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Phylogeny of Bromelioideae (Bromeliaceae) inferred from nuclear and plastid DNA loci reveals the evolution of the tank habit within the subfamily

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ABSTRACT

Phylogenetic relationships within subfamily Bromelioideae (Bromeliaceae, Poales) were inferred using DNA sequence data from the low-copy nuclear gene phosphoribulokinase (PRK) and five plastid loci (*mat*K gene, 3′*trn*K intron, *trn*L intron, *trn*L-*trn*F spacer, *atp*B-*rbc*L spacer). The PRK dataset exhibited a considerably higher proportion of potentially informative characters than the plastid dataset (16.9% vs. 3.1%), leading to a higher resolution and improved nodal support of the resulting phylogenies. *Bromelia* is resolved as sister to the remainder of the subfamily, albeit this relationship receives only weak nodal support. The basal position of *Bromelia*, as well as *Deinacanthon*, *Greigia*, *Ochagavia*, *Fascicularia* and *Fernseea* within the subfamily is corroborated and the remainder of the subfamily forms a highly supported clade (the eu-bromelioids). By the inclusion of nuclear data the sister group position of *Fernseea* to the eu-bromelioids is now highly supported. Within the eu-bromelioids the resolution of the clade representing the more advanced core bromelioids has increased and further demonstrates the highly problematic generic concept of *Aechmea* as well as *Quesnelia*.

Moreover, the data were used to examine the evolution of sepal symmetry and the tank habit. Tracing of character transitions onto the molecular phylogeny implies that both characters have undergone only few transitions within the subfamily and thus are not as homoplasious as previously assumed. The character state reconstruction reveals the great importance of the evolution of the tank habit for the diversification of the core bromelioids.

1. Introduction

Repeated invasion of related lineages of organisms into similar environments, as for example those characterized by extreme drought and nutrient deficiency, can result in parallel as well as convergent evolution. The multiple and independent origin of similar features within closely related lineages is one of the important challenges in taxonomy, as it renders the recognition of phylogenetic relationships among those lineages difficult. In Bromelioideae similar abiotic and biotic pressures of the colonized habitats in the different regions of tropical and subtropical America have been postulated as one possible reason for the considerable degree of homoplasies displayed by the subfamily, as for example in inflorescence and flower morphology (Faria et al., 2004; Schulte and Zizka, 2008).

The Bromelioideae, with 32 genera and approx. 800 species (Smith and Till, 1998; Luther 2006), are one of eight subfamilies currently recognized within Bromeliaceae (Poales) (Givnish et al. 2007) and display striking ecological versatility, occupying a wide range of terrestrial, lithophytic and epiphytic habitats. They are distributed throughout tropical and subtropical America, with a centre of diversity in southeastern Brazil, particularly the Atlantic rain forest (Smith and Downs, 1979). Whereas the monophyly of Bromelioideae is strongly supported by both, morphological and molecular data (e.g. Terry et al., 1997; Crayn et al., 2004; Givnish et al., 2004, 2007; Schulte et al., 2005), the inter- and infrageneric relationships of the subfamily are the most poorly understood within the family (Benzing, 2000; Brown and Leme, 2000). The generic delimitation within the subfamily is regarded as especially problematic because morphological characters often prove to be homoplastic and hence fail to delimit natural groups and because several genera are defined by unique combinations of characters, rather than by traditional synapomorphies. The arising problems are especially evident in the *Aechmea* alliance (e.g. Faria et al., 2004; Horres et al., 2007; de Oliveira et al., 2007; Schulte and Zizka, 2008). Furthermore, potentially useful characters are often inaccessible in herbarium material and thus their variability is hardly understood. Frequent changes of generic limits within Bromelioideae reflect the considerable uncertainties concerning the taxo-

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nomic value of morphological characters (e.g. Smith and Kress, 1989, 1990; Read and Baensch, 1994; Brown and Leme, 2005; Betancur and Salinas, 2006; de Sousa and Wendt, 2008). Furthermore, since the last comprehensive monograph of the subfamily (Smith and Downs, 1979) the number of described species has increased by more than one third (Luther, 2006), and a modern taxonomic revision is urgently needed.

Several molecular studies employing different plastid markers have dealt with the phylogenetic relationships of Bromelioideae (e.g. Terry et al., 1997; Horres et al., 2000, 2007; Givnish et al., 2004, 2007; Barfuss et al. 2005; Schulte et al., 2005; Schulte and Zizka, 2008). They consistently resolved *Puya* (Pitcairnioideae *s.l.*), a genus of terrestrial plants with a principally Andean distribution, as sister group to the Bromelioideae.

Due to an extraordinary low amount of sequence divergence within plastid DNA regions in Bromelioideae in general (Horres et al., 2000, 2007; Crayn et al., 2004; Schulte et al., 2005, Schulte and Zizka, 2008), resolution among bromelioid genera based on only one or two plastid regions has remained poor (e.g. Horres et al., 2000, 2007; Crayn et al., 2004). By the combination of several plastid regions, resolution was increased and several genera of putatively basal position (*Bromelia*, *Deinacanthon*, *Greigia*, *Ochagavia*/*Fascicularia* and *Fernseea*) were identified, but without resolving relationships between those genera. The remaining Bromelioideae formed a highly supported group with *Fernseea* as putative sister group, but this relationship received only weak bootstrap support. Among the former, the majority of genera (e.g. *Aechmea*, *Billbergia*, *Neoregelia*, *Nidularium*) formed a poorly resolved clade, representing the core bromelioids (Schulte et al., 2005; Schulte and Zizka, 2008).

Within the family a progression towards an increasing independence from water and nutrition supply from the soil is found in different evolutionary lines (e.g. Tietze, 1906; Pittendrigh, 1948; Benzing, 2000). Unique trichomes facilitating water uptake via the leaf surface, a tank habit allowing for water and nutrient capture in external reservoirs formed by the leaf sheaths, and the crassulacean acid metabolism (CAM), which diminishes water loss during photosynthesis, are regarded as key innovations that allow for the successful colonization of xeric and nutrition deficient environments by the family (Tietze, 1906; Pittendrigh, 1948; Medina, 1974; Crayn et al., 2004; Givnish et al., 1997; Givnish et al., 2007). Within the family different eco-morphological types can be discerned reflecting an increasing independence from the substrate (Tietze, 1906; Pittendrigh, 1948; Benzing, 2000) on the basis of the characteristics regarded as mainly responsible for this development (e.g. increasing differentiation of leaf trichomes, increasing importance of water uptake via leaf trichomes, formation of external water reservoirs, reduction of root system, photosynthetic mode: C3/CAM).

In all of the concepts the more primitive types are characterized by a well developed root system, reliance on water and nutrition uptake via soil roots and little or no external water storage capacity (every leaf sheath forms a distinct phytotelm). In the more advanced types the leaf trichomes become more important for water and nutrition uptake. The development of a central tank, formed by the leaf sheaths of the rosulate plant is seen as an important progression. The lack of a central tank and the complete reliance on the indumentum for the water and nutrient supply characterizes the most advanced type realized within the extreme atmospheric Tillandsioideae. Due to the poor phylogenetic resolution within Bromelioideae the lines of evolution of the eco-morphological types within the subfamily have remained unclear. The last comprehensive systematic treatment of Smith and Downs (1979) implies a high evolutionary lability of these types within the subfamily.

The lack of phylogenetically informative markers and the sole reliance on information from the plastid genome have been up to now principal weaknesses of phylogenetic research in Bromeliaceae. Independent attempts by a number of workers to use the nuclear ribosomal DNA-region ITS (internal transcribed spacer) have failed due to amplification difficulty and insufficient phylogenetic variability (Barfuss, unpublished data).

Low-copy nuclear loci provide some of the most variable and phylogenetically informative molecular markers available. They are especially advantageous in obtaining resolution among rapidly diversifying lineages or at low taxonomic levels, particularly where universal markers such as plastid and nuclear ribosomal DNA fail to resolve relationships due to low sequence variability (Sang, 2002; Mort and Crawford, 2004; Small et al., 2004). However, their scarce use in phylogenetic analysis is due to practical and theoretical complications, e.g. in obtaining the target regions, the differentiation between paralogous and orthologous regions and the presence of heterozygosity, which requires cloning (Sang, 2002; Mort and Crawford, 2004; Small et al., 2004). Low-copy nuclear regions have been employed with considerable success at a range of taxonomic levels, for example in Arecaceae (Lewis and Doyle, 2001, 2002; Norup et al., 2006; Gunn, 2004; Roncal et al., 2005; Thomas et al., 2006; Loo et al., 2006).

PRK is a low-copy nuclear gene that encodes phosphoribulokinase, a key regulatory enzyme of the Calvin cycle for photosynthetic carbon dioxide assimilation. Thus far its use in phylogenetic research has been restricted to the monocot family Arecaceae, where it was useful to resolve relationships at generic as well as species level (e.g. Loo et al., 2006; Norup et al., 2006; Thomas et al., 2006). Based on the promising results of these studies, PRK was considered potentially useful in the reconstruction of relationships within the Bromelioideae.

The goals of this study were (a) to explore the phylogenetic utility of PRK in resolving relationships within Bromelioideae, (b) to elucidate the intergeneric relationships within the subfamily by combining plastid and nuclear genetic markers, and (c) to examine character transformation patterns in key morphological features to discuss character evolution within Bromelioideae.

2. Materials and methods

2.1. Taxon sampling

In total, DNA sequences of 48 species from 24 genera were analyzed in the present study. Of subfamily Bromelioideae 43 species from 23 genera were sampled, representing all principal lineages within the subfamily according to previous molecular studies (Schulte et al., 2005; Schulte and Zizka, 2008). Within *Aechmea* 13 species were studied representing all seven subgenera (*Aechmea*, *Lamprococcus* (Beer) Baker, *Macrochordion* (de Vriese) Baker, *Ortgiesia* (Regel) Mez, *Platyaechmea* (Baker) Baker, *Podaechmea* Mez, and *Pothuava* (Baker) Baker) recognized by Smith and Till (1998). Five representatives of the genus *Puya* (Pitcairnioideae *s.l.*), consistently revealed as sister group of Bromelioideae by molecular studies (e.g. Givnish et al., 2004, 2007; Crayn et al., 2004; Schulte et al., 2005, Schulte and Zizka, 2008), were chosen as outgroup.

Sequences from one low-copy nuclear gene (phosphoribulokinase, PRK) and five plastid regions (*atp*B-*rbc*L spacer, *trn*L intron, *trn*L-*trn*F spacer, *mat*K gene, and part of the adjacent 3′*trn*K intron) were analyzed. The PRK sequence data were generated specifically for this study, and combined with cpDNA sequence data largely taken from our previous studies (see Table 1). Plant material was derived from the Palmengarten Frankfurt/Main, the Botanical Gardens of the Universities Heidelberg, Berlin-Dahlem, Kassel and from the Royal Botanic Gardens, Kew. Vouchers are deposited in one of the following herbaria: B, FR, FRP, HEID, K. Information on

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Table 1
Studied material. References: Ref. 1: this study; Ref. 2: Schulte and Zizka (2008); Ref. 3: Horres et al. (2000); Ref. 4: Horres et al. (2007); Ref. 5: Schulte et al. (2005). *Abbreviations:* B, Herbarium Berlin-Dahlem; BGB, Botanical Garden Berlin-Dahlem; GHB, Herbarium of the Botanical Garden Berlin-Dahlem; HEID, Botanical Garden and Herbarium of the University of Heidelberg; FR, Herbarium Senckenbergianum; FRP, Botanical Garden and Herbarium Palmengarten, Frankfurt; K, Herbarium of the Royal Botanic Gardens, Kew; KEW: Royal Botanic Gardens Kew.

Species	Accession no. living collection/ herbarium specimen	DNA-isolate no.	GenBank No./reference-No.					
			PRK	Clone (PRK)	*atp*B-*rbc*L spacer	*trn*L intron	*trn*L-*trn*F spacer	*mat*K, 3′*trn*K
Bromelioideae								
Acanthostachys strobilacea (Schult.f.) Klotzsch	FRP 98-16986-0/Horres 019 (FR)	H 019	EU780812/ Ref. 1	K4	EU219694/ Ref. 2	AF188765/ Ref. 3	DQ084606/ Ref. 4	AY950021/ Ref. 5
Aechmea calyculata E.Morren ex Baker	HEID 103296/Schulte 240203-9 (FR)	H 184			EU219713/ Ref. 2	DQ084674/ Ref. 4	DQ084593/ Ref. 4	AY950040/ Ref. 5
Aechmea calyculata E.Morren ex Baker	HEID 103296/Schulte 270404-6 (FR)	J 034	EU780818/ Ref. 1	K5				
Aechmea distichantha Lem.	FRP 88-16753-2/Zizka 1549 (FRP), Horres 008 (FR)	H 008	EU780823/ Ref. 1	K2	EU219714/ Ref. 2	DQ084643/ Ref. 3	DQ084579/ Ref. 4	AY950041/ Ref. 5
Aechmea drakeana André	FRP 98-16955-2/ Zizka 1100 (FRP)	H 042	EU780814/ Ref. 1	K8	EU219716/ Ref. 2	AF188772/ Ref. 3	DQ084588/ Ref. 4	AY950043/ Ref. 5
Aechmea farinosa (Regel) L.B.Sm.	FRP 98-16961-3/Zizka 1108 (FRP)	H 272	EU780820/ Ref. 1	K7	EU219704/ Ref. 2	DQ084677/ Ref. 4	DQ084586/ Ref. 4	AY950031/ Ref. 5
Aechmea filicaulis (Griseb.) Mez	FRP 98-16863-0/Horres & Schulte 180701-6 (FR)	H 248	EU780822/ Ref. 1	K1	EU219709/ Ref. 2	DQ084679/ Ref. 4	DQ084576/ Ref. 4	AY950036/ Ref. 5
Aechmea gracilis Lindm.	FRP 98-16949-3/Schulte 280203-1 (FR)	H 043	EU780816/ Ref. 1	K4	EU219711/ Ref. 2	DQ084682/ Ref. 4	DQ084594/ Ref. 4	AY950038/ Ref. 5
Aechmea kertesziae Reitz	FRP 98-16935-3/Zizka 1177 (FRP)	H 270	EU780817/ Ref. 1	K4	EU219712/ Ref. 2	DQ084683/ Ref. 4	DQ084595/ Ref. 4	AY950039/ Ref. 5
Aechmea lamarchei Mez	BG Berlin-Dahlem 118-37-74-86/11309 (GHB)	H 242	EU780824/ Ref. 1	K2	EU219717/ Ref. 2	DQ084684/ Ref. 4	DQ084590/ Ref. 4	AY950044/ Ref. 5
Aechmea lueddemanniana (K.Koch) Mez	FRP 95-14215-0/Schulte 100203-3 (FR); Schulte 010305-1 (FR)	H 150	EU780827/ Ref. 1	K1	EU219702/ Ref. 2	DQ084685/ Ref. 4	DQ084596/ Ref. 4	AY950029/ Ref. 5
Aechmea mertensii (G.Mey.) Schult.f.	FRP 98-16873-0/Zizka 1572 (FRP)	H 044	EU780821/ Ref. 1	K6	EU219708/ Ref. 2	DQ084686/ Ref. 4	DQ084575/ Ref. 4	AY950035/ Ref. 5
Aechmea mexicana Baker	HEID 104025/Schulte 240203-12 (FR); Schulte 171103-25 (FR)	H 256	EU780826/ Ref. 1		EU219701/ Ref. 2	DQ084688/ Ref. 4	DQ084597/ Ref. 4	AY950028/ Ref. 5
Aechmea pimenti-velosoi Reitz	FRP 98-16878-0/Schulte 230305-1 (FR)	J 013	EU780815/ Ref. 1	K1	EU780879/ Ref. 1	EU780855/ Ref. 1	EU780867/ Ref. 1	EU780843/ Ref. 1
Aechmea racinae L.B.Sm.	FRP 98-16934-3/Schulte 120203-1 (FR)	H 257	EU780819/ Ref. 1	K1	EU219703/ Ref. 2	DQ084691/ Ref. 4	DQ084583/ Ref. 4	AY950030/ Ref. 5
Ananas nanus (L.B.Sm.) L.B.Sm.	FRP s.n./Horres & Schulte 050401-9 (FR)	H 040	EU780810/ Ref. 1	K1	EU219727/ Ref. 2	DQ084695/ Ref. 4	DQ084573/ Ref. 4	AY950054/ Ref. 5
Ananas fritzmuelleri Carmago	KEW 1972-9/Chase 23823 (K)	KEW 23823	EU780811/ Ref. 1		EU780880/ Ref. 1	EU780856/ Ref. 1	EU780868/ Ref. 1	EU780844/ Ref. 1
Androlepis skinneri (K.Koch) Brongn. ex Houllet	FRP 97-16793-2/Schulte 140105-12 (FR)	H 048	EU780829/ Ref. 1	K3	EU219678/ Ref. 2	AF188780/ Ref. 3	DQ084610/ Ref. 4	AY950005/ Ref. 5
Araeococcus flagellifolius Harms	KAS s. n./Rex 260105-1 (FR)	K 9	EU780834/ Ref. 1	K1	EU219676/ Ref. 2	DQ084696/ Ref. 4	DQ084629/ Ref. 4	AY950003/ Ref. 5
Araeococcus goeldianus L.B.Sm.	FRP 99-18256-2/Schulte 100203-1 (FR)	H 206	EU780833/ Ref. 1		EU219675/ Ref. 2	DQ084697/ Ref. 4	DQ084630/ Ref. 4	AY950002/ Ref. 5
Bromelia serra Griseb.	FRP 98-17751-0/Horres 029 (FR)	H 029	EU780799/ Ref. 1	K2	EU219692/ Ref. 2	DQ084699/ Ref. 4	DQ084622/ Ref. 4	AY950019/ Ref. 5
Canistrum fosterianum L.B.Sm.	FRP 86-16991-3/Zizka 927 (FRP)	H 047	EU780840/ Ref. 1	K6	EU219697/ Ref. 2	AF188773/ Ref. 3	DQ084618/ Ref. 4	AY950024/ Ref. 5
Chevaliera cariocae (L.B.Sm.) L.B.Sm. & W.J. Kress	KEW 2001-1722/Chase 23820 (K)	KEW 23820	EU780825/ Ref. 1		EU780881/ Ref. 1	EU780857/ Ref. 1	EU780869/ Ref. 1	EU780845/ Ref. 1
Cryptanthus glaziovii Mez	HEID 102583/Schulte 010601-3 (FR)	H 215	EU780806/ Ref. 1		EU219683/ Ref. 2	DQ084701/ Ref. 4	DQ084635/ Ref. 4	AY950010/ Ref. 5
Deinacanthon urbanianum (Mez) Mez	FRP 98-17786-0/Horres 018 (FRP)	H 018	EU780800/ Ref. 1	K1	EU219690/ Ref. 2	AF188781/ Ref. 3	DQ084607/ Ref. 4	AY950017/ Ref. 5
Edmundoa lindenii (Regel) Leme	HEID 105009/Schulte 010601-4 (FR)	H 213	EU780831/ Ref. 1		EU219685/ Ref. 2	DQ084704/ Ref. 4	DQ084631/ Ref. 4	AY950012/ Ref. 5
Fascicularia bicolor (Ruiz & Pav.) Mez	FRP 98-16846-3/Zizka 1790 (FR)	H 006a	EU780802/ Ref. 1	K3	EU219696/ Ref. 2	AF188775/ Ref. 3	DQ084605/ Ref. 4	AY950023/ Ref. 5
Fernseea itatiaiae (Wawra) Baker	HEID 102174/Horres 067 (FR)	H 067b	EU780801/ Ref. 1	K9	EU219672/ Ref. 2	DQ084705/ Ref. 4	DQ084633/ Ref. 4	AY949999/ Ref. 5
Greigia spec. nov.	FRP 99-19040/Grant 19040 (FR)	H 157	EU780804/ Ref. 1		EU219687/ Ref. 2	DQ084710/ Ref. 4	DQ084601/ Ref. 4	AY950014/ Ref.1
Greigia mulfordii L.B.Sm.	-/Till 13090 (W)	H 111	EU780805/ Ref. 1		EU219689/ Ref. 2	DQ084709/ Ref. 3	DQ084600/ Ref. 3	AY950016/ Ref. 5
Hohenbergia eriostachia Mez	KEW 1972-1470/Chase 23818 (K)	KEW 23818	EU780841/ Ref. 1		EU780882/ Ref. 1	EU780858/ Ref. 1	EU780870/ Ref. 1	EU780846/ Ref. 1
Neoglaziovia variegata (Arruda) Mez	FRP 97-16794-3/ Zizka 1105 (FRP)	H 052	EU780813/ Ref. 1	K7	EU219724/ Ref. 2	AF188763/ Ref. 3	DQ084614/ Ref. 4	AY950051/ Ref. 5
Ochagavia elegans R.Phil.	FRP 98-16852-3/Horres 23a (FR)	H 23a	EU780803/ Ref. 1		EU219679/ Ref. 2	AF 188778/ Ref. 3	DQ084603/ Ref. 4	AY950006/ Ref. 5
Orthophytum disjunctum L.B.Sm.	KEW 1975-3132/Chase 23816 (K)	KEW 23816	EU780808/ Ref. 1	K1	EU780883/ Ref. 1	EU780859/ Ref. 1	EU780871/ Ref. 1	EU780847/ Ref. 1

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Table 1 (*continued*)

Species	Accession no. living collection/ herbarium specimen	DNA-isolate no.	GenBank No./reference-No.					
			PRK	Clone (PRK)	*atp*B-*rbc*L spacer	*trn*L intron	*trn*L-*trn*F spacer	*mat*K, 3′*trn*K
Orthophytum maracasense L.B.Sm.	KEW 1979-3587/Chase 23817 (K)	KEW 23817	EU780809/ Ref. 1	K1	EU780884/ Ref. 1	EU780860/ Ref. 1	EU780872/ Ref. 1	EU780848/ Ref. 1
Orthophytum supthutii E.Gross & Barthlott	HEID 102160/Barthlott & Supthut 10315 (HEID)	H 223	EU780807/ Ref. 1	K10	EU219695/ Ref. 2	DQ084713/ Ref. 4	DQ084572/ Ref. 4	AY950022/ Ref. 5
Portea grandiflora Philcox	KEW 1977-760/Chase 23822 (K)	KEW 23822	EU780839/ Ref. 1		EU780885/ Ref. 1	EU780861/ Ref. 1	EU780873/ Ref. 1	EU780849/ Ref. 1
Portea leptantha Harms	FRP 99-18222-3/Schulte 060901-1 (FR); Zizka 1055 (FRP)	H 239	EU780838/ Ref. 1	K1	EU219725/ Ref. 2	DQ084714/ Ref. 4	DQ084621/ Ref. 4	AY950052/ Ref. 5
Quesnelia arvensis Mez	KEW 1975-3122/Chase 23821 (K)	KEW 23821	EU780835/ Ref. 1	K1	EU780886/ Ref. 1	EU780862/ Ref. 1	EU780874/ Ref. 1	EU780850/ Ref. 1
Quesnelia edmundoi L.B.Sm.	FRP 92-10483-3/Zizka 964 (FRP)	H 050	EU780837/ Ref. 1	K2	EU219719/ Ref. 2	AF188769/ Ref. 4	DQ084616/ Ref. 4	AY950046/ Ref. 5
Quesnelia lateralis Wawra	FRP 90-10484-0/Zizka 1554 (FRP)	H 051	EU780836/ Ref. 1	K3	EU219720/ Ref. 2	AF188771/ Ref. 3	DQ084615/ Ref. 4	AY950047/ Ref. 5
Streptocalyx poeppigii Beer	FRP 94-13845-4/Horres & Schulte 201101-5 (FR)	H 267	EU780830/ Ref. 1	K1	EU219677/ Ref. 2	DQ084719/ Ref. 4	DQ084598/ Ref. 4	AY950004/ Ref. 5
Ursulaea tuitensis (Magana & E.J.Lott) Read & Baensch	FRP s.n./Horres 033 (FR) voucher DNA	H 033	EU780828/ Ref. 1	K3	EU219700/ Ref. 2	DQ084720/ Ref. 4	DQ084625/ Ref. 4	AY950027/ Ref. 5
Wittrockia superba Lindm.	FRP 93-12641-0/Horres & Schulte 050401-8 (FR)	H 049	EU780832/ Ref. 1	K7	EU219698/ Ref. 2	AF188767/ Ref. 3	DQ084611/ Ref. 4	AY950025/ Ref. 5
Puyoideae								
Puya chilensis Molina	KEW 1988-8221/Chase 23824 (K)	KEW 23824	EU780798/ Ref. 1		EU780887/ Ref. 1	EU780863/ Ref. 1	EU780875/ Ref. 1	EU780851/ Ref. 1
Puya densiflora Harms	HEID 103568/Horres 076 (FR)	H 076	EU780797/ Ref. 1	K2	EU219670/ Ref. 2	DQ084716/ Ref. 4	DQ084564/ Ref. 4	AY949997/ Ref. 5
Puya ferruginea (Ruiz & Pav.) L.B.Sm.	KEW 1992-880/Chase 23826 (K)	KEW 23826	EU780796/ Ref. 1	K1	EU780889/ Ref. 1	EU780865/ Ref. 1	EU780877/ Ref. 1	EU780853/ Ref. 1
Puya mirabilis (Mez) L.B.Sm.	HEID 103731/Horres 060 (FR)	H 060	EU780794/ Ref. 1		EU219671/ Ref. 2	AF188793/ Ref. 3	DQ084562/ Ref. 4	AY949998/ Ref. 5
Puya raimondii Harms	KEW 1988-133/Chase 23825 (K)	KEW 23825	EU780795/ Ref. 1		EU780888/ Ref. 1	EU780864/ Ref. 1	EU780876/ Ref. 1	EU780852/ Ref. 1

the vouchered taxa analyzed (including GenBank Accession numbers) is presented in Table 1.

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from fresh or lyophilized leaf material using a modified cetyl trimethylammonium bromide (CTAB) procedure (Doyle and Doyle, 1987) as described in Horres et al. (2000). To a large extent, raw genomic DNA from our former studies, stored at the Grunelius Möllgaard laboratory at the Senckenberg Research Institute, Frankfurt/Main, was used to generate the sequence data. For 10 taxa genomic DNA was obtained from the DNA Bank at the Royal Botanic Garden, Kew, UK (Table 1).

For amplification of PRK final reaction volumes of 25 μl were prepared from 22.5 μl Taq Master Mix containing 2.5 mM $MgCl_2$ (ReddyMix, ABgene), 0.25 μl of each primer [0.1 μg/μl], 0.5 μl BSA [0.04%], 1 μl DNA template [25 ng/μl], and 0.5 μl double-distilled water. Thermal cycling conditions were as follows: 1 cycle of 2 min at 94 °C, followed by 38 cycles of 1 min at 94 °C, 1 min at 50 °C, and 2 min at 72 °C, followed by one cycle of 7 min at 72 °C.

PRK primers (prk622f: 5′-CAG CAA TGA GGT TAA ATT TGC ATG GA-3′ and prk1069r: 5′-GAA AAT CTG C(AG)T GCT TCA GCA TTT G-3′) were designed to be fairly specific for one paralog of PRK (paralog 1) of Bromeliaceae (Barfuss et al., unpublished data). Primer prk622f is nested near the 3′ end of PRK exon 2 and prk1069r is positioned within exon 5 (Fig. 1). Products were cleaned from solution by using a PCR purification kit (NucleoSpin Extract, Macherey & Nagel) according to the manufacturer's protocol and eluted in 30 μl elution buffer. Sequencing reactions were performed according to the ABI Prism Big Dye 3.1 terminator cycle sequencing protocol (Applied Biosystems). Amplification primers were used as sequencing primers. In several cases internal primers (prk735f: 5′-CTG CAG ATC CGC AGA AGA AAT ATG C-3′; prk889r: 5′-GGG TAT GAG CAT GTC AAT TTC CTC CC-3′) positioned in exon 4 were used additionally. A study assessing the phylogenetic utility of PRK in Bromeliaceae including primer design, PCR optimization and analyses of different portions of the gene will be published elsewhere (Barfuss et al., unpublished data). The sequences were generated on an ABI 377 automated sequencer at the Royal Botanic Gardens, Kew. PCR and sequencing of the plastid regions were according to the protocols described earlier (*trn*L: Horres et al., 2000; *trn*L-*trn*F: Horres et al., 2007; *mat*K, 3′*trn*K: Schulte et al., 2005; *atp*B-*rbc*L: Schulte and Zizka, 2008).

In 70.8% of the accessions PRK raw electropherogram data obtained from direct sequencing included overlapping signals, which indicated the presence of heterozygotes, duplicated loci, or contamination with unspecific other PCR products. In these cases,

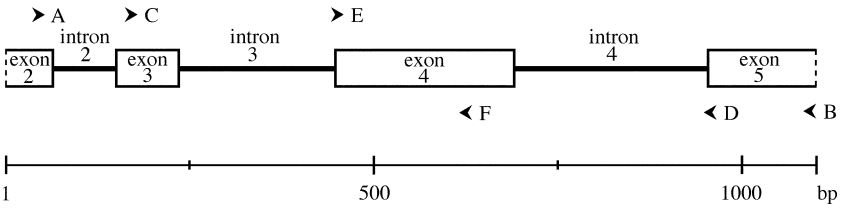

Fig. 1. Diagram of phosphoribulokinase (PRK) gene, paralog 1, exon 3 to exon 5. The location of primers is indicated by arrows. (A) prk622f; (B) prk1069r; (C) prk672f; (D) prk961r; (E) prk735f; (F) prk889r.

cloning was carried out. Amplified fragments of PRK were cloned using pGEM-T Easy Vector System (Promega) following the manufacturer's protocol. Successfully transformed colonies were sampled for PCR by inoculating PCRs with transformed cells as template. PCR products were cleaned and sequenced as described above.

For *Acanthostachys strobilacea* the target sequence could not be obtained employing the primers prk622f and prk1069r. For this taxon more specific primers were designed by the first author against the sequences already obtained from the other bromeliad taxa. Primer prk672f (5′-CAC AGC CTT GAA AGC ATC AAA′-3′) is nested near the 5′ end of exon 3, primer prk961r (5′-CAT CTC CAG CAT CGA AAC CT-3′) is located near the 5′ end of exon 5 (Fig. 1). Sequences generated using these primers were approx. 250 bp shorter than those obtained with prk622f and prk1069r.

2.3. Paralogy and heterozygosity

In spite of the use of fairly specific primers, an additional paralog of the target copy of PRK was identified in six species of Bromelioideae (*Aechmea warasii*, *Ananas comosus*, *Cryptanthus bahianus*, *Cryptanthus glaziovii*, *Hohenbergia stellata* and *Quesnelia liboniana*). Although being similar in length to the target copy of PRK, the paralog was easily distinguished in the sequence alignment from the target copy, since the two copies were hardly alignable due to considerable differences within both the intron and the exon parts. Nevertheless, BLAST searches (Altschul et al., 1997) of the paralog found it most similar to the PRK gene in *Triticum aestivum* and other Poaceae, followed by dicot PRK sequences (e.g. *Geranium dissectum*, *Acer saccharum*). In taxa where only this paralog of the target copy was obtained initially, a further PCR effort was performed in which additional clones were sequenced. In doing so, the target copy could be recovered in only one case (*Cryptanthus glaziovii*). The remaining taxa were successfully replaced by other representatives of the respective genera.

To assess the degree of variability of the target copy of PRK (paralog 1) within clones of the same species, 5 clones each of 9 species (*Aechmea distichantha*, *Aechmea drakeana*, *Aechmea farinosa*, *Aechmea mertensii*, *Canistrum fosterianum*, *Deinacanthon urbanianum*, *Fernseea itatiaiae*, *Orthophytum supthutii*, and *Wittrockia superba*) were sequenced and analyzed. Comparisons between the clones revealed that although different versions of the target copy were retrieved in the majority of species, they differed in only a small number of point mutations (caused by the presence of heterozygots or *Taq* errors during PCR) and for several taxa in a few indels (caused by length difference of the two alleles of one locus; i.e. *Aechmea mertensii*, *Canistrum fosterianum*, *Fernseea itatiaiae*). Indels were confined to intron 3 and intron 4, ranging from 1 to 18 nt, with indels of 1 to 3 nt length prevailing. The different copies of the target copy were all resolved as monophyletic groups in preliminary analyses and thus most likely represented alleles or artificial PCR products (e.g. *Taq* errors). Therefore, for the purpose of this study, one of the cloned sequences of the target copy was selected at random for inclusion in the analyses.

2.4. Alignment of sequences, data congruence and phylogenetic analysis

Contiguous alignments were assembled and edited using DNAStar Seqman II, version 5.07 (Lasergene, Madison, Wisconsin, USA). Sequences were aligned with ClustalX version 1.81 (Thompson et al., 1997) followed by manual adjustments in MacClade 4.06 for OS X (Maddison and Maddison, 2003). Regions of uncertain homology were excluded from analysis. Indels were coded in a binary matrix applying the simple indel coding method (Simmons and Ochoterena 2000) using SeqState version 1.36 (Müller, 2005, 2006). The same program was used to calculate sequence statistics (e.g. sequence divergence, number of parsimony informative characters).

For phylogenetic reconstruction all genomic regions were analyzed individually (PRK intron 2; PRK intron 3; PRK intron 4; PRK exon 3; PRK exon 4; PRK exon 5; *mat*K; 3′*trn*K; *trn*L; *trn*L-*trn*F; *atp*B-*rbc*L) and in different combinations (PRK introns; PRK exons; PRK gene; all 5 plastid regions: 5cp; nuclear and plastid regions combined: PRK5cp), also considering the respective indel characters.

Congruence between the individual data partitions as well as between the different combinations was tested using the incongruence length difference (ILD) test (Farris et al., 1995) implemented as the partition homogeneity test in PAUP Version 4.0b10 (Swofford, 2002) employing 100 replicates (heuristic search, 10 random addition replicates, TBR branch swapping), saving a maximum of 1000 most parsimonious trees per replicate.

Maximum parsimony ratchet analyses (Nixon, 1999) were conducted in PAUP with command files generated with PRAP (Müller, 2003). For each of the twenty random addition replicates, 200 ratchet iterations were performed. Each iteration comprised 10 rounds of TBR swapping, saving one shortest tree, and the most parsimonious trees were used to compute the consensus trees. Statistic support of the clades was estimated by nonparametric bootstraping as implemented in PAUP. Bootstrap proportions (BP) were based on 1000 pseudoreplicates, each with 10 random taxon addition replicates followed by tree-bisection-reconnection (TBR) branch swapping saving no more than 1000 trees per pseudo replicate. Tree length, consistency indices (CI), and retention indices (RI) were calculated for trees from the separate and combined analyses.

2.5. Bayesian analysis

Bayesian inference of phylogeny was accomplished using Metropolis-coupled Markov chain Monte Carlo (MCMCMC) estimation of posterior probability distributions as implemented in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). For the combined analysis the alignment (excluding ambiguously aligned regions) was divided into eight partitions (PRK exons; PRK introns; *mat*K; 3′*trn*K; *trn*L; *trn*L-*trn*F; *atp*B-*rbc*L; indelmatrix). To avoid overparameterization of the combined model the individual PRK exon and PRK intron regions were analyzed as a combined partition each. For each of the seven DNA partitions the best-fit model of evolution was inferred using ModelTest version 3.6 (Posada and Crandall 1998) based on the Akaike Information Criterion AIC (Table 2). For Bayesian analysis the best-fit model according to ModelTest that could be implemented in MrBayes was assigned to each of the seven DNA partitions (Table 2). These as well as the combined datasets (PRK, 5cp, PRK5cp) were analyzed considering the respective indel matrices. In the partitioned models the overall evolutionary rate was allowed to be different across partitions. Furthermore, the nucleotide models were allowed to be unique

Table 2
Best-fit models of DNA substitutions based on the Akaike Information Criterion for the DNA partitions analyzed with Baysian inference. MT, best-fit model selected by Modeltest. BI, best-fit model selected by Modeltest that could be implemented in MrBayes.

Data partition	MT	BI
PRK exons	K80+I+G	K80+I+G
PRK introns	HKY+G	HKY+G
*mat*K gene	TIM+I+G	GTR+I+G
3′*trn*K intron	K81 uf	HKY
*trn*L intron	K81 uf+I+G	GTR+I+G
*trn*L-*trn*F spacer	GTR+G	GTR+G
*atp*B-*rbc*L spacer	GTR+I+G	GTR+I+G

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for each partition, i.e. the substitution model parameters were enabled to be independent across partitions, only branch length and topology remained linked between partitions.

For each analysis three independent MCMCMC runs were conducted starting all chains from different, randomly chosen trees. Four Metropolis-coupled chains with incremental heating were used. After initial preruns the heating parameter was set to 0.1 to improve mixing behavior of the chains. All MCMC analyses were performed for 1,000,000 generations, sampling values every 100th generation. As default, the first 2500 sampled generations from each run were discarded from the analysis as burnin (i.e. the number of generations before apparent stationarity). After each analysis run statistics were considered to explore whether convergence had been reached after the designated burnin phase. In all runs the plots of model likelihood against generation had leveled off after the preset burnin phase and were fluctuating around a stable value. In all cases, the potential scale reduction factors (Gelman and Rubin, 1992) were very close to one (to the second decimal), indicating that the runs had adequately converged. The standard deviation of split frequencies between the three independent runs had dropped far below 0.1, indicating that the tree samples from the different runs were sufficiently similar.

Calculations of the 50% majority rule consensus trees and the posterior probabilities (PP) of each branch were based upon all tree samples of the three independent runs gained after the designated burnin phase.

2.6. Evaluation of character transformation patterns

Character transitions of sepal symmetry (symmetric, asymmetric) and habit (central tank present or absent) were examined to explore the evolution of these characters in the subfamily. The information was mainly compiled from literature (for sepal symmetry: Smith and Downs, 1974, 1979; Smith 1988; Leme 1997; for habit: Smith and Downs, 1974, 1979; Benzing 2000; supplemented by own observations). The selected characters were traced by overlying them onto the strict consensus tree of the parsimony analysis based on the overall datamatrix comprising nuclear as well as plastid data, including the coded indels.

3. Results

3.1. Variability of nuclear and plastid regions

The length of the analyzed PRK sequences from paralog 1 ranged between 713 (*Deinacanthon urbanianum*) and 1106 nt (*Aechmea mertensii*) due to considerable length mutations within the intron parts. Intron 3 and intron 4 were especially variable in sequence length, ranging from 113 to 416 nt and 148 to 416 nt, respectively. Within the introns three regions were identified, in which an accurate primary homology assessment was not possible and which therefore were excluded from analysis (intron 3: one region, intron 4: two regions), resulting in an overall alignment of the PRK sequence data of 888 nt length. Sequence statistics are summarized in Table 3.

Within the five plastid regions studied, the two spacer regions (*atp*B-*rbc*L, *trn*L-*trn*F) showed the highest variability in sequence length ranging from 614 to 793 and 188 to 346, respectively. Within the *atp*B-*rbc*L spacer a length variable polyT/polyA stretch precluded an unambiguous alignment and was therefore excluded from the analysis, leaving 846 characters in the alignment of the region. Likewise, a hypervariable region within the *trn*L-*trn*F region was excluded from the analysis, resulting in an alignment of 314 characters for the region. The combined alignment from all five plastid regions comprised 3393 characters in the final dataset (i.e. excluding regions of ambiguous alignment).

The combination of the nuclear region PRK and the five plastid regions led to an overall alignment of 4281 characters, excluding ambiguous regions. In total, the overall alignment contained 0.3% missing data. The proportion of variable and potentially informative characters in the analyzed data matrices was highest within the PRK introns with 44.7% and 20.6%, respectively, followed by the PRK exons with 26.4% and 12.4%, respectively. Among the five plastid regions, the proportion of variable characters ranged from 7.1% to 11.7%, being highest within the introns (*trn*K, *trn*L).The proportion of potentially informative characters within the plastid dataset was lowest within *mat*K and *atp*B-*rbc*L with 2.6% and highest within the intron regions (*trn*K, *trn*L) with 5.4% and 4.7%, respectively. Nevertheless, *mat*K

Table 3
Comparison of sequence statistics between all nuclear and chloroplast regions analyzed individually (Part A) and in different combinations (Part B) for the 48 taxon set of Bromelioideae. Char., number of characters in the alignment matrix (excluding hotspots); length range, actual sequence length in nucleotides (minimal and maximal observed value, including hotspots); mean length, mean of all observed sequence lengths (standard deviation given in brackets); % divergence, pairwise sequence distance in percent (uncorrected *P* distance), overall mean, lowest and highest scores in brackets); % var., percentage of variable characters; % inform., percentage of parsimony informative indels. [*]Minimal value due to missing data for *Acanthostachys strobilacea* (A.c.). The sequence was obtained using primer pairs nested within exon 3 and exon 5. Minimal values without A.c.: 33, 86, 64 for intron 2, exon 3 and exon 5, respectively.

Data partition	Char.	Length range	Mean length	% Divergence	% Var.	% Inf.	No. indels	% Inf. indels
(A) Individual								
	a	b	b	b	a	a	a	a
PRK intron 2	74	0*–56	50.4 (8.0)	4.7 (0.0–17.6)	37.8	14.9	33	33.3
PRK intron 3	142	113–416	213.4 (36.5)	10.1 (0.6–25.8)	48.6	22.5	7	28
PRK intron 4	270	148–310	264.0 (42.9)	8.2 (0.0–20.3)	44.4	21.1	39	35.9
PRK exon 3	86	9*–86	84.4 (11.0)	2.8 (0.0–22.2)	20.9	14	0	0
PRK exon 4	244	241–244	243.0 (0.4)	3.6 (0.0–7.8)	29.5	13.5	2	50
PRK exon 5	72	0*–72	69.8 (10.4)	2.2 (0.0–8.7)	22.2	6.9	4	50
*mat*K	1554	1524–1545	1533.7 (3.9)	0.7 (0.0–1.7)	7.5	2.6	5	20
*trn*K intron	185	169–185	179.9 (1.7)	1.4 (0.0–3.3)	11.4	5.4	2	0
*trn*L intron	494	467–484	474.4 (3.5)	1.0 (0.0–2.6)	11.7	4.7	10	30
*trn*L-*trn*F spacer	314	188–346	286.3 (19.0)	0.9 (0.0–3.7)	9.6	3.5	15	20
*atp*B-*rbc*L spacer	846	614–793	757.8 (34.2)	0.9 (0.0–2.0)	7.1	2.6	19	47.4
(B) Combined								
PRK introns	486	312–705	527.8 (60.0)	8.6 (0.5–18.1)	44.7	20.6	79	34.2
PRK exons	402	252–402	397.2 (21.3)	3.2 (0.0–6.2)	26.4	12.4	6	50
PRK	888	713–1106	925.0 (66.6)	6.0 (0.3–11.6)	36.4	16.9	85	35.3
5cp	3393	3091–3296	3232.1 (40.0)	0.8 (0.0–1.6)	8.4	3.1	51	31.4
PRK5cp	4281	3960–4345	4157.0 (75.1)	1.9 (0.2–3.5)	14.2	6	136	33.8

[a] Data refer to the matrix used in tree inference and exclude regions of ambiguous alignment.
[b] Data refer to complete matrix, including mutational hotspots.

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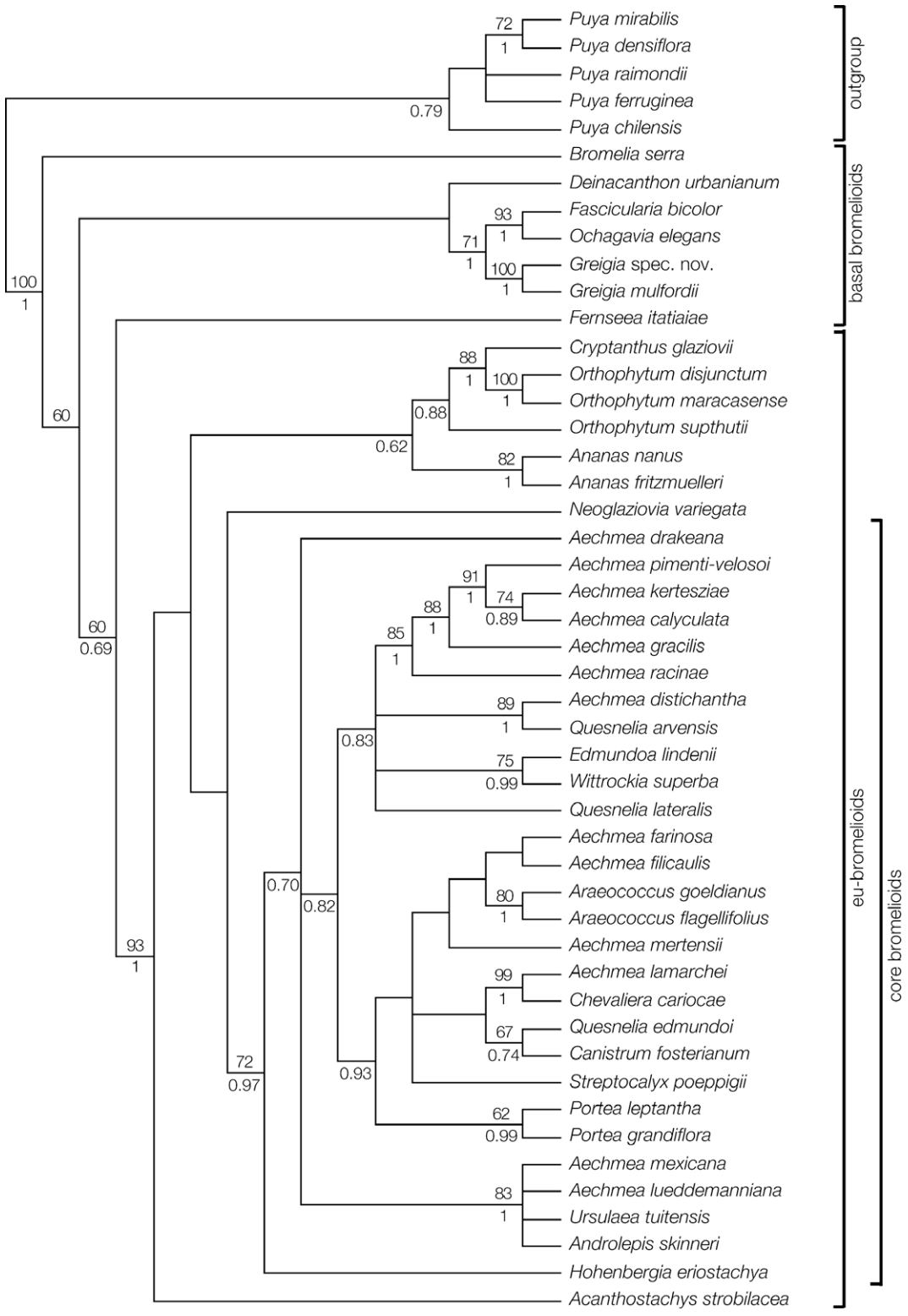

Fig. 2. Strict consensus tree of a parsimony ratchet analysis of the PRK region (intron 2 to exon 5) including coded indels. The analysis yielded 109 most parsimonious trees of 705 steps length (consistency index CI 0.67, retention index RI 0.70). Bootstrap support is given above branches, posterior probabilities from Bayesian analysis are indicated below branches.

contributed most to the total amount of potentially informative characters within the plastid dataset (40 of 106) due to its substantial length (1554 nt). The proportion of potentially informative characters was considerably higher within the combined PRK dataset than within the combined plastid dataset (16.9% vs. 3.1%). Of the 3393 characters of the combined dataset of nuclear and plastid regions, 14.2% were variable and 6.0% were parsimony informative.

Most indels were observed in the PRK introns, followed by the analyzed spacers and introns of the plastid genome and the lowest

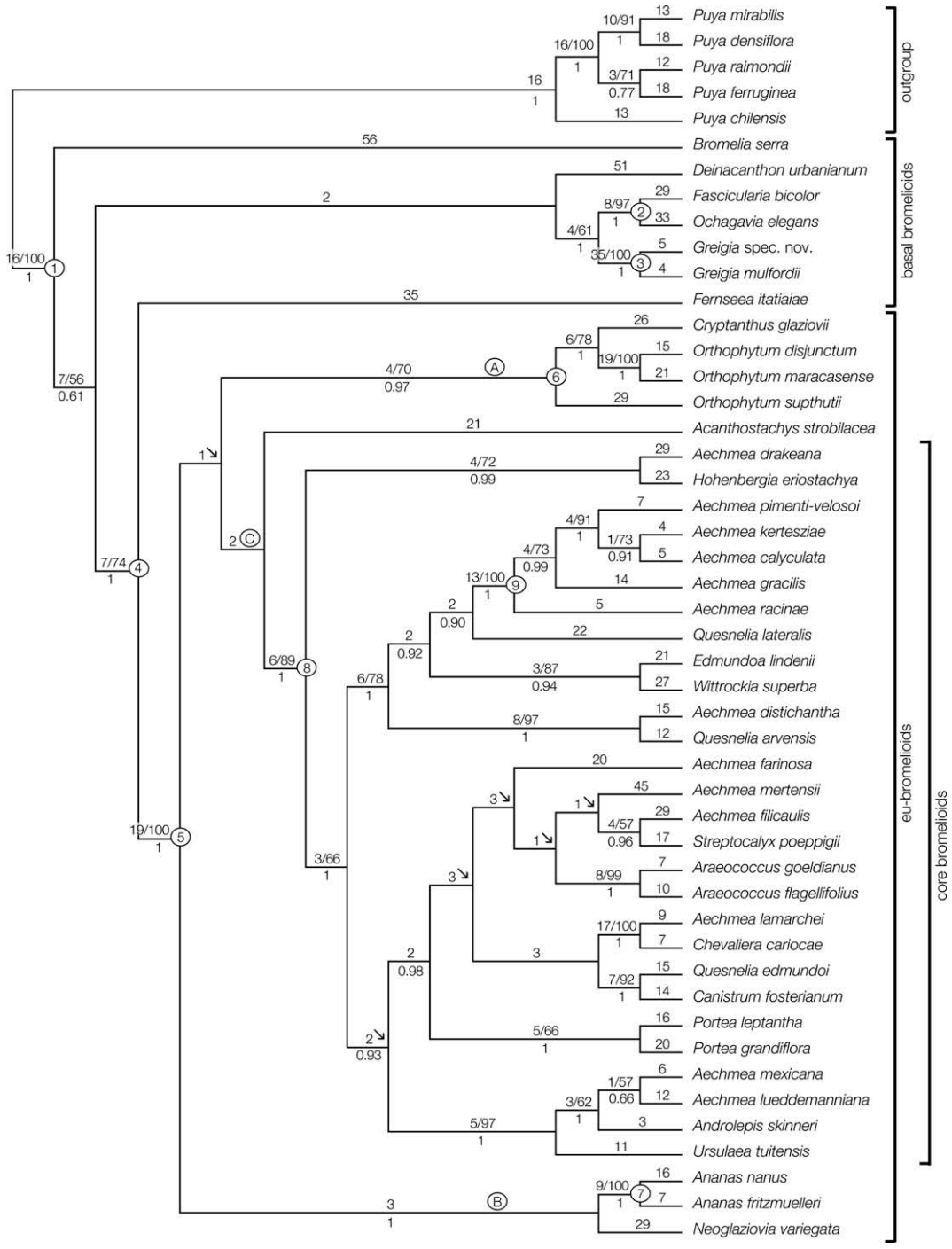

Fig. 3. One of 77 most parsimonious trees of a maximum parsimony analysis of PRK and five plastid regions (*mat*K gene, 3′*trn*K intron, *atp*B-*rbc*L spacer, *trn*L intron, *trn*L-*trn*F spacer), including coded indels, of 1168 steps length (consistency index CI 0.71, retention index RI 0.72). Branch length and bootstrap support are given above branches, posterior probabilities from Bayesian analysis are indicated below branches. Branches, that collapse in the strict consensus are marked with an arrow.

number of indels was found in the exon regions. Only 33.8% of the 136 indels of the overall matrix were potentially parsimony informative (Table 3).

3.2. Phylogenetic relationships

Partition homogeneity tests indicated that the different data partitions of the PRK region are not significantly incongruent (*P*-values in all pairwise comparisons >0.05) whereas within the plastid dataset two pairwise comparisons were significantly incongruent (*P*-values <0.01 for *atp*B-*rbc*L vs. *mat*K and *atp*B-*rbc*L vs. *trn*L). However, comparison of the topologies obtained from analysis of the different data partitions on a node-by-node basis, which may give a better assessment of congruence than the sometimes unreliable partition homogeneity test (Wiens, 1998; Dolphin et al., 2000; Reeves et al., 2001) did not result in any highly supported, incongruent relationships (BV > 70). Due to the general high level of congruence between the different data partitions, they were combined. Incongruence homogeneity tests for the different combined matrices (e.g. PRK exons vs. PRK introns, PRK vs. five plastid

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regions) yielded no significant incongruence between the combined partitions (*P*-values > 0.08).

The trees obtained from the different datasets differed mainly in the degree of resolution, being lowest in the individual data partitions and highest in the combined dataset of the PRK region (Fig. 2) and the combined analysis of PRK and the five plastid regions (Fig. 3 and Tables 4, 5). The increased number of well-supported clades resulting from the analysis of the combined datasets relative to the separate data partitions gives further evidence to our assertion that the different datasets are broadly congruent. Therefore, the trees (MP, BI) obtained from the combined analysis of the total dataset of one nuclear and five plastid regions are considered as the best approximation of genetic relationships, and the MP strict consensus tree is described in the following (Fig. 3).

The 20 parsimony ratchet replicates of 200 iterations conducted with the overall dataset (PRK5cp) yielded a total of 77 most parsimonious trees with a length of 1,168 steps, a consistency index excluding uninformative characters of 0.71, and a rescaled consistency index of 0.51 (Table 4).

The phylogenetic analyses corrobate the monophyly of subfamily Bromelioideae (BV 100, PP 1). Within the sister group of Bromelioideae *Puya* (Pitcairnioideae *s.l.*), *Puya chilensis* (subgen. *Puya*) is found in sister group position to a strongly supported clade comprising representatives of both subgenera (*Puya* and *Puyopsis*) (BV 100, PP 1), not supporting the current subgeneric concept of *Puya*.

The monophyly of Bromelioideae is highly supported (BV 100, PP 1, Fig. 3: node 1). *Bromelia* is resolved in sister group position to all other Bromelioideae, neverthess this relationship receives only weak nodal support (BV 56/PP 0.61). On the next branch, *Deinacanthon*, *Fascicularia*, *Ochagavia* and *Greigia* form a clade sister to a moderately supported branch comprising the remaining Bromelioideae (BV 74, PP 1). The position of *Deinacanthon* remains unclear as the depicted sister group relationship to *Fascicularia*/*Ochagavia* and *Greigia* receives no statistical support. *Fascicularia* together with *Ochagavia* (BV 97, PP 1, Fig. 3: node 2) as well as *Greigia* (BV 100, PP 1, Fig. 3: node 3) form highly supported clades, but the sister group position of the two clades is only weakly supported in the bootstrap analysis (BV 61, PP 1).

Fernseea is resolved as sister to the remaining Bromelioideae (informally referred to as eu-bromelioids in the following) with high statistical support (BV 100, PP 1, Fig. 3: node 4). The eu-bromelioids split into a trichotomy. Branch A depicts *Orthophytum* as paraphyletic, with *Cryptanthus* being nested within the moderately supported clade (BV 70, PP 0, 97, Fig. 3: node 6). *Orthophytum supthutii* is found in sister group position to the remainder of the clade (BV 78, PP 1).

Branch B shows a highly supported *Ananas* clade (BV 100, PP 1, 3: node 7) together with *Neoglaziovia*, without gaining statistical support. Branch C depicts *Acanthostachys* in sister group position to a large and well-supported clade representing the core bromelioids (BV 89, PP 1, Fig. 3: node 8). The core bromelioids are well resolved, nevertheless relationships between supported clades remain largely uncertain due to a lack of nodal support. *Aechmea* and *Quesnelia* are shown as highly polyphyletic, their representatives being found in different positions within the core bromelioids. Among the different moderately to highly supported clades the following ones are noteworthy: (1) A moderately supported *Ortgiesia* clade (BV 73, PP 1, Fig. 3, node 9), consisting of *Aechmea* subgen. *Ortgiesia* with *Aechmea racinae* (subgen. *Lamprococcus*) in sister group position. (2) A highly supported "Nidularioid"-clade comprising *Edmundoa* and *Wittrockia* (BV 87, PP 0, 94). (3) A highly supported *Podaechmea* clade (BV 97) uniting *Ursulaea* and *Androlepis* with two species of *Aechmea* subgen. *Podaechmea* (BV 97, PP 1).

Table 4
Results of maximum parsimony analysis of different data partitions. Trees, number of most parsimonious trees; TL, length of shortest trees; CI, consistency index; RC, rescaled consistency index; No. of nodes, number of nodes present in the strict consensus tree.

Data partition	Trees	TL	CI	RC	No. of nodes
(A) Individual					
PRK intron 2	2379	85	0.82	0.60	2
PRK intron 3	3438	123	0.76	0.24	8
PRK intron 4	3798	271	0.71	0.53	7
PRK exon 3	1184	25	0.72	0.58	4
PRK exon 4	3524	121	0.65	0.50	7
PRK exon 5	1159	26	0.81	0.70	0
*mat*K	8	144	0.88	0.79	18
*trn*K intron	21	26	0.89	0.83	6
*trn*L intron	966	92	0.80	0.62	10
*trn*L-*trn*F spacer	1912	53	0.89	0.88	3
*atp*B-*rbc*L spacer	1379	101	0.8	0.68	14
(B) Combined					
PRK introns	1692	505	0.70	0.50	23
PRK exons	366	186	0.63	0.47	17
PRK	705	109	0.67	0.48	37
5cp DNA	827	449	0.78	0.62	27
PRK5cp	77	1168	0.71	0.51	39

Table 5
Statistical support of selected nodes in the phylogenetic analysis of different datasets. Combined, PRK and 5 cp regions; nuclear, PRK; plastid, 5 cp regions (*mat*K gene, 3′*trn*K intron, *atp*B-*rbc*L spacer, *trn*L intron, *trn*L-*trn*F spacer).

Node[a]	Statistical support (BV/PP)		
	Combined	Nuclear	Plastid
1. Monophyly of Bromelioideae	100/1	100/1	99/1
2. Monophyly of *Ochagavia/Fascicularia* clade	97/1	93/1	<50/ n.p.
3. Monophyly of *Greigia*	100/1	100/1	100/1
4. *Fernseea* sister to eu-bromelioids	74/1	60/0.69	n.p.
5. Monophyly of eu-bromelioids	100/1	93/1	98/1
6. Monophyly of *Orthophytum/Cryptanthus* clade	70/0.97	<50/ 0.88	n.p.
7. Monophyly of *Ananas*	100/1	82/1	100/1
8. Monophyly of core bromelioids	89/1	72/0.97	n.p.
9. Monophyly of *Ortgiesia* clade (including *Ae. racinae*)	100/1	85/1	99/1

[a] Node numbers refer to nodes in Fig. 3. n.p., node not present in the respective tree.

4. Discussion

4.1. Phylogenetic utility of PRK

Previous molecular studies in Bromeliaceae relied on plastid DNA data only, which exhibited an extraordinary low sequence divergence within subfamily Bromelioideae (Horres et al., 2000, 2007; Schulte et al., 2005). The low-copy nuclear gene PRK exhibits a significantly higher level of variation than that of any plastid region used in phylogenetic studies of Bromeliaceae so far. For example, the proportion of potentially parsimony informative characters obtained for the five plastid regions used in this study ranged from 2.6% to 5.4% whereas PRK yielded 16.9%. The estimates of phylogeny of Bromelioideae based on PRK data alone (888 characters) exhibited a higher resolution and support than those based on single plastid regions as well as the combination of up to five plastid regions (comprising 3393 characters). This study demonstrates that the examined PRK regions are of high utility in resolving phylogenetic relationships within Bromelioideae and are well capable of resolving relationships at intergeneric level. The intron regions are also expected to be of great value in resolving intrageneric relationships due to their high variability.

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As has been demonstrated within this study special care has to be taken in discriminating between paralogous and orthologous regions. In Bromelioideae two highly divergent paralogs of PRK have been retrieved that were easily distinguishable in the assessment of homology. Furthermore cloning revealed highly similar copies of the target copy of PRK (paralog 1) within single accessions which could stem from either heterozygosity, PCR artifacts or recent paralogy. Preliminary analysis demonstrated that interspecific variation exceeded intraspecific variation in all cases, i.e. the copies of PRK formed monophyletic groups concordant with the species they were retrieved from. Thus, even if the different copies represented recent paralogy, the analyses indicate that possible gene duplication events postdated speciation. Therefore, the analysis of a random choice of these copies is regarded as appropriate approach to infer the phylogeny of Bromelioideae. The phylogenetic reconstructions obtained from the nuclear region PRK are fully congruent with results obtained from the five plastid regions, not indicating any conflicts, and possess a higher resolution, thus making the target region a valuable phylogenetic tool.

4.2. Phylogenetic relationships

One severe obstacle in the understanding of the evolution of Bromelioideae has been the difficulty in assigning the phylogenetic position of their genera, especially the assignment of putatively basal and advanced groups within the subfamily (e.g. Smith, 1934; Pittendrigh, 1948; Smith and Downs, 1979; Benzing, 2000). Molecular studies so far all had to contend with an exceedingly low sequence variability of plastid markers leading to poorly resolved phylogenies and often suffered from poor taxonomic sampling. Based on molecular studies with a thorough sampling relying on more than two plastid markers several putatively basal genera within the subfamily could be distinguished as well as a core group comprising the more advanced bromelioids (Schulte et al., 2005; Schulte and Zizka, 2008).

The inclusion of nuclear sequence data in the phylogenetic reconstruction of Bromelioideae significantly improves resolution as well as support for the revealed intergeneric relationships. The phylogenetic reconstructions including the nuclear region PRK now give reasonable evidence that the six genera *Bromelia*, *Deinacanthon*, *Greigia*, *Ochagavia/Fascicularia* and *Fernseea* represent early divergent lineages within the subfamily, and thus confirm the results of our earlier studies based on different plastid loci (Schulte et al., 2005; Schulte and Zizka, 2008).

The sister group relationship of *Bromelia*, a genus comprising terrestrial xerophytes, to the remainder of the subfamily is resolved for the first time in a molecular study with a thorough sampling, especially of putatively basal bromelioids, albeit receiving only weak statistical support. Although the genus has been regarded as an early divergent line within the subfamily before (e.g. Pittendrigh, 1948; Givnish et al., 2004, 2007; Schulte et al., 2005; Schulte and Zizka, 2008), up to now its position among the basal bromelioids has remained speculative, either because other putatively basal lineages of the subfamily where not represented in the molecular studies or because of a lack of resolution (Givnish et al., 2004, 2007; Horres et al., 2000, 2007; Schulte et al., 2005; Schulte and Zizka, 2008). Nevertheless, the relationships between early divergent bromelioids as indicated by the inclusion of the nuclear marker PRK in the molecular dataset still need to be corroborated by additional molecular evidence.

According to the molecular phylogeny the monotypic genus *Deinacanthon* represents a distinct lineage within the basal bromelioids and its separation from *Bromelia* (Mez, 1896; Smith, 1988) appears justified.

The molecular tree shows an affinity between the xeric genera *Ochagavia*/*Fascicularia* and the mesophytic *Greigia*. The three genera share several traits with *Puya*, the sister group of Bromelioideae, which are discussed as ancestral in the subfamily (e.g. C3 photosynthesis, distribution types; terrestrial life form; Schulte et al., 2005).

The sister group position of *Fernseea* to the remainder of the subfamily, as already indicated by plastid data alone (Schulte et al., 2005), is further corroborated by the inclusion of nuclear data and now is highly supported. *Fernseea*, a small genus of terrestrial plants of high mountain habitats in southeastern Brazil, has been discussed earlier as possible remnant of the migration of Bromelioideae between the Andes and eastern Brazil, the latter being today's center of diversity within the subfamily (Schulte et al., 2005).

Within the highly supported clade comprising the remaining Bromelioideae, several lineages can be discerned from the more advanced core bromelioids.

The *Orthophytum/Cryptanthus* clade gives the first evidence for the paraphyly of *Orthophytum*, with *Cryptanthus* being nested within the clade. Both genera are endemic to southeastern Brazil and comprise terrestrial or lithophytic xerophytes. Recent morphological studies within the genus *Orthophytum* indicate that *Orthophytum supthutii* belongs to a more primitive group that merits its own generic status (Louzada, 2008). Within our molecular phylogeny including nuclear data, *Orthophytum supthutii* is found in sister group position to a clade comprising *Cryptanthus* and the remaining *Orthophytum* species, thus supporting the view of Louzada (2008).

The morphologically well characterized genus *Ananas* forms another highly supported clade, whereas the sister group position of *Neoglaziovia* to the *Ananas* clade receives no nodal support. The phylogenetic position of *Acanthostachys*, which is depicted as sister group of the core bromelioids in the consensus trees of the analysis of the overall data matrix, remains unclear.

Within the core bromelioids resolution has improved compared to the molecular studies based on plastid data alone but relationships between the different moderately to highly supported clades still remain uncertain. Once again, the polyphyly of the large and highly problematic genus *Aechmea* becomes evident, with members of the genus being found in different positions of the clade, grouping together with members of several genera such as *Quesnelia*, *Streptocalyx*, *Chevaliera*, *Androlepsis* and *Ursulaea*. This supports findings based on AFLPs (Horres et al., 2007) and plastid data alone (Schulte et al., 2005; Schulte and Zizka, 2008) as well as morphological studies (Faria et al., 2004). Two highly supported groups, the *Ortgiesia* clade and the *Podaechmea* clade have been consistently found in the AFLP and the plastid study as well and merit further investigation (Schulte et al., 2005). According to Givnish et al. (2004, 2007), the clade representing the core bromelioids is rather young with an estimated age of the crown group of around 5 Ma. This clade obviously underwent a rapid diversification, especially within the Atlantic rain forest of southeastern Brazil, leading to the current taxonomic difficulties. Due to the low sequence divergence displayed by the group, relationships are still not revealed satisfactorily. The integration of further informative nuclear regions into the phylogenetic reconstruction promises to shed more light on phylogeny and evolution of this highly diverse clade.

4.3. Evolution of sepal symmetry

Sepals in Bromelioideae are often asymmetric, with one sepal lobe larger than the other, the enlarged lobe often gaining a wing-like appearance. Most genera comprise species that possess either distinctly asymmetric or rather symmetric sepals. Smith (1988) used sepal symmetry in the identification of bromelioid genera without attaching further taxonomic value to the character. The inferred evolution of sepal symmetry indicates that symmetric se-

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pals are ancestral within the subfamily. As the reconstruction of character evolution based on the molecular MP strict consensus tree shows, the sepals of the basal lineages of Bromelioideae can be regarded as primarily symmetric (Fig. 4). Asymmetric sepals are found within the *Ananas* clade and within the core bromelioids. To elucidate whether the occurence of asymmetric sepals within these two clades stem from a common ancestor or were gained independently, the position of the *Ananas* clade needs to be further resolved by the inclusion of additional molecular data. Nevertheless, tracing of character transitions onto the molecular tree indicates that asymmetric sepals are ancestral within the core bromelioids and that several reversals to symmetric sepals occurred within this lineage. Interestingly several clades are found in which more than one genus possesses symmetric sepals indicating that these reversals stem from common ancestry. This is the case for the genera *Edmundoa*, *Wittrockia*, *Nidularium* which belong to the Nidularioid clade (Schulte et al., 2005; Schulte and Zizka, 2008, this study), *Ursulaea* and *Hohenbergiopsis* belonging to the Central American *Podaechmea* clade (Schulte et al., 2005; Schulte and Zizka, 2008, this study), and *Canistrum fosterianum* and *Quesnelia edmundoi* which form another clade (Fig. 4). Within the genus *Quesnelia* symmetric sepals are the exception and might indicate wrong taxonomic concepts as the molecular data suggests. The evolutionary explanation for the occurrence of symmetric and asymmetric sepals within *Araeococcus* requires further investigation with a broader taxon sampling.

4.4. Evolution of the tank habit

The eco-morphological types of Tietze (1906), Pittendrigh (1948) and Benzing (2000) reflect the progression of a suite of characters that are partly evolving interdependently (e.g. reduction of root system and increase of water uptake capacity via leaf trichomes). Within Bromelioideae the more primitive eco-morphological types can be discerned from the most advanced type by the absence/presence of a central tank, which allows the plant to collect large quantities of water and organic material in an external repository. Whereas representatives of the more primitive eco-morphological types are almost exclusively terrestrials or lithophytes, representatives of the most advanced type (type III in all classifications) exhibit the highest flexibility in life form (epiphytic, lithophytic, less often terrestrial).

The reconstruction of the evolution of a central tank within the subfamily shows an unexpectedly clear pattern. The absence of a central tank is inferred as ancestral within the subfamily. The molecular phylogeny implies a single origin of the central tank within the subfamily namely ancestral to core bromelioids. Within the core bromelioids all genera are characterized by the presence of the central tank, which thus can be regarded as a synapomorphy for the clade. Singular exceptions are found within the clade in genera that are predominantly tank-forming. The character reconstruction implies that the tank habit was lost again in some taxa, as for example in *Araeococcus flagellifolius*, where the rosette forms an apically closed pseudobulb. The tank habit thus possesses a much lower evolutionary lability within the Bromelioideae than previously assumed, on the contrary, it appears to be highly conserved. This demonstrates the considerable advantage of the evolution of the central tank within the group. By gaining this key innovation, bromelioids promptly gained a high ecological flexibility. The rapid diversification of the core bromelioids, which is indicated by the molecular data, may have been triggered by the evolution of the central tank, enabling the group to conquer the most diverse hab-

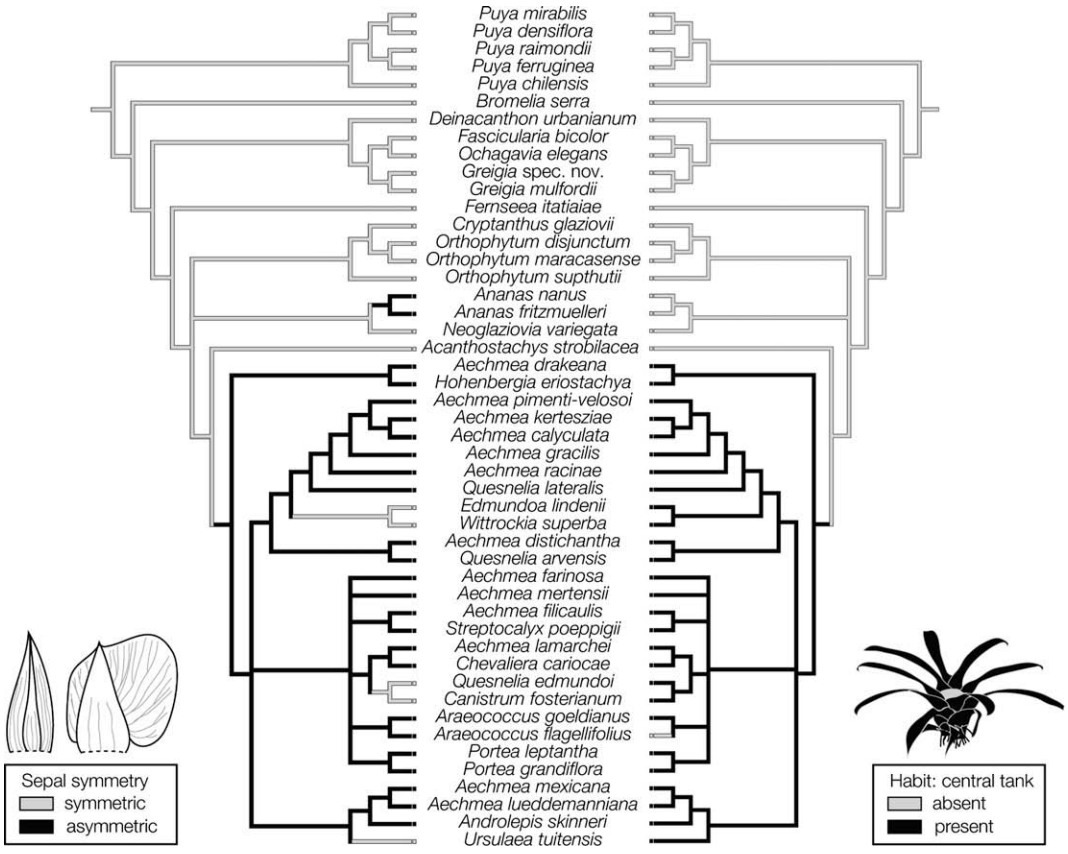

Fig. 4. Most parsimonious reconstruction of the evolution of sepal symmetry (on the left) and tank habit (on the right) in Bromelioideae, based on the strict consensus tree from a parsimony ratchet analysis of PRK and five plastid regions (*mat*K gene, 3′*trn*K intron, *atp*B-*rbc*L spacer, *trn*L intron, *trn*L-*trn*F spacer), including indels.

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itats, being highly independent of water and nutrition supply from the substrate.

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References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PDI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Barfuss, M.H.J., Samuel, R., Till, W., Stuessy, T.F., 2005. Phylogenetic relationships in subfamily Tillandsioideae (Bromeliaceae) based on DNA sequence data from seven plastid regions. Am. J. Bot. 92, 337–351.

Benzing, D.H., 2000. Bromeliaceae: Profile of an Adaptive Radiation. Cambridge University Press, New York.

Betancur, J., Salinas, N.R., 2006. El ocaso de *Pseudaechmea* (Bromeliaceae: Bromelioideae): the *Pseudaechmea* (Bromeliaceae: Bromelioideae) twilight. Caldasia 28, 157–164.

Brown, G.K., Leme, E.M.C., 2000. Cladistic analysis in the Nidularioid complex. In: Leme, E.M.C. (Ed.), *Nidularium* – Bromeliads of the Atlantic Forest. Sexante Artes, Rio de Janeiro, pp. 240–247.

Brown, G.K., Leme, E.M.C., 2005. The re-establishment of *Andrea* (Bromeliaceae: Bromelioideae), a monotypic genus from Southeastern Brazil threatened with extinction. Taxon 54, 63–70.

Crayn, D.M., Winter, K., Smith, J.A.C., 2004. Multiple origins of crassulacean acid metabolism and the epiphytic habit in the Neotropical family Bromeliaceae. Proc. Natl. Acad. Sci. USA 101, 3703–3708.

de Oliveira, L., de Sousa, F., Wendt, T., Brown, G.K., Tuthill, D.E., Evans, T.M., 2007. Monophyly and phylogenetic relationships in *Lymania* (Bromeliaceae: Bromelioideae) based on morphology and chloroplast DNA sequences. Syst. Bot. 32, 264–270.

De Sousa, L.D.F., Wendt, T., 2008. Taxonomy and conservation of the genus *Lymania* (Bromeliaceae) in the southern Bahian Atlantic Forest of Brazil. Bot. J. Linn. Soc. 157, 47–66.

Dolphin, K., Belshaw, R., Orme, C.D.L., Quicke, D.L.J., 2000. Noise and incongruence: interpreting results of the incongruence length difference test. Mol. Phylogenet. Evol. 17, 401–406.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Faria, A.P.G., Wendt, T., Brown, G.K., 2004. Cladistic relationships of *Aechmea* (Bromeliaceae, Bromelioideae) and allied genera. Ann. MO Bot. Gard. 91, 303–319.

Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1995. Constructing a significance test for incongruence. Syst. Biol. 44, 570–572.

Gelman, A., Rubin, D.B., 1992. Inference from iterative simulation using multiple sequences. Stat. Sci. 7, 457–472.

Givnish, T.J., Milliam, T.M., Evans, T.M., Hall, J.C., Berry, P.E., Terry, R.G., 2004. Ancient vicariance or long-distance dispersal? Inferences about phylogeny and South American-African disjunctions in Rapateaceae and Bromeliaceae based on *ndh*F sequence data. Int. J. Plant Sci. 165 (4, Suppl.), 35–54.

Givnish, T.J., Milliam, K.C., Berry, P.E., Sytsma, K.J., 2007. Phylogeny, adaptive radiation, and historical biogeography of Bromeliaceae inferred from *ndh*F sequence data. Aliso 23, 3–26.

Givnish, T.J., Sytsma, K.J., Smith, J.F., Hohn, W.J., Benzing, D.H., Burkhardt, E.M., 1997. Molecular evolution and adaptive radiation in *Brocchinia* (Bromeliaceae: Pitcairnioideae) atop tepuis of the Guayana shield. In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, New York, pp. 259–311.

Gunn, B.F., 2004. The phylogeny of the Cocoeae (Arecaceae) with emphasis on *Cocos nucifera*. Ann. MO Bot. Gard. 91, 505–522.

Horres, R., Zizka, G., Kahl, G., Weising, K., 2000. Molecular phylogenetics of Bromeliaceae: evidence from *trnL*(UAA) intron sequences of the chloroplast genome. Plant Biol. 2, 306–315.

Horres, R., Schulte, K., Weising, K., Zizka, G., 2007. Systematics of Bromelioideae (Bromeliaceae) – evidence from molecular and anatomical studies. Aliso 23, 27–43.

Leme, E.M.C., 1997. *Canistrum* – Bromeliads of the Atlantic Forest. Salamandra, Rio de Janeiro.

Lewis, C.E., Doyle, J.J., 2001. Phylogenetic utility of the nuclear gene malate synthase in the palm family (Arecaceae). Mol. Phylogenet. Evol. 19, 409–420.

Lewis, C.E., Doyle, J.J., 2002. A phylogenetic analysis of tribe Areceae (Arecaceae) using two low-copy nuclear genes. Plant Syst. Evol. 236, 1–17.

Loo, A.H.B., Dransfield, J., Chase, M.W., Baker, W.J., 2006. Low-copy nuclear DNA, phylogeny and the evolution of dichogamy in the betel nut palms and their relatives (Arecinae; Arecaceae). Mol. Phylogenet. Evol. 39, 598–618.

Louzada, R.B., 2008. Taxonomia e citogenética das espécies de inflorescência séssil do gênero *Orthophytum* Beer (Bromeliaceae). Instituto de Botânica, São Paulo. pp. 1–102.

Luther, H.D., 2006. An Alphabetical List of Bromeliad Binomials. The Bromeliad Society International, Sarasota, Florida.

Maddison, D.R., Maddison, W.P., 2003. MacClade 4.05 OS X. Analysis of Phylogeny and Character Evolution. Sinauer, Sunderland, Massachusetts.

Medina, E., 1974. Dark CO2 fixation, habitat preferences and evolution within the Bromeliaceae. Evol. 28, 677–686.

Mez, C., 1896. Bromeliacées. In: de Candolle, C. (Ed.), Monographiae Phanerogamarum. Prodromi nunc continuatio nunc revisio, vol. 9. Masson & Cie, Paris, pp. 1–990 (i-lxxxvii).

Mort, M.E., Crawford, D.J., 2004. The continuing search: low-copy nuclear sequences for lower-level plant molecular phylogenetic studies. Taxon 53, 257–261.

Müller, K.F., 2003. PRAP-computation of Bremer support for large data sets. Mol. Phylogenet. Evol. 31, 780–782.

Müller, K.F., 2005. SeqState – primer design and sequence statistics for phylogenetic DNA data sets. Appl. Bioinf. 4, 65–69.

Müller, K.F., 2006. Incorporating information from length-mutational events into phylogenetic analysis. Mol. Phylogenet. Evol. 38, 667–676.

Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15, 407–414.

Norup, M.V., Dransfield, J., Chase, M.W., Barfod, A.S., Fernando, E.S., Baker, W.J., 2006. Homoplasious character combinations and generic delimitation: a case study from the Indo-Pacific arecoid palms (Arecaceae: Areceae). Am. J. Bot. 93, 1065–1080.

Pittendrigh, C.S., 1948. The Bromeliad–Anopheles–Malaria complex in Trinidad. I – the Bromeliad flora. Evolution 2, 58–89.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Read, R.W., Baensch, H.U., 1994. *Ursulaea*; a new genus of Mexican Bromeliads. J. Bromeliad Soc. 44, 205–211.

Reeves, G., Chase, M.W., Goldblatt, P., Rudall, P., Fay, M.F., Cox, A.V., Lejeune, B., Souza-Chies, T., 2001. Molecular systematics of Iridaceae: evidence from four plastid DNA regions. Am. J. Bot. 88, 2074–2087.

Roncal, J., Francisco-Ortega, J., Asmussen, C.B., Lewis, C.E., 2005. Molecular phylogenetics of tribe Geonomeae (Arecaceae) using nuclear DNA sequences of phosphoribulokinase and RNA polymerase II. Syst. Bot. 30, 275–283.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Sang, T., 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. Crit. Rev. Biochem. Mol. Biol. 37, 121–147.

Schulte, K., Horres, R., Zizka, G., 2005. Molecular phylogeny of Bromelioideae and its implications on biogeography and the evolution of CAM in the family (Poales, Bromeliaceae). Senckenbergiana Biol. 85, 113–125.

Schulte, K., Zizka, G., 2008. Multi locus plastid phylogeny of Bromelioideae (Bromeliaceae) and the taxonomic utility of petal appendages and pollen characters. Candollea 63, 209–255.

Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49, 369–381.

Small, R.L., Cronn, R.C., Wendel, J.F., 2004. Use of nuclear genes for phylogeny reconstruction in plants. Aust. Syst. Bot. 17, 145–170.

Smith, L.B., 1934. Geographical evidence on the lines of evolution in the Bromeliaceae. Bot. Jahrb. Syst. Pflanzengesch. Pflanzengeogr. 66, 446–468.

Smith, L.B., Downs, R.J., 1974. Flora Neotropica Monograph No. 14 Part 1: Pitcairnioideae (Bromeliaceae). In: Wurdack, J.J. (Ed.), Flora Neotropica. Organization for Flora Neotropica, Hafner, New York, pp. 1–658 (Reprint: The New York Botanical Garden 1986).

Smith, L.B., Downs, R.J., 1979. Flora Neotropica Monograph No. 14 Part 3: Bromelioideae (Bromeliaceae). In: Rogerson, C.T. (Ed.), Flora Neotropica. Organization for Flora Neotropica, New York Botanical Garden, New York, pp. 1493–2142 (Reprint: The New York Botanical Garden 1983).

Smith, L.B., 1988. New key to the genera of the Bromeliaceae. Beitr. Biol. Pflanz. 63, 403–411.

Smith, L.B., Kress, W.J., 1989. New or restored genera of Bromeliaceae. Phytologia 66, 70–79.

Smith, L.B., Kress, W.J., 1990. New genera of Bromeliaceae. Phytologia 69, 271–274.

Smith, L.B., Till, W., 1998. Bromeliaceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants. Springer, Berlin, pp. 74–100.

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Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods). [4.0b10]. Sinauer Associates, Sunderland, Massachusetts.

Terry, R.G., Brown, G.K., Olmstead, R.G., 1997. Examination of subfamilial phylogeny in Bromeliaceae using comparative sequencing of the plastid locus *ndh*F. Am. J. Bot. 84, 664–670.

Thomas, M.M., Garwood, N.C., Baker, W.J., Henderson, S.A., Russell, S.J., Hodel, D.R., Bateman, R.M., 2006. Molecular phylogeny of the palm genus *Chamaedorea*, based on the low-copy nuclear genes PRK and RPB2. Mol. Phylogenet. Evol. 38, 398–415.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Tietze, M., 1906. Die Entwicklung der wasseraufnehmenden Bromeliaceen-Trichome. Z. Naturwiss. 78, 1–49.

Wiens, J.J., 1998. Combining data sets with different phylogenetic histories. Syst. Biol. 47, 568–581.

Please cite this article in press as: Schulte, K., et al. Phylogeny of Bromelioideae (Bromeliaceae) inferred from nuclear and plastid ... Mol. Phylogenet. Evol. (2009), doi:10.1016/j.ympev.2009.02.003