Aliso: A Journal of Systematic and Evolutionary Botany

Volume 23 | Issue 1 Article 5

2007

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Aliso 23, pp. 27–43

SYSTEMATICS OF BROMELIOIDEAE (BROMELIACEAE)—EVIDENCE FROM MOLECULAR AND ANATOMICAL STUDIES

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ABSTRACT

A reconstruction of the phylogeny of Bromeliaceae based on sequence data from three noncoding chloroplast DNA markers (*trn*L intron, *trn*T–*trn*L, and *trn*T–*trn*F intergenic spacer [IGS]) is presented, including 26 genera and 33 species. Relationships of Bromelioideae and phylogeny within this subfamily were analyzed in more detail on the basis of two of these markers (*trn*L intron and *trn*L–*trn*F IGS) using a set of 37 genera/74 species of Bromeliaceae, including 28 genera/60 species of Bromelioideae. Sister group relationships of Bromelioideae were not resolved with sufficient reliability, but the most likely candidates are the genera *Fosterella* and *Puya.* The basal phylogeny of Bromelioideae also was not resolved. *Greigia, Ochagavia/Fascicularia/Fernseea, Deinacanthon, Bromelia,* and a ''core group'' of the remaining Bromelioideae formed a basal polytomy. Within Bromelioideae, the AFLP technique was applied to assess relationships among selected groups of genera. In the *Ochagavia/Fascicularia* group (5 species and subspecies/16 accessions), AFLP data fully confirmed the systematic relationships based on morphological and anatomical characters. Investigation of 30 *Aechmea* species (33 accessions), including all subgenera and one species each from the related genera *Ursulaea, Portea, Chevaliera,* and *Streptocalyx* produced no resolution for several of the species. Clades that received good bootstrap support generally did not correspond with the delimitation of subgenera of *Aechmea.* Additionally, leaf blade anatomy of these species was investigated. The results corresponded partly with those of the AFLP analysis. Generic rank for *Ursulaea* and *Portea* was not supported.

Key words: *Aechmea,* AFLPs, Bromeliaceae, *Fascicularia, Greigia, Ochagavia,* phylogeny, *trn*L intron, *trn*L–*trn*F, *trn*T–*trn*L IGS.

INTRODUCTION

Bromeliaceae is a medium-sized family comprising 56 genera with more than 2600 species (Smith and Till 1998). The almost exclusively Neotropical bromeliads have been very successful as colonizers of epiphytic as well as terrestrial habitats. Unique trichomes capable of water absorption and the development of various strategies to deal with water stress (succulence, foliar impoundment, CAM photosynthesis) allow an extraordinary ecological versatility in the family. In spite of the species richness of bromeliads, their abundance in many Neotropical habitats, and their importance as ornamentals, updated revisions are lacking for most of the genera, especially for the most species-rich ones.

Different generic concepts have been proposed resulting in many new combinations, usually without being based on new revisional work. The elevation of the eight subgenera of *Aechmea* Ruiz & Pav. (Bromelioideae) to generic rank by Smith and Kress (1989, 1990), as well as the sinking of *Streptocalyx* Beer in *Aechmea* proposed by Smith and Spencer in 1992 was rigorously criticized by Brown et al. (1993), as it was not accompanied by a thorough reevaluation of the affected taxa.

The weaknesses of the delimitations of genera such as *Aechmea* have become clear, but still there is no convincing and commonly accepted concept for (sub)generic delimitation in most groups. Molecular data are expected to provide the most valuable information in this respect, but of equal importance is further research on morphology and anatomy and the variability of relevant characters within bromeliads.

Several molecular studies have dealt with the phylogeny of the family (see Table 1) and provided important new insights. Nevertheless, most of these studies suffered from limited taxon sampling and from the fact that they were often based on single markers, usually of the chloroplast genome. Another weakness is the unusually low sequence variability of all markers investigated in Bromeliaceae so far, leading to low phylogenetic resolution, especially in the subfamilies Tillandsioideae and Bromelioideae.

The monophyly of Bromeliaceae, which was commonly accepted on the basis of morphological and anatomical features before (e.g., unique epidermal trichomes, flower morphology, silica bodies of epidermal cells, as in Dahlgren et al. 1985), has been confirmed by all molecular studies.

While in Angiosperm Phylogeny Group (APG) 1998, Bromeliaceae are placed within a commelinoid clade without identifying their sister group and closer relationships, the later phylogenies proposed by Chase et al. (2000) and APG II (2003) reveal the family as part of the order Poales. Among this order, Rapateaceae is sister to a clade with all the other families, including Bromeliaceae. The latter is sis-

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Table 1. Recently published studies on the phylogeny of Bromeliaceae based on molecular data. T: Tillandsioideae, B: Bromelioideae, P: Pitcairnioideae (sensu Smith and Till 1998).

Authors	Molecular Data	
	Marker	Genera/species of Bromeliaceae
Ranker et al. (1990)	restriction sites (cp)	9/10 (T: 4/5, P: 3/3, B: 2/2)
Givnish et al. (1990)	restriction sites (cp)	7/7
Clark and Clegg (1990)	*rbc*L	3/3
Clark et al. (1993)	*rbc*L	7/7
Duvall et al. (1993)	*rbc*L	7/7 (T: 3/3, P: 2/2, B: 2/2)
Terry and Brown (1996)	*ndh*F	30/51 (T: 7/28, P: 8/8, B: 15/15)
Givnish et al. (1997)	restriction sites (nr + cp)	4/19 (mostly *Brocchinia*; P: 4/19)
Terry et al. (1997*a*)	*ndh*F	29/30 (T: 6/7, P: 8/8, B: 15/15)
Terry et al. (1997*b*)	*ndh*F	9/28 (mostly Tillandsioideae)
Horres et al. (2000)	*trn*L intron	32/62 (T: 7/23, P: 9/19, B: 16/20)
Behnke et al. (2000)	*rbc*L	11/11 (T: 2/2, P: 5/5, B: 4/4)
Crayn et al. (2000)	*mat*K	15/40 (mostly Pitcairnioideae; T: 3/3, P: 11/36, B: 1/1)
Reinert et al. (2003)	*mat*K	11/35 (analysis of data by Crayn et al. 2000, P: 11/35)
Crayn et al. (2004)	*mat*K & *rps*16 intron	24/51 (T: 7/10, P: 9/33, B: 8/8)
Givnish et al. (2005)	*ndh*F	25/35 (T: 5/5, P: 14/24, B: 6/6)

ter to a branch with Poaceae, Anarthriaceae, Restionaceae, Flagellariaceae, Xyridaceae, Cyperaceae, Juncaceae, Thurniaceae, and Mayacaceae (Chase et al. 2000). In a study of *ndh*F cpDNA data analysis, Givnish et al. (2006) found that members of Typhaceae are sister to Bromeliaceae at the base of the order Poales sensu APG II (2003), with Rapateaceae next divergent.

Our molecular studies of the *trn*L intron alone (Horres et al. 2000) revealed a clade comprising *Brocchinia* Schult. & Schult. f. and *Ayensua* L. B. Sm. as sister to the remainder of the family. Among the latter, three principal branches can be discerned: one lineage comprising the members of Tillandsioideae; a second formed by the representatives of *Hechtia;* and a third containing all Bromelioideae and the remaining Pitcairnioideae (except *Brocchinia, Ayensua,* and *Hechtia* Klotzsch). In this third lineage, Bromelioideae are monophyletic, but the phylogeny of *Puya* Molina, *Fosterella* L. B. Sm., and the other Pitcairnioideae is not resolved. These results correspond with those obtained from the *ndh*F gene by Terry et al. (1997*a*) as far as the basal position of *Brocchinia* is concerned (*Ayensua* was not studied by these authors). However, the *ndh*F tree differs in identifying *Puya* as sister to Bromelioideae and *Fosterella* as part of a clade of Pitcairnioideae s.s.

The analysis of *mat*K sequence data by Crayn et al. (2000) resulted in insufficient low resolution, but the combined analysis of *mat*K and *rps*16 sequence data (Crayn et al. 2004) revealed a much better resolution in many ways. This improved study, aimed at an understanding of the evolution of CAM and epiphytism, included 25 genera/51 species (see Table 1). The data of Crayn et al. (2004) supported many findings based on *ndh*F (Givnish et al. 2006) except for a different basal resolution, as *Brocchinia serrata* L. B. Sm., *Lindmania* Mez, and *Brewcaria* L. B. Sm., Steyerm. & H. Rob. were not included. *Brocchinia acuminata* and *B. micrantha* (Baker) Mez are sister to an unresolved polytomy comprising *Navia phelpsiae* L. B. Sm. and *Cottendorfia* Schult. f. on a well-supported clade (94% bootstrap support); *Hechtia* and Tillandsioideae are on a branch with low bootstrap support (54%). A ''DFPPB-clade,'' comprising *Deuterocohnia* Mez/*Dyckia* Schult. f./*Encholirium* Mart. ex Schult., *Fosterella,* and *Navia igneosicola* L. B. Sm., Steyerm. & H. Rob., occurs on a clade with the majority of *Pitcairnia* L'Hér. species (''Pitcairnia 2''), while *P. burle-marxii* Braga & Sucre, *P. carinata* Mez, and *P. heterophylla* (''Pitcairnia 1'') are well supported on a separate clade, and *Puya* is sister to Bromelioideae (68% bootstrap support).

Based on the analysis of *ndh*F data by Givnish et al. (2006; see Table 1) the monophyly of Tillandsioideae is supported with 57% bootstrap support only. The *mat*K and *rps*16 intron data by Crain et al. (2004) support this finding with bootstrap values of 85%. Although all of these studies provide new insights, especially for Pitcairnioideae, there remain partly conflicting topologies so that phylogeny within Bromeliaceae is far from clear. Pitcairnioideae was the only subfamily that had been grouped into tribes (Brocchineae, Puyeae, and Pitcairnieae), based on the studies of Varadarajan and Gilmartin (1988*a, b*; see also Smith and Till 1998). The molecular data at hand contradict such a systematic concept by showing that the subfamily in its traditional circumscription is paraphyletic (Givnish et al. 1997, 2006; Terry et al. 1997*a*; Crayn et al. 2000, 2004; Horres et al. 2000).

Monophyly for Tillandsioideae has been confirmed by all molecular studies, but the genera *Vriesea* Lindl.*, Alcantarea* (E. Morren ex Mez) Harms*, Werauhia* J. R. Grant, *Tillandsia* L.*, Racinaea* M. A. Spencer & L. B. Sm., and *Guzmania* Ruiz & Pav. do not appear to be natural groups (Horres et al. 2000, unpubl. data; also see Barfuss et al. 2003). However, phylogenetic resolution was generally not sufficient to define new generic boundaries.

Bromelioideae also form a weakly supported monophyletic group in all published studies, but no reliable conclusions could be drawn about its sister group and its principal evolutionary lineages. Smith (1934: 468) commented on this: ''Bromelioideae differ from the other two subfamilies . . . in the mystery that surrounds their evolution.'' He suggested that the group might have originated in the Amazon Basin. Today, the diversity of Bromelioideae is concentrated in Brazil, especially in the Atlantic Forest and adjacent habitats that house a considerable number of endemics (Benzing

2000; Leme 2000). Still, we have no commonly accepted concept, where Bromelioideae originated, how they evolved, and in which way the present distribution developed. Moreover, the generic concept in this subfamily is the most problematic and most poorly understood among bromeliads. Brown and Leme (2000: 244) state, ''that generic-level relationships and thus sister-taxon relationships, are virtually unknown within the Bromelioideae.''

An important step toward a better understanding of the phylogeny of Bromelioideae has been the cladistic analyses in the Nidularioid complex by Brown and Leme (2000) based on morphological, micromorphological, and anatomical characters. The taxon selection was based on the molecular studies of Terry et al. (1997*a*), in which a ''Nidularoid clade'' had been identified. The analyses of Brown and Leme (2000) included the genera *Nidularium* Lem. s.s., *Wittrockia* Lindm. s.s., *Edmundoa* (Regel) Leme, *Canistropsis* (Mez) Leme, *Neoregelia* L. B. Sm., and *Canistrum* E. Morren s.s., all principally occurring in the Atlantic Forest of eastern Brazil. Six species each of *Aechmea* and *Cryptanthus* Otto & A. Dietr. were also included, but were not the focus of the analysis. Monophyly was supported for *Nidularium, Canistropsis, Canistrum,* and *Edmundoa,* but not for *Neoregelia* and *Wittrockia.* Other important information was provided by Ramírez-Morillo and Brown (2001). Their studies of chromosome numbers and nuclear DNA content revealed that *Cryptanthus* is derived within the subfamily.

We still have no good idea about the relationships in other groups of Bromelioideae. This is especially true for the ''core-group'' of the subfamily formed by *Aechmea* and related genera. Several taxonomic changes in this group—principally lifting subgenera or parts of subgenera to generic rank or sinking genera—have taken place in the last two decades (e.g., Smith and Read 1982; Read 1984; Smith and Kress 1989, 1990; Smith and Spencer 1992; Read and Baensch 1994) with most of these changes based on different weighting of morphological characters. Molecular data and the use of yet underexploited characters, such as leaf blade anatomy, offer an opportunity to develop a more stable generic concept. However, especially in critical groups like *Aechmea,* a complete taxonomic treatment remains essential and is very much needed.

Other interesting groups, although comparatively poor in species, are those genera of Bromelioideae that are adapted to cooler habitats: *Fernseea* Baker*, Ochagavia* Phil., *Fascicularia* Mez, and *Greigia* Regel. Representatives of the latter two genera also extend to the humid, temperate Valdivian Forest in southern Chile, which is the southwestern limit of Bromeliaceae distribution. Provided that Bromeliaceae originated in the Guayana Shield, as molecular data suggest (see the thorough discussion on this in Givnish et al. 2006), how did bromeliads spread to southern Chile and radiate there?

We examined the phylogenetic relationships within Bromeliaceae using DNA sequences of the *trn*L intron as well as *trn*T–*trn*L, and *trn*L–*trn*F IGS, concentrating especially on the subfamily Bromelioideae. Relationships between and within closely related genera were also examined with the AFLP method (Vos et al. 1995) that is increasingly applied to assess genetic similarity at the interspecific level (e.g., Aggarwal et al. 1999; Mace et al. 1999; Gimenes et al. 2002; Beardsley et al. 2003; Després et al. 2003). Within Bromelioideae, our special interest is in *Aechmea* and related genera and in the smaller genera forming the southwestern limit of the family distribution (*Ochagavia, Fascicularia,* and *Greigia*).

The objectives of this study were to (1) contribute to the hypothesis of the phylogeny of the family, especially concerning basal branching patterns, (2) identify the sister group of Bromelioideae and principal lineages in this subfamily, (3) assess the monophyly of selected genera and subgenera of Bromelioideae, and (4) compare the results obtained from leaf anatomical studies in the genus *Aechmea* with the AFLP tree.

MATERIALS AND METHODS

Plant material was derived from the living collections of the Palmengarten Frankfurt/Main and the Botanical Gardens of the Universities of Heidelberg, Bonn, Munich, Frankfurt/Main, and Berlin, Germany. Voucher specimens were deposited in the Herbarium Senckenbergianum (FR) and in the Herbarium of the Palmengarten (FRP). The investigated species and accessions are listed in Table 2. Nomenclature of genera follows Smith and Till (1998). Among *Aechmea,* the following subgenera are recognized: *Aechmea, Lamprococcus* (Beer) Baker, *Macrochordion* (De Vriese) Baker, *Ortgiesia* (Regel) Mez, *Platyaechmea* (Baker) Baker, *Podaechmea* Mez, and *Pothuava* (Baker) Baker.

DNA Methods

Isolation and purification of genomic DNA, amplification of *trn*T–*trn*L, *trn*T–*trn*F IGS and the *trn*L intron as well as processing and sequencing of polymerase chain reaction (PCR) products were performed as described in Horres et al. (2000). Amplification of the *trn*T–*trn*L IGS, the *trn*L intron and the *trn*L–*trn*F IGS was performed with primers ''a'' and ''b,'' ''c'' and ''d,'' ''e'' and ''f,'' or ''c'' and ''f,'' after Taberlet et al. (1991).

AFLPs

AFLP (amplified fragment length polymorphism) analyses were performed with the ''AFLP Core Reagent Kit'' and the ''AFLP Small Genome Primer Kit'' (Life Technologies, Gaithersburg, Maryland, USA) according to the protocol provided by the kit manufacturer with minor modifications. In short, 60 to 65 ng of genomic DNA were digested with *Eco*RI and *Mse*I in a total volume of 6.25 μl, and adapters were ligated to the restriction fragments in a final volume of 12.5 μl. Two consecutive PCR amplifications with primers containing either no (+0), one (+1), two (+2) or three (+3) selective nucleotides at their 3′-ends were carried out in an Eppendorf Mastercycler, essentially following the instructions of the kit manufacturer. For pre-amplification, one-tenth of the restriction-ligation assay was used as a template and *Mse*I+C/*Eco*RI+0 served as primers. The efficiency of pre-amplification was checked by agarose electrophoresis. Selective amplifications were performed with a 1 : 20 diluted pre-amplification mix and several combinations of *Mse*I+3/*Eco*RI+2 primers. Prior to selective PCR, *Eco*RI primers were end-labelled with [^{32}P]γ-ATP using T4 polynucleotide kinase following standard procedures. After PCR, one vol-

Table 2. List of investigated species, accessions, and herbarium vouchers. Generic concept and nomenclature follows Smith and Till (1998). Accession numbers in living collections: BGB = BG Berlin-Dahlem; BGBN = BG University Bonn; BGFR = BG University Frankfurt/Main; FAN = Fundación Amigos de la Naturaleza, Santa Cruz, Bolivia; FRP = Palmengarten Frankfurt/Main; HEID = BG University Heidelberg; KAU = University Kassel; WU = BG University Vienna Herbarium vouchers are deposited in the Herbarium Senckenbergianum (FR) or in the herbarium of the Palmengarten (FRP). Project No. 1 = sequences of the *trn*T–*trn*L IGS; 2 = sequences of the *trn*L intron; 3 = sequences of the *trn*L–*trn*F IGS; 4 = AFLPs and leaf anatomy of the *Aechmea*-complex; 5 = AFLPs of *Fascicularia–Ochagavia–Greigia*.

Taxon	Accession no.	Voucher	Project no.	GenBank accession no.
Acanthostachys strobilacea (Schult. f.) Klotzsch	FRP 98-16986-0	*RH H 019* (FR)	1, 2, 3	DQ084642, AF188765, DQ084606
Aechmea aciculosa Mez & Sodiro	HEID 103739	*KS 280802-1* (FR)	4	
A. aquilega (Salisb.) Griseb.	FRP 98-16956-2	*KS 120203-4* (FR); *KS 220503-2* (FR)	4	
A. bambusoides L. B. Sm. & Reitz	FRP s. n.	*KS 170203-1* (FR)	4	
A. blumenavii Reitz	HEID 104588	*KS 180901-12* (FR)	4	
A. bromeliifolia (Rudge) Baker	FRP 99-17993-3	*KS 051202-4* (FR)	4	
A. calyculata (E. Morren) Baker	HEID 103296	*KS 240203-9* (FR)	2, 3, 4	DQ084674, DQ084593
A. chantinii (Carrière) Baker	HEID 130293	*KS 280802-2* (FR)	4	
A. chantinii	KAU s. n.	*RS 250203-1* (RS)	2, 3	DQ084675, DQ084581
A. distichantha Lem.	FRP 2-1211-88-2	*RH 19.03.99*; *H 008* (FR)	1, 2, 3, 4	DQ084643, AF188761, DQ084579
A. distichantha	HEID 104948	*KS 180901-14* (FR)	2, 3, 4	DQ084676, DQ084580
A. drakeana André	FRP 98-16955-2	*Z 1100* (FRP)	2, 3	AF188772, DQ084588
A. eurycorymbus Harms	FRP 98-16942-3	*RH H205* (FR)	4	
A. eurycorymbus	HEID 102588	*KS 280802-3* (FR); *KS 240203-11* (FR)	4	
A. farinosa (Regel) L. B. Sm.	FRP 98-16961-3	*KS 280801-5* (FR); *Z 1108* (FR)	2, 3, 4	DQ084677, DQ084586
A. fasciata (Lindl.) Baker	KAU s. n.	*RS 250203-2* (FR)	2, 3	DQ084678, DQ084582
A. fendleri André	FRP 95-14217-0	*KS 060901-5* (FR)	4	
A. filicaulis (Griseb.) Mez	FRP 98-16863-0	*H/S 180701-6* (FR)	2, 3, 4	DQ084679, DQ084576
A. fulgens Brongn.	FRP s. n.	*KS 100203-1* (FR)	2, 3	DQ084680, DQ084587
A. gamosepala Wittm.	HEID 130384	*KS 280301-7* (FR)	2, 3	DQ084681, DQ084591
A. gracilis Lindm.	FRP 98-16949-3	*KS 280203-1* (FR)	2, 3, 4	DQ084682, DQ084594
A. gracilis	HEID 103595	*KS 280301-9* (FR); *RT 18.11.1999-12* (FR)	4	
A. kertesziae Reitz	FRP 98-16935-3	*H/S 201101-1* (FR); *Z 1177* (FRP)	2, 3, 4	DQ084683, DQ084595
A. lamarchei Mez	BGB 118-37-74-86	*11309* (GHB)	2, 3, 4	DQ084684, DQ084590
A. lueddemanniana (K. Koch) Brongn. ex Mez	FRP 95-14215-0	*KS 100203-3* (FR)	2, 3, 4	DQ084685, DQ084596
A. lueddemanniana	HEID 130149	*KS 240203-10* (FR)	4	
A. mariae-reginae H. Wendl.	HEID 103651	*KS 280301-5* (FR); *KS 180901-4* (FR)	4	
A. mariae-reginae	FRP 98-16926-0	*KS 260401-1* (FR)	4	
A. mertensii Schult. f.	FRP 98-16873-0	*KS 120203-6* (FR); *Z 1572* (FRP)	2, 3, 4	DQ084686, DQ084575
A. mertensii	FRP 98-16873-0 (Ind. b)	*RH H249* (FR)	2, 3	DQ084687, DQ084577
A. mexicana Baker	HEID 104025	*KS 240203-12* (FR); *RT 12.04.00-35* (FR)	2, 3, 4	DQ084688, DQ084597
A. miniata (Beer) hort. ex Baker	BGB 118-45-7483	*GH 11149* (B)	4	
A. miniata	FRP 98-16947-0	*KS 280801-7* (FR); *RT 03.09.1999-5* (FR)	4	
A. mulfordii L. B. Sm.	FRP 93-12136-2	*KS 280801-3* (FR); *KS 090603-2* (FR)	4	
A. nidularioides L. B. Sm.	HEID 103652	*KS 180901-5* (FR); *RT 18.11.99-15* (FR)	4	
A. nudicaulis (L.) Griseb.	HEID 103783	*KS 240203-6* (FR)	2, 3, 4	DQ084689, DQ084589
A. nudicaulis	FRP 98-16924-3	*KS 200603-1* (FR)	4	
A. paniculata Ruiz & Pav.	FRP s. n.	*H/S 210601-11* (FR)	4	
A. pimenti-velosoi Reitz (Ortgiesia)	FRP 90-1144-4	*RH H271*	2, 3	DQ084690, DQ084592
A. racinae L. B. Sm.	FRP 98-16934-3	*KS 120203-1* (FR)	2, 3, 4	DQ084691, DQ084583

Table 2. Continued.

Taxon	Accession no.	Voucher	Project no.	GenBank accession no.
A. racinae	FRP 86-16933-3	*KS 190203-4* (FR)	4	
A. rubens (L. B. Sm.) L. B. Sm.	FRP 98-16939-2	*KS 130901-1* (FR); *KS 030602-2* (FR)	4	
A. warasii E. Pereira	HEID 130354	*KS 240203-17* (FR)	2, 3, 4	DQ084692, DQ084584
A. weberbaueri Harms	FRP 98-16952-0	*KS 280801-1* (FR); *RT 03.02.00-21* (FR)	4	
A. weberbaueri	FRP 98-17981-2	*KS 130303-1* (FR)	4	
A. weilbachii Didr.	FRP 2-1467-87-80	*KS 051202-1* (FR)	2, 3, 4	DQ084693, DQ084585
A. weilbachii	FRP 99-18220-0	*KS 190203-1* (FR); *Z 1507* (FRP)	4	
A. winkleri Reitz	HEID 103605	*KS 180901-8* (FR)	4	
A. winkleri	FRP 98-16954-2	*KS 051202-3* (FR)	4	
Alcantarea regina Harms	FRP 97-16784-0	*RH H 015* (FR)	1, 2, 3	DQ084657, AF188814, DQ089025
Ananas comosus (L.) Merrill	BGFR s. n.	*RH H 136* (FR)	2, 3	DQ084694, DQ084574
A. nanus (L. B. Sm.) L. B. Sm.	FRP s. n.	*RH H 040* (FR)	1, 2, 3	DQ084662, DQ084695, DQ084573
Androlepis skinneri (K. Koch) Brongn. ex Houllet	FRP 97-16793-2	*RH H 048* (FR)	1, 2, 3	DQ084638, AF188780, DQ084610
Araeococcus flagellifolius Harms	KAU s. n.	*RH K9* (FR)	2, 3	DQ084696, DQ084629
A. goeldianus L. B. Sm.	FRP 99-18256-2	*RH H 206* (FR)	2, 3	DQ084697, DQ084630
Ayensua uaipanensis (Maguire) L. B. Sm.	FRP 92-9510-2	*RH H 011* (FR)	1, 2, 3	DQ084671, AF188760, DQ089026
Barbacenia elegans (Oliv. ex Hook. f.) Pax (Velloziaceae)	FRP 95-15078 0	*RH H 059* (FR)	1, 2, 3	DQ084672, AF188755, DQ084637
Billbergia decora Poepp. & Endl.	FRP 90-733-2-4	*RH H 129* (FR)	2, 3	DQ084698, DQ084624
B. nutans H. Wendl. ex Regel	FRP 99-18405-0	*RH H 036* (FR)	1, 2, 3	DQ084651, AF188766, DQ084623
Brocchinia acuminata L. B. Sm.	FRP 92-10169-4-0	*RH H 001* (FR)	1, 2, 3	DQ084646, AF188758, DQ089027
B. tatei L. B. Sm.	FRP 95-13566-4-2	*RH H 003* (FR)	1, 2, 3	DQ084668, AF188759, DQ089028
Bromelia serra Griseb.	FRP 98-17751-0	*RH H 029* (FR)	2, 3	DQ084699, DQ084622
Canistrum fosterianum L. B. Sm.	FRP 86-347-2-3-3	*Z 927* (FRP)	1, 2, 3	DQ084650, AF188773, DQ084618
Catopsis morreniana Mez	FRP 92-10229-4-0	*Z 1040* (FRP); *Z 1440* (FRP)	2, 3	AF188816, DQ084559
C. nitida (Hooker) Griseb.	FRP 2-1304-84-4	*RH H 073* (FR)	1, 2, 3	DQ084647, AF188817, DQ089029
Chevaliera sphaerocephala (Baker) L. B. Sm. & W. J. Kress	FRP 99-18245-3		4	
C. sphaerocephala	FRP 90-8561-3	*KS 310702-2* (FR)	4	
C. sphaerocephala	FRP 90-1183-4-3	*Z 1104* (FRP)	1, 2, 3, 4	DQ084669, AF188770, DQ084578
Cryptanthus bahianus L. B. Sm.	HEID 103794	*RH H 214* (FR)	2, 3	DQ084700, DQ084634
C. glaziovii Mez	HEID 102583	*RH H 215* (FR)	2, 3	DQ084701, DQ084635
Deinacanthon urbanianum (Mez) Mez	FRP 98-17786-0	*RH H 018* (FR)	1, 2, 3	DQ084648, AF188781, DQ084607
D. urbanianum	BGFR H140		2, 3	DQ084702, DQ084608
Deuterocohnia glandulosa E. Gross	HEID 103854	*RH H 090* (FR)	1, 2, 3	DQ084652, AF188784, DQ089030
D. lotteae (Rauh) M. A. Spencer & L. B. Sm.	HEID 103817	*RH H 084* (FR)	2, 3	AF188783, DQ084566
D. scapigera (Rauh & L. Hrom.) M. A. Spencer & L. B. Sm.	HEID 130020	*RH H 085* (FR)	2, 3	AF188787, DQ089031
Dyckia goehringii Rauh	HEID 105013	*RH H 118* (FR)	2, 3	DQ084703, DQ084565
Edmundoa lindenii (Regel) Leme	HEID 105009	*RH H 213* (FR)	2, 3	DQ084704, DQ084631
Fascicularia bicolor (Ruiz & Pav.) Mez ssp. *bicolor*	FRP 98-16846-3	*RH H 006a* (FR)	1, 2, 3, 5	DQ084665, AF188775, DQ084605
F. bicolor ssp. *bicolor*	FRP 98-16845-2	*RH H 004a* (FR)	5	
F. bicolor spp. *bicolor*	FRP 98-16847-2	*RH H 009a* (FR)	5	
F. bicolor spp. *bicolor*	FRP 98-16848-3	*RH H 010a* (FR)	5	
F. bicolor spp. *bicolor*	FRP 98-16849-0	*RH H 011a* (FR)	5	

Table 2. Continued.

Taxon	Accession no.	Voucher	Project no.	GenBank accession no.
F. bicolor ssp. *canaliculata* E. C. Nelson & Zizka	FRP 93-12574-3	*RH H 005a* (FR)	1, 2, 3, 5	DQ084666, AF188776, DQ084604
F. bicolor ssp. *canaliculata*	FRP 90-16851-3	*RH H 014a* (FR)	5	
F. bicolor ssp. *canaliculata*	FRP 90-17118-3	*RH H 016a* (FR)	5	
F. bicolor ssp. *canaliculata*	FRP 90-17119-3	*RH H 017a* (FR)	5	
Fernseea itatiaiae (Wawra) Baker	HEID 102174	*RH H 067* (FR)	2, 3	DQ084705, DQ084633
Fosterella albicans (Griseb.) L. B. Sm.	FRP 98-18320-1	*KS 130901-3* (FR), *RH H 156*	2, 3	DQ084706, DQ084570
F. caulescens Rauh	FRP 99-18434-3	*RH H 158* (FR)	2, 3	DQ084707, DQ084569
F. floridensis Ibisch, R. Vásquez & E. Gross	FAN PI 97.83	*Ibisch 97.83* (FR)	2, 3	DQ084708, DQ084568
F. penduliflora (C. H. Wright) L. B. Sm.	HEID 103655	*RH H 086* (FR)	1, 2, 3	DQ084660, AF188782, DQ084571
Glomeropitcairnia erectiflora Mez	FRP 99-18392-2	*RH H 002* (FR)	1, 2, 3	DQ084658, AF188818, DQ084558
Greigia mulfordii L. B. Sm.	WU WT 13090	*RH H 111* (FR)	2, 3	DQ084709, DQ084600
G. sp. nov.	FRP 99-19040-0	*Grant 19040* (FR)	2, 3	DQ084710, DQ084601
G. sphacelata (Ruiz & Pav.) Regel	FRP 92-10171-4-3	*RH H 004* (FR)	1, 2, 3, 5	DQ084645, AF188779, DQ084599
G. sphacelata	FRP 98-16855-1	*RH H 027a* (FR)	5	
Guzmania monostachia (L.) Rusby ex Mez	FRP 89-18406-0	*RH H 016* (FR)	1, 2, 3	DQ084670, AF188795, DQ089032
G. wittmackii (André) André ex Mez	FRP 99-18407-3	*RH H 017* (FR)	1, 2, 3	DQ084659, AF188797, DQ084560
Hechtia guatemalensis Mez	HEID 103967	*RH H 088* (FR)	1, 2, 3	DQ084656, AF188821, DQ089033
H. stenopetala Klotzsch	FRP 92-10661-4-2	*RH H 005* (FR)	1, 2, 3	DQ084655, AF188819, DQ089034
Hohenbergia stellata Schult. f.		*RH H 037* (FR)	1, 2, 3	DQ084663, AF188774, DQ084609
Hohenbergiopsis guatemalensis (L. B. Sm.) L. B. Sm. & Read	FRP 91-1227-8-3	*RH H 138* (FR)	2, 3	DQ084711, DQ084627
Lymania alvimii (L. B. Sm. & Read) Read	FRP 0-19135-3	*RH H 087* (FR)	2, 3	AF188768, DQ084619
Neoglaziovia variegata (Arruda) Mez	FRP 97-16794-3	*RH H 052* (FR)	2, 3	AF188763, DQ084614
Neoregelia binotii (Antoine) L. B. Sm.	FRP 98-16967-3	*RH H 081* (FR)	2, 3	AF188764, DQ084613
N. laevis (Mez) L. B. Sm.	FRP 98-16962-3	*RH H 080* (FR)	1, 2, 3	DQ084654, AF188762, DQ084612
Nidularium procerum Lindm.	FRP 99-18618-3	*RH H 137* (FR)	2, 3	DQ084712, DQ084628
Ochagavia carnea (Beer), L. B. Sm. & Looser	FRP 94-14614-3b	*RH H 115* (FR)	5	
O. carnea	FRP 94-12461-4-8	*RH H 117* (FR)	5	
O. elegans Phil.	FRP 98-16852-3	*RH H 023a* (FR)	1, 2, 3, 5	DQ084667, AF188778, DQ084603
O. elegans	FRP 98-16852-3b	*RH H 077* (FR)	5	
O. litoralis (Phil.) Zizka, Trumpler & Zoellner	FRP 98-16853-2	*RH H 015a* (FR)	1, 2, 3	DQ084664, AF188777, DQ084602
O. litoralis	FRP 98-16853-2	*RH H 112* (FR)	5	
O. litoralis	FRP 94-14614-3a	*RH H 113* (FR)	5	
O. litoralis	FRP 98-16854-2	*RH H 114* (FR)	5	
Orthophytum supthutii E. Gross & Barthlott	HEID 102160	*RH H 223* (FR)	2, 3	DQ084713, DQ084572
Pitcairia feliciana (A. Chev.) Harms & Mildbr.	BGBN 12804	*BGBN 12804*	1, 2, 3	DQ084644, AF188792, DQ084567
P. heterophylla (Lindl.) Beer	FRP 93-11378-4	*RH H 024* (FR)	1, 2, 3	DQ084649, AF188789, DQ089035
Portea leptantha Harms	FRP 99-18222-3	*KS 060901-1* (FR): *Z 1055* (FR)	2, 3, 4	DQ084714, DQ084621
P. leptantha	HEID 103164	*KS 010601-8* (FR)	4	
P. petropolitana Mez var. *extensa* L. B. Sm.	FRP 99-18000-0	*Z 1056* (FRP)	2, 3	DQ084715, DQ084620
Puya alpestris (Poepp.) Gay	HEID 103731	*RH H 060* (FR)	1, 2, 3	DQ084640, AF188793, DQ084562
P. densiflora Harms	HEID 103568	*RH H 076* (FR)	2, 3	DQ084716, DQ084564
P. laxa L. B. Sm.	FRP 94 12923-4-00	*RH H 006* (FR)	1, 2, 3	DQ084639, AF188794, DQ084563
Quesnelia edmundoi L. B. Sm.	FRP 92-10483-3	*Z 964* (FRP)	2, 3	AF188769, DQ084616
Q. lateralis Wawra	FRP 90-10484-0	*Z 1554* (FRP)	2, 3	AF188771, DQ084615

Table 2. Continued.

Taxon	Accession no.	Voucher	Project no.	GenBank accession no.
Q. liboniana (De Jonghe) Mez	FRP 99-17934-0	*Z 1384* (FRP)	2, 3	DQ084717, DQ084617
Rapatea paludosa Aubl. (Rapateaceae)	BGBN 8522	*RH H 072* (FR)	1, 2, 3	DQ084673, AF188756, DQ084636
Ronnbergia petersii L. B. Sm.	FRP 99-17997-3	*RH H 120* (FR)	2, 3	DQ084718, DQ084632
Streptocalyx poeppigii Baker	FRP 94-13845-4	*H/S 050401-6* (FR)	2, 3, 4	DQ084719, DQ084598
Tillandsia pretiosa Mez	FRP 90-6592-4-2	RH H 007 (FR); *Z 1376* (FRP)	1, 2, 3	DQ084661, AF188813, DQ089036
T. tragophoba M. O. Dillon	FRP 93-11900-4-2	*Z 1521* (FRP)	2, 3	AF188807, DQ084561
Ursulaea macvaughii (L. B. Sm.) Read & Baensch	WU WT 255; HEID 105622	*KS 240203-21* (FR); *RH H 127* (FR)	2, 3, 4	DQ084720, DQ084625
U. tuitensis (Magaña & E. J. Lott) Read & Baensch	FRP s. n.	*RH H 033* (FR)	1, 2, 3	DQ084641, DQ084721, DQ084561
Wittrockia superba Lindm.	FRP 93-12641-0	*RH H 049* (FR)	1, 2, 3	DQ084653, AF188767, DQ084611

ume of loading buffer containing 98% formamide, 10 mM EDTA, 0.025% bromophenol blue, and 0.025% xylene cyanol were added; samples were denatured for 3 min at 96°C and aliquots separated on 4% sequencing gels in 1 × TBE at 100 W and 45–50°C. Gels were dried on a vacuum gel dryer and exposed to X-ray film for autoradiography. AFLP banding patterns were scored by eye, and the data were entered into a binary matrix. All fragments with faint or fuzzy bands for any OTU were ignored.

Data Analysis

Multiple sequence alignments were performed with CLUSTAL_X (Thompson et al. 1997). Parsimony analyses were performed with PAUP*: vers. 4.0 b10 (Swofford 2002). For more details see Horres et al. (2000). For the combined analysis of *trn*L intron and the two IGS, *Rapatea paludosa* (Rapateaceae) and *Barbacenia elegans* (Velloziaceae) were used as outgroups. A second analysis based on sequence data from *trn*L intron and *trn*L–*trn*F IGS focused on Bromelioideae. In this case, four species of Tillandsioideae were selected as outgroup. Full alignments are available from the corresponding author upon request; all sequences are available from GenBank (accession numbers listed in Table 2).

The 0/1-matrices obtained from the AFLP banding patterns were converted into distance matrices based on either the Jaccard index or the Nei-Li (Nei and Li 1979) index of similarity using the software packages NTSYS-pc (Rohlf 1993) and PAUP* vers. 4.0b10. Phenograms were generated by cluster analysis using the unweighted pair group method of arithmetic means (UPGMA) and the Neighbor Joining (NJ) option implemented in PAUP* vers. 4.0b10. Statistical support for individual clusters was evaluated by bootstrap analyses using the same software. Details on primer combinations used as well as 0/1-matrices are available from the first and second author upon request.

Leaf Blade Anatomy

Complete transverse hand sections of the leaf blade (about halfway between leaf tip and base) were prepared from unstained fresh material. For more detailed analysis microtome sections were obtained from material embedded either in Paraplast and stained with safranine-astrablue, or embedded in HEMA (Igersheim and Cichocki 1996) and stained with toluidine. The sections were investigated and documented by light microscopy (Leitz Dialux 22), a digital camera system (Leica DC 300), and by camera lucida drawings. For measuring and analyzing the sections the software IM 1000 (Leica) was applied.

RESULTS

Sequence Data

The intention of our study was to increase the number of characters and the sample size as compared with preceding studies. We now have sequenced the *trn*L intron of 46 genera and 124 species of Bromeliaceae. However, this considerably larger taxon sampling did not increase the resolution of a parsimony tree, as compared to our earlier study based on 32 genera and 62 species (Horres et al. 2000). We therefore

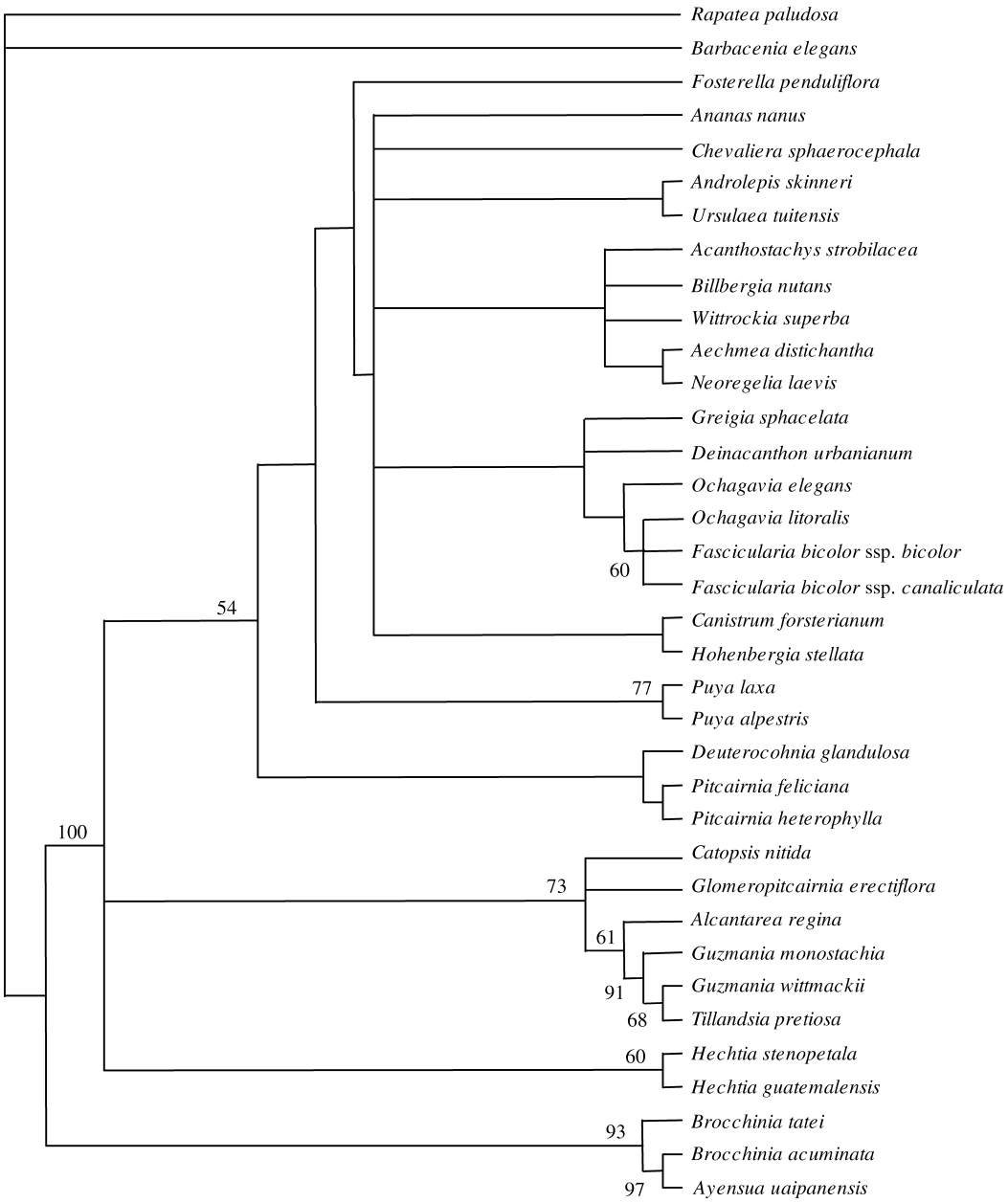

Fig. 1.—Combined analysis of *trn*L intron, *trn*T–*trn*L, and *trn*T–*trn*F IGS for 26 genera and 33 species of Bromeliaceae, *Rapatea paludosa* (Rapateaceae), and *Barbacenia elegans* (Velloziaceae) were used as outgroup. Strict consensus tree of 5170 most parsimonious trees of 499 steps (CI = 0.87; RI = 0.736). Length of alignment: 1159 bp, variable positions: 367, synapomorphies: 87. Bootstrap values >50% are depicted above the branches.

performed a combined analysis of *trn*L intron and *trn*L–*trn*F IGS including 103 bromeliad species, of which 74 were included in our analysis focusing on Bromelioideae (see below). The third marker studied, the *trn*T–*trn*L IGS, proved to be so highly size variable that a reasonable alignment (including only a small part of *trn*T–*trn*L IGS) could be produced only for a reduced set of 26 genera and 33 species of Bromeliaceae. The combined analysis of these three markers (Fig. 1) agreed with the results of Horres et al. (2000), as far as the basal position of the *Brocchinia/Ayensua* clade and the monophyly of Tillandsioideae and Bromelioideae were concerned. Noteworthy is the fact that *Ayensua uaipanensis* is part of a well-supported clade together with *Brocchinia acuminata,* both being sister to *B. tatei.* This casts doubt on the generic status of *Ayensua* and *Brocchinia*; (for the evolution of *Brocchinia,* see Givnish et al. 1997). The taxonomic consequence of sinking *Ayensua* in *Brocchinia* was already considered by Givnish et al. (2006).

The remainder of the family is split into a polytomy of one moderately and two weakly supported clades. The first clade solely consists of two species of *Hechtia,* the second comprises Tillandsioideae (including *Glomeropitcairnia* (Mez) Mez and *Catopsis* Griseb.) and the third largest clade is composed of all Bromelioideae together with *Fosterella, Puya,* and Pitcairnioideae s.s. (*Deuterocohnia* Mez and *Pitcairnia*). Within the latter, statistical support is low for any

of the subclades. The sister position of *Fosterella* and Bromelioideae is surprising, for *Fosterella* is usually regarded as part of Pitcairnioideae, where Smith and Till (1998) had placed it in the tribe Pitcairnieae. This interesting genus of mesophytic, terrestrial plants seems to have undergone speciation in interandine arid to semi-humid valleys, especially in Bolivia, but also includes representatives from the (semi)-humid central South American lowlands (Ibisch et al. 1999, 2002). *Fosterella* deserves further detailed investigation that should also provide insights into the evolution and biogeography of Bromelioideae as a whole.

Among Pitcairnioideae s.s., the West African species *Pitcairnia feliciana* groups together with *P. heterophylla.* Phylogenies, including four *Pitcairnia* and two *Pepinia* Brongn. ex André species and based on *trn*L intron and *trn*L–*trn*F IGS sequences alone (Horres et al. unpubl. data), reveal a close relationship between *Pitcairnia feliciana* and other *Pitcairnia* species. This supports the hypothesis of Porembski and Barthlott (1999) for a comparatively recent long-distance dispersal of the ancestral species to the Fouta Djallon highlands of Guinea. The separation of *Pitcairnia* and *Pepinia* seems to be artificial, as Taylor and Robinson (1999) pointed out on the basis of their leaf anatomical studies.

Hechtia, with its functionally unisexual flowers on different plants, has long been recognized as morphologically distinct. The genus was placed with *Puya* (and *Brewcaria, Deuterocohnia, Dyckia,* and *Encholirium*) in the tribe Puyeae, subfamily Pitcairnioideae (Smith and Till 1998). Our molecular data does not support a closer relationship between *Hechtia* and *Puya.* There also is no support for the placement of *Deuterocohnia, Dyckia, Encholirium,* and *Hechtia* in a tribe Dyckieae, as proposed by Robinson and Taylor (1999).

As our focus was the phylogeny of the subfamily Bromelioideae and the identification of its sister group, a larger set of 74 species was analyzed on the basis of *trn*L intron and *trn*L–*trn*F IGS sequence data only. Four species of Tillandsioideae (*Glomeropitcairnia erectiflora, Catopsis morreniana, Guzmania wittmackii,* and *Tillandsia tragophoba*) were selected as outgroups. Besides 28 genera and 60 species of Bromelioideae, the following taxa were also included: *Fosterella* (4 species), *Puya* (3 species), *Pitcairnia feliciana, Dyckia goehringii,* and *Deuterocohnia lotteae.* The 80% majority rule consensus tree (Fig. 2) shows numerous polytomies and only little resolution. The sister group of Bromelioideae is not resolved. The investigated *Puya* and *Fosterella* species each form well-supported clades and group together with the investigated Bromelioideae in a clade with basal polytomy. Within Bromelioideae, only the following few clades receive good bootstrap support >80%:

Greigia.—The genus comprises 32 morphologically very distinct species (Luther 2000) with lateral, sessile inflorescences. The genus also displays an extraordinarily disjunct distribution. The majority of species are distributed in humid, cool, high elevation habitats (mostly Andean cloud forest and páramo) in Colombia, Ecuador, Venezuela, Peru, Bolivia, Central America, and Mexico. Four species endemic to Chile grow terrestrially in humid evergreen forests at low altitudes—more than 2000 km away from the rest of the genus (Will and Zizka 1999). *Greigia sphacelata* endemic to southern Chile, *G. mulfordii* from high Andean habitats of Colombia and Ecuador, and *Greigia* sp. nov. from Bolivia were included in the study and form a distinct clade, with *G. sphacelata* as sister to the other two species. Morphology, ecology, and molecular data agree in defining *Greigia* as a distinct lineage within Bromelioideae. Further molecular analyses of relationships within this genus, e.g., with AFLPs, and a taxonomic revision nevertheless are needed.

Ochagavia *and* Fascicularia.—These two genera are morphologically very similar and have often been confused with each other. The few species (*Fascicularia*: 1, *Ochagavia*: 4) are endemic to central and southern Chile (between 31° and 42° S). Four taxa (*F. bicolor* subsp. *bicolor,* and subsp. *canaliculata, O. carnea, O. elegans,*) have been included in the analysis. While the latter three form a distinct clade with moderate bootstrap support, the morphologically close *O. elegans,* endemic to Robinson Crusoe Island, is sister to the former. This topology, however, receives no bootstrap support. AFLP studies have been performed to assess generic delimitation in *Ochagavia* and *Fascicularia* in more detail (see below). Surprisingly, *Fernseea itatiaiae* is sister to *Ochagavia/Fascicularia.* This species, one of the few bromeliads restricted to cooler habitats, is endemic to Mt. Itatiaia and its vicinity in southeastern Brazil, growing terrestrially above 2000 m.a.s.l. (meters above sea level).

Another clade with low bootstrap support is formed by two accessions of *Deinacanthon urbanianum,* the only species of the genus. *Deinacanthon* Mez, which displays distinct leaf anatomical characters (Horres and Zizka 1995), might well represent a distinct lineage within Bromelioideae.

The remaining ''core group'' of Bromelioideae, comprises 23 genera and 51 species and is hardly resolved at all. The two accessions of *Ananas* Mill. (*A. comosus* and *A. nanus)* receive good bootstrap support. *Ananas* is also morphologically well defined by its densely strobiliform inflorescence bearing a large, conspicuous coma and developing into a syncarp. *Aechmea lamarchei* and *P. petropolitana* (Wawra) Mez form another clade with good bootstrap support, but are morphologically quite distinct from each other. Noteworthy is the fact that all investigated accessions of *Aechmea* subgen. *Ortgiesia* group together in one clade with *Aechmea* (*Lamprococcus*) *racinae,* these being sister to *Neoregelia laevis.* All these representatives are restricted to southeastern Brazil.

AFLPs

The low chloroplast DNA sequence variability in Bromeliaceae resulting in insufficient phylogenetic resolution prompted us to the search for additional molecular markers in the nuclear genome. An obvious choice was the ribosomal ITS region, which has been applied in many plant studies at the interspecific level. However, all our attempts to amplify and sequence ITS from Bromeliaceae have failed so far. We, therefore, adopted the amplified fragment length polymophism (AFLP) technique developed by Vos et al. (1995). This method combines the advantages of PCR with the robustness of RFLPs (restriction fragment length polymorphism). The application of AFLPs in phylogenetic studies and especially at the intergeneric level is not widely used so far. We agree with the review by Després et al. (2003) on the potential of the AFLP technique. Although one has to consider that

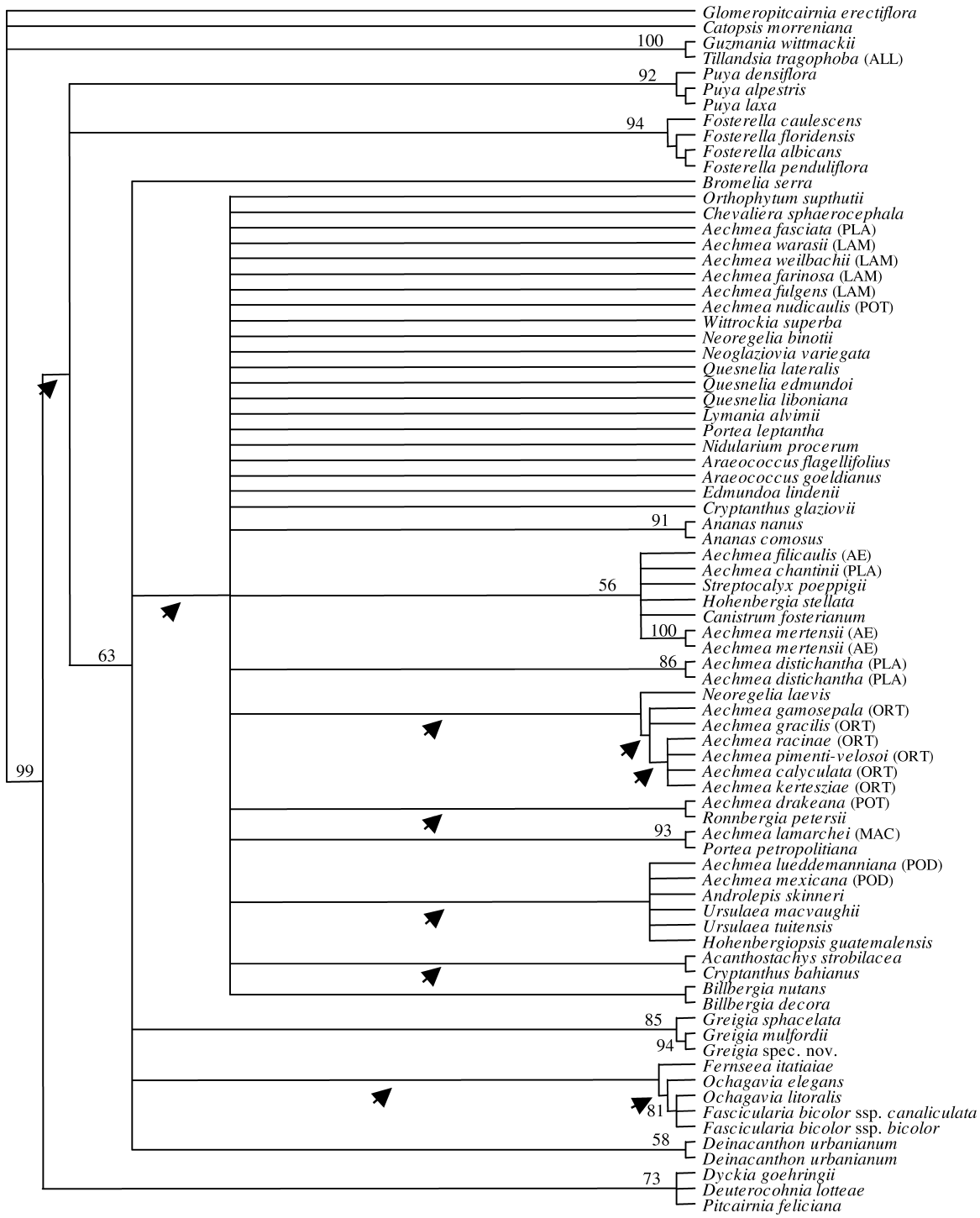

Fig. 2.—Phylogenetic reconstruction based on *trn*L intron and *trn*L–*trn*F IGS sequence data of 37 genera/74 species of Bromeliaceae, including 28 genera/60 species of Bromelioideae (all genera of the subfamily except *Disteganthus, Pseudaechmea,* and *Pseudananas* Hassl. & Harms). 80% majority rule consensus tree of 3690 most parsimonious trees 289 steps long (CI: 0.754; RI: 0.781). Length of alignment: 1174 bp + 21 indels, variable positions: 193, synapomorphies: 88. Arrows indicate nodes that collapse in the strict consensus tree.

AFLP data can be highly homoplasious, the application of AFLPs is justified when: (a) enough common fragments still remain in all OTUs examined, (b) a high number of characters are considered, and (c) the AFLP data set is analyzed in comparison to findings from more data sets, such as sequence data and leaf blade anatomy.

Ochagavia/Fascicularia.—The closely related genera *Ochagavia* and *Fascicularia* have been revised for the Flora de Chile, as well as the Chilean species of the genus *Greigia* (Will and Zizka 1999; Zizka et al. 1999, 2002). The aim of the AFLP studies was to assess genetic relationships among species of *Ochagavia* and *Fascicularia* and to compare them

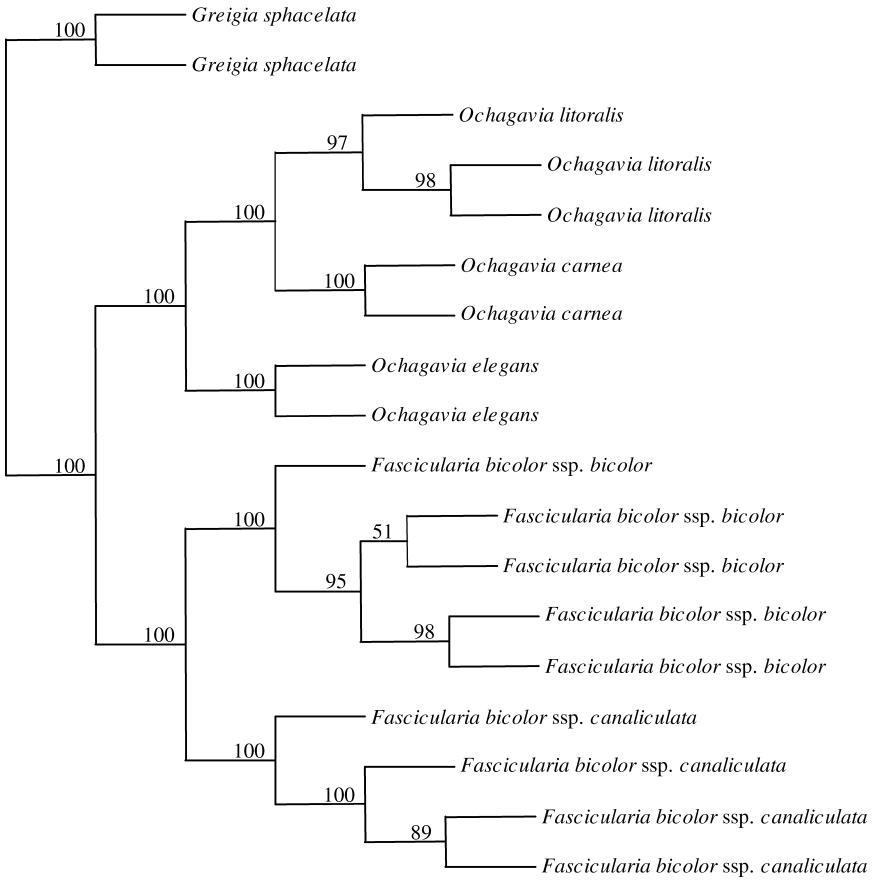

Fig. 3.—*Fascicularia, Ochagavia,* and *Greigia.* Bootstrap 50% majority-rule consensus tree of UPGMA analysis of the AFLP data from 18 accessions (six species and subspecies) of the genera *Fascicularia, Ochagavia,* and *Greigia* (Bromelioideae), based on 648 positions of AFLP fragments generated by seven primer pair combinations. Pairwise genetic distances were calculated using the Nei-Li (1979) dissimilarity coefficient implemented in PAUP* vers. 4.0 b10. Figures are bootstrap values (1000 replicates). The same topology with similar bootstrap support was obtained with a neighbor-joining (NJ) analysis.

to the concept obtained from morphological and anatomical studies, especially concerning the position of *O. elegans. Ochagavia andina* (Phil.) Zizka, Trumpler & Zoellner was not included because no living material could be obtained. One representative of *Greigia* (*G. sphacelata*) occurring sympatric with *Fascicularia bicolor* (Ruiz & Pav.) Mez was included, although the genus is not closely related, according to morphological and cpDNA sequence data.

Seven out of 16 primer combinations initially tested resulted in distinct banding patterns of appropriate complexity and were selected for further study. These seven primer pair combinations were analyzed across 16 accessions of three genera/five species and subspecies of Bromelioideae, generating a total of 648 scorable bands. The number of AFLP fragments obtained with each primer varied from 78 to 145. A 50% majority rule bootstrap tree of an unweighted pair-group method with arithmetic averaging (UPGMA) cluster analysis based on the Nei-Li coefficient is shown in Fig. 3 (an underlying 0/1-matrix as well as pairwise genetic distances calculated by the Nei-Li index can be obtained from the authors [R. H. or G. Z.]).

The topology of the bootstrap tree agrees very well with the taxonomic treatment based on morphology and leaf-blade anatomy alone. All recognized taxa form separate clades. Most clusters received high bootstrap support. Differences between the *Ochagavia* species are comparatively small. The morphological distinct endemic *O. elegans* from Robinson Crusoe Island clearly groups together with the two other *Ochagavia* species, a topology that does not receive bootstrap support in the phylogeny based on sequence data. In *Fascicularia,* the two infraspecific groups discerned by their leaf anatomy are also clearly distinguished by the AFLP analysis. Surprisingly, AFLP variation between the two morphologically very similar subspecies of *Fascicularia* exceeds the AFLP variation among the morphologically distinct species of *Ochagavia.* The groups found in *Fascicularia* as well as in *Ochagavia* also differ ecologically; both comprise elements of coastal habitats (*O. litoralis*; *F. bicolor* subsp. *bicolor*) as well as elements from more humid habitats further inland (*O. carnea*; *F. bicolor* subsp. *canaliculata*). In correspondence with the morphological data, *Ochagavia* and *Fascicularia* are regarded as a monophyletic group, whose ancestor probably reached the present range via cooler Andean habitats. The ancestor of *O. elegans* seems to have reached Robinson Crusoe Island by long-distance dispersal.

Aechmea *and related genera*.—The AFLP analysis comprised 37 accessions, including 30 *Aechmea* species from seven subgenera recognized by Smith and Downs (1974,

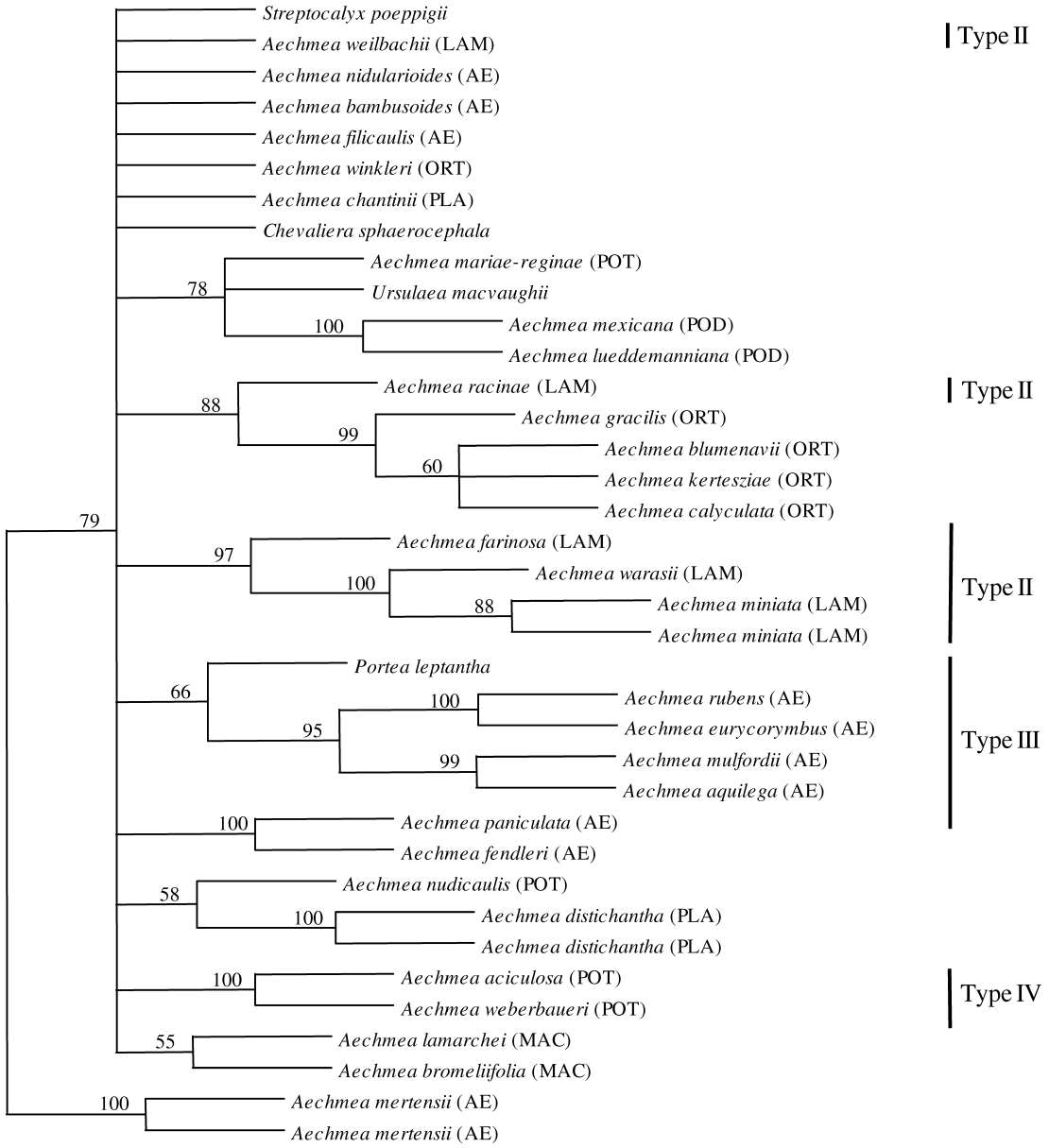

Fig. 4.—*Aechmea* and allied genera. Bootstrap 50% majority-rule consensus tree of UPGMA analysis of the AFLP data from 37 accessions (34 species; branches receiving <50% bootstrap support are collapsed), based on 843 positions of AFLP fragments generated by five primer pair combinations. Included are the genera *Aechmea* (30 species from all subgenera); *Portea* (1); *Ursulaea* (1); and *Streptocalyx* (1). Pairwise genetic distances were calculated using the Nei-Li (1979) dissimilarity coefficient implemented in PAUP*: vers. 4.0 b10. Figures are bootstrap values (1000 replicates). The vertical lines on the right indicate the occurrence of the recognized leaf anatomy types. Abbreviations in brackets refer to the subgenera of *Aechmea* (AE = *Aechmea*; LAM = *Lamprococcus*; MAC = *Macrochordion*; ORT = *Ortgiesia*; PLA = *Platyaechmea*; POD = *Podaechmea*; POT = *Pothuava*).

1977, 1979) (*Aechmea*: 10 species; *Lamprococcus*: 5; *Macrochordion*: 2; *Ortgiesia*: 5; *Platyaechmea*: 2; *Podaechmea*: 2; *Pothuava*: 4; *Chevaliera* (Gaudich. ex Beer) Baker: see *C. sphaerocephala*) and representatives from related genera (*Streptocalyx poeppigii, Ursulaea macvaughii, Chevaliera sphaerocephala,* and *Portea leptantha*). The 50% majority rule bootstrap tree of an UPGMA analysis (Fig. 4) provides only limited resolution. It displays a basal dichotomy with one branch formed by the two accessions of *A. mertensii,* an epiphytic species occurring from Colombia to Peru and Amazonian Brazil (Smith and Downs 1974, 1977, 1979) and is reported to grow as part of ant gardens. The other branch comprises the remaining species and is divided into a large polytomy. Whereas relationships between the groups are not resolved, some of the branches receive moderate-to-strong bootstrap support and are briefly listed below:

Podaechmea *group* (Ursulaea macvaughii, Aechmea mexicana, A. lueddemanniana, A. mariae-reginae).—These species are confined to Mexico and Central America; *Aechmea mexicana* and *A. lueddemanniana* are representatives of subgen. *Podaechmea,* while *A. mariae-reginae* is placed in subgen. *Pothuava.* The inflorescence of the latter is simple and densely spicate, while those of the other species are amply

compound as is typical for subgen. *Podaechmea.* All species are medium-sized plants growing epiphytically or epilithically from about sea level to well over 1000 m.a.s.l. The genus *Ursulaea* was described by Read and Baensch (1994) and comprises two species (*U. macvaughii* and *U. tuitensis*) that were formerly placed in *A.* subgen. *Podaechmea* (Smith and Downs 1974, 1977, 1979).

Ortgiesia *group* (Aechmea racinae, A. kertesziae, A. gracilis, A. blumenavii, A. calyculata).—The representatives of this group belong to subgen. *Ortgiesia,* except *A. racinae,* that is placed in subgen. *Lamprococcus.* The medium-sized plants growing epiphytically or terrestrially in forests have a restricted distribution area in southeastern Brazil. The relationships of *A. winkleri,* another species from this subgenus included in the study, are not resolved.

Lamprococcus *group* (Aechmea farinosa, A. warasii, A. miniata *[two accessions]*).—These species occur in eastern to northeastern Brazil, probably growing as epiphytes. Two additional species of *A.* subgen. *Lamprococcus* were studied: *A. weilbachii* and *A. racinae.* Both occur in the same area and apparently in similar habitats. For *A. weilbachii,* relationships are not resolved. *Aechmea racinaea* grouped together with the species of subgen. *Ortgiesia* from southeastern Brazil (see above).

Gravisia *group* (Portea leptantha, Aechmea rubens, A. eurycorymbus, A. mulfordii, A. aquilega).—Three species of this group have formerly been placed in the separate genus *Gravisia,* which Smith and Downs (1974, 1977, 1979) regarded as synonymous to *Aechmea.* The representatives are medium-sized to large, terrestrial or epiphytic plants, occurring principally in northeastern Brazil (*A. aquilega* extending north to Costa Rica). *Portea leptantha,* which also is distributed in northeastern Brazil groups together with these species. *Portea* is usually maintained as a separate genus in more recent publications, comprising nine species from eastern Brazil (Luther 2000). It was separated from *Aechmea* by Smith and Till (1998) on the basis of the combination of pedicellate flowers with connate, long mucronate sepals. The present AFLP analysis casts doubt on the generic rank of *Portea.*

A well-supported group is also formed by *A. aciculosa* and *A. weberbaueri,* representing two taxa from northwestern South America, west of the Andes. The two other species from subgen. *Pothuava* included in this study display completely different distribution types and also occupy separate positions in the tree: *A. mariae-reginae* (clustering with the *Podaechmea* group, see above) is restricted to Costa Rica, while *A. nudicaulis* (clustering with *A. distichantha,* subgen. *Platyaechmea*) is one of the most widespread Bromelioideae, extending from Mexico to southern Brazil. Another branch is formed by *A. fendleri* and *A. paniculata* from Peru and Venezuela. For further interpretation it will be necessary to include additional species with these distribution types, especially from subgen. *Pothuava* and *Aechmea.*

Leaf Blade Anatomy

Transverse sections of leaf blades from 31 genera and 103 species of Bromelioideae (all genera except *Pseudaechmea* L. B. Sm. & Read and *Disteganthus* Lem.) are presently being studied by K. Schulte. Leaf anatomy of the set of species investigated with the AFLP technique (see above) as well as of two species of *Fosterella* were analyzed and compared to the molecular data. We observed considerable variability in anatomical characters. Four distinct anatomical types could be discerned, which are characterized as follows (Fig. 5):

Type I: Vascular bundles protruding into the water storage tissue adaxially. Extra-fascicular fibrous strands absent. Water storage tissue adaxially well developed, much more prominent in the middle of the leaf than towards the margin. Air lacunae well developed.

Type II: Vascular bundles totally embedded in chlorenchyma. Extra-fascicular fibrous strands absent. Water storage tissue adaxially poorly developed, evenly distributed from the middle towards the margin. Air lacunae poorly developed.

Type III: Vascular bundles totally embedded in chlorenchyma. Extra-fascicular fibrous strands adaxially and abaxially present, within the chlorenchyma. Water storage tissue adaxially well developed, evenly distributed from the middle towards the margin, abaxially in cushion-like groups. Air lacunae well developed.

Type IV: Vascular bundles totally embedded in chlorenchyma. Extra-fascicular fibrous strands absent. Water storage tissue adaxially well developed, being much more prominent in the middle of the leaf than towards the margin. Air lacunae well developed.

The occurrence of anatomical types and the AFLP results display the following correspondences (see also Fig. 4): Type I was found only in *Fosterella* (not included in the AFLP study) and is quite distinct from all types present in the *Aechmea* species. Type II was only found in the six investigated species of *A.* subgen. *Lamprococcus,* four of which formed a well-supported clade in the AFLP analysis (with the exception of *A. weilbachii* and *A. racinae*). Type III anatomy was found in four species of *A.* subgen. *Aechmea* and in *Portea leptantha,* which also grouped together in the AFLP analysis. Six additional species from *A.* subgen. *Aechmea* included in the analysis lack this distinct type of leaf anatomy, and their relationships were not resolved on the basis of the AFLP data.

Finally, Type IV characterizes two species of *A.* subgen. *Pothuava* distributed in Peru and Ecuador west of the Andes. These two species also form a distinct branch in the AFLP analysis, whereas the other two representatives of the subgenus with different leaf anatomy also occupy distant positions in the AFLP tree.

DISCUSSION

The molecular phylogenies at hand as well as our studies of three noncoding markers from cpDNA clearly indicate that the classical division of Bromeliaceae into three subfamilies needs to be revised. Pitcairnioideae in their current circumscription are clearly paraphyletic and *Brocchinia* and *Ayensua* occupy a basal position in the family, but as long as representatives of the other genera from subfamily Pitcairnioideae sensu Smith and Till (1998) such as *Connellia* N. E. Br., *Cottendorfia,* and *Steyerbromelia* L. B. Sm. are not included, the drawing of taxonomic and nomenclatural

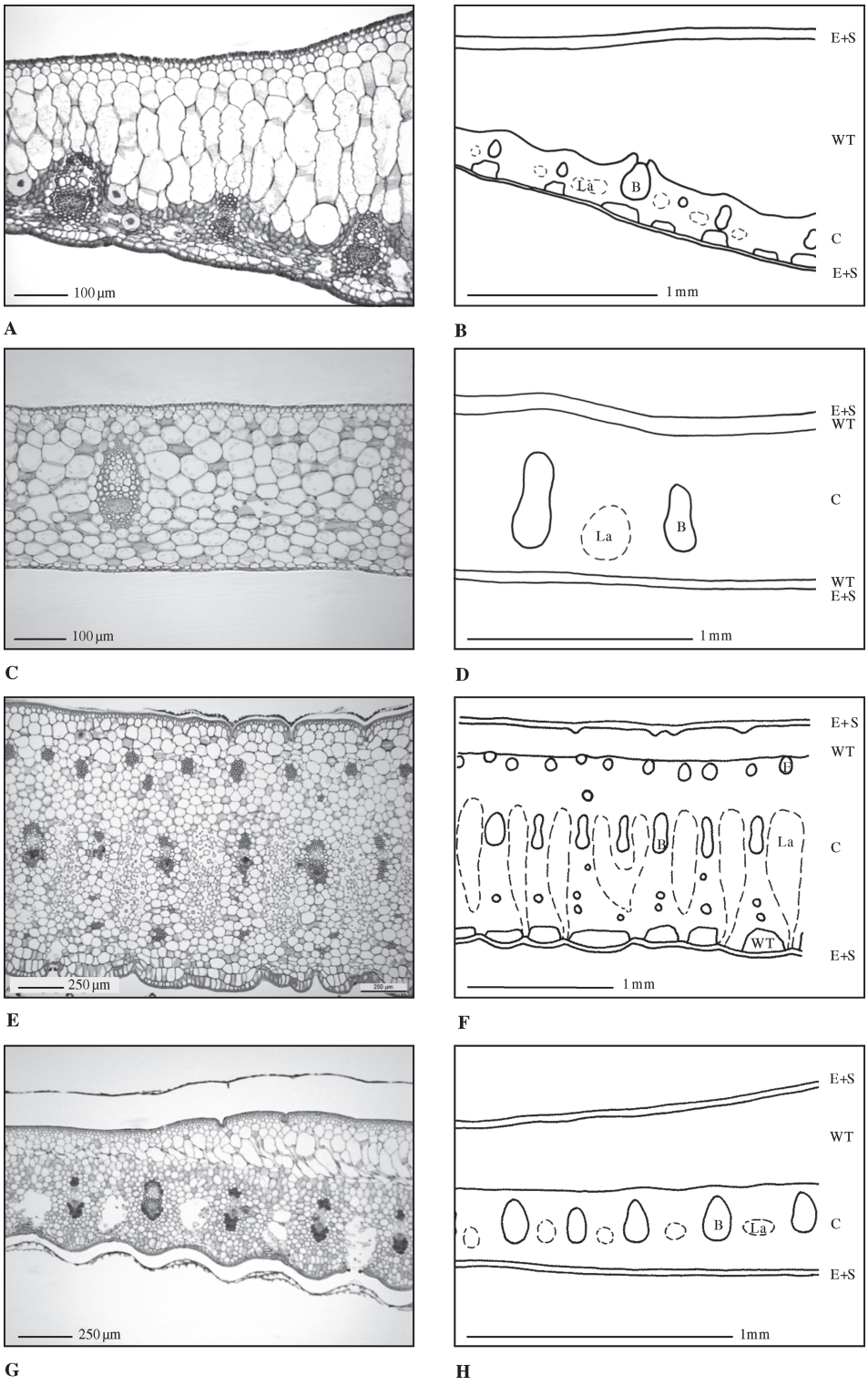

Fig. 5.—Leaf anatomy types observed in *Aechmea* species and *Fosterella.* A, B: Type I, *Fosterella albicans.* C, D: Type II, *Aechmea racinae.* E, F: Type III, *Aechmea rubens.* G, H: Type IV, *Aechmea weberbaueri.* B = vascular bundle; C = chlorenchyma; E = epidermis; F = extra-fascicular fibrous strands; La = air lacunae; S = sclerotic layer of hypodermis; WT = water storage tissue.

consequences would be premature. A solid delimitation of subfamilies in Bromeliaceae will also require better resolution of the position of the genus *Hechtia,* which appears to be a distinct lineage. Monophyly of Tillandsioideae and Bromelioideae is supported by all molecular studies performed so far. A sister group relationship of *Fosterella* and Bromelioideae, as suggested by our three-marker phylogeny, does not receive statistical support and needs to be substantiated by additional studies. In the more extended two-marker analysis, *Fosterella, Puya,* and Bromelioideae form sep-

arate clades, but relationships among these clades are not resolved.

There have been few hypotheses about the origin of Bromelioideae, which today display a center of diversity in eastern and southeastern Brazil. *Fosterella* and *Puya,* as possible sister groups, are both concentrated in Andean habitats, supporting a hypothesis that Bromelioideae evolved from ancestors with principally Andean distribution. Our molecular phylogenies do not resolve the basal phylogeny of Bromelioideae, but it is noteworthy, that genera with an isolated distribution in temperate South America west of the Andes (*Ochagavia, Fascicularia*) or a principally Andean distribution also extending far south (*Greigia*) form distinct clades, while most of the remaining Bromelioideae (including the putatively derived *Cryptanthus*) form an unresolved core group.

Fosterella, Puya, Ochagavia, Fascicularia, and *Greigia* comprise predominantly terrestrial species (the latter two also include epiphytic representatives) with absorptive soil roots and shoots not developing phytotelma (possibly with exceptions in *Greigia*), most of these genera belong to the ecophysiological Type I sensu Benzing (2000). This also holds true for *Bromelia* L. and *Deinacanthon*

Looking at the distribution of C_3 and CAM metabolism (Martin 1994; Benzing 2000; Crayn et al. 2000, 2004), we find records of C_3 for *Fosterella, Puya* (also records for CAM), and *Greigia,* while in the core group of Bromelioideae CAM metabolism is dominant. Speculating about the ancestral stock of Bromelioideae we could characterize it as a group of predominantly terrestrial plants of ecophysiological Type I sensu Benzing (2000) with C_3 metabolism and principally Andean distribution. Bromelioideae then could have spread to lowland areas and also to the Atlantic Forest of eastern and southeastern Brazil (possibly via the ''coastal gate'', as Winkler 1980 suggested), where successful and rapid radiation led to the present center of diversity.

As far as *Ochagavia* and *Fascicularia* are concerned, molecular as well as morphological and anatomical data correspond very well with each other and give evidence that the presently recognized species are natural groups (Zizka et al. 1999; this study). Both genera are closely related, could very well have evolved from one ancestral species, and might as well be united. We nevertheless kept them separate to minimize nomenclatural changes. All in all, we are still far away from a consistent general concept of generic delimitations in Bromeliaceae. This can only be achieved when updated revisions and convincing concepts including molecular data are at hand for the boundaries of the larger genera such as *Tillandsia, Vriesea, Aechmea,* and *Pitcairnia.*

Among the core group of Bromelioideae, *Aechmea* and related genera form the largest alliance. Lack of updated revisions and molecular data make this group the taxonomically most problematic among Bromelioideae. Our cpDNA sequence data do not provide enough resolution to support or reject present generic concepts. The AFLP technique provides more genetic variation, but relationships are not resolved for many of the investigated species, and the bootstrap tree (Fig. 4) displays a basal polytomy. Only three clusters receive fairly good bootstrap support, but these unite species of different subgenera of *Aechmea* or even species from different genera (*Ursulaea* Read & Baensch, *Aechmea*). Clustering patterns seem to correspond to distribution types (Mexico/Central America; southeastern Brazil; eastern Brazil) rather than to the morphological characters used to differentiate subgenera and genera. The AFLP data support the results of Izquierdo and Pinero (1998), which could not confirm generic rank for the species of *Ursulaea* on the basis of allozyme divergence. The generic rank of *Portea* Brongn. ex. K. Koch is doubtful, too. This corresponds with the results of Böhme (1988) who, based on a comparative study of the septal nectaries, recommended that *Gravisia* Mez be reinstated and united with *Portea.* While *Aechmea* subgen. *Ortgiesia* appears to be a natural group this is not confirmed for subgen. *Aechmea* and *Lamprococcus.*

Leaf anatomical studies have also recently provided relevant information to assess relationships among Bromeliaceae (Horres and Zizka 1995; Sajo et al. 1998; Robinson and Taylor 1999). In our opinion, however, leaf anatomical characters are still underexploited in Bromeliaceae systematics. As was demonstrated in *Fascicularia* (Nelson and Zizka 1997), leaf blade anatomy can even serve to discern groups on subspecies level. Our anatomical studies in *Aechmea* and allied genera correspond in part with the AFLP studies, supporting the clade of the ''*Gravisia* group.'' Partly in conflict with the AFLP data, all representatives of subgen. *Lamprococcus* were specifically characterized by the same leaf anatomy type. Noteworthy is the fact that two species from subgen. *Pothuava,* with a distinct distribution type (western Peru and Ecuador), share a peculiar leaf blade anatomy (Type IV, Fig. 4).

The results presented here are still fragmentary, but give new insights in the relationships of genera within Bromelioideae. For resolving the basal phylogeny of Bromeliaceae and Bromelioideae, additional molecular markers will have to be studied, preferably of the nuclear genome and including representatives of all genera. As far as *Aechmea* and allied taxa are concerned, the AFLP technique appears appropriate to assess genetic relationships, but sampling will have to be increased. Combining AFLP and anatomical data appears to be a rewarding approach. Nevertheless, a stable concept for genera and subgenera can only be achieved when updated revisions are provided, as recently done by Leme (1997, 1998, 2000) and Wendt (1997), and which are underway for *Chevaliera* and *Portea.*

ACKNOWLEDGMENTS

We acknowledge support by grants from the Deutsche Forschungsgemeinschaft (DFG Zi 557/2-1, 2-2 and 5-1) and the ''Freunde und Förderer der J.W. Goethe-Universität.'' Plant material was kindly supplied by the Palmengarten der Stadt Frankfurt/Main as well as the Botanical Gardens of the universities of Bonn, Heidelberg, Frankfurt, Munich, and Berlin.

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