tend to have wider latitudinal ranges than high-elevation species. High-elevation species (3000 m and above) are rare south of the Tropic of Capricorn. Species found north of the Equator tend to inhabit high elevations and have smaller latitudinal and elevational ranges, with the exception of the widespread *P. floccosa* E.Morren ex Baker. Nearly one-third of the species are known only from single (type) localities. Species numbers increase with elevation, with > 40 species found between 2600 and 3300 m, followed by a decline in species number at higher elevations. The highest numbers of species are found at several central Andean latitudes (9°S, 17°S), with species number decreasing both north of the equator and south of the Tropic of Capricorn. When distribution across latitudinal space was depicted as distance from the equator (e.g. the number of species found at 5°N and 5°S were added together to give the number of species 5° from the equator), the increase in species number towards the equator is roughly linear (Fig. 3). A standard R^2 linear regression found a moderately good fit of the data to a linear model ($R^2 = 0.725$).

PHYLOGENETIC ANALYSES

Both distance and Bayesian inference methods were used to analyse the 885 AFLP fragments generated. Fragments generated from independent rounds of laboratory work from the same DNA samples were nearly identical. The resultant topologies from the two analyses were highly congruent, as was the overall topology of the NeighborNet analysis (Fig. 4). In this latter analysis, taxa resolve into four major clusters, corresponding to the clades 'Central & Northern Andes', 'Central & Southern Andes', 'Zygomorphic' and 'Blue *Puya*'. *Puya aequatorialis* André and *P. atra* L.B.Sm. were placed in an intermediate position relative to the two largest clusters.

In the Bayesian inference phylogeny (Fig. 5), rooted with the functional outgroup 'Blue *Puya*' (Chilean species with blue flowers), a well-supported [posterior probability (PP) 97] clade comprising the widespread *P. ferruginea* (Ruiz & Pav.) L.B.Sm. and the narrow endemic *P. mima* L.B.Sm. & Read is placed as sister to the remainder of the genus. These two species both have large zygomorphic flowers (Fig. 1A). The remaining *Puya* spp. are placed in two main well-supported sister clades: a Central & Northern Andes clade (PP 96) and a Central & Southern Andes clade

A
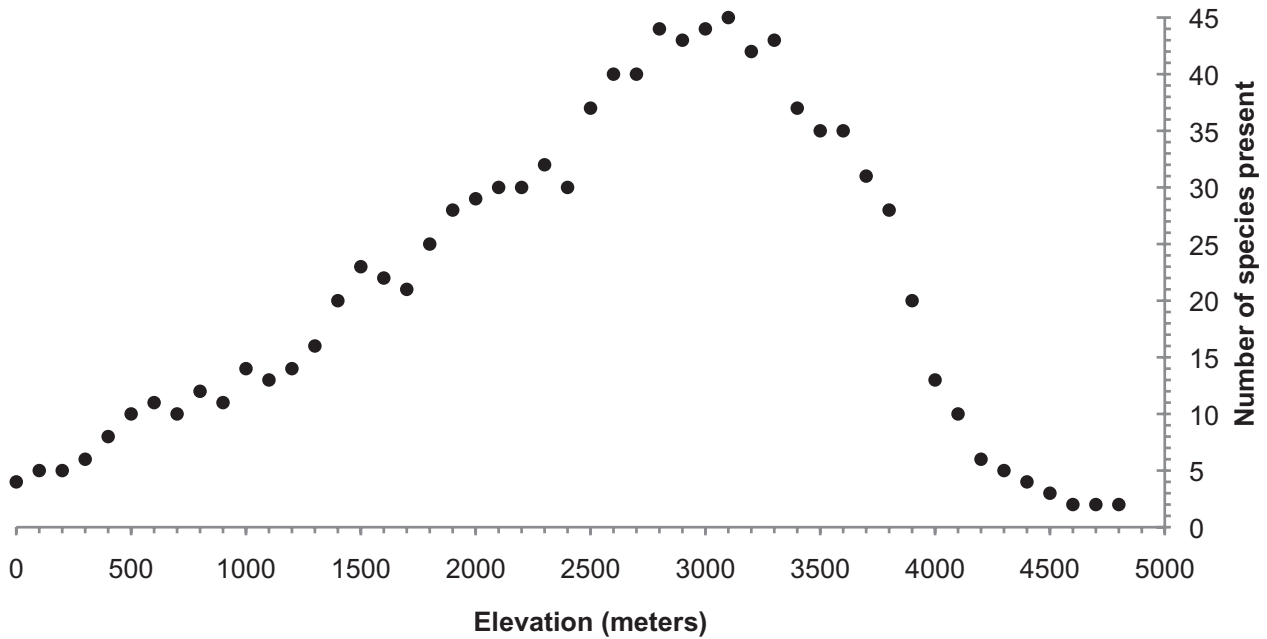
B
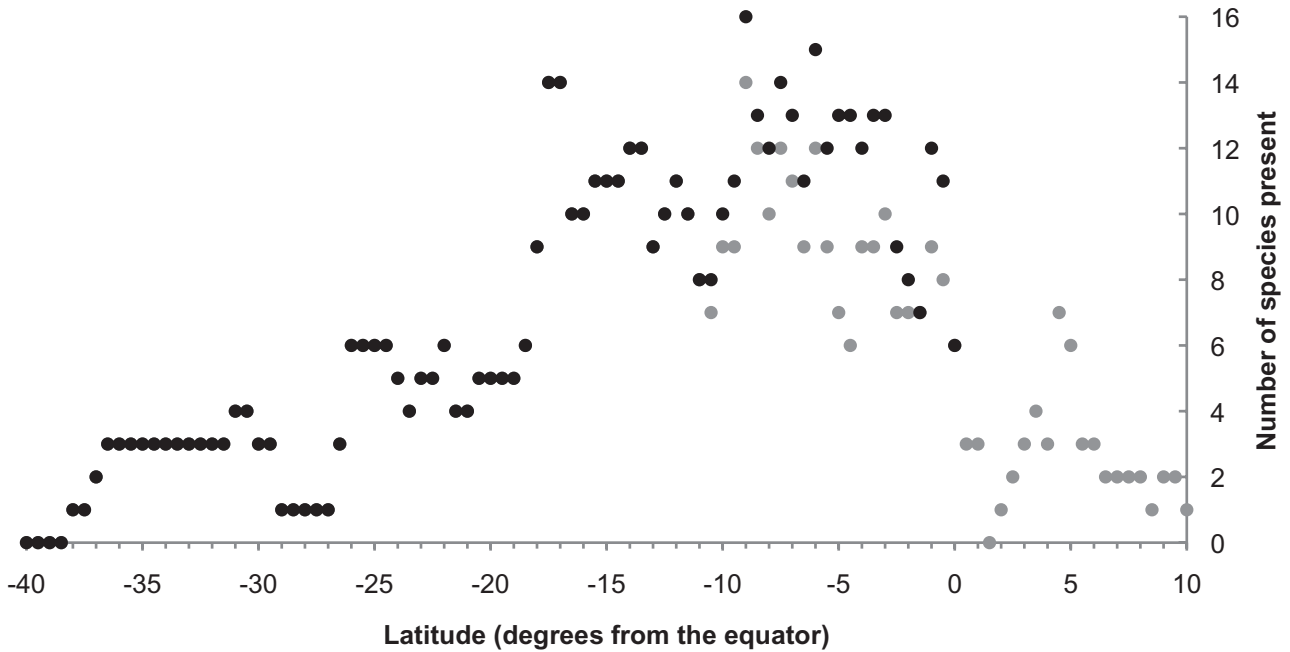
Figure 3. Numbers of species of *Puya* with ranges at A, each 100 m of elevation and B, each 0.5 degree of latitude. Negative values are south of the equator, positive north of the equator. Black dots correspond to presence by latitude from the equator irrespective of hemisphere, and are therefore additive from −10 to 0; grey dots correspond to latitudinal position in both hemispheres.

(PP 100). *Puya atra* L.B.Sm from central Bolivia is sister to the remainder of a Central & Northern Andes clade (PP 98), which form two subclades. The 'Yellow *Puya*' clade (Chilean taxa with yellow flowers) is nested in a well-supported (PP 100), primarily high-elevation clade from both the Cordilleras Occidental and Oriental composed of *P. yakespala* A.Cast., *P. herrerae* Harms, *P. angusta* L.B.Sm., *P. weberbau-*
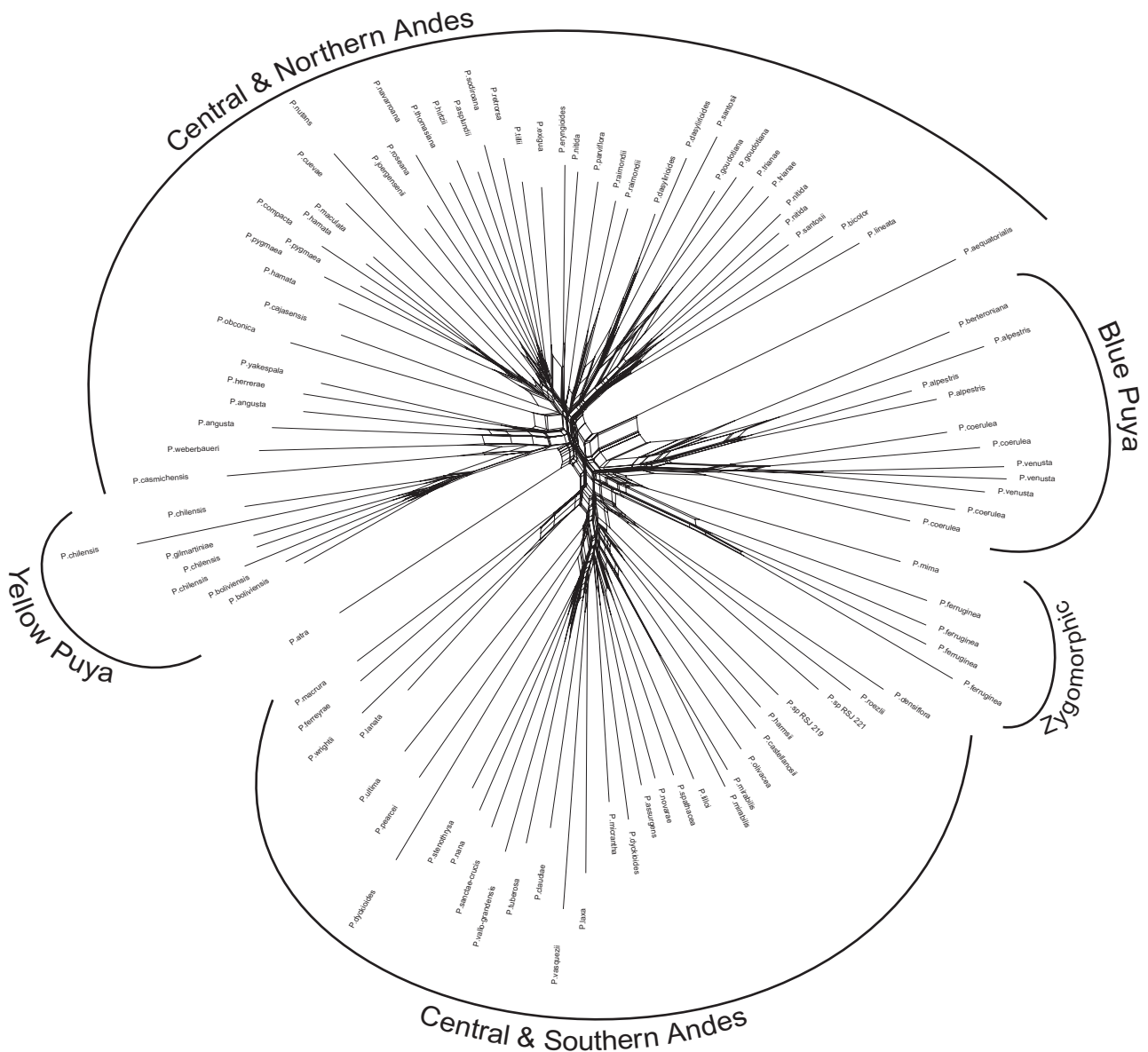
Figure 4. Phylogenetic network of 98 *Puya* accessions from 75 species based on NeighborNet analysis of Nei–Li distances generated from 885 AFLP fragments.

eri Mez and *P. casmichensis* L.B.Sm. In 'Yellow *Puya*', *P. boliviensis* Baker is sister to *P. chilensis* Molina and *P. gilmartiniae* G.S.Varad. & A.R.Flores, which form a monophyletic clade (PP 100). The other poorly supported (PP 73) subclade of Central & Northern Andes places *P. raimondii* Harms and *P. parviflora* L.B.Sm stepwise as sister to all taxa from the Northern Andes (PP 96). Phylogenetic support is high (PP 100) for the clade comprising Costa Rican *P. dasylirioides* Standl. and species from the Eastern Cordillera of Colombia: *P. goudotiana* Mez, *P. trianae* Baker, *P. nitida* Mez, *P. santosii* Cuatrec., *P. lineata* Mez and *P. bicolor* Mez. Support is lower (PP 68) for the sister clade of pri-

marily Ecuadorian species from the Western and Central Colombia Cordilleras found in similar higher and lower elevation habitats. *Puya parviflora* L.B.Sm. and *P. cajasensis* Manzan. & W.Till from Ecuador are not part of this monophyletic Ecuadorian clade, but the positions of these taxa lack support. In the Ecuadorian clade, relationships between species from mid elevations (e.g. *P. tillii* Manzan., *P. roseana* L.B.Sm., *P. retrorsa* Gilmartin) are poorly defined, in contrast to a well-supported (PP 100) lineage of high-elevation taxa (*P. hamata* L.B.Sm., *P. maculata* L.B.Sm., *P. compacta* L.B.Sm., *P. pygmaea* L.B.Sm. and *P. cuevae* Manzan. & W.Till).
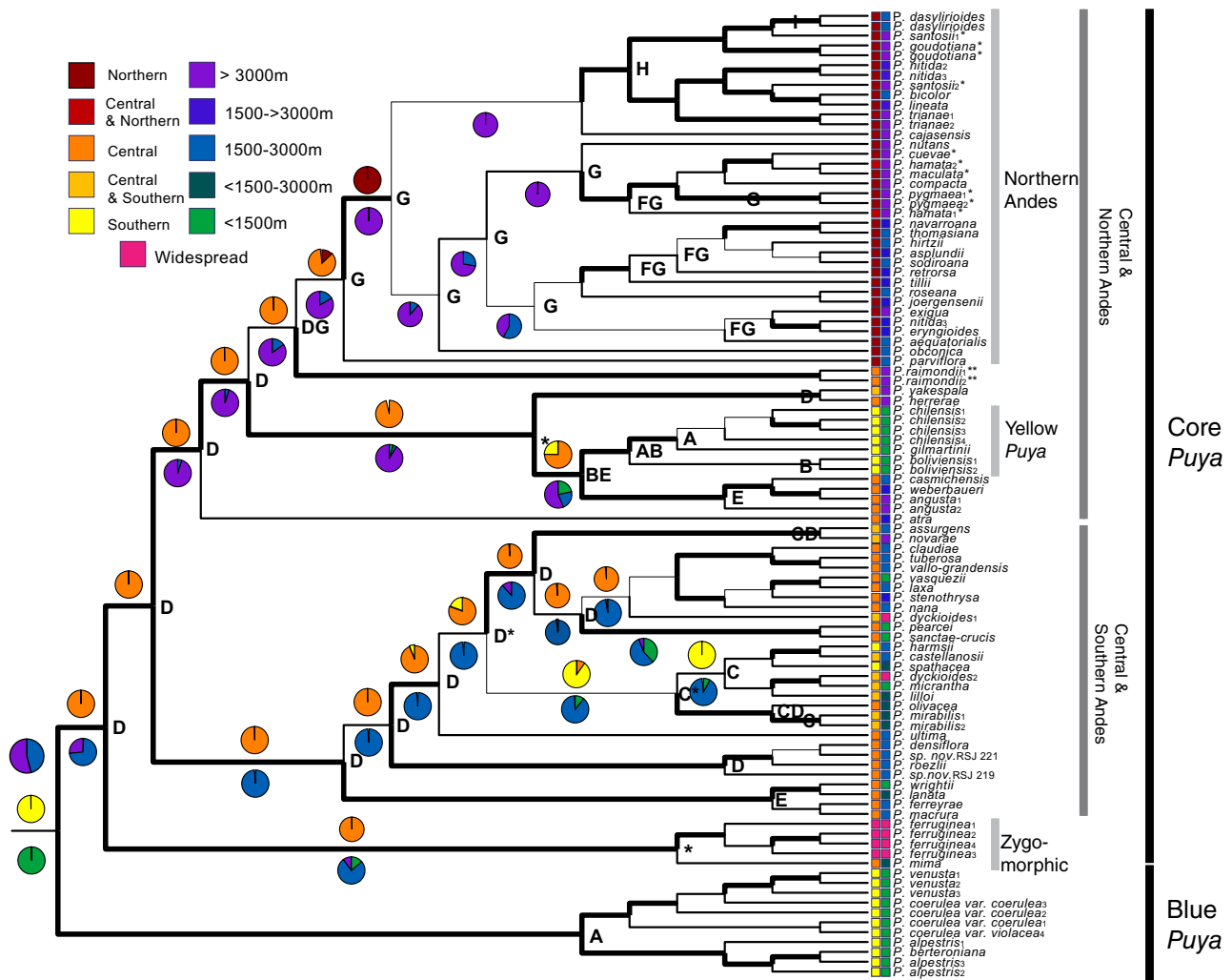
Figure 5. Priority rule consensus phylogeny of *Puya*. Branch thickness corresponds to phylogenetic support: thickest lines represent posterior probability (PP) of 80 and above, medium lines represent PP of 60–79 and thin represent less than 60 PP. Life-history types are denoted as **semelparous, *semi-semelparous. Coloured boxes next to terminal names and pie charts above and below branches represent latitude and elevational zones. Pie charts represent the ancestral latitudinal and elevational zones as percentages of probability determined in BayesTraits. Letters at nodes correspond to ancestral area cordillera (*sensu* Simpson, 1975) as determined in S-DIVA. A, Coastal; B, Principal; C, Pampean; D, Oriental; E, Occidental; F, Western Cordillera of Colombia; G, Central Cordillera of Colombia; H, Eastern Cordillera of Colombia; I, Cordillera Talamanca of Costa Rica. An asterisk at nodes indicates widespread ancestor (more than two ancestral areas). For clarity, pie charts and ancestral areas were not placed tip-ward if all resultant clades from a node had the same value.

The backbone of the second major clade, Central & Southern Andes, is generally less resolved, but strong support is found for several smaller clades. A clade of species with simple inflorescences from the Cordillera Occidental of the central Andes (*P. macrura* Mez, *P. ferreyrae* L.B.Sm., *P. lanata* Schult.f and *P. wrightii* L.B.Sm.) is well supported (PP 93) as sister to the remaining Central & Southern Andes clade. Several undescribed taxa from Apurimac, Peru, were placed in a clade with *P. roezlii* E.Morren and *P. densiflora*

Harms. Well-supported subclades tend to include species found in close geographical proximity (e.g. *P. harmsii* A.Cast., *P. castellanosii* L.B.Sm., *P. spathacea* Mez, *P. lilloi* A.Cast. and *P.micrantha* Mez from the Pampean range of north-western Argentina; *P. claudiae* Ibisch, R.Vásquez & E.Gross, *P. tuberosa* Mez, *P. vallo-grandensis* Rauh, *P. vasquezii* Ibisch & E.Gross, *P. laxa* L.B.Sm., *P. stenothrysa* Mez and *P. nana* Wittm. from the Cordillera Oriental of central Bolivia).

Table 1. Parsimony reconstruction of the distribution of *Puya* spp. coded as different discrete characters

Characters coded	Tree-steps	Consistency index	Retention index
Andean Cordilleras	19	0.368	0.810
Latitude (two-state)	1	1.000	1.000
Elevation (two-state)	14	0.071	0.567
Latitude (three-state)	8	0.250	0.891
Elevation (three-state)	16	0.125	0.661

ANCESTRAL STATE RECONSTRUCTION OF BIOGEOGRAPHICAL CHARACTERS

Dispersal/vicariance analysis (Fig. 5) recovered the Cordillera Oriental as the ancestral area for the deepest ancestral nodes in the Andes. Multiple dispersals into the Cordillera Occidental and the Pampean Ranges were found. *Puya ferruginea* and *P. boliviensis* independently dispersed into the Cordillera Principal and Atacama Desert region. All analyses determined a single dispersal event across the Western Andean Portal into the Northern Andes, with no subsequent transitions back south. Subsequently, taxa in the Eastern Cordillera of Colombia were segregated from those of the Western and Central Cordilleras. Transitions between the Western and Central Cordilleras were frequent. The Cordillera de Talamanca of Costa Rica was colonized by the ancestor of *P. dasylirioides* from within the Eastern Cordillera.

Range shifts between elevational zones were much more frequent and less consistent in direction than transitions between latitudinal zones (Tables 1, 2). Likewise, there is more uncertainty surrounding the ancestral states (Fig. 5) and a greater magnitude of character state transitions in general in elevation compared with latitude (Tables 1, 2). Multiple transitions into both high- and low-elevation zones from mid-elevational zones occur in both the Central & Southern Andes and the Central & Northern Andes clades. BayesDiscrete did not favour a model of correlated evolution between latitude and elevation states over character independence ($P = 0.1$, $\chi^2 = 7.92$, d.f. = 4). The independent model found that evolutionary transitions between elevational zones, regardless of latitude, were more frequent than transitions in latitude (Fig. 6).

EVOLUTION OF LIFE-HISTORY VARIABILITY

Field observations clarified three life-history categories within *Puya* (Fig. 5, Table 3). All individuals of *P. raimondii* surveyed were composed of a single rosette, and are deemed semelparous (Fig. 1C). Most

Table 2. Relative transition rates (q) of *Puya* spp. in latitude (1–6) and elevation (7–12) and significance of selected hypotheses

	qxz = Relative transition rate (q) from x to y	q values calculated from data	Hypothesis testing proposals	Likelihood ratio test
1	$q02$	0.047	$q02 = q20$	0.076
2	$q01$	0.061	$q01 = q10$	1.315
3	$q20$	0.092	$q21 = 0$	**15.416**
4	$q10$	0.435	$q12 = q21$	0.066
5	$q12$	0.480	$q20, q02 = 0$	0.076
6	$q21$	0.482	$q01 = 0$	0.0008
7	$q02$	0.262	$q02 = q20$	0.397
8	$q01$	0.858	$q01 = q10$	0.466
9	$q20$	0.246	$q21 = 0$	**20.023**
10	$q10$	1.028	$q12 = q21$	**6.322**
11	$q12$	0.886	$q20, q02 = 0$	0.362
12	$q21$	2.078	$q01 = 0$	0.118

Characters are 0 = north of WAP, 1 = WAP to Tropic of Capricorn, 2 = south of Tropic of Capricorn; 0 = < 1500 m, 1 = 1500–3000 m, 2 = > 3000 m; bold type indicates significant at $P = 0.001$.
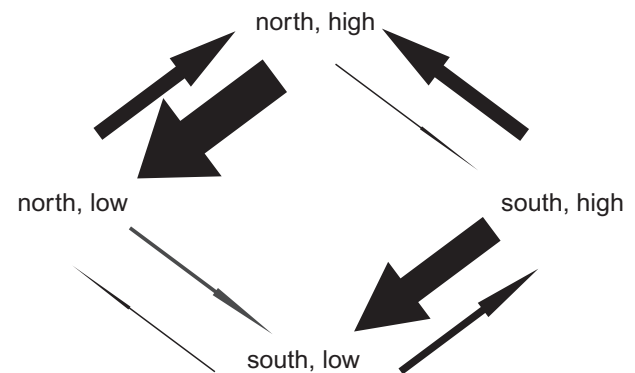
Figure 6. Relative frequencies of evolutionary transitions between latitudinal and elevational zones of *Puya* lineages. For latitude, 'north' denotes north of the Western Andean Portal and 'south' denotes south of the Western Andean Portal. For elevation, 'low' indicates less than 3000 m, and 'high' indicates 3000 m and above. Arrow thickness is proportional to relative rate of transition calculated under the independent model of BayesTraits Discrete.

other *Puya* spp. observed in the field were found to be iteroparous, with mature individuals composed of multiple, attached rosettes by the age of inflorescence production (Fig. 1D). Iteroparous species are composed of several to thousands of interconnected rosettes by the age of sexual reproduction. An intermediate category, 'semi-semelparous', is used to describe several species with attached rosette pups

Table 3. Life-history categories, example species and descriptions for *Puya*

Category	Definition	Elevational range	Habitat description	Inflorescence	Indument density
Semelparous, e.g. *Puya raimondii*	All individuals composed of a single rosette at all points in the life cycle, and never produce vegetative pup rosettes				
Semi-semelparous, e.g. *Puya hamata, P. goudotiana, P. clava-herculis, P. santosii, P. pygmaea*	Some individuals in a population produce vegetative pup rosettes before or after inflorescence production but others do not	High	Primarily wet; often in rich, waterlogged soil; terrain can be flat	Densely strobiliform/ highly conspicuous	Ample
Iteroparous, e.g. *Puya trianae, P. chilensis, P. dyckioides, P. ferruginea*	All flowering individuals composed of multiple interconnected rosettes	Low to high	Primarily dry; often on steep and rocky slopes	Lax/less conspicuous	Minimal

occurring in only a subset of reproductive-aged individuals in a population. Exemplar semi-semelparous species *P. goudotiana*, *P. santosii* and *P. hamata* are all relatively tall (1.5–*c*. 3.0 m including the inflorescence) and found commonly in high-elevation wet páramo habitats in Colombia and Ecuador.

BayesMultistate recovered the ancestral life-history state of *Puya* as iteroparous with 90% probability. Semelparous *P. raimondii* is placed as sister to a clade containing all of the sampled semi-semelparous taxa and many iteroparous taxa from the northern Andes (Fig. 5). In the northern Andean clade, semi-semelparity apparently evolved independently several times.

DISCUSSION

AFLP data were employed to produce a well-resolved expanded phylogenetic tree for *Puya* that corroborates and enhances the emerging picture of evolution of the genus from nuclear and plastid DNA sequences and previous AFLP analysis (Jabaily & Sytsma, 2010; Schulte *et al*., 2010; Givnish *et al*., 2011). Despite lack of resolution due to low sequence variation in Andean 'Core *Puya*', Jabaily & Sytsma (2010) found some evidence for major clades corresponding to broad geographical areas. The results presented here offer an even more detailed but similar picture in terms of broad geographical structure across the phylogeny.

THE ROLE OF HYBRIDIZATION IN THE EVOLUTION OF *PUYA*

Schulte *et al*. (2010) sampled extensively throughout the distribution of the seven Chilean *Puya* spp. and used AFLP data to investigate the prevalence of inter-specific hybridization. Hybridization at various levels was noted between and putatively within the phylogenetically well-defined major groups of Chilean *Puya* ('*chilensis*': *P. chilensis*, *P. gilmartiniae*, *P. boliviensis*; '*alpestris*': *P. alpestris*, *P. berteroniana* Mez; '*coerulea*': *P. venusta* Phil. in Baker and *P. coerulea* Miers and associated varieties), particularly where species occur in sympatry. The NeighborNet analysis presented here resolves the same three major Chilean groups, and suggests greater frequency of non-bifurcation events in these groups than between other clusters of species from elsewhere in the Andes. This could indicate that hybridization is particularly common among the Chilean *Puya* spp. or could be an artefact of the sparser taxon and within-species sampling in the Andes compared with Chile in this study.

Reproductive isolating factors among Chilean *Puya* spp. are apparently limited, in line with weak pre- and post-zygotic barriers across Bromeliaceae (Wendt *et al*., 2001, 2008). Multiple species in the presented

phylogeny were recovered as non-monophyletic (e.g. *P. santosii*, *P. dyckioides* Mez), which could be indicative of a putative hybrid origin of these accessions, incomplete lineage sorting or species delimitation problems. Population-level sampling of widespread, morphologically variable and/or putatively non-monophyletic species would be necessary to investigate the extent of introgression in populations of *Puya* in the Andes. In one such study, interpopulation AFLP analysis of *Puya raimondii* found markedly low levels of polymorphism and high levels of inbreeding (Sgorbati *et al.*, 2004), indicating that introgression may not be ubiquitous in *Puya*. The nearest relatives of most of the species included in the present study often occur in close geographical proximity. As with Chilean *Puya* (Schulte *et al.*, 2010), geographical isolation at a local scale, such as species endemic to different elevational zones on the same mountain range, may be the predominant prezygotic reproductive barrier among the Andean *Puya* spp. In plant communities of the páramos, e.g. in Colombia, *P. goudotiana*, *P. trianae* Baker, *P. santosii* and *P. nitida* occur in true sympatry.

Incongruence between maternally inherited and biparentally inherited gene trees in earlier phylogenetic analyses of Jabaily & Sytsma (2010) and morphological and ecological evidence corroborate the study of Schulte *et al.* (2010) suggesting that several ancient interspecific hybridization events were probably involved in formation of the seven extant Chilean *Puya* spp., including the origin of the '*alpestris*' group as potential homoploid hybrid species (polyploidy is not known in *Puya* and is very rare in Bromeliaceae). These events were discerned by analyzing the discordance between phylogenies derived from maternal and biparental loci, in combination with morphological and ecological information. Homoploid hybrid speciation is often mediated by strong ecological selection and spatial segregation (Rieseberg & Willis, 2007). These conditions are probably common across elevational gradients in narrow inter-Andean valleys, where unique biotic communities occur at different elevations and aspects in close spatial proximity, and may well have been factors in the early hybrid-mediated evolutionary events in *Puya*.

HISTORICAL BIOGEOGRAPHY SCENARIO FOR *PUYA*

The Bromeliaceae-wide time-calibrated plastid DNA phylogeny of Givnish *et al.* (2011) included eight *Puya* spp. and estimated the divergence time of *Puya* from its sister clade, Bromelioideae, at 10.1 Mya (range for 100 random trees 8.37–12.64 Mya), with the crown radiation of extant taxa in the Andes estimated at 3.5 Mya, and in Chile at 2.5 Mya. These estimates,

based on the best available data in this rapidly evolving group with low levels of molecular evolution, place the origin of the major clades and extant *Puya* spp. in the timeframe of the final uplift of the Andes and subsequent Pleistocene glaciation cycles. The AFLP phylogenetic tree presented here and distribution patterns of most taxa further suggest that both late Neogene and early Quaternary geological timeframes and associated processes were important in shaping the evolution of the group. The combination of ancestral state reconstructions, elevational and latitudinal distributions, and the expanded phylogeny of *Puya* provide four key pieces of evidence about the evolutionary history of *Puya*: (1) the monophyletic northern Andean lineage is derived from within a broad central Andean clade, with no transitions back across the Western Andean Portal; (2) subsequent cladogenesis between adjacent cordilleras and different elevational zones were common and multidirectional; (3) the number of *Puya* spp. increases towards the equator, but fewer *Puya* spp. are found at and especially north of the equator; and (4) the greatest number of species and most narrow endemics are found at mid-elevations, above the moist forest and below the high-elevation habitats.

These results suggest that *Puya* originated in central Chile, where many early branching lineages of the sister subfamily Bromelioideae are also endemic, along with the Chilean clade that is sister to the rest of the primarily Andean 'Core *Puya*' clade. Early divergence of the major clades of Bromelioideae and the two major clades of *Puya* is indicated by the short branch lengths in the analyses of Givnish *et al.* (2011) and Jabaily & Sytsma (2010). Species from south of the Tropic of Capricorn including *Puya* of Chile and the lowlands of Argentina generally have much broader elevational and latitudinal ranges than those from tropical latitudes, with the number of narrow endemics generally increasing north towards the equator (Fig. 2). Temperate latitudes and associated high seasonality may also represent the limits of the climatic niche for *Puya*, with few species known from higher elevations at temperate latitudes in the Andes. However, newly discovered species from high-elevation habitats in western Argentina indicate that further fieldwork is needed to establish the range limits of the genus with greater certainty (Aráoz & Grau, 2008; Gómez Romero & Grau, 2009).

The Andean orogeny proceeded generally from south to north and the broad biogeographical pattern of *Puya* cladogenesis, also from south to north, also reflects this overall progression of Andean uplift. The movement of *Puya* into the Cordillera Oriental, whether via dispersal as suggested by S-DIVA or via the vicariant process of mountain uplift, effectively segregated the Chilean lineage of *Puya* from the

Andean (before the later secondary reintroduction of the 'Yellow *Puya*' lineage back into Chile, as discussed by Jabaily & Sytsma, 2010). Major mountain uplift events were punctuated by long periods of relative stability, with the various cordilleras at half the average and maximum height between 23 and 11 Mya and an additional uplift of over 2000 m occurring later in the Quaternary (Graham, 2010). Common vegetation types in the emerging Andes prior to the late Miocene include lowland rainforest and montane broadleaved forest (Graham, 2010), which today are generally devoid of *Puya*, a lineage generally intolerant of extensive shading. Seasonally dry tropical forests have been present since before the putative time of the *Puya* crown radiation (Pennington *et al.*, 2010; Särkinen *et al.*, 2011) and today many *Puya* spp. of mid to lower elevations are endemic to steep valleys where these forests dominate and rocky outcrops are common. Thus, suitable dry habitats lacking dense tree cover may have been present for *Puya* spp. to invade the Andes, with extensive cladogenesis hypothesized to have occurred in the Cordillera Oriental before *Puya* moved into adjacent ranges to the north.

Puya apparently traversed the WAP zone from the central Andes only once, with no subsequent transitions back south. The WAP presented a potentially important barrier to many groups of organisms (Vuilleumier, 1969; Duellman, 1979; Ayers, 1999; Andersson, 2006; Antonelli *et al.*, 2009; Cosacov *et al.*, 2009) and is an area exhibiting high endemism for many other groups (Berry, 1982; Weigend, 2002; Smith & Baum, 2006). The inland incursion of the ocean at the WAP receded with the uplift of the Andes, and was gone by the mid-Miocene, suggesting a dispersal rather than vicariant explanation for the distribution of extant *Puya*, given current divergence time estimates for the group. The WAP region today houses a large number of narrowly endemic *Puya* spp., both to the north and to the south, with just two widespread species (*P. hamata*, *P. lanata*) present on both sides, presumably the results of subsequent dispersal events across the WAP.

The northern Andes (particularly Colombia and Venezuela) generally have fewer *Puya* spp. than similar habitats in the central Andes, perhaps indicative of the relatively recent colonization of these younger parts of the Andes or the smaller physical area of high-elevation or dry inter-Andean habitats suitable for *Puya* compared with the more extensive Cordilleras Occidental and Oriental south of the WAP. One of the most prominent patterns in global biogeography is increased species richness towards the equator (Wiens & Donoghue, 2004; Weir, 2006; Mittelbach *et al.*, 2007). The number of *Puya* spp. increases roughly linearly from temperate latitudes towards the equator, but fewer *Puya* spp. are found at and especially north of the equator than would be predicted by a linear model. The actual latitudinal zone with the greatest number of species is not at the equator, but is rather from central Peru into central Bolivia, where most species are found in inter-Andean valleys. This region with highest diversity of *Puya* is also the same for Andean *Solanum* L. (Knapp, 2002). The northernmost *Puya* lineages are thus postulated to be some of the most recently derived.

The evolutionary progression of *Puya* along the Andes was most probably primarily, but not unidirectionally, from south to north. The lower-elevation Pampean region of Argentina was colonized multiple times from neighbouring regions of the Cordillera Oriental. Once these central Andean derived lineages moved into lower elevations on the eastern slope of the Andes, there were no subsequent transitions in elevation or latitude, with the probable caveat that the recently discovered high-elevation taxa (Gómez Romero & Grau, 2009) were not sampled. This may suggest that these are also more recently colonized areas, or alternatively that these lowland chaco habitats mark the ecological limits of where the lineage can live.

ALLOPATRIC SPECIATION DRIVEN BY PLEISTOCENE GLACIAL CYCLES

The apparently frequent evolutionary transitions of *Puya* both up and down in elevation, indicated today by the occurrences of closely related species at different elevations in the same latitudinal zone, and frequent transitions between adjacent cordilleras (e.g. Western and Central Colombian Cordilleras, Fig. 5) provide possible evidence for speciation via a glacial 'pump' during the Pleistocene. Once the major lineages of *Puya* were in place throughout the central and northern Andes, glacially driven cyclical fragmentation of populations could have been a driver of allopatric speciation in *Puya*. Multiple glacial cycles in the tropical Andes occurred during the Pleistocene, causing tropical and montane forests to move down by as much as 1200–1500 m during glacial maxima and move up in elevation during interglacial periods (van der Hammen, 1974). Cooler ecosystems such as the puna and páramo are postulated to have expanded and contracted (Haffer & Prance, 2001). *Puya* spp. have relatively poor seed dispersal capabilities and relatively long life spans (Benzing, 2000), suggesting that populations may not have been as mobile to track a given climate envelope during the Pleistocene glacial cycles. If populations were subsequently isolated, adaptation to the regional temperature and moisture regimes of the valley or mountain range where the lineages remained may have followed,

leading to allopatric speciation. *Puya* is the only bromeliad genus to have evolved CAM photosynthesis multiple times (Martin, 1994; Benzing, 2000; Crayn, Winter & Smith, 2004; Givnish *et al.*, 2007) and thus was adapted to a wide variety of both xeric and mesic habitats in the Neotropics. A more precisely scaled molecular dated phylogeny of the group beyond that of Givnish *et al.* (2011) would be crucial to testing the putative timing suggested by this scenario, as the crown diversification of many species-rich South American plants and animals has been determined to have occurred prior to the onset of Pleistocene glaciation cycles (Hoorn *et al.*, 2010).

The greatest number of species in *Puya* is found at mid elevations (2600–3300 m) in the central Andes. This mid-elevational species diversity 'hump' fits with broader global trends in species richness with elevation (Rahbek, 1995; Kluge, Kessler & Dunn, 2006; Lomolino, Riddle & Brown, 2006). Colwell & Hurtt (1994) postulated that mid-elevations should have the highest species richness in a given group because these elevations are the maximum distance from the 'hard boundaries' imposed by elevation-dependent environmental factors at both the upper and the lower limits for a group of organisms. A lower elevational boundary for most *Puya* may be the dominance of various kinds of Neotropical forest, particularly on the eastern slope of the Andes as lower elevation species are frequent along the western slope in seasonally dry valleys and matorral habitats. At the upper elevational boundary, the diurnally harsh high-elevation páramo and puna habitats present unique challenges that only a limited number of *Puya* lineages have been able to overcome. Expansion of ecological niche modelling to Andean *Puya* (currently only available for Chilean taxa; Zizka *et al.*, 2009) would allow for more explicit identification of ecological factors that shape current distributional patterns and more detailed biogeographical scenarios to be hypothesized (Nakazato, Warren & Moyle, 2010).

Many groups of Andean organisms show similar phylogenetic and biogeographical patterns to *Puya* and evolved in the same late Cenozoic time period. Major systematic divisions in Andean groups often correspond to major latitudinal divisions (e.g. northern Andes/central Andes; Ezcurra, 2002; Smith & Baum, 2006; Amico, Vidal-Russell & Nickrent, 2007). More recently diverged species are often found in the geologically younger northern Andean cordilleras and older lineages are found further south, corresponding to the south-to-north pattern of Andean uplift (Moritz *et al.*, 2000; Emshwiller, 2002; Torres-Carvajal, 2007; Soejima *et al.*, 2008; Cosacov *et al.*, 2009; Simpson *et al.*, 2009). Widespread lowland taxa have given rise repeatedly to localized, high-elevation groups (Simpson, 1979; Emshwiller, 2002; Hall, 2005; Fjeldsa

& Rahbek, 2006; Brumfield & Edwards, 2007; Ribas *et al.*, 2007; Bonaccorso, 2009), although in some cases mid to high elevations can be the source for lowland taxa (Elias *et al.*, 2009). Uplift of the Andes and subsequent climate change, whether increased aridity or shifting vegetation belts in response to glaciation, were major events in *Heliotropium* L. (Luebert *et al.*, 2011), *Chuquiraga* Juss. (Ezcurra, 2002), *Lepechinia* Willd. (Drew & Sytsma, 2012) and many others.

LIFE-HISTORY CLASSIFICATION IN *PUYA*

Field observations corroborate clear differences in the production of vegetative ('pup') rosettes among *Puya* spp. (most species do, and few species do not) and this life-history trait is apparently fixed within species. The majority of species are iteroparous, readily producing pups before and after production of inflorescences. Although each individual rosette produces a single terminal inflorescence (hapaxanthic), sympodially branched hapaxanthic plants are considered to be iteroparous (Young & Auspurger, 1991; Benzing, 2000). Iteroparous *Puya* spp. grow in the coastal matorral of central Chile, steep cliff-faces of inter-Andean valleys and in sympatry with semi-semelparous and semelparous species in high-elevation páramo and puna habitats (Fig. 1A). Individuals within a species can vary greatly in the number of rosettes that make up their body at reproductive age and in overall plant size (Augspurger, 1985) and iteroparous species differ greatly in habitat and broad morphology, including inflorescence types (simple, compound, strobiliform etc.).

Non-iteroparous species are few, with only *P. raimondii* from the high-elevation puna of Bolivia and Peru being apparently entirely semelparous. Individuals appear not to produce pup rosettes before or after production of the terminal inflorescence and total senescence follows seed dispersal (Fig. 5C). Semi-semelparous species from high-elevation páramo habitats have a similar, but not as extreme, life history (e.g. *P. hamata* and *P. goudotiana*) with some, but not all, individuals producing pup rosettes before and/or after production of the terminal inflorescence; clonal individuals are generally composed of many fewer rosettes than related iteroparous species. The current phylogenetic analysis suggests that non-iteroparity has evolved multiple times, with no apparent trend of semi-semelparity as an intermediate step leading to true semelparity. More complete taxon sampling, including *P. weberiana* E.Morren ex Mez and *P. bravoi* Aráoz & A.Grau, recently identified as monocarpic in the new treatment of *Puya* in Argentina (Gómez Romero & Grau, 2009), is needed to provide new insight into the evolution of this curious

life-history strategy. Similarity in overall morphology and habitat preferences amongst non-iteroparous *Puya* spp. suggests that life-history type may be predicted for other taxa (Table 3).

Semelparity has convergently evolved in many long-lived, rosette-forming taxa from tropical montane ecosystems, including several Hawaiian *Argyroxiphium* DC (Robichaux *et al.*, 1990), *Ruilopezia* Cuatrec. (Cuatrecasas, 1968), some species of *Espeletia* Nutt. (Berry & Calvo, 1989) and *Lobelia telekii* Schweinf. ex Engl. from east Africa (Young, 1984). The repeated derivation of non-iteroparity in *Puya* is similar to patterns observed within *Aeonium* Webb & Berthel. (Jorgensen & Olesen, 2001), but differs from the single derivation in *Agave* L. (Good-Avila *et al.*, 2006) and island-dwelling *Echium* L. (Böhle, Hilger & Martin, 1996). Non-iteroparity is relatively rare in *Puya*, as it is in *Yucca* L. (Schaffer & Schaffer, 1977; Huxman & Loik, 1997), and may represent a more local adaptation to harsh conditions in high-elevation habitats, rather than a key innovation prompting diversification, as found for monocarpy in the genera *Agave* and *Furcraea* Vent. (Good-Avila *et al.*, 2006).

Puya raimondii is one of the most striking and well-studied species of *Puya* (Sgorbati *et al.*, 2004) and one of the most wide-ranging species latitudinally, but is restricted to high-elevation puna habitats. Importantly, it is the largest and most likely the longest lived bromeliad species, with heights to > 12 m and age estimates of flowering individuals of 60–100 years (Hartman, 1981; Hornung-Leoni & Sosa, 2004). Semi-semelparous high-elevation *P. hamata* and *P. goudotiana* from the northern Andes páramos are the next tallest *Puya* spp. and can reach heights of > 5 m, taking many decades to reach maturity (Smith & Downs, 1974; Manzanares, 2005). Delayed reproduction, slow growth and massive inflorescences are the hallmarks of semelparity in long-lived rosette plants, which incur trade-offs between increased resource allocation in current fecundity at the expensive of future, subsequent fecundity (Young & Auspurger, 1991).

Evolution of semelparous life history has been explained by two different models: (1) the reproductive effort model and (2) the demographic model. The reproductive effort or 'big bang' model of evolution of semelparity predicts that increasing rewards of greater fecundity for each additional investment of resources in reproduction will drive the evolution of semelparity (Schaffer & Schaffer, 1977). For example, if the number of pollinators is relatively low compared with the number of flowers, pollinators may select for larger floral displays to optimize foraging. For semelparous *P. raimondii*, the sheer size of the inflorescence, often with > 100 000 flowers and copious nectar, attracts many species of hummingbirds and passerine birds (Hornung-Leoni, Sosa & Lopez, 2007) lending anecdotal support to this theory, as do the relatively large size of semi-semelparous *P. hamata* and *P. goudotiana* inflorescences. In many other plant groups, larger inflorescences and a significantly shorter post-flowering half-life are found in semelparous species than in closely related iteroparous species (Young, 1984; Rocha, Valera & Eguiarte, 2005).

The demographic or bet-hedging model (reviewed by Young, 1990) predicts that semelparity should be favoured in habitats where climates are harsh and future reproduction is less likely or infrequent. In other plant groups, including the giant bromeliad *Alcantarea* Harms (Barbará *et al.*, 2009), iteroparous species are found primarily at low to mid elevations, and semelparous species are found in more arid or harsh high elevations (Young, 1984; Good-Avila *et al.*, 2006). In support of this model in *Puya*, all non-iteroparous species are restricted to high elevations in the Andes in habitats that experience strong diurnal temperature fluctuations, ice crystal formation, solifluction and intense solar radiation (Balslev & Luteyn, 1992), factors that limit seedling establishment. Miller & Silander (1991) reported that seedlings of 'monocarpic' *P. clava-herculis* Mex & Sodiro in the Ecuadorian páramos almost exclusively establish next to grass tussocks that protect the seedling from the elements and help to prevent solifluction.

Other giant rosette plants of high-elevation tropical montane ecosystems branch less frequently and in turn produce a greater number of leaves per rosette that serve to insulate the meristem from freezing nocturnal temperatures (Monasterio, 1986). The rosettes of *P. raimondii* and other non-iteroparous taxa appear to be composed of a much greater number of leaves than iteroparous relatives (Fig. 1), which may serve to insulate the meristem. The leaves of high-elevation *Puya* are typically glabrous or only sparsely pubescent, with more direct insulation from the dense lanate hairs that cover the inflorescence axes, bracts and sepals (Fig. 1B) providing more direct insulation to regulate the temperature of the ovaries and promote seed set (Miller, 1986). Numerous leaves may also be beneficial during fires because marcescent leaves at the rosette base protect the meristem from fire, and also serve to elevate the apical meristem higher above the ground (Givnish, McDiarmid & Buck, 1986). As with *Espeletia* in the northern Andes (Cuatrecasas, 1968), mature *P. raimondii* rosettes with blackened lower leaves are commonly seen in the high puna, although human-caused fires of the inflorescence are a major conservation concern (Hornung-Leoni & Sosa, 2004; Sgorbati *et al.*, 2004).

FUTURE AREAS OF RESEARCH

Phylogenetic analysis of many Andean plant groups is rapidly progressing, allowing for broad patterns across the massive geographical space and geological time to be deciphered. With next-generation sequencing methodologies becoming more commonplace in species-level systematics (Egan, Schlueter & Spooner, 2012; Fay, 2012), well-resolved and robustly supported phylogenetic trees will become available for species-rich high-elevation Andean clades such as *Puya*. The next iteration of phylogenetic analysis in *Puya* will need to be based on larger DNA sequence data sets and should target additional species from high-elevation habitats with different life histories. This will allow for rigorous time-calibration of the *Puya* phylogeny using modern molecular dating approaches (which are currently nascent for AFLP datasets; Kropf *et al*., 2009) and enable the incorporation of *Puya* into meta-analyses of the biota of the Andes (Hoorn *et al*., 2010; Antonelli & Sanmartín, 2011; Rull, 2011; Särkinen *et al*., 2011; Sklenář *et al*., 2011), further enhancing our understanding of the importance of geological events in the Cenozoic. Denser taxon sampling is needed to corroborate the emerging historical biogeographical narrative.

More extensive field and herbarium data are also needed to fully develop these ideas in a comparative phylogenetic framework. Precise species distributions are often not known for Andean plant taxa because of the paucity of collections from many remote locations and difficulties with species delimitation in evolutionary young lineages. *Puya* is certainly not without these issues, and the distribution patterns discussed here reflect the current incomplete state of collecting efforts and taxonomy in the various Andean regions (Betancur & Callejas, 1997; Manzanares, 2005; Gómez Romero & Grau, 2009; Cano Flores & Jabaily, 2010).

Many collectors of *Puya* specimens (including types) did not record information on key life-history traits, which cannot be scored from the specimens themselves. Furthermore, life-history traits were not recorded for the vast majority of *Puya* species descriptions or ecological observations with the notable exception of Monasterio (1980), Laegaard (1992), Manzanares (2005) and Gómez Romero & Grau (2009). Beyond *P. raimondii*, however, caution is urged in assigning other *Puya* to the semelparous or monocarpic life-history category. Additional field observations, ecological data and detailed demographic surveys of populations for species with differing life-history strategies are needed, particularly in high-elevation Andean habitats. Hopefully, these efforts will encourage careful observation and further studies of life history in the field in the primarily Neotropical Bromeliaceae and other groups.

Beyond assessing taxonomic strategies and determining biogeographical history, phylogenies of Andean taxa should be used as a framework in which to develop and test evolutionary hypotheses. For example, pinpointing the exact relationship between iteroparous and non-iteroparous *Puya* spp. living in sympatry in northern Andean páramos would help shape an evolutionary ecological study of the energetic trade-offs in life-history evolution. This phylogenetic information would be coupled with intensive demographic surveys of the populations, as the bet-hedging model predicts that the demography of low adult survivorship, long periods between reproductive events and early senescence would tend to evolve semelparity. Multi-year demographic information is only available for *Puya dasylirioides* of Costa Rica (Augspurger, 1985) and much more long-term population monitoring effort should be expanded to other species.

Additional field studies of life-history evolution in the Equatorial Andes are a high priority for future work because of the potential implication for conservation. The long life spans and reliance upon seed production for perpetuation make non-iteroparous taxa specifically vulnerable to grazing, fire and climate change pressures, predicted to increase in tropical alpine habitats (Balslev & Luteyn, 1992; Williams, Jackson & Kutzbach, 2007). The revision of Manzanares (2005) is notable for considering life-history status when assigning IUCN conservation status to Ecuadorian *Puya*. When choosing what to prioritize for study in a mega-diverse area such as the tropical Andes, taxa at greatest risk for extinction because of their distribution or evolutionary history should be given immediate attention.

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APPENDIX 1

Voucher information and localities for specimens used in AFLP analysis.

Puya aequatorialis André, RSJ 097 (QCNE) Ibarra, Ecuador. ***Puya alpestris*** Poepp., 1. RSJ 007 (WIS) Huntington Bot. Gar. USA; 2. RSJ 177 (WIS) Constitution, Chile; 3. RSJ 174 (WIS) Curacavi, Chile. ***Puya***

angusta L.B.Sm., 1. RSJ 226 (WIS) Ancash, Peru; 2. RSJ 230 (WIS) Ancash, Peru. *Puya asplundii* L.B.Sm., RSJ 098 (QCNE) Cotacachi, Ecuador. *Puya assurgens* L.B.Sm., RSJ 026 (WIS) Huntington Bot. Gar., USA. *Puya atra* L.B.Sm., RSJ 974 (WIS) Comarapa, Bolivia. *Puya berteroniana* Mez, RSJ 168 (WIS) Fray Jorge, Chile. *Puya bicolor* Mez, RSJ 202 (COL) Villa de Leyva, Colombia. *Puya boliviensis* Baker 1. M. Rosas s.n. (WIS) Chile; 2. D. Stanton s.n. (WIS) Chile. *Puya cajasensis* Manzan. & Till, RSJ 128 (QCNE) Cajas, Ecuador. *Puya casmichensis* L.B.Sm., RSJ 223 (WIS) Otuzco, Peru. *Puya castellanosii* L.B.Sm., RSJ 148 (WIS) Lago Brealito, Argentina. *Puya chilensis* Molina, 1. RSJ 164 (WIS) Zapallar, Chile; 2. RSJ 171 (WIS) La Serena, Chile; 3. RSJ 172 (WIS) Mineral de Talca, Chile; 4. RSJ 170 (WIS) Totoralillo, Chile. *Puya claudiae* Ibisch, Vásquez & E. Gross, RSJ 065 (WIS) Samaipata, Bolivia. *Puya coerulea var. coerulea* Miers, RSJ 085 (WIS) Huntington Bot. Gard., USA; 2. RSJ 175 (WIS) Cauquenes, Chile; 3. RSJ 176 (WIS) Constitution, Chile. **var. violacea,** 4. RSJ 057 (WIS) Huntington Bot. Gard, USA**.** *Puya compacta* L.B.Sm., RSJ 129 (QCNE) Cajas, Ecuador. *Puya cuevae* Manzan. & Till, RSJ 110 (QCNE) Cerro Toledo, Ecuador. *Puya dasylirioides* Standl. F 2141915; B. Berger s.n. (WIS) Cerro de la Muerte, Costa Rica. *Puya densiflora* Harms, RSJ 213 (WIS) Cusco, Peru. *Puya dyckioides* Mez, 1. RSJ 067 (WIS) Samaipata, Bolivia; 2. RSJ 150 (WIS) Salta, Argentina. *Puya eryngioides* André, RSJ 114 (QCNE) Podocarpus, Ecuador. *Puya exigua* Mez, RSJ 134 (QCNE) Matanga, Ecuador. *Puya ferreyrae* L.B.Sm., RSJ 222 (WIS) Trujillo, Peru. *Puya ferruginea* (Ruiz & Pav.) L.B.Sm., 1. RSJ 059 (WIS) Huntington Bot. Gard.; 2. RSJ 209 (WIS) C'orao, Peru; 3. RSJ 210 (WIS) Pisac, Peru; 4. RSJ 214 (WIS) Tres Cruces, Peru; *Puya gilmartiniae* G.S.Varad. & A.R.Flores. *Puya goudotiana* Mez, RSJ 182 (COL) Cruz Verde, Colombia; RSJ 207 (COL), Colombia. *Puya hamata* L.B.Sm., 1. RSJ 090 (QCNE) El Angel, Ecuador; 2. RSJ 122 (QCNE) Fierro Urcu, Ecuador. *Puya harmsii* (A.Cast.)A.Cast., RSJ 145 (WIS) Tafi del Valle, Argentina. *Puya herrerae* Harms, RSJ 212 (WIS) Urubamba, Peru. *Puya hirtzii* Manzan. & Till, RSJ 096 (WIS) Buenos Aires, Ecuador. *Puya lanata* Schult., RSJ 105 (QCNE) Catamayo, Ecuador. *Puya laxa* L.B.Sm., RSJ 190 (WIS) Comarapa, Bolivia. *Puya lilloi* A.Cast., Till B134 (WU). *Puya lineata* Mez, RSJ 180 (COL) Cruz Verde, Colombia. *Puya macrura* Mez, RSJ 230 (WIS) Caraz, Peru. *Puya*

maculata L.B.Sm., RSJ 120 (QCNE) Fierro Urcu, Ecuador. *Puya micrantha* Mez, RSJ 151 (WIS), Salta, Argentina. *Puya mima* L.B.Sm. & Read, RSJ 228 (WIS) Caraz, Peru. *Puya mirabilis* (Mez) L.B.Sm., 1. RSJ 153 (WIS) Salta, Argentina; 2. RSJ 161 (WIS) La Candelaria, Argentina. *Puya nana* Wittm., RSJ 062 (WIS) El Fuerte, Bolivia. *Puya navarroana* Manzan. & Till, RSJ 137 (QCNE) Matanga, Ecuador. *Puya nitida* Mez, 1. RSJ 112 (QCNE) Podocarpus, Ecuador; 2. RSJ 179 (COL) Tablaso, Colombia; 3. RSJ 206 (COL) Chingaza, Colombia. *Puya novarae* G.S.Varad. ex Gómez Rom. & A. Grau, RSJ 156 (WIS) Santa Victoria, Argentina. *Puya nutans* L.B.Sm., RSJ 133 (QCNE) Matanga, Ecuador. *Puya obconica* L.B.Sm., RSJ 106 (QCNE) Cerro Toledo, Ecuador. *Puya olivacea* Wittm., RSJ 068 (WIS) El Portal, Bolivia. *Puya parviflora* L.B.Sm., RSJ 103 (WIS) Catamayo, Ecuador. *Puya pearcei* Mez, RSJ 038 (WIS) Huntington Bot. Gard., USA. *Puya pygmaea* L.B.Sm., 1. RSJ 121 (QCNE) Fierro Urcu, Ecuador; 2. RSJ 135 (QCNE) Matanga, Ecuador. *Puya raimondii* Harms, 1. RSJ 048 (WIS) Huntington Bot. Gar. USA; 2. RSJ 230 (WIS) Ancash, Peru. *Puya roezlii* E.Morr., RSJ 220 (WIS) Abancay, Peru. *Puya roseana* L.B.Sm., RSJ 115 (QCNE) Saraguro, Ecuador. *Puya sanctae-crucis* (Baker)L.B.Sm., RSJ 060 (WIS) Santa Cruz, Bolivia. *Puya santosii* Cuatrec., 1. RSJ 186 (COL) Laguna Verde, Colombia; 2. RSJ 194 (COL), Colombia. *Puya sodiroana* Mez, RSJ 100 (QCNE) Calacali, Ecuador. *Puya* **sp. nov., RSJ** 221 (WIS) Abancay, Peru. *Puya* **sp. nov., RSJ** 219 (WIS) Cunyac, Peru. *Puya spathacea* Mez, RSJ 163 (WIS) Cordoba, Argentina. *Puya stenothyrsa* Mez, RSJ 073 (WIS) Comarapa, Bolivia. *Puya thomasiana* André, RSJ 104 (QCNE) Catamayo, Ecuador. *Puya tillii* Manzan., RSJ 143 (QCNE) Tandapi, Ecuador. *Puya trianae* Baker, 1. RSJ 183 (COL) Laguna Verde, Colombia; 2. RSJ 192 (COL) Villa de Leyva, Colombia. *Puya tuberosa* Mez, RSJ 063 (WIS) El Fuerte, Bolivia. *Puya ultima* L.B.Sm., RSJ 051 (WIS) Huntington Bot. Gard., USA. *Puya vallo-grandensis* Rauh. RSJ 070 (WIS) Vallegrande, Bolivia. *Puya vasquezii* Ibisch & Gross R. Vasquez s.n. (USZ). *Puya venusta* Phil., 1. RSJ 006 (WIS), Huntington Bot. Gard., USA; 2. RSJ 165 (WIS) Valparaiso, Chile; 3. RSJ 166 (WIS) Coquimbo, Chile. *Puya weberbaueri* Mez, RSJ 217 (WIS) Aguas Calientes, Peru. *Puya wrightii* L.B.Sm., RSJ 039 (WIS). Huntington Bot. Gard., USA. *Puya yakespala* Castallanos, RSJ 157 (WIS) Santa Victoria, Argentina.