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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