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Multi locus plastid phylogeny of Bromelioideae (Bromeliaceae) and the taxonomic utility of petal appendages and pollen characters

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Abstract

SCHULTE, K. & G. ZIZKA (2008). Multi locus plastid phylogeny of Bromelioideae (Bromeliaceae) and the taxonomic utility of petal appendages and pollen characters. *Candollea* 63: 209-225. In English, English and French abstracts.

For the first time a molecular phylogeny based on five plastid markers is presented for subfamily *Bromelioideae (Bromeliaceae)*. The species set includes 40 genera / 81 species of *Bromeliaceae* representing all subfamilies: *Bromelioideae* (29 genera / 58 species), *Tillandsioideae* (6 genera / 8 species) and *Pitcairnioideae* s.l. (5 genera / 14 species). Basal clades among the *Bromelioideae* are identified, nevertheless the "Core Bromelioids" comprising the majority of the species display low resolution. The phylogeny obtained makes evident, that the generic concept for *Aechmea* Ruiz & Pav. and its allied taxa does not describe monophyletic groups. The same holds true for several subgenera of *Aechmea.* The phylogeny allows the assessment of the systematic value of two characters that have been regarded as systematic valuable for generic delimitation in the (sub)family, 1) the petal appendages and 2) the pollen morphology. Basal *Bromelioideae* are characterized by sulcate pollen, while the more derived *Bromelioideae* display three different pollen types and several transitions between the character states.

Key-words

BROMELIOIDEAE – *Aechmea* – Molecular phylogeny – Plastid markers – Character evolution

Résumé

SCHULTE, K. & G. ZIZKA (2008). Phylogénie des Bromelioideae (Bromeliaceae) basée sur l'analyse de locus plastidiques et utilité taxonomique des caractères asssociés aux pétales et au pollen. *Candollea* 63: 209-225. En anglais, résumés anglais et français.

Pour la première fois, une phylogénie moléculaire de la sous-famille *Bromelioideae (Bromeliaceae)* est établie, basée sur 5 marqueurs plastidiques. L'échantillonnage d'espèces inclut 40 genres / 81 espèces de *Bromeliaceae* représentant toutes les sous-familles: *Bromelioideae* (29 genres / 58 espèces), *Tillandsioideae* (6 genres / 8 espèces) et *Pitcairnioideae* s.l. (5 genre / 14 espèces). Des clades basaux sont identifiés au sein des *Bromelioideae*, bien que leur noyau central («core bromelioids»), comprenant la majorité des espèces, montre une faible résolution. La phylogénie obtenue montre que la présente conception générique d'*Aechmea* Ruiz & Pav. et de ses taxons affines ne délimite pas des groupes monophylétiques. La même observation peut être faite pour certains sous-genres reconnus au sein d'*Aechmea*. La phylogénie permet d'évaluer la valeur systématique de deux caractères estimés être d'une importance taxonomique pour la délimitation des genres dans la (sous-)famille: 1) les appendices des pétales, et 2) la morphologie du pollen. Les *Bromelioideae* primitifs sont caractérisés par un pollen sulqué, tandis que les *Bromelioideae* les plus dérivés montrent trois types différents de pollen et plusieurs caractères de transition.

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Introduction

The almost exclusively neotropical family *Bromeliaceae* (*Poales*) comprises more than 3000 species in 56 genera (Smith & Till, 1998; Luther, 2004). The family displays a striking ecological versatility, occupying a wide range of terrestrial, lithophytic and epiphytic habitats. Unique leaf trichomes capable of water absorption, tank habit, succulence, and CAM photosynthesis are seen as key innovations to allow for a successful adaptation to xeric conditions (Pittendrigh, 1948; Medina, 1974; Crayn & al., 2004). *Bromeliaceae* not only constitute the second most diverse family of flowering plants among neotropical epiphytes (after the orchids), but are also of considerable economic importance (*Ananas comosus*, many ornamental plants).

Bromeliaceae has traditionally been divided into the three subfamilies – *Pitcairnioideae*, *Tillandsioideae*, and *Bromelioideae* – based on flower, fruit and seed characters. Molecular studies of different plastid regions have consistently confirmed the monophyly of *Tillandsioideae* and *Bromelioideae*, respectively, whereas *Pitcairnioideae* are clearly polyphyletic and here referred to as *Pitcairnioideae* s.l. (Terry & al., 1997; Horres & al., 2000, 2007; Givnish & al., 2004; Crayn & al., 2004; Schulte & al., 2005).

Subfamily *Bromelioideae* currently comprises 32 genera with more than 800 species of predominantly rosulate herbs (Smith & Till, 1998; Luther, 2004). They are distributed throughout Central and South America with a centre of diversity in eastern Brazil (Smith & Downs, 1974-1979).

Phylogenetic relationships and character evolution of *Bromelioideae* are the most poorly understood within the family (e.g. Benzing, 2000; Brown & Leme, 2000). This is in great part due to the high extent of morphological, ecological and physiological variation exhibited by the subfamily, rendering the recognition of homoplasies difficult. The delimitation of genera is considered especially problematic, because it often relies on only a few characters, partly being of uncertain systematic value. Moreover, since the last comprehensive monograph of the subfamily by Smith & Downs (1974-1979), the number of described species has increased by more than one third (Luther, 2004). Numerous generic level changes (e.g. Smith & Kress, 1989, 1990; Smith & Spencer, 1992; Read & Baensch, 1994; Brown & Leme, 2005; Betancur & Salinas, 2006) have been proposed since then, further illustrating the problematic concept of the subfamily. Although urgently needed, an updated generic concept for the subfamily is not in sight mainly due to severe uncertainties concerning the taxonomic value of morphological characters. Recently it has become obvious, that intergeneric relationships in *Bromeliaceae* can best be inferred from molecular data (e.g. Barfuss & al., 2005; Rex & al., 2007).

To date only few molecular studies about the phylogeny of *Bromelioideae* exist (e.g. Horres & al., 2000, 2007; Givnish & al., 2004; Crayn & al., 2004; Schulte & al., 2005). They have to contend with an exceedingly low sequence variability of plastid markers investigated and often suffer from poor taxonomic sampling. Nevertheless, one principal problem for the understanding of the phylogeny of *Bromelioideae* has recently been resolved: the identification of the sister group of the subfamily, *Puya* Molina, a genus of terrestrial plants with principally Andean distribution (Terry & al., 1997; Crayn & al., 2004; Givnish & al., 2004; Schulte & al., 2005).

The more comprehensive molecular studies in the subfamily, including most bromelioid genera and relying on more than two plastid markers (Schulte & al., 2005; Horres & al., 2007), revealed several putatively basal genera forming well supported distinct groups *(Bromelia* L., *Deinacanthon* Mez, *Greigia* Regel, *Ochagavia* Phil. / *Fascicularia* Mez). Furthermore, a scarcely resolved core group comprising the more advanced "Core Bromelioids" was identified here. However, relationships between the recognized clades remained unclear.

In the past, systematic concepts of *Bromelioideae* differed considerably because strong emphasis has been placed on different diagnostic characters. In his treatment, Wittmack (1888) relied on the form of the sepals and the type of placentation, whereas Baker (1889) utilized different floral and inflorescence characters. Mez (1891-1894, 1896, 1934) placed great importance on floral features and introduced palynological characters into the classification of *Bromeliaceae*, which was adopted by Harms (1930). According to the type of aperture the tribe *Bromelieae* (from 1930 on:subfamily *Bromelioideae)* was subdivided into three groups:

1. pollen grains without aperture: *Archaeobromeliae* (Mez, 1894, 1896), later renamed as *Integrae* (Mez, 1934);
2. pollen grains with pores: *Poratae;*
3. pollen grains with a furrow: *Sulcatae.*

With the advent of the scanning electron microscopy several of Mez´s observations had to be revised (e.g. Ehler & Schill, 1973; Erdtman & Praglowski, 1974; Halbritter, 1992). Smith & Downs (1974-1979) doubted the utility of palynological characters for the classification of *Bromelioideae.* Instead, they assigned great taxonomic value to petal appendages, secondarily stressing inflorescence characters. The use of the presence/absence of petal appendages as diagnostic character at the generic level in *Bromeliaceae* taxonomy has been questioned repeatedly (e.g. Gilmartin, 1983; Brown & Terry, 1992; Leme, 1997). Up to now, potentially diacritic characters for *Bromelioideae* have not been evaluated within a phylogenetic framework based on DNA-data. Thus, the utility of characters used in previous classification systems of *Bromelioideae* remained speculative.

The main objectives of our study were:

- to broaden the molecular database for the phylogenetic reconstruction of subfamily *Bromelioideae* by analyzing data from four non-coding plastid regions and the coding plastid region *mat*K gene;
- to resolve character transformation patterns of two key morphological features (petal appendages and pollen types) used in former classification systems to subdivide subfamily *Bromelioideae* and thus evaluate the diagnostic potential of these characters.

Materials and methods

Taxon sampling and plant material

DNA sequences of 81 species (82 accessions) from 40 genera of *Bromeliaceae* were analysed in this study. Within subfamily *Bromelioideae* 58 species (59 accessions) were included representing 29 (of 32) genera (see Appendix 1). Within *Aechmea* Ruiz & Pav., 17 species were sampled comprising all seven subgenera *(Aechmea, Lamprococcus* (Beer) Baker, *Macrochordion* (de Vriese) Baker, *Ortgiesia* (Regel) Mez, *Platyaechmea* (Baker) Baker, *Podaechmea* Mez, and *Pothuava* (Baker) Baker). Additionally, six genera (eight species) of subfamily *Tillandsioideae* (see Appendix 2) and five genera (14 species) of subfamily *Pitcairnioideae* (see Appendix 3) were included to represent further principal clades of the family. According to previous molecular studies (Horres & al., 2000; Givnish & al., 2004; Crayn & al., 2004) we used *Brocchinia* Schult. & Schult. f. and *Hechtia* Klotzsch as the outgroup with which phylogenetic trees were rooted.

In total, sequences from five genomic regions were analysed: the *atp*B-*rbc*L spacer, the *trn*L intron, the *trn*L-*trn*F spacer, the *mat*K gene and part of the adjacent 3′*trn*K intron. A large portion of the *atp*B-*rbc*L sequences were generated specifically for this study, and combined with our sequence data on the *trn*L intron (Horres & al., 2000, 2007), *trn*L-*trn*F spacer (Horres & al., 2007), *mat*K gene and 3′*trn*K intron (Schulte & al., 2005). Additionally 63 sequences originally published by Barfuss & al. (2005) were downloaded from GenBank to complement the dataset. Plant material was derived from the Palmengarten Frankfurt/Main, and the Botanical Gardens of the Universities Frankfurt/Main, Heidelberg, Berlin-Dahlem, Bonn, Vienna and Kassel. In great part, the original DNA samples from our former studies, stored at the DNA archive of the Grunelius Möllgaard laboratory at the Research Institute Senckenberg, Frankfurt/Main, were used to generate the *atp*B-*rbc*L data. Vouchers were deposited in the Herbarium Senckenbergianum (FR) and the Palmengarten Herbarium, Frankfurt/M (FRP).

DNA isolation, amplification, and sequencing

Total genomic DNA was isolated from fresh or lyophilized leaves as described in Horres & al. (2000). The *atpB-rbc*L spacer was amplified employing the universal primers *atp*B1 and *rbc*L1 (Chiang & al., 1998). The following polymerase chain reaction (PCR) protocol was used: Initial denaturation for 2 min at 94° C, followed by 34 cycles of 1 min at 94° C for denaturation, 1 min for primer annealing at 52° C, and 2 min at 72° C for elongation, followed by a final elongation period of 7 min at 72° C. Reactions were performed with 0.4 pmol/µl of each primer, 0.2 mM of each dNTP, 2 mM $MgCl_2$, 1x PCR-buffer, 0.01 uM/µl *Taq* DNA polymerase, and 25 ng of template DNA per 50 µl reaction volume. PCR products were purified with NucleoSpin extract (Macherey & Nagel) according to the manufacturer´s protocol. Cycle sequencing was performed with BigDye Terminator Premix V 3.0 (Applied Biosystems) using the amplification primers. The sequencing protocol consisted of an initial denaturation for 1 min at 96° C, followed by 24 cycles of 10 sec denaturation at 96° C, 5 sec annealing at 50° C, and 4 min elongation at 60° C.

The cleaned cycle-sequencing products were analyzed on an ABI 377 automated sequencer, according to the manufacturer´s protocols. Sequencing was performed by Scientific Research and Developement GmBH, Oberursel, Germany. For all taxa both strands of the region were sequenced.

Alignment and phylogenetic analyis

Sequences were edited and assembled using Seqman 5.07 software (DNASTAR). Sequences were initially aligned using ClustalX 1.81 (Thompson & al., 1997) followed by manual adjustments in BioEdit 5.0.9 (Hall, 1999). For parsimony analysis indels were coded in a presence/absence matrix and appended to the alignment (see Table 1). Ambiguous parts of the alignment (one region each within *atp*B-*rbc*L and within *trn*L-*trn*F) were excluded from the analysis. For phylogenetic analysis we applied a pluralistic approach using maximum parsimony (MP), maximum likelihood (ML), and Bayesian methods as advocated by Thornton & Kolaczkowski (2005). The datasets were first analyzed separately and then simultaneously on a PowerPC G5 computer (Macintosh). Parsimony analyses were conducted with PAUP version 4.0b10 (Swofford, 2001). All character changes were treated as unordered and equally weighted (Fitch, 1971) and gaps were treated as missing data. Heuristic search for most parsimonious trees was conducted keeping only the best trees. The initial tree was generated by stepwise addition with 100000 random replicates keeping one tree per step. Subsequent tree bisection-reconnection (TBR) branch swapping was performed saving no more than 100000 trees per replicate, the MULTREES-option not being in effect. The COLLAPSED-option was switched off and polytomous trees condensed after branch swapping by collapsing branches of a maximal length of zero.

Table 1. – Description of the sequence data.

Region	Length of alignment [in basepairs]	Number of variable characters	Number of parsimony-informative characters *[B: Bromelioideae; P: Pitcairnioideae; T: Tillandsioideae]*	Number of coded indels / parsimony informative coded indels
*atp*B-*rbc*L spacer	901	132 (B: 71; P: 63; T: 28)	73 (B: 30; P: 39; T: 15)	46/24
*mat*K gene	1554	252 (B: 131; P: 87; T: 91)	133 (B: 55; P: 55; T: 40)	4/1
3′ *trn*K intron	185	49 (B: 22; P: 22; T: 15)	31 (B: 14, P: 14; T: 5)	3/2
*trn*L intron	600	103 (B: 63; P: 37; T: 19)	46 (B: 23; P: 18; T: 7)	24/8
*trn*L-*trn*F spacer	404	88 (B: 54; P: 35; T: 20)	43 (B: 19; P: 21; T: 9)	39/18
5 plastid regions	3644	621 (B: 341; P: 244; T: 173)	326 (B: 141; P: 147; T: 76)	116/53

Multiple parsimonious trees were combined to form a strict consensus (not shown) and a 50% majority rule (MR) consensus tree. Support for trees was assessed using non-parametric bootstrapping (FELSENSTEIN, 1985) as implemented in PAUP. Bootstrap values (bv) were assessed analizing 1000 bootstrap replicates using 100 replicates of random taxon addition and TBR branch swapping with a limit of 1000 trees saved in each replicate.

The evolutionary model for ML and Bayesian analyses was selected from 56 models using Modeltest 3.7 (POSADA & CRANDALL, 1998), employing the Akaike information criterion (AKAIKE, 1974). For the final combined data set (5 plastid markers), the best fit model was the general time-reversible (GTR) model plus a gamma shape parameter (G) and a proportion of invariable sites (P-invar). The selected model and parameter estimates were then used for tree searches. ML analyses were performed using PAUP. Settings of the heuristic search were largely similar to the parsimony analysis, exept for the number of random addition replicates (50) and the settings for the TBR branch swapping (MULTREES option in effect, without restriction in the number of saved trees).

Bayesian analyses were conducted with MrBayes 3.0 (HUELSENBECK & RONQUIST, 2001; RONQUIST & HUELSENBECK, 2003). The combined dataset was partitioned according to the five plastid regions and unlinked to allow divergence of parameters and rates of evolution. For the final analysis, four Markov chains starting at random trees were run simultaneously for 5000000 generations, with samples of one tree taken from every 100th generation, with a heating temperature of 0.05. Trees that preceded the stabilization of the same likelihood value found in all four Markov chains (the burn-in) were excluded, and the remaining trees (49400) were used to calculate posterior probabilities and to construct a MR consensus tree. The final analyses were run three times independently starting from random trees.

Evaluation of major morphological transitions

We explored the diversification of petal appendages (absent, present: scales, lateral folds) and pollen apertures (sulcate, porate, inaperturate). We focused on these traits not only because they have traditionally been considered important in the classification of the subfamily, but also to hypothesize the evolution of those characters in the subfamily. The information was compiled from literature (for petal appendages: SMITH & DOWNS, 1974-1979; for pollen types: EHLER & SCHILL, 1973; ERDTMAN & PRAGLOWSKI, 1974; HALBRITTER, 1992; HALBRITTER & TILL, 1998; SMITH & TILL, 1998; FARIA & al., 2004). The character states of the petal appendages were scored for each species represented in the phylogeny. Data on pollen types could not be obtained for each species except for the very heterogeneous genus *Aechmea*. For the remaining genera, available information on character states was summed up and scored for each genus as a whole. We used MacClade 4.06 (MADDISON & MADDISON, 2003) to trace the selected characters by overlying them onto one selected most parsimonious tree of the parsimony analysis of the five plastid markers.

Results

Sequence data

For the 81 accessions (81 species) of *Bromeliaceae*, the total alignment of the sequenced plastid regions *atp*B-*rbc*L spacer, *trn*L intron, *trn*L-*trn*F spacer, *mat*K and 3′*trn*K comprised 3644 positions. From 621 variable positions within *Bromeliaceae*, 326 (8.9% of all positions) were potentially parsimony informative. Within *Bromelioideae*, 341 positions were variable and 141 (3.9% of all positions) were potentially parsimony informative. The alignment included 904 gap positions which were scored as 116 indels. From the 116 coded indels, 53 were potentially parsimony informative (see Table 2). Pairwise sequence divergence (uncorrected for multiple hits) reached a maximum of 3.5% (between *Brocchinia tatei* and *Fosterella albicans).* Within *Pitcairnioideae* maximal sequence divergence was highest, within *Tillandsioideae* it reached 2.7% (between *Catopsis nutans* and *Tillandsia fasciculata)* and it was lowest within *Bromelioideae* with 1.7% (between *Bromelia plumieri* and *Aechmea mertensii*).

Phylogenetic relationships

Parsimony analysis of the combined dataset found 18344 most parsimonious trees of 1143 steps with a consistency index CI of 0.710 and a retention index RI of 0.815. Results from

maximum likelihood (tree not shown) and Bayesian analyses (Fig. 1, the branch length reflect changes per site) are largely congruent with the results from the parsimony analysis. Differences concern nodes that were only poorly supported. In the following we refer to the 50% MR consensus tree of the parsimony analysis (Fig. 2, *Cryptanthus glaziovii* and *Orthophytum supthutii* receive a bootstrap support of 56) unless mentioned otherwise.

The MR consensus tree shows *Brocchinia* (bv 100) and *Hechtia* in sister group position to the rest of the family (bv 61). The latter forms a dichotomy with one branch comprising all representatives of the subfamily *Tillandsioideae* (bv 94) and the other the remaining *Pitcairnioideae* together with the *Bromelioideae* (bv 93). *Pitcairnia* L'Hér. and *Fosterella* L. B. Sm. as representatives of the *Pitcairnioideae* s.s. form a clade sister to a strongly supported clade comprising *Puya* and the *Bromelioideae* (bv 99). The strict consensus and the MR consensus tree of the parsimony analysis are identical in the described topology.

Puya forms a strongly supported monophyletic group (bv 100). The MR consensus tree depicts the genus as sister to *Bromelioideae*, a relationship that receives weak statistical support (bv 61). In the strict consensus, *Puya* falls at a polytomy with the *Bromelioideae*.

In the 50% MR consensus the *Bromelioideae* split into a first polytomy of five separate clades, four of which receive high support values: 1) *Bromelia* (bv 100), 2) *Deinacanthon* (bv 100), 3) *Greigia* (bv 100), 4) a branch uniting the genera *Ochagavia* and *Fascicularia* (bv 93), and 5) the remaining *Bromelioideae* (bv 51). Within the latter, *Fernseea* Baker is found in sister group position to the remaining *Bromelioideae*, which form a highly supported clade (bv 93) and are termed "Eu-Bromelioids" hereafter (Fig. 1). Nevertheless, this branching collapses in the strict consensus and *Fernseea* falls at a polytomy together with *Puya*, *Bromelia*, *Deinacanthon*, *Greigia*, *Ochagavia* / *Fascicularia* and the branch comprising the Eu-Bromelioids.

Relationships within the Eu-Bromelioids are poorly resolved. The following three groups are identified:

1. *Ananas* Mill. (bv 100). The sister group relationship to *Neoglaziovia* Mez does not receive statistical support and collapses in the strict consensus.
2. *Orthophytum supthutii* and *Cryptanthus glaziovii* (BV 56). The sister group relationship to *C. bahianus* is also found within the strict consensus tree, but does not receive statistical support.
3. the more advanced "Core Bromelioids" (Fig. 1).

Within the "Core Bromelioids", the following groups are noteworthy:

- a well supported clade uniting *Ronnbergia petersii* and *Aechmea drakeana* (bv 92);
- nidularioid clade (bv 88) comprising the genera *Nidularium* Lem., *Neoregelia* L. B. Sm., *Wittrockia* Lindm. and a taxon whose determination recently has become doubtful and therefore is listed as Gen. spec. 1;
- an Ortgiesian clade comprising several *Aechmea* species of the subgenus *Ortgiesia* Regel and *Aechmea racinae* (subgenus *Lamprococcus);*
- a *Podaechmea* clade. This taxonomical heterogeneous clade comprises two *Aechmea* species of the subgenus *Podaechmea (Aechmea lueddemanniana, A. mexicana)* and *Androlepis* Houllet (bv 78), with *Hohenbergiopsis* L. B. Sm. & Read and *Ursulaea* Read & Baensch in sister group position. *Ursulaea* has formerly been placed in *Aechmea* subgenus *Podaechmea*.

Discussion

Phylogenetic relationships

The five marker phylogeny principally confirms the results of former studies based on plastid markers (Schulte & al., 2005; Horres & al., 2000, 2007). The sister group of *Bromelioideae*, the genus *Puya*, and the basal clades among the subfamily principally comprise terrestrial species, whereas the "Core Bromelioids" are in great part epiphytic. The latter group remains insufficiently resolved, nevertheless documenting, that the present generic delimitation of *Aechmea* and allied genera does not reflect monophyletic groups. The same holds true for several subgenera of *Aechmea* (*Aechmea* subg. *Aechmea,* subg. *Chevaliera* Beer, subg. *Pothuava*, subg. *Lamprococcus*, subg. *Platyaechmea).* Interesting clades among the "Core Bromelioids" are:

- the *Podaechmea* clade comprising members from Central America (*Hohenbergiopsis, Ursulaea, Androlepis* and two species of *Aechmea* subg. *Podaechmea);*
- the "Nidularioid clade" with *Nidularium*, *Wittrockia*, and *Neoregelia;*
- the species of *Aechmea* subg. *Ortgiesia* plus *Aechmea racinae* (*Aechmea* subg. *Lamprococcus).*

While the latter two clades have been already recognized up to now (Smith & Downs, 1974-1979; Leme, 1997, 1998, 2000) there has been no evidence or suggestion that at least the major part of the Central American bromelioid genera might form a monophyletic group. Our molecular data do not support the subgeneric concept in *Aechmea* with the exception of the subgenus *Ortgiesia*. Moreover, no evidence is provided for lifting one of the subgenera to generic rank.

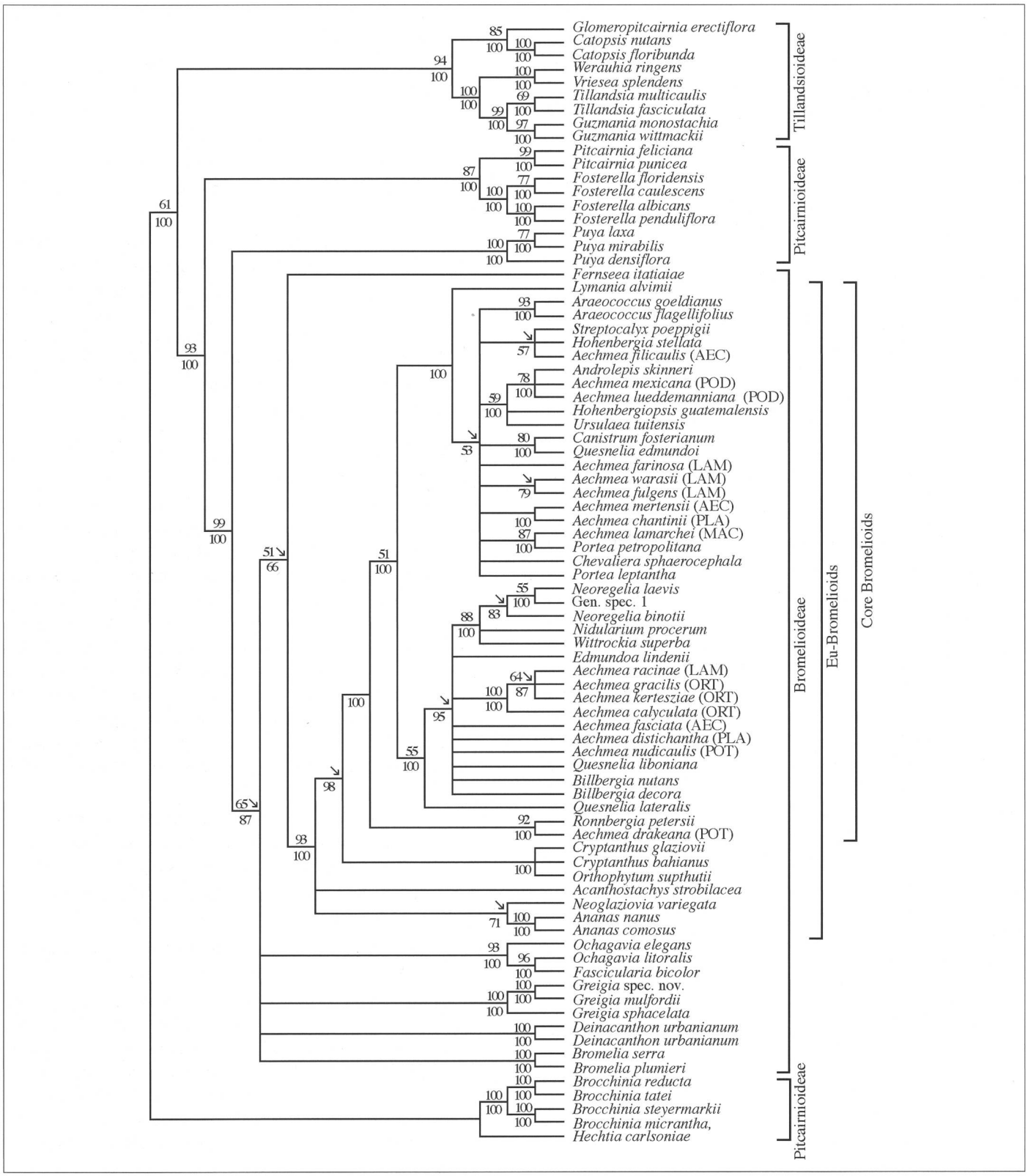

Fig. 1. – 50% Majority rule consensus tree of 49400 trees obtained from four runs of Bayesian analysis of the combined dataset implementing the GTR+G+I model. Posterior probabilites are given above branches. (Abbreviations: *Aechmea* subgenera, AEC: *Aechmea*; POD: *Podaechmea*; PLA: *Platyaechmea*; LAM: *Lamprococcus*; ORT: *Ortgiesia*; POT: *Pothuava*; MAC: *Macrochordion*).

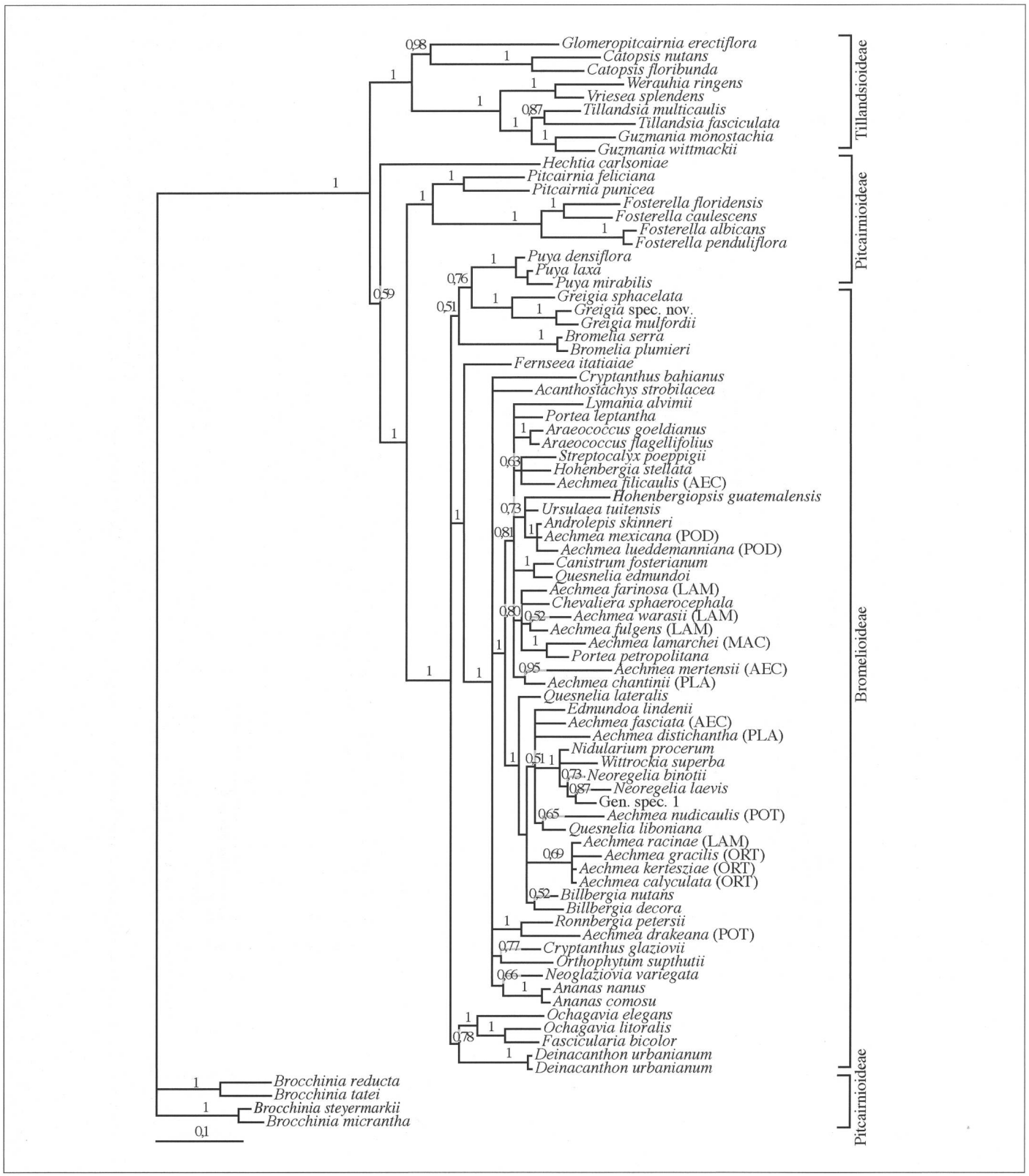

Fig. 2. – One single tree selected from the set of 18344 equally parsimonious trees of 1143 steps length of the combined dataset. Bootstrap values are given above branches, clade frequencies are given below branches. Nodes that are not present in the strict consensus tree are marked with an arrow. (Abbreviations: *Aechmea* subgenera, AEC: *Aechmea*; POD: *Podaechmea*; PLA: *Platyaechmea*; LAM: *Lamprococcus*; ORT: *Ortgiesia*; POT: *Pothuava*; MAC: *Macrochordion*).

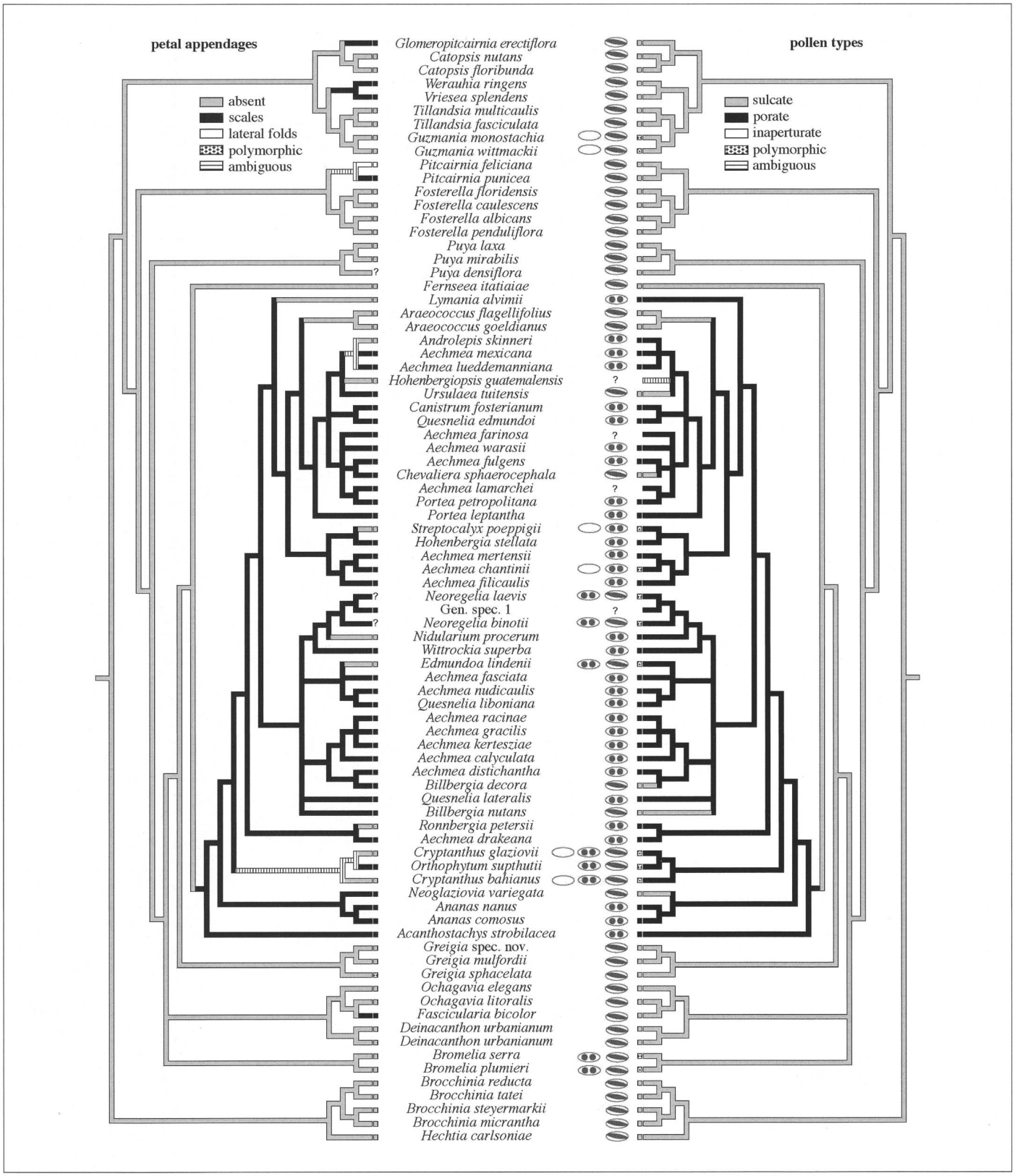

Fig. 3. – Most-parsimonious reconstruction of the evolution of petal appendages and pollen types in Bromelioideae, based on relationships revealed by maximum parsimony analysis of five plastid markers (*atp*B-*rbc*L, *mat*K, 3´*trn*K, *trn*L, *trn*L-*trn*F).

Reconstruction of character evolution

Tracing character state transitions for petal appendages on one selected most parsimonious tree (of 18344) of the parsimony analysis of the five plastid markers exhibits high levels of homoplasy for the character (Fig. 3). Petal appendages show several independent origins within the family as well as within subfamily *Bromelioideae*. The inferred evolution of the character indicates that the lack of petal appendages is ancestral in *Bromelioideae* and that petal appendages have evolved at least three times independently within *Bromelioideae* (within the clade that comprises *Ochagavia/Fascicularia*, in *Greigia*, and the Eu-Bromelioids). Furthermore, the reconstruction indicates that petal appendages were lost multiple times within the Eu-Bromelioids.

Interestingly, several well supported clades unite genera that are separated mainly on the basis of the presence/absence of petal appendages (e.g. *Ronnbergia* E. Morr. & Andre / *Aechmea*, *Cryptanthus* Otto & A. Dietr. / *Orthophytum* Beer, *Ochagavia/Fascicularia*, and *Nidularium/Wittrockia* within the Nidularioid clade). Recent revisions in the case of *Ochagavia* and *Fascicularia* (Zizka & al., 1999, 2002) have underlined their morphological and genetic similarity and casted additional doubts on the value of this floral character. In the systematic concept of Smith & Downs (1974-1979) the close relationships between the above mentioned groups are obscured due to the artificial separation of genera according to presence/absence of petal appendages. Based on ontogenetic studies of this character in *Bromeliaceae*, Brown & Terry (1992) questioned the prominent role of petal appendages in the circumscription of bromeliad genera. They argued that since these are formed late in the ontogeny of the flower they represent terminal ontogenetic characters that are more susceptible to modifications (Løvtrup, 1978). Our results make evident that the character is too homoplasious to be used in higher level classification of *Bromelioideae*.

Character transitions from presence/absence of petal appendages are observed in various terminal nodes of the phylogeny (Fig. 3) indicating that the character is inappropriate for generic delimitation, too.

The inferred evolution of pollen types displays a progression from sulcate *(Pitcairnioideae* s.l., *Tillandsioideae*, basal lineages of *Bromelioideae)* to porate pollen (especially within the Eu-Bromelioids) within *Bromeliaceae*. Tracing character transitions on one selected most parsimonious tree of the parsimony analysis indicates that the pollen of the genera *Greigia*, *Fascicularia*, *Ochagavia*, *Deinacanthon*, *Bromelia*, and *Fernseea* is primarily sulcate and supports the assumption of this condition being primitive in *Bromelioideae* (Mez, 1894, 1896, 1934; Harms, 1930; Halbritter, 1992). However, the inferred character transitions also show a secondary origin of sulcate pollen within the subfamily, especially within the Core Bromelioids (i. e. *Billbergia, Canistrum, Neoregelia, Araeococcus, Aechmea, Ursulaea).*

According to the reconstruction of character transitions, porate pollen presumably evolved only a few times and was lost again multiple times. Porate pollen arose independently at least twice, in *Bromelia* and in the Eu-Bromelioids. Among several basal Eu-Bromelioids porate pollen occurs (e.g. *Ananas, Orthophytum, Cryptanthus, Acanthostachys* Link), indicating an early origin of this character state within the clade. Moreover, the examination of pollen type transitions reveals several independent origins of inaperturate pollen within several lineages *(Guzmania* Ruiz & Pav., *Aechmea, Cryptanthus, Streptocalyx* Beer). Thus, the assumption that inaperturate pollen is a derived condition within the family (Mez, 1894, 1896, 1934; Harms, 1930; Halbritter, 1992) is supported.

Comparing the tribal classification for *Bromelioideae* of Mez (1934) which he based on the three pollen types with the results of our molecular phylogeny is especially interesting. Mez´s tribes *Integrae* and *Sulcatae* unify the basal lineages of Bromelioideae as well as several of the basal Eu-Bromelioids *(Crypthanthus, Orthophytum, Neoglaziovia).* On the other hand, the tribe *Poratae* includes mainly representatives of the "Core Bromelioids". Although Mez´s observations on aperture types had to be revised in part (e.g. Halbritter, 1992), it is remarkable, that this author already succeeded in recognizing major relationships within Bromelioideae based on pollen characters. In the light of our results, the concerns of Smith & Downs (1974-1979) about the taxonomic utility of pollen characters are intelligible. The rejection of Mez´s tribal division of the subfamily and the introduction of a classification system based on the highly homoplasious character of petal appendages (Smith, 1967; Smith & Downs, 1974-1979) rather hampered the understanding of phylogenetic relationships and character evolution within *Bromelioideae* and has to be refused.

Conclusion

The molecular phylogeny presented here provides a first framework to evaluate character transformation within *Bromelioideae*, thus allowing the assessment of traits regarding their systematic value. The evaluation of putative diacritic characters revealed high levels of homoplasy for morphological characters used in previous classification systems of the subfamily. Cladistic analysis of an extensive morphological dataset for *Aechmea* and closely related genera by Faria & al. (2004) also indicated high levels of homoplasy for characters previously used in bromeliad taxonomy. This underlines the need for further studies in character transitions within the subfamily. The results of

molecular studies in *Bromeliaceae* at hand point towards an underestimation of the morphological variability and an overestimation of the systematic value of morphological characters in the past. Our phylogenetic reconstruction gives also the surprising evidence, that groups of genera with similar geographic distribution (and possibly origin) might be closer related than up to now suggested – in spite of considerable morphological differences.

Petal appendages are obviously inappropriate for higher level classification of *Bromelioideae*, displaying character transitions even among closely related taxa. It is evident, that the results of recent molecular analyses suggest systematic and nomenclatural consequences. In bromeliads, due to the lack of updated "classical" revision, drawing systematic and nomenclatural consequences is difficult and often premature. This refers especially to the genus *Aechmea* and allied genera.

However, even in cases where the necessary morphological investigations are at hand like in the genera *Ochagavia* and *Fascicularia* and molecular data suggest uniting the two genera, the decision is not straightforward. Practical reasons give evidence to maintain the present generic concept recognizing two morphologically similar but well recognizable groups, at least as long as detailed molecular studies of all species are at hand.

In order to improve our understanding of character evolution within the subfamily it remains crucial to broaden the morphological database as well as to improve resolution and statistical support of phylogenetic estimates by using additional molecular markers. For the latter purpose, regions from the nuclear genome seem to be most rewarding (Sang, 2002; Small & al., 2004), but remain to be established for *Bromelioideae* yet.

Acknowledgements

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Appendix 1. – Taxa represented in the study, source, voucher information and GenBank accession numbers for *Bromelioideae*. Nomenclatu = Botanical Garden Berlin-Dahlem; BG FR = Botanical Garden of the University of Frankfurt/Main; FRP = Herbarium and living collections of the University of Kassel.

Species	Accession no. living collection/ herbarium specimen	DNA-Isolat No
Bromelioideae		
Acanthostachys strobilacea (Schult f.) Klotzsch	FRP 98-16986-0 / Horres 019 (FR)	H 019
Aechmea calyculata Baker	HEID 103296 / Schulte 240203-9 (FR)	H 184
Aechmea chantinii (Carrière) Baker	KAS s.n. / Rex 260105-3 (FR)	K 4
Aechmea distichantha Lem.	FRP 88-16753-2 / Zizka 1549 (FRP), Horres 008 (FR)	H 008
Aechmea drakeana André	FRP 98-16955-2 / Zizka 1100 (FRP)	H 042
Aechmea farinosa (Regel) L. B. Sm.	FRP 98-16961-3 / Zizka 1108 (FRP)	H 272
Aechmea fasciata (Lindl.) Baker	KAS s.n. / Rex 260105-2 (FR)	K 5
Aechmea filicaulis (Griseb.) Mez	FRP 98-16863-0 / Horres & Schulte 180701-6 (FR)	H 248
Aechmea fulgens Brongn.	FRP s.n. / Schulte 130105-5 (FR)	H 144
Aechmea gracilis Lindm.	FRP 98-16949-3 / Schulte 280203-1 (FR)	H 043
Aechmea kertesziae Reitz	FRP 98-16935-3 / Zizka 1177 (FRP)	H 270
Aechmea lamarchei Mez	BG Berlin-Dahlem 118-37-74-86 / Gartenherbar 11309 (B)	H 242
Aechmea lueddemanniana (K. Koch) Mez	«FRP 95-14215-0 / Schulte 100203-3 (FR); Schulte 010305-1 (FR)»	H 150
Aechmea mertensii (G. Mey.) Schult. f.	FRP 98-16873-0 / Zizka 1572 (FRP)	H 044
Aechmea mexicana Baker	«HEID 104025 / Schulte 240203-12 (FR); Schulte 171103-25 (FR)»	H 256
Aechmea nudicaulis (L.) Griseb.		MB 118
Aechmea racinae L. B. Sm.	FRP 98-16934-3 / Schulte 120203-1 (FR)	H 257
Aechmea warasii E. Pereira	HEID 130354 / Schulte 240203-17 (FR)	H 185
Ananas comosus (L.) Merr.	BG FR s.n. / Horres & Schulte 220601-1 (FR)	H 136
Ananas nanus (L. B. Sm.) L. B. Sm.	FRP s.n. / Horres & Schulte 050401-9 (FR)	H 040
Androlepis skinneri (K. Koch) Houllet	FRP 97-16793-2 / Schulte 140105-12 (FR)	H 048
Araeococcus flagellifolius Harms	KAS s. n. / Rex 260105-1 (FR)	K 9
Araeococcus goeldianus L. B. Sm.	FRP 99-18256-2 / Schulte 100203-1 (FR)	H 206
Billbergia decora Poepp. & Endl.	«FRP 90-733-2-4 / Horres 129 (FR); Zizka 882 (FRP)»	H 129
Billbergia nutans Regel	FRP 97-16791-0 / Zizka 1528 (FRP)	H 280
Billbergia nutans Regel	FRP 99-18405-0 / Horres 036 (FRP)	H 036
Bromelia plumieri (E. Morren) L. B. Sm.		MB 119
Bromelia serra Griseb.	FRP 98-17751-0 / Horres 029 (FR)	H 029
Canistrum fosterianum L. B. Sm.	FRP 86-16991-3 / Zizka 927 (FRP)	H 047
Chevaliera sphaerocephala (Baker) L. B. Sm. & W. J. Kress	FRP 90-835-3 / Zizka 1104 (FRP)	H 030
Chevaliera sphaerocephala (Baker) L. B. Sm. & W. J. Kress	FRP 99-18245-3 / Horres 030b (FR) (voucher DNA)	H 030b
Cryptanthus bahianus L. B. Sm.	HEID 103794 / Gartenherbar 11060a (B)	H 214
Cryptanthus glaziovii Mez	HEID 102583 / Schulte 010601-3 (FR)	H 215
Deinacanthon urbanianum (Mez) Mez	FRP 98-17786-0 / Horres 018 (FRP)	H 018
Deinacanthon urbanianum (Mez) Mez	BG FR s.n. / Horres 140 (FR)	H 140
Edmundoa lindenii (Regel) Leme	HEID 105009 / Schulte 010601-4 (FR)	H 213
Fascicularia bicolor (Ruiz & Pav.) Mez	FRP 98-16846-3 / Zizka 1790 (FR)	H 006a
Fernseea itatiaiae (Wawra) Baker	HEID 102174 / Horres 067 (FR)	H 067b
Genus sp. 1	FRP 90-1144-4-00 / Zizka 1193 (FRP)	H 271
Greigia mulfordii L. B. Sm.	- / Till 13090 (W)	H 111
Greigia sp.	FRP 99-19040 / Grant 19040 (FR)	H 157
Greigia sphacelata (Ruiz & Pav.) Regel	FRP 92-9513-3 / Schulte 230305-4 (FR)	H 004
Hohenbergia stellata Schult. f.	FRP 95-14252-0 / Horres 037 (FRP)	H 037
Hohenbergiopsis guatemalensis (L. B. Sm.) L. B. Sm. & Read	FRP 8-1991-1227-52 / Schulte 130901-6 (FR)	H 138
Lymania alvimii (L. B. Sm. & Read) Read	HEID 103784 / Horres & Schulte 050401-4 (FR)	H 087
Neoglaziovia variegata (Arruda) Mez	FRP 97-16794-3 / Zizka 1105 (FRP)	H 052

follows SMITH & DOWNS (1974-1979) and LUTHER (2004). For the living collections, following abbreviations are used: BG Berlin the Palmengarten Frankfurt/Main; HEID = Herbarium and Botanical Garden of the University of Heidelberg; KAS = Greenhouses

GenBank no./ atpB -rbcL spacer	**trnL intron reference-no.**	**trnL -trnF spacer**	**matK, 3´ trnK**
EU219694	AF188765	DQ084606	AY950021
EU219713	DQ084674	DQ084593	AY950040
EU219715	DQ084675	DQ084581	AY950042
EU219714	DQ084643	DQ084579	AY950041
EU219716	AF188772	DQ084588	AY950043
EU219704	DQ084677	DQ084586	AY950031
EU219707	DQ084678	DQ084582	AY950034
EU219709	DQ084679	DQ084576	AY950036
EU219706	DQ084680	DQ084587	AY950033
EU219711	DQ084682	DQ084594	AY950038
EU219712	DQ084683	DQ084595	AY950039
EU219717	DQ084684	DQ084590	AY950044
EU219702	DQ084685	DQ084596	AY950029
EU219708	DQ084686	DQ084575	AY950035
EU219701	DQ084688	DQ084597	AY950028
AY614390	AY614268.1	AY614268	AY614024.1
EU219703	DQ084691	DQ084583	AY950030
EU219705	DQ084692	DQ084584	AY950032
EU219728	DQ084694	DQ084574	AY950055
EU219727	DQ084695	DQ084573	AY950054
EU219678	AF188780	DQ084610	AY950005
EU219676	DQ084696	DQ084629	AY950003
EU219675	DQ084697	DQ084630	AY950002
EU219723	DQ084698	DQ084624	AY950050
EU219722			AY950049
	AF188766	DQ084623	
AY614389.1	AY614267.1	AY614267.1	AY614023.1
EU219692	DQ084699	DQ084622	AY950019
EU219697	AF188773	DQ084618	AY950024
	AF188770		
EU219718		DQ084578	AY950045
EU219684	DQ084700	DQ084634	AY950011
EU219683	DQ084701	DQ084635	AY950010
EU219690	AF188781	DQ084607	AY950017
EU219691	DQ084702	DQ084608	AY950018
EU219685	DQ084704	DQ084631	AY950012
EU219696	AF188775	DQ084605	AY950023
EU219672	DQ084705	DQ084633	AY949999
EU219710	DQ084690	DQ084592	AY950037
EU219689	DQ084709	DQ084600	AY950016
EU219687	DQ084710	DQ084601	AY950014
EU219688	AF188779	DQ084599	AY950015
EU219699	AF188774	DQ084609	AY950026
EU219693	DQ084711	DQ084627	AY950020
EU219673	AF188768	DQ084619	AY950000
EU219724	AF188763	DQ084614	AY950051

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Species	Accession no. living collection/ herbarium specimen	DNA-Isolat No.
Bromelioideae		
Neoregelia binotii (Antoine) L. B. Sm.	FRP 98-16967-3 / Zizka 1418 (FRP)	H 081
Neoregelia laevis (Mez) L. B. Sm.	FRP 98-16962-3 / Horres & Schulte 220601-3 (FR)	H 080
Nidularium procerum Lindm.	FRP 99-18619-0 / Horres & Schulte 220601-8 (FR)	H 137
Ochagavia elegans R. Phil.	FRP 98-16852-3 / Horres 23a (FR)	H 23a
Ochagavia litoralis(Phil.) Zizka, Trumpler & Zoellner	FRP 98-16853-2 / Horres 15a (FR) (voucher DNA)	H 15a
Orthophytum supthutii E.Gross & Barthlott	HEID 102160 / Barthlott & Supthut 10315 (HEID)	H 223
Portea leptantha Harms	«FRP 99-18222-3 / Schulte 060901-1 (FR); Zizka 1055 (FRP)»	H 239
Portea petropolitana (Wawra) Mez .	«FRP s.n. / Zizka 1056 (FRP); Schulte 060901-2 (FR)»	H 053
Quesnelia *edmundoi* L. B. Sm.	FRP 92-10483-3 / Zizka 964 (FRP)	H 050
Quesnelia lateralis Wawra	FRP 90-10484-0 / Zizka 1554 (FRP)	H 051
Quesnelia liboniana (De Jonghe) Mez	FRP 99-17934-0 / Zizka 1384 (FRP)	H 220
Ronnbergia petersii L. B. Sm.	FRP 99-17997-3 / Schulte 170203-5 (FR)	H 120
Streptocalyx poeppigii Beer	FRP 94-13845-4 / Horres & Schulte 201101-5 (FR)	H 267
Ursulaea tuitensis (Magana & E. J. Lott) Read & Baensch	FRP s.n. / Horres 033 (FR) (voucher DNA)	H 033
Wittrockia superba Lindm.	FRP 93-12641-0 / Horres & Schulte 050401-8 (FR)	H 049

follows SMITH & DOWNS (1974-1979) and LUTHER (2004). For the living collections, following abbreviations are used: BG Berlin the Palmengarten Frankfurt/Main; HEID = Herbarium and Botanical Garden of the University of Heidelberg; KAS = Greenhouses

GenBank no./ atpB -rbcL spacer	**trnL intron reference-no.**	**trnL -trnF spacer**	**matK, 3´ trnK**
EU219682	AF188764	DQ084613	AY950009
EU219681	AF188762	DQ084612	AY950008
EU219686	DQ084712	DQ084628	AY950013
EU219679	AF 188778	DQ084603	AY950006
EU219680	AF188777	DQ084602	AY950007
EU219695	DQ084713	DQ084572	AY950022
EU219725	DQ084714	DQ084621	AY950052
EU219726	DQ084715	DQ084620	AY950053
EU219719	AF188769	DQ084616	AY950046
EU219720	AF188771	DQ084615	AY950047
EU219721	DQ084717	DQ084617	AY950048
EU219674	DQ084718	DQ084632	AY950001
EU219677	DQ084719	DQ084598	AY950004
EU219700	DQ084720	DQ084625	AY950027
EU219698	AF188767	DQ084611	AY950025

Appendix 2. – Taxa represented in the study, source, voucher information and GenBank accession numbers for *Tillandsioideae*. Nomenclature living collections of the Palmengarten Frankfurt/Main.

Species	Accession no. living collection/ herbarium specimen	DNA-Isolat No.
Tillandsioideae		
Catopsis floribunda L. B. Sm.		MB 106
Catopsis nutans (Sw.) Griseb.		MB 2
Glomeropitcairnia erectiflora Mez	FRP 99-18392-2 / Horres 002 (FRP)	H 002
Glomeropitcairnia erectiflora Mez		MB 30
Guzmania monostachia (L.) Mez	FRP 89-18406-0 / Horres 016 (FR) (voucher DNA)	H 016
Guzmania monostachia (L.) Mez		MB 22
Guzmania wittmackii (André) Mez	FRP 99-18407-3 / Schulte 170305-4 (FR)	H 017
Tillandsia fasciculata Sw.		MB 76
Tillandsia multicaulis Steud.		MB 107
Vriesea splendens (Brongn.) Lem.		MB 37
Werauhia ringens (Griseb.) J. R. Grant		MB 19

Appendix 3. – Taxa represented in the study, source, voucher information and GenBank accession numbers for *Pitcairnioideae*. Nomenclature Garden of the University of Bonn; FRP = Herbarium and living collections of the Palmengarten Frankfurt/Main; HEID = Herbarium and

Species	Accession no. living collection/ herbarium specimen	DNA-Isolat No.
Pitcairnioideae		
Brocchinia micrantha (Baker) Mez		MB 115
Brocchinia reducta Baker		MB 113
Brocchinia steyermarkii L. B. Sm.		MB 114
Brocchinia tatei L. B. Sm.		MB 116
Fosterella albicans (Griseb.) L. B. Sm.	FRP 98-18320-1 / Schulte 130901-3 (FR), Horres 156 (FR)	H 156
Fosterella caulescens Rauh	FRP 99-18434-3 / Rauh 40579a (HEID)	H 158
Fosterella floridensisIbisch R. Vásquez & E. Gross	- / Ibisch & Ibisch 97-83 (FR)	H 204
Fosterella penduliflora (C. H. Wright) L. B. Sm.	HEID 103655 / Horres 086 (FR)	H 086
Hechtia carlsoniae Burt-Utley & Utley		MB 79
Pitcairnia feliciana (A. Chev.) Harms & Mildbr.	BG Bonn 12804 / Porembski 12804 (BONN)	P1
Pitcairnia punicea Scheidw.		MB 77
Puya mirabilis (Mez) L. B. Sm.	HEID 103731 / Horres 060 (FR)	H 060
Puya densiflora Harms	HEID 103568 / Horres 076 (FR)	H 076
Puya laxa L. B. Sm.	FRP 94-12923-4 / Horres 006 (FRP)	H 006
Puya laxa L. B. Sm.		MB 78

follows SMITH & DOWNS (1974-1979) and LUTHER (2004). For the living collections, following abbreviation is used: FRP = Herbarium and

GenBank no./ atpB -rbcL spacer	**trnL intron reference-no.**	**trnL -trnF spacer**	**matK, 3´ trnK**
AY614391	AY614269.1	AY614269.1/Ref.4	AY614025.1
AY614392.1	AY614270.1	AY614270.1	AY614026.1
	AF188818	DQ084558	
AY614395.1			AY614029.1
			AY949990
AY614420.1	AY614298.1	AY614298.1	
EU219664	AF188797	DQ084560	AY949991
AY614466	AY614344.1	AY614344.1	AY614100.1
AY614478.1	AY614356.1	AY614356.1	AY614112.1
AY614411.1	AY614289.1	AY614289.1	AY614045.1
AY614413.1	AY614291.1	AY614291.1	AY614047.1

follows SMITH & DOWNS (1974-1979) and LUTHER (2004). For the living collections, following abbreviations are used: BG Bonn = Botanical Botanical Garden of the University of Heidelberg.

GenBank no./ atpB -rbcL spacer	**trnL intron reference-no.**	**trnL -trnF spacer**	**matK, 3´ trnK**
AY614381.1	AY614259.1	AY614259.1	AY614015.1
AY614384.1	AY614262.1	AY614262.1	AY614018.1
AY614382.1	AY614260.1	AY614260.1	AY614016.1
AY614383.1	AY614261.1	AY614261.1	AY614017.1
EU219667	DQ084706	DQ084570	AY949994
EU219668	DQ084707	DQ084569	AY949995
EU219666	DQ084708	DQ084568	AY949993
EU219669	AF 188782	DQ084571	AY949996
AY614386.1	AY614264.1	AY614264.1	AY614020.1
EU219665	AF188792	DQ084567	AY949992
AY614387.1	AY614265.1	AY614265.1	AY614021.1
EU219671	AF188793	DQ084562	AY949998
EU219670	DQ084716	DQ084564	AY949997
	AF188794	DQ084563	
AY614388.1			AY614022.1