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Molecular phylogeny of Bromelioideae and its implications on biogeography and the evolution of CAM in the family

(Poales, Bromeliaceae)

Katharina Schulte, Ralf Horres & Georg Zizka

Abstract

Phylogenetic analysis of Bromeliaceae with focus on subfamily Bromelioideae was performed based on *mat*K, *trn*L intron and *trn*L-*trn*F intergenic spacer sequence data. A total of 40 genera and 81 species of Bromeliaceae was studied, the majority of them (29 genera/ 58 species) belonging to subfamily Bromelioideae. *Brocchinia* and *Hechtia* species were used as outgroup in the analyses. Representatives of the genus *Puya* are sister to the Bromelioideae, the latter being monophyletic. Among Bromelioideae, *Greigia* is putative sister to the remainder of the subfamily. The latter displays an unresolved trichotomy with branches formed by (a) *Bromelia*, (b) *Ochagavia, Fascicularia* and *Deinacanthon*, and (c) the remaining Bromelioideae. The evolution and distribution of Bromelioideae is discussed in the light of geographical distribution and occurrence of C3 photosynthesis and Crassulacean Acid Metabolism (CAM). Our data indicate that early Bromelioideae had an Andean distribution, and were terrestrials with C3 photosynthesis, lacking water-impounding phytotelmata. The genus *Greigia* forms a basal clade among the subfamily. Molecular data support the hypothesis that colonisation of the Atlantic Forest of southeastern South America by early representatives of Bromelioideae originated from the southern Andes. The poorly resolved "core Bromelioideae" are divided into two weakly supported clades, one comprising species restricted to southeastern Brazil, the other species of different distribution types.

K e y w o r d s : *Puya, Greigia, mat*K, *trn*L intron, *trn*L-*trn*F intergenic spacer

Introduction

The Bromeliaceae are a medium sized family comprising more than 2600 species in 56 genera (Smith & 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. Despite the fact that Bromeliaceae are the second-most diverse family of flowering plants among neotropical epiphytes and their economic importance as ornamentals, we still lack updated revisions for most of the genera. As far as hypotheses about phylogeny and evolution of the family

Authors' address:

Katharina Schulte, Dr. Ralf Horres, Prof. Dr. Georg Zizka, Abteilung Botanik und molekulare Evolutionsforschung, Forschungsinstitut Senckenberg & Johann Wolfgang Goethe-Universität, Senckenberganlage 25; D-60325 Frankfurt am Main, Germany; email of the corresponding author: georg.zizka@senckenberg.de

are concerned, these have been speculative and/or suffered from the high extent of morphological, physiological and ecological variation that renders the recognition of homoplasy difficult (e.g. SMITH 1934, WINKLER 1980, 1986, VARADARAJAN & GILMARTIN 1988 a, 1988 b, BENZING 2000). This situation changed with molecular data becoming available. Several molecular studies dealing with the phylogeny of the family have by now been published (e.g. TERRY et al. 1997 a, b, HORRES et al. 2000, CRAYN et al. 2000, CRAYN et al. 2004, GIVNISH et al. 2004, BARFUSS et al. 2004). In spite of the remaining problems, the following results are supported by all recent publications: Bromeliaceae as a whole as well as subfamilies Bromelioideae and Tillandsioideae are monophyletic. Pitcairnioideae are clearly paraphyletic. *Brocchinia* and *Ayensua* form a basal clade sister to the remaining Bromeliaceae. Among these, a study based on *ndh*F sequence data and an extraordinary good sampling of genera from the Guayana Shield by GIVNISH et al. (2004) identifies a basal "*Lindmania*-clade" formed by 2 *Lindmania* species. The remainder displays a trichotomy with (a) Tillandsioideae, (b) the genus *Hechtia* and (c) Bromelioideae, *Puya* and remaining Pitcairnioideae forming separate clades. Among the latter group, Bromelioideae, *Puya* and Pitcairnioideae s.str. on one hand are sister to a "Cratonic clade" composed of *Navia, Brewcaria, Cottendorfia* and *Brocchinia serrata. Puya* is identified with high bootstrap support as sister group of the Bromelioideae, a relationship that was also found by HORRES et al. (2000) and CRAYN et al. (2004) but then received no or weak statistical support.

Bromelioideae have usually been represented only by few taxa in the molecular studies and thus the evolution of this subfamily has remained enigmatic.

The here presented study is focussing on the subfamily Bromelioideae. Although DNA-variability and thus resolution is notoriously low in Bromelioideae, we aim at retrieving information from the combination of the phylogenetic reconstruction based on three markers from the chloroplast genome, present distribution data and the occurrence of C3 and CAM photosynthesis to discuss the evolution and historical biogeography of the Bromelioideae.

The molecular data support the hypothesis already published by SMITH (1934) that the bromeliads originated in the Guayana Shield. According to the calculation of a molecular clock by GIVNISH et al. (2004), the stem lineage of Bromeliaceae arose 84 Ma ago, while the crown radiation of Bromeliaceae occurred at 9.4 Ma. The invasion of the entire Andean cordillera by the genus *Puya* began at least at 10 Ma. The invasion of the Brazilian Shield by higher Bromelioideae is calculated at ca. 7 Ma. As far as the evolution of Bromelioideae is concerned, the inclusion of 5 genera/5 species into the analysis prevented a more detailed hypothesis on the origin and evolution of the subfamily by these authors. As we like to show, the taxon sampling plays a crucial role for the interpretation of the biogeographic history of Bromelioideae: Inclusion of Andean or more southern genera like *Greigia, Fascicularia* and *Ochagavia* adds important information about the putative ancestors of the subfamily.

Materials and methods

Taxon sampling and plant material: This study includes 81 species (82 accessions) of Bromeliaceae from 40 genera, subfamily Bromelioideae is represented here by 29 genera (of 31) and 58 species (59 accessions). Additionally, 6 genera (of 9) and 8 species of Tillandsioideae and 5 genera (of 16) and 14 species of Pitcairnioideae were included to represent further principal clades of the family. Relying on the results of previous phylogenetic analyses of Bromeliaceae (HORRES et al. 2000, CRAYN et al. 2004, GIVNISH et al. 2004, HORRES et al. 2005), representatives of the basal genera *Brocchinia* (4 species) and *Hechtia* (1 species) were selected as outgroup.

Sequence data of the chloroplast loci *mat*K gene, part of its adjacent 3´*trn*K intron, *trn*L intron and *trn*L-*trn*F spacer were analysed. A large proportion of the *mat*K sequences were generated specifically for this study, and combined with our sequence data on the *trn*L intron (HORRES et al. 2000) and *trn*L-*trn*F spacer (HORRES et al. 2005). The data set was complemented with 46 sequences for 17 taxa obtained from GenBank provided by BARFUSS et al. (2004). Plant material was derived from the living collections of the Palmengarten Frankfurt/Main and the Botanical Gardens of the Universities of Heidelberg, Bonn, Frankfurt/Main, Vienna and Berlin. Voucher specimens were deposited in the Herbarium Senckenbergianum (FR) and the Palmengarten Herbarium, Frankfurt (FRP). Nomenclature of genera follows SMITH & TILL (1998). Among *Aechmea* RUIZ & PAVON, 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.

Investigated species, herbarium vouchers and living collections as well as GenBank accession numbers are listed in Tab. 1.

DNA isolation, polymerase chain reaction (PCR) amplification and sequencing: Total cellular DNA from fresh or lyophilized leaf material was isolated and purified as described in HORRES et al. (2000). Gene amplification was conducted in two steps.

First the 3´ two thirds of *mat*K and its flanking 3´ *trn*K Intron were amplified using the primers TO*mat*K 480 F (HILU et al. 2003) and *trn*K2 R (JOHNSON & SOLTIS 1995). An internal sequencing primer (BRO*mat*K 860 F: 5´-GCA ATT CTG GCT TCA AAA GG-3´) located ap-

Tab. 1: Taxa represented in the study and information on the aligned sequences. — References: Ref. 1: this study; Ref. 2: HORRES et al. 2000; Ref. 3: HORRES et al. (2005); Ref. 4: BARFUSS et al. (2004). — Abbreviations: BG Berlin = Botanical Garden Berlin-Dahlem; B = Herbarium Berlin-Dahlem; BG Bonn = Botanical Garden of the University of Bonn, BG FR = Botanical Garden of the University of Frankfurt am Main; FR = Herbarium Senckenbergianum, FRP = Herbarium and living collections of the Palmengarten Frankfurt/Main; H = R. HORRES; HEID = Herbarium and Botanical Garden of the University of Heidelberg; HS = R. HORRES/K. SCHULTE; KAS= Herbarium and Botanical Garden of the University of Kassel; KS = K. SCHULTE; W = Herbarium of the Natural History Museum, Vienna; Z = G. ZIZKA. Nomenclature follows SMITH & DOWNS (1974–1979) and LUTHER (2002).

Genus and species	**Accession no. living collection/herbarium specimen**	**GenBank no./reference no.**		
		***mat*K, *trn*K**	***trn*L Intron**	***trn*L-*trn*F Spacer**
Bromelioideae:				
Acanthostachys strobilacea (SCHULTES filius) KLOTZSCH 1840	FRP 98-16986-0/FR H 019	AY950021/Ref. 1	AF188765/Ref.2	Ref. 3
Aechmea calyculata (E. MORREN) BAKER 1879	HEID 103296/FR KS 240203-9	AY950040/Ref. 1	Ref. 3	Ref. 3
Aechmea chantinii (CARRIÈRE) BAKER 1889	KAS s.n./ FR Rex 260105-3	AY950042/Ref. 1	Ref. 3	Ref. 3
Aechmea distichantha LEMAIRE 1853	FRP 88-16753-2/FRP Z 1549, FR H 008	AY950041/Ref. 1	AF188761	Ref. 3
Aechmea drakeana ANDRÉ 1888	FRP 98-16955-2/FRP Z 1100	AY950043/Ref. 1	AF188772/Ref.2	Ref. 3
Aechmea farinosa (REGEL) L. B. SMITH 1966	FRP 98-16961-3/FRP Z 1108	AY950031/Ref. 1	Ref. 3	Ref. 3
Aechmea fasciata (LINDLEY) BAKER 1879	KAS s.n./ FR Rex 260105-2	AY950034/Ref. 1	Ref. 3	Ref. 3
Aechmea filicaulis (GRISEBACH) MEZ 1894	FRP 98-16863-0/ FR HS 180701-6	AY950036/Ref. 1	Ref. 3	Ref. 3
Aechmea fulgens BRONGNIART 1841	FRP s.n./FR KS 130105-5	AY950033/Ref. 1	Ref. 3	Ref. 3
Aechmea gracilis LINDMAN 1891	FRP 98-16949-3/FR KS 280203-1	AY950038/Ref. 1	Ref. 3	Ref. 3
Aechmea kertesziae REITZ 1952	FRP 98-16935-3/FRP Z 1177	AY950039/Ref. 1	Ref. 3	Ref. 3
Aechmea lamarchei MEZ 1892	BG Berlin-Dahlem 118-37-74-86/B GH 11309	AY950044/Ref. 1	Ref. 3	Ref. 3
Aechmea lueddemanniana (K. KOCH) MEZ 1934	FRP 95-14215-0/FR KS 100203-3; FR KS 010305-1	AY950029/Ref. 1	Ref. 3	Ref. 3
Aechmea mertensii (G. MEYER) SCHULTES filius 1809	FRP 98-16873-0/FRP Z 1572	AY950035/Ref. 1	Ref. 3	Ref. 3
Aechmea mexicana BAKER 1879	HEID 104025/FR KS 240203-12; FR KS 171103-25	AY950028/Ref. 1	Ref. 3	Ref. 3
Aechmea nudicaulis (LINNEAUS) GRISEBACH 1864		AY614024.1/Ref. 4	AY614268.1/Ref. 4	AY614268.1/Ref. 4
Aechmea pimenti-velosoi REITZ 1952	FRP 90-1144-4-00/FRP Z 1193	AY950037/Ref. 1	Ref. 3	Ref. 3
Aechmea racinae L. B. SMITH 1941	FRP 98-16934-3/FR KS 120203-1	AY950030/Ref. 1	Ref. 3	Ref. 3
Aechmea warasii E. PEREIRA 1972	HEID 130354/FR KS 240203-17	AY950032/Ref. 1	Ref. 3	Ref. 3
Ananas comosus (LINNEAUS) MERRILL 1917	BG FR s.n./FR HS 220601-1	AY950055/Ref. 1	Ref. 3	Ref. 3
Ananas nanus (L. B. SMITH) L. B. SMITH 1962	FRP s.n./FR HS 050401-9	AY950054/Ref. 1	Ref. 3	Ref. 3
Androlepis skinneri (K.KOCH) BRONGNIART ex HOULLET 1870	FRP 97-16793-2/FR KS 140105-12	AY950005/Ref. 1	AF188780/Ref. 2	Ref. 3
Araeococcus flagellifolius HARMS 1929	KAS s. n./FR Rex 260105-1	AY950003/Ref. 1	Ref. 3	Ref. 3
Araeococcus goeldianus L. B. SMITH 1955	FRP 99-18256-2/FR KS 100203-1	AY950002/Ref. 1	Ref. 3	Ref. 3
Billbergia decora POEPPIG & ENDLICHER 1838	FRP 90-733-2-4/FR H 129; FRP Z 882	AY950050/Ref. 1	Ref. 3	Ref. 3
Billbergia nutans H.WENDLAND ex REGEL 1869	FRP 99-18405-0/FRP H 036		AF188766/Ref. 2	Ref. 3
— — —	FRP 97-16791-0/FRP Z 1528	AY950049/Ref. 1		
Bromelia plumieri (E. MORREN) L. B. SMITH 1967		AY614023.1/Ref. 4	AY614267.1/Ref. 4	AY614267.1/Ref. 4
Bromelia serra GRISEBACH 1879	FRP 98-17751-0/FR H 029	AY950019/Ref. 1	Ref. 3	Ref. 3
Canistrum fosterianum L. B. SMITH 1952	FRP 86-16991-3/FRP Z 927	AY950024/Ref. 1	AF188773	Ref. 3
Chevaliera sphaerocephala BAKER 1879	FRP 90-835-3/FRP Z 1104		AF188770/Ref. 2	
— — —	FRP 99-18245-3/FR H 030b (Voucher DNA)	AY950045/Ref. 1		Ref. 3
Cryptanthus bahianus L. B. SMITH 1943	HEID 103794/B GH 11060a	AY950011/Ref. 1	Ref. 3	Ref. 3
Cryptanthus glaziovii MEZ 1891	HEID 102583/FR KS 010601-3	AY950010/Ref. 1	Ref. 3	Ref. 3
Deinacanthon urbanianum (MEZ) MEZ 1896	FRP 98-17786-0/FRP H 018	AY950017/Ref. 1	AF188781/Ref. 2	Ref. 3
Deinacanthon urbanianum (MEZ) MEZ 1896	BG FR s.n./FR H 140	AY950018/Ref. 1	Ref. 3	Ref. 3

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		*mat*K, *trn*K	*trn*L Intron	*trn*L-*trn*F Spacer
Bromelioideae:				
Edmundoa lindenii (REGEL) LEME 1997	HEID 105009/FR KS 010601-4	AY950012/Ref. 1	Ref. 3	Ref. 3
Fascicularia bicolor (RUIZ & PAVON) MEZ 1896	FRP 98-16846-3/FR Z 1790	AY950023/Ref. 1	AF188775/Ref. 2	Ref. 3
Fernseea itatiaiae (WAWRA) BAKER 1889	HEID 102174/FR H 067	AY949999/Ref. 1	Ref. 3	Ref. 3
Greigia spec. nov.	FRP 99-19040/FR Grant 19040	AY950014/Ref.1	Ref. 3	Ref. 3
Greigia sphacelata (RUIZ & PAVON) REGEL 1865	FRP 92-9513-3/FR KS 230305-4	AY950015/Ref. 1	AF188779/Ref. 2	Ref. 3
Greigia mulfordii L. B. SMITH 1949	-/W Till 13090	AY950016/Ref. 1	Ref. 3	Ref. 3
Hohenbergia stellata SCHULTES FILIUS 1830	FRP 95-14252-0/FRP H 037	AY950026/Ref. 1	AF188774/Ref. 2	Ref. 3
Hohenbergiopsis guatemalensis (L. B. SMITH) L. B. SMITH & R. W. READ 1976	FRP 8-1991-1227-52/FR KS 130901-6	AY950020/Ref. 1	Ref. 3	Ref. 3
Lymania alvimii (L. B. SMITH & R. W. READ) R. W. READ 1984	HEID 103784/FR HS 050401-4	AY950000/Ref. 1	AF188768/Ref. 2	Ref. 3
Neoglaziovia variegata (ARRUDA DA CAMARA) MEZ 1894	FRP 97-16794-3/ FRP Z 1105	AY950051/Ref. 1	AF188763/Ref. 2	Ref. 3
Neoregelia binotii (ANTOINE) L. B. SMITH 1936	FRP 98-16967-3/FRP Z 1418	AY950009/Ref. 1	AF188764/Ref. 2	Ref. 3
Neoregelia laevis (MEZ) L. B. SMITH 1934	FRP 98-16962-3/FR HS 220601-3	AY950008/Ref. 1	AF188762/Ref. 2	Ref. 3
Nidularium procerum LINDMAN 1891	FRP 99-18619-0/ FR HS 220601-8	AY950013/Ref. 1	Ref. 3	Ref. 3
Ochagavia elegans R.PHILIPPI 1856	FRP 98-16852-3/ FR H 23a	AY950006/Ref. 1	AF 188778/Ref. 2	Ref. 3
Ochagavia litoralis (PHILIPPI) ZIZKA, TRUMPLER & ZOELLNER 2002	FRP 98-16853-2/ FR H 15a (Voucher DNA)	AY950007/Ref. 1	AF188777/Ref. 2	Ref. 3
Orthophytum supthutii E.GROSS & BARTHLOTT 1990	HEID 102160/HEID Barthlott & Supthut 10315	AY950022/Ref. 1	Ref. 3	Ref. 3
Portea leptantha HARMS 1929	FRP 99-18222-3/FR KS 060901-1; FRP Z 1055	AY950052/Ref. 1	Ref. 3	Ref. 3
Portea petropolitana (WAWRA) MEZ 1892	FRP s.n./FRP Z1056; FR KS 060901-2	AY950053/Ref. 1	Ref. 3	Ref. 3
Quesnelia edmundoi L. B. SMITH 1955	FRP 92-10483-3/FRP Z 964	AY950046/Ref. 1	Ref. 3	Ref. 3
Quesnelia lateralis WAWRA 1880	FRP 90-10484-0/FRP Z 1554	AY950047/Ref. 1	AF188771/Ref. 2	Ref. 3
Quesnelia liboniana (DE JOHNGE) MEZ 1922	FRP 99-17934-0/FRP Z 1384	AY950048/Ref. 1	Ref. 3	Ref. 3
Ronnbergia petersii L. B. SMITH 1973	FRP 99-17997-3/FR KS 170203-5	AY950001/Ref. 1	Ref. 3	Ref. 3
Streptocalyx poeppigii BEER 1856	FRP 94-13845-4/FR HS 201101-5	AY950004/Ref. 1	Ref. 3	Ref. 3
Ursulaea tuitensis (MAGANA & E. J. LOTT) R. W. READ & H. U. BAENSCH 1994	FRP s.n./ FR H 033 (Voucher DNA)	AY950027/Ref. 1	Ref. 3	Ref. 3
Wittrockia superba LINDMAN 1891	FRP 93-12641-0/FR HS 050401-8	AY950025/Ref. 1	AF188767	Ref. 3
Tillandsioideae:				
Catopsis floribunda L. B. SMITH 1937		AY614025.1/Ref. 4	AY614269.1/Ref. 4	AY614269.1/Ref.4
Catopsis nutans (SWARTZ) GRISEBACH 1887		AY614026.1/Ref. 4	AY614270.1/Ref. 4	AY614270.1/Ref. 4
Glomeropitcairnia erectiflora MEZ 1905	FRP 99-18392-2/ FRP H 002	AY614029.1/Ref. 4	AF188818/Ref. 2	Ref. 3
— — —				
Guzmania monostachia (LINNEAUS) RUSBY ex MEZ 1896	FRP 89-18406-0/ FR H 016 (Voucher DNA)	AY949990/Ref. 1		
— — —			AY614298.1/Ref. 4	AY614298.1/Ref. 4
Guzmania wittmackii (ANDRÉ) ANDRÉ ex MEZ 1896	FRP 99-18407-3/ FR KS 170305-4	AY949991/Ref. 1	AF188797/Ref. 2	Ref. 3
Tillandsia fasciculata SWARTZ 1788		AY614100.1/Ref. 4	AY614344.1/Ref. 4	AY614344.1/Ref. 4
Tillandsia multicaulis STEUDEL 1841		AY614112.1/Ref. 4	AY614356.1/Ref. 4	AY614356.1/Ref. 4
Vriesea splendens (BRONGNIART) LEMAIRE 1850–51		AY614045.1/Ref. 4	AY614289.1/Ref. 4	AY614289.1/Ref. 4
Werauhia ringens (GRISEBACH) J. R. GRANT 1995		AY614047.1/Ref. 4	AY614291.1/Ref. 4	AY614291.1/Ref. 4
Pitcairnioideae:				
Brocchinia micrantha (BAKER) MEZ 1894		AY614015.1/Ref. 4	AY614259.1/Ref. 4	AY614259.1/Ref. 4
Brocchinia reducta BAKER 1882		AY614018.1/Ref. 4	AY614262.1/Ref. 4	AY614262.1/Ref. 4
Brocchinia steyermarkii L. B. SMITH 1951		AY614016.1/Ref. 4	AY614260.1/Ref. 4	AY614260.1/Ref. 4
Brocchinia tatei L. B. SMITH 1946		AY614017.1/Ref. 4	AY614261.1/Ref. 4	AY614261.1/Ref. 4

Genus and species	Accession no. living collection/herbarium specimen	GenBank no./reference no.		
		***mat*K, *trn*K**	***trn*L Intron**	***trn*L-*trn*F Spacer**
Bromelioideae:				
Fosterella albicans (GRISEBACH) L. B. SMITH 1960	FRP 98-18320-1/FR KS 130901-3; FR H 156	AY949994/Ref. 1	Ref. 3	Ref. 3
Fosterella caulescens RAUH 1979	FRP 99-18434-3/HEID Rauh 40579a	AY949995/Ref. 1	Ref. 3	Ref. 3
Fosterella floridensis IBISCH, R. VASQUEZ & E. GROSS 1999	-/Ibisch & Ibisch 97-83 (FR)	AY949993/Ref. 1	Ref. 3	Ref. 3
Fosterella penduliflora (C. H. WRIGHT) L. B. SMITH 1960	HEID 103655/FR H086	AY949996/Ref. 1	AF 188782/Ref. 2	Ref. 3
Hechtia carlsoniae BURT-UTLEY & J. UTLEY 1988		AY614020.1/Ref. 4	AY614264.1/Ref. 4	AY614264.1/Ref. 4
Pitcairnia feliciana (A. CHEVALIER) HARMS & MILBRAED 1938	BG Bonn 12804/Porembski 12804 (BONN)	AY949992/Ref. 1	AF188792/Ref. 2	Ref. 3
Pitcairnia punicea SCHEIDWEILER 1842		AY614021.1/Ref. 4	AY614265.1/Ref. 4	AY614265.1/Ref. 4
Puya alpestris (POEPPIG) GAY 1952	HEID 103731/FR H060	AY949998/Ref. 1	Ref. 3	Ref. 3
Puya densiflora HARMS 1929	HEID 103568/FR H076	AY949997/Ref. 1	Ref. 3	Ref. 3
Puya laxa L. B. SMITH 1958	FRP 94-12923-4/FRP H006		AF188794/Ref. 2	Ref. 3
— — —		AY614022.1/Ref. 4		

proximately 860 bp into the coding region was designed after amplifying and sequencing first with the amplification primers. In a second step the 5´ first third of *mat*K was amplified using the primers *mat*K5 F (CRAYN et al. 2000) and BROM1 (5´-GGT TCC AGA AGA TGT TAA TCG-3´), the latter being designed for this study using sequence information obtained from the first sequencing step.

Amplification, processing of amplification products, cycle sequencing and electrophoresis were carried out as described in HORRES et al. (2000) except for the PCR conditions. The PCR regime for the amplification of the 3´-two thirds of *mat*K and its flanking 3´ *trn*K intron was 1 min at 95° C, followed by 35 cycles of 30 sec at 95° C, 1 min at 50° C and 1.5 min at 72° C, and a final step of 20 min at 72° C. The PCR regime of the sequencing reaction was 29 cycles of 5 sec at 96° C and 4 min at 50° C. The PCR regimes for amplification and sequencing reaction of the 5´end of *mat*K followed CRAYN et al. (2000) and were performed by Scientific Research and Development GmbH, Oberursel, as well as the sequencing of both parts.

Sequence alignment and phylogenetic analysis: *Mat*K sequences were aligned by hand using the software BioEdit (HALL 1999). *Trn*L intron and *trn*L-*trn*F spacer were aligned using Clustal X (THOMPSON et al. 1997) followed by manual adjustments. Indels were coded in a presence/absence matrix and appended to the alignment. One region of problematic alignment within the *trn*L-*trn*F spacer was excluded from the analysis. Parsimony analyses were conducted using PAUP 4.0 b 10 (SWOFFORD 2002) on a PowerPC G5 computer (Macintosh). The data sets were first analysed separately and then simultaneously. Fitch-Parsimony (FITCH 1971) was applied, all characters being unordered. All aligned positions were given equal weight and gaps were treated as missing data. Character state optimization was conducted under the ancillary criterium of accelerated transformation (ACCTRAN). Rooting of trees was accomplished by outgroup rooting, *Brocchinia* and *Hechtia* being chosen as outgroups. Heuristic search for most parsimonious trees was conducted keeping only the best trees. The intitial tree was generated by stepwise addition with 10.000 random replicates keeping 3 trees at each step, thus allowing a search for islands of optimal trees. Subsequent tree bisection-reconnection (TBR) branch swapping was performed saving no more than 1000 trees per replicate, the MULTREES-option not being in effect. The COLLAPSE-option was switched off and polytomous trees condensed after branch swapping by collapsing branches of zero maximum length. Multiple parsimonious trees were combined to form both a strict consensus (not shown) and a 50 % majority rule consensus tree. Levels of support were tested with 1000 bootstrap replicates (FELSENSTEIN 1985) with 100 random starting trees each.

Photosynthetic pathway: Assignment of pathway is based on the detailed study of CRAYN et al. (2004 and supplement http: //www.plants.ox.ac.uk/bromeliaceae), where the photosynthetic pathway of 1873 bromeliad species was determined from tissue carbon-isotope ratio $\delta^{13}C$. Where information of the photosynthetic pathway of species was missing, it was deduced by the information available of the respective genus. In all but one cases these genera exhibited either purely CAM or C3 photosynthesis thus allowing a reliable assignment for those species. In the case of *Orthophytum supthutii* a photosynthetic pathway was not assigned because of the occurence of both photosynthetic pathways within the genus.

Biogeography: Distribution of the species and genera is based on SMITH & DOWNS (1974-1979) and for later described taxa on the Missouri Botanical Garden's VAST database (http: //www.mobot.mobot.org/W3 T/ Search/vast.html). After a survey of the distribution

areas of Bromelioideae and *Puya* the following main distribution types were categorized to subsume the main distribution patterns: Central American and Caribbean (Fig. 3 a), northern South American (Fig. 3 b), northern– (Central American/Northern South American) southeastern South American disjunction (Fig. 3 c), Andean (Fig. 3 d), Chilean (Fig. 3 e), southeastern South American (Fig. 3 f), and widespread (Fig. 3 g). The authors are well aware that these types are rough simplifications ignoring many differences in the distribution of species and genera. Nevertheless, we regard them as helpful to display biogeographic patterns among the subfamily. Photosynthetic pathway and distribution of the species were mapped on the 50 % majority rule (MR) consensus tree (Fig. 2).

Results

Sequence data

For the 82 accessions (81 species) of Bromeliaceae, the total alignment of the sequenced chloroplast regions *mat*K, its flanking 3´ *trn*K intron, *trn*L intron and *trn*L-*trn*F spacer comprised 2877 positions, including 590 gap positions, the latter being scored as 75 indels. One region of problematic alignment within the *trn*L-*trn*F spacer, accounting for 134 positions was excluded from the analysis. The analysed dataset thus comprised in total 2743 positions, including 456 gap positions, scored as 70 indels. Of 489 variable positions, 252 were parsimony-informative (9.2 % of all positions); of the 70 coded indels, 30 were parsimony-informative. Pairwise sequence divergence (uncorrected for multiple hits) reached a maximum of 3.6 % (between *Brocchinia micrantha* and *Werauhia ringens*) and 3.2 % within the ingroup (*Hohenbergiopsis guatemalensis* and *Tillandsia fasciculata*) (see Tab. 2). Parsimony analysis of the combined dataset found 2830 most parsimonious trees of 860 steps length. One tree is shown in Fig. 1. The MR consensus of these trees is shown in Fig. 2 with photosynthetic pathway and species distribution mapped on.

Molecular phylogeny

Among Bromeliaceae four principle clades can be discerned. *Brocchinia* forms a strongly supported monophyletic group (bootstrap value [= bv] 100). Its position as sister to the rest of the family has been shown in former molecular analyses, including various closely related families as outgroups (Terry et al. 1997 a, Horres et al. 2000, Crayn et al. 2004, Givinish et al. 2004). (a) *Hechtia*, (b) Tillandsioideae and (c) *Pitcairnia*, *Fosterella*, *Puya* and Bromelioideae form a trichotomy, the latter two major clades being well supported (bv 90 and 94 respectively), the position of *Hechtia* however receives no bootstrap support. Among the clade (c) including Bromelioideae, *Pitcairnia* and *Fosterella* as representatives of Pitcairnioideae s.str. are basal and receive moderate statistical support. *Puya* forms a strongly supported clade and is sister to Bromelioideae, although this topology is only weakly supported (bv 65). The Bromelioideae form a monophyletic group of comparatively low resolution. The 50 % MR consensus tree depicts a strongly supported *Greigia*-clade (bv 100) as sister to the remaining Bromelioideae, but this position receives no bootstrap support and collapses in the strict consensus tree (not shown). The subsequent node exhibits a trichotomy of (a) a strongly supported *Bromelia*-clade (bv 100), (b) a *Deinacanthon*, *Ochagavia*, and *Fascicularia*-clade, and (c) a larger, moderately supported bromelioid clade (bv 84) comprising the remainder of the Bromelioideae. Among the latter, the 50 % MR consensus tree depicts *Fernseea* as sister to the remaining Bromelioideae, nevertheless this branching collapses in the strict consensus. The subsequent polytomy is formed by 5 separate sublineages (see below). One lineage we refer to as “core Bromelioideae” encompasses all investigated species of *Aechmea* together with 21 other bromelioid genera. Within this lineage the following relationships are noteworthy: The genus *Aechmea* is polyphyletic. Four of five investigated members of *Aechmea* subgenus

Tab. 2: Description of the sequence data.

Region	Length of alignment [bp]	Number of indels	Number of parsimony-informative characters (+ parsimony-informative indels)
*mat*K gene	1554	4	133 (+ 1)
*trn*K 3´ intron	185	3	31 (+ 2)
*trn*L intron	600	24	46 (+ 8)
*trn*L-*trn*F spacer	538	44	43 (+ 19)
*trn*L-*trn*F spacer excluding problematic region	403	39	43 (+ 18)

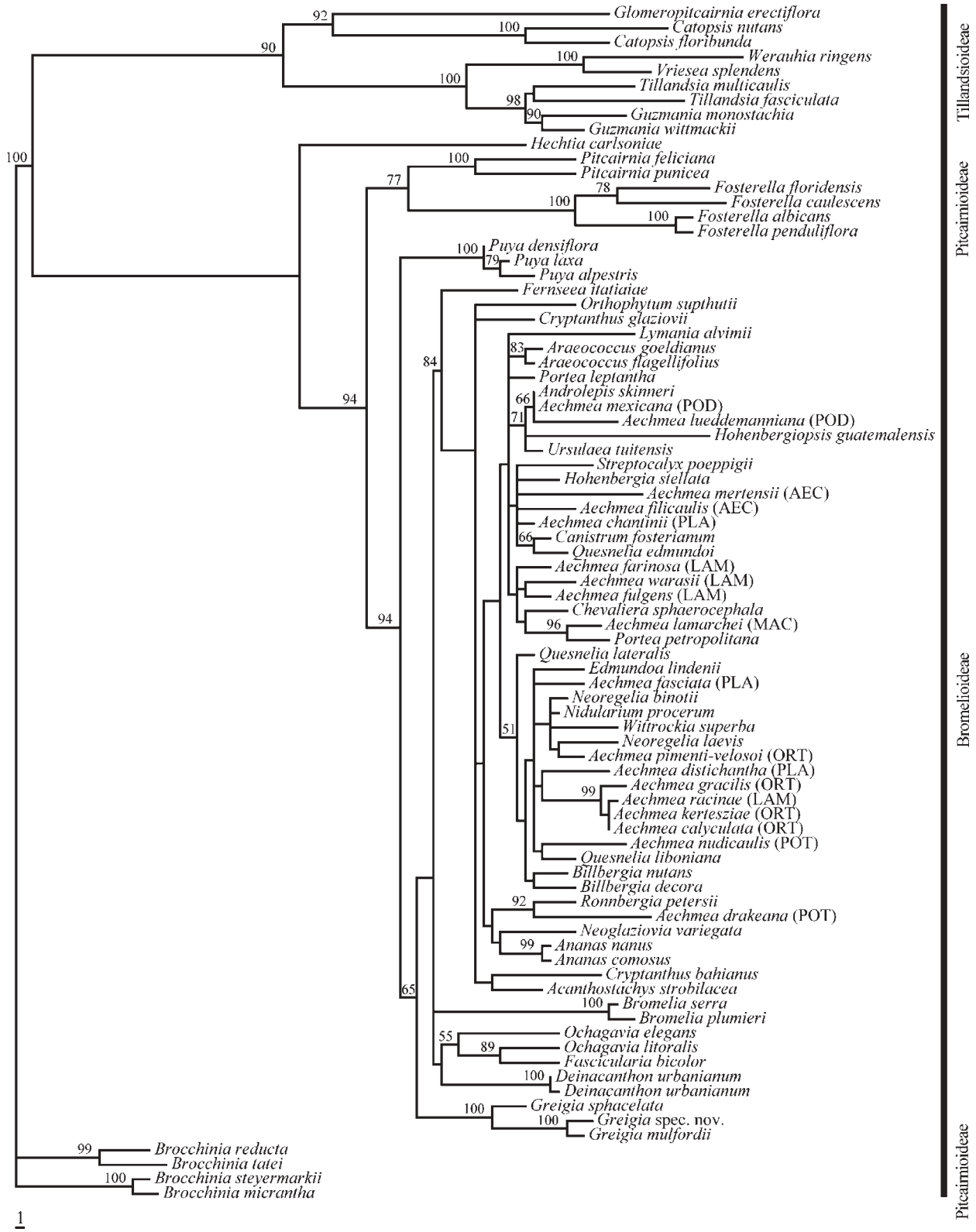

Fig. 1: One randomly chosen of the 2830 equally parsimonious phylograms of 860 steps length obtained from combined analysis of the chloroplast regions *mat*K and its flanking 3´ *trn*K intron, *trn*L intron and *trn*L-*trn*F spacer. Bootstrap values above 50 are indicated above the branches. CI (Consistency Index, excluding uninformative characters) = 0.720, RI (Retention Index) = 0.818 and RCI (Rescaled Consistency Index) = 0.590. Subgenera of *Aechmea* are given in brackets. Abbreviations: AEC = *Aechmea*; POD = *Podaechmea*; PLA = *Platyaechmea*, LAM = *Lamprococcus*, ORT = *Ortgiesia*, POT = *Pothuav*a, MAC = *Macrochordion*.

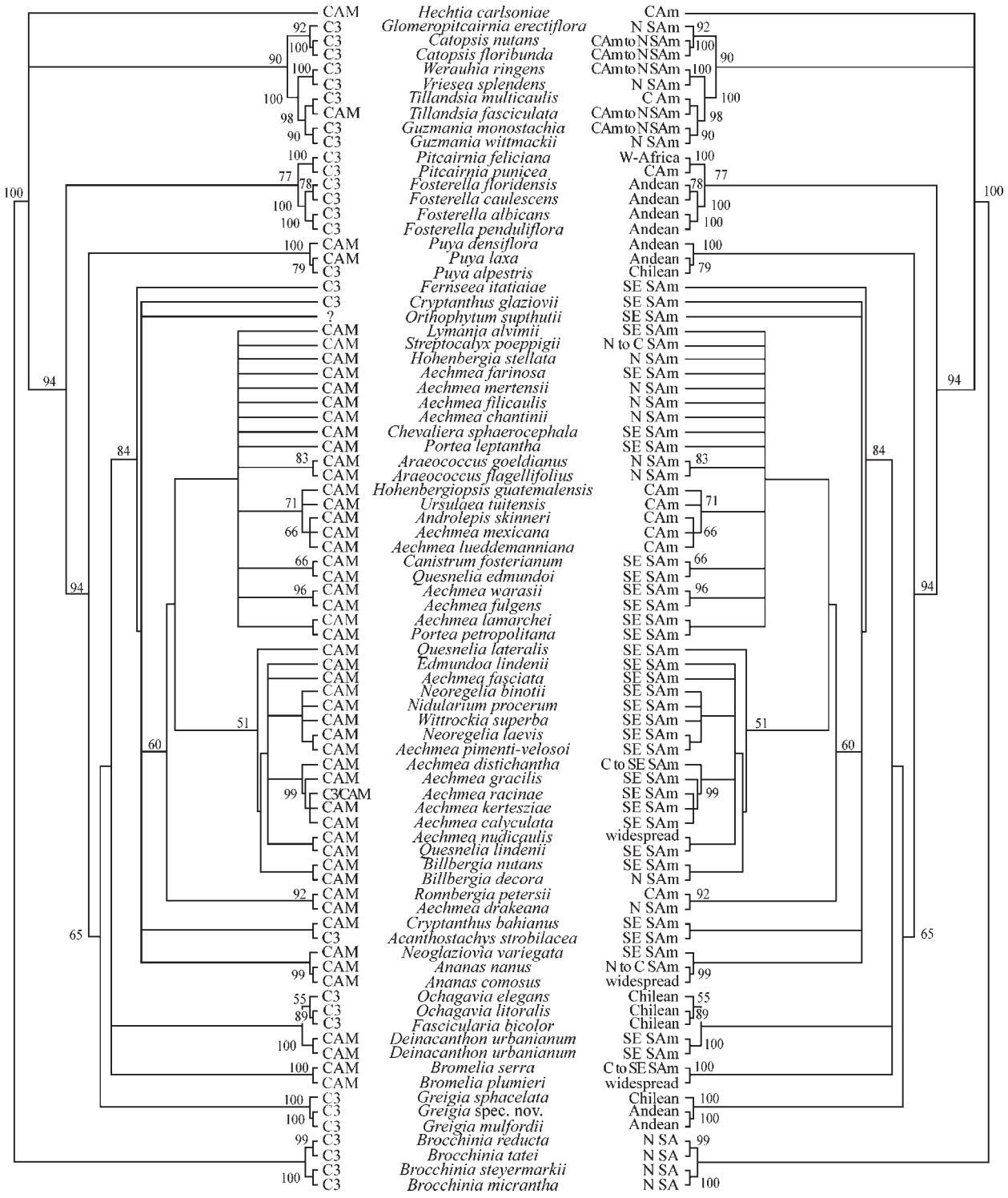

Fig. 2: 50 % Majority Rule Consensus cladogram of 2830 equally parsimonious trees of 860 steps length. Bootstrap values are indicated above the branches. Mapped on the trees are photosynthetic pathway (tree left) and distribution types of the species (tree right). Photosynthetic pathway information is based on Crayn et al. (2004) and distribution on Smith & Downs (1979) and the Missouri Botanical Garden's VAST database (http: //www.mobot.mobot.org/W3 T/Search/vast.html), distribution types are illustrated in Fig. 3. — Abbreviations: CAM = Crassulacean acid metabolism; C3 = C3 photosynthesis; CAm = Central America, N SAm = northern South America; SE SAm = southeastern South America.

Ortgiesia form a strongly supported "Ortgiesian clade" (bv 99) together with *Aechmea racinae* (subgenus *Lamprococcus*). *Neoregelia, Nidularium, Wittrockia* and *Aechmea pimenti-velosoi* (subgenus *Ortgiesia*) form a "Nidularioid clade" lacking further resolution and bootstrap support. *Hohenbergiopsis, Ursulaea, Androlepis, Aechmea mexicana* und *Aechmea lueddemanniana* (both members of the subgenus *Macrochordion*) form a moderately supported Central American clade (bv 71). Further groups within the core bromelioids that receive moderate to high bootstrap support are *Araeococcus* (bv 83), *Aechmea warasii* and *Aechmea fulgens* (2 of 4 investigated members of the subgenus *Lamprococcus*) with strong bootstrap support (bv 96), and a clade comprising *Ronnbergia petersii* and *Aechmea drakeana* (subgenus *Pothuava*) (bv 92).

Photosynthetic pathway

Within Bromeliaceae four different lineages that developed CAM photosynthesis were recognized by Crayn et al. (2004), three of which are included in the present study (*Hechtia*, Tillandsioideae, *Puya* and Bromelioideae; not included is the clade comprising *Dyckia, Encholirium* and *Deuterocohnia*). Noteworthy is that the most derived "core Bromelioideae" are characterised by exclusively CAM photosynthesis. The only exception is *Aechmea racinae*, having a carbon isotope value of –19.7 somewhat intermediate between CAM and C3. Among the remaining Bromelioideae, forming clades basal to the "core Bromelioideae", lineages with CAM or C3 photosynthesis or both are found: The *Greigia* clade displays exclusively C3. Another distinct clade is formed by *Bromelia* with CAM photosynthesis, which is placed at the first trichotomy within the Bromelioideae in the 50 % MR consensus tree. The other branch at this node comprises *Ochagavia, Fascicularia* (both C3) and *Deinacanthon* (CAM), but the node that combines the members of the two different photosynthetic pathways receives no bootstrap support. The third and largest clade of this trichotomy separates into a basal clade formed by *Fernseea itatiaiae* (C3) sister to 5 clades forming a polytomy: (a) *Cryptanthus* (C3), (b) *Orthophytum supthutii* (metabolism unknown), (c) "core Bromelioideae (CAM), (d) *Cryptanthus bahianus* (CAM) and *Acanthostachys* (C3), and (e) *Neoglaziovia* and *Ananas* (both CAM) (Fig. 2).

Biogeography

The combination of molecular phylogeny with todays distribution of the species and genera reveals some interesting patterns. The biogeographic restriction of *Brocchinia* to the ancient Guayana Shield and its position as sister to all other bromeliads has been recognized before. The early divergence and isolated position of *Hechtia* has been also revealed by several molecular analyses. According to the molecular data at hand, *Puya* with Andean distribution is sister to Bromelioideae. Early divergent *Greigia* also has a clearly Andean distribution. Another distinct lineage comprises *Deinacanthon, Ochagavia* and *Fascicularia*, the latter two genera being distributed in coastal and forest habitats of Chile. *Deinacanthon* occurs in dry habitats in southeastern South America. *Bromelia*, another genus of terrestrials, has a much wider distribution within South America and Central America and thus does not provide information about possible migration routes. The remaining bromelioids are found predominantly in southeastern South America, many genera being endemic to that region and demonstrating close relationships.

Interesting is the fact that within the core bromelioids a very weakly supported subclade (bv 51) comprises nearly exclusively species with southeastern South American distribution, whereas the other subclade also includes species with northern South American and Central American distribution. Within this subclade the moderately supported clade of Central American species is found including the genera *Hohenbergiopsis*, *Ursulaea*, *Androlepis* and two species of *Aechmea* subgenus *Podaechmea.*

Discussion

One principal problem for the reconstruction of the phylogeny of Bromelioideae could not be solved until recently: the identification of the sister group of the subfamily. Here *Puya* is identified as sister to Bromelioideae, a topology congruent with the findings of Terry et al. (1997 a), Horres et al. (2000), Crayn et al. (2004), and Givnish et al. (2004). Although in all cases bootstrap support is low to moderate (<90), it now appears justified to base further evolutionary interpretations on the sister relation of the baccate fruited bromeliads with this genus of clearly Andean distribution. Several authors have stressed the morphological and biogeographic peculiarities of *Puya* before and postulated a basal or isolated position within the family (e.g. Smith 1934, Winkler 1980, 1986). Nevertheless, only on the base of molecular data the surprisingly close relationship with Bromelioideae could be disclosed. The distribution type and the occurrence of C3 as well as CAM photosynthesis

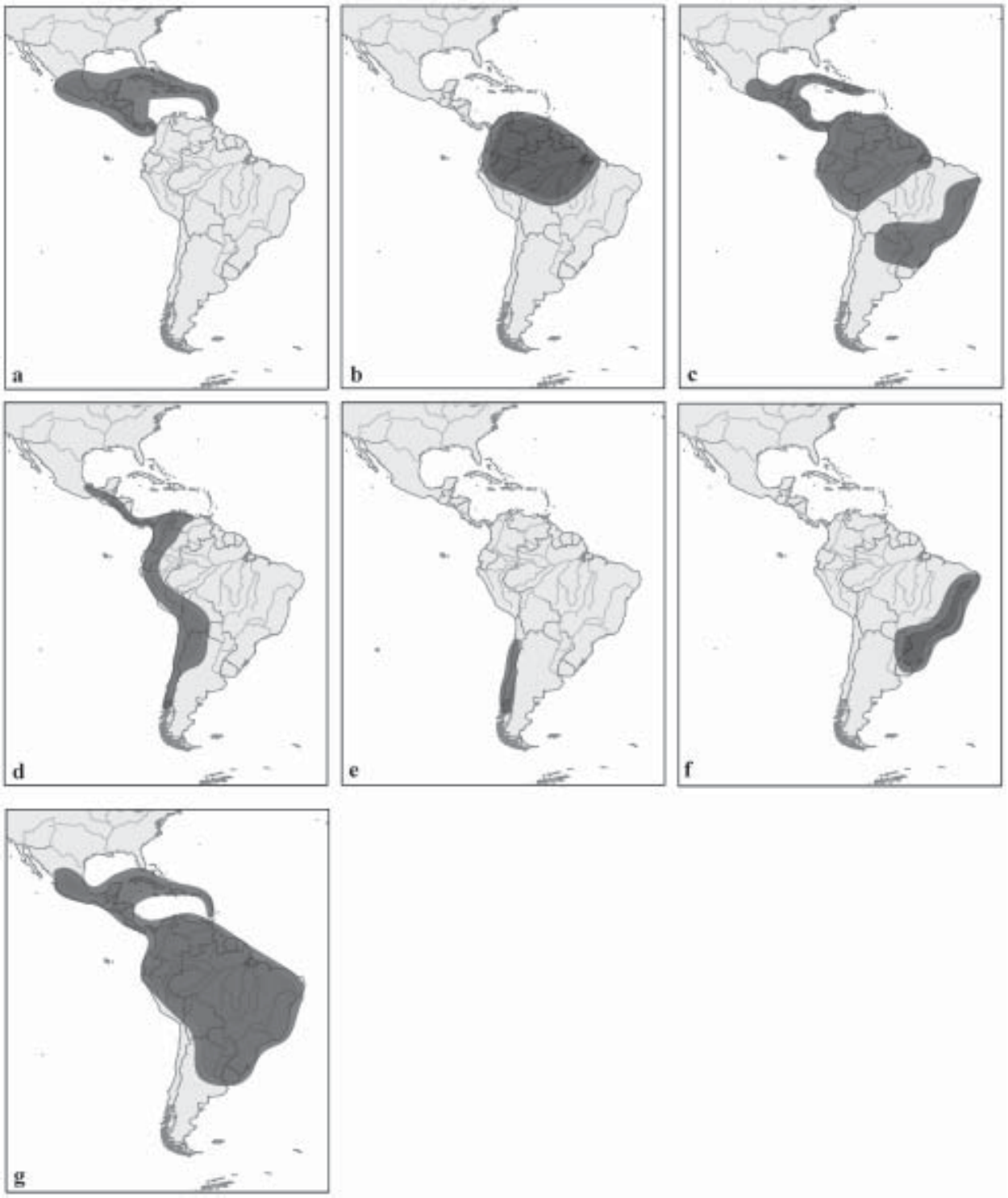

Fig. 3: Main distribution types of Bromelioideae and *Puya* based on distribution data provided by Smith & Downs (1979) and the Missouri Botanical Garden's VAST database (http: //www.mobot.mobot.org/W3 T/Search/vast.html). a) Central American and Caribbean. *Androlepis*, *Hohenbergiopsis*, *Ursulaea* (all 3 restricted to Central America); *Hohenbergia* subgen. *Wittmackiopsis* (Caribbean); *Aechmea* subgen. *Podaechmea* and *Ronnbergia* (reaching into northern South America). b) Northern South American. *Disteganthus*, *Neoregelia* subgen. *Hylaeaicum; Araeococcus* subgen. *Araeococcus* (extending to Costa Rica). c) North/Southeast disjuction: *Aechmea* subgen. *Lamprococcus*, *Ae.* subgen. *Pothuava, Araeococcus* subgen. *Pseudaraeococcus*, *Billbergia* subgen. *Billbergia*, *Chevaliera*, *Hohenbergia* subgen. *Hohenbergia*, *Pseudananas,* and *Streptocalyx.* d) Andean. *Greigia*, *Puya.* e) Chilean. *Fascicularia*, *Ochagavia.* f) Southeastern South American. *Acanthostachys*, *Aechmea* subgen. *Ortgiesia*, *Canistrum*, *Cryptanthus*, *Edmundoa*, *Fernseea*, *Lymania*, *Neoglaziovia*, *Nidularium*, *Orthophytum*, *Portea*, *Quesnelia*, and *Wittrockia.* f) Widespread. *Aechmea* subgen. *Aechmea*, *Ae*. subgen. *Macrochordion* and *Ae*. subgen. *Platyaechmea*, *Bromelia*, *Billbergia* subgen. *Helicodea,* and *Ananas.*

(found in 24 % of species sampled by CRAYN et al. 2004) in *Puya* provide important information for the reconstruction of the evolution of Bromelioideae.

Looking at the Bromelioideae clade, the addition of *mat*K sequence data improved resolution compared to the analysis of *trn*L intron and *trn*L-*trn*F spacer alone (HORRES et al. 2005). Nevertheless, polytomies and weak statistical support could not be eliminated, especially for the core group of Bromelioideae comprising most of the genera and species. Among Bromelioideae, principal branches of the clade could be identified and include (a) *Greigia*, putative sister to the remainder of the subfamily, (b) *Ochagavia, Fascicularia*, and *Deinacanthon* and (c) *Bromelia*, the latter two forming a polytomy together with the remaining Bromelioideae (Figs. 1, 2). Although not resolved in our study, a basal position of *Bromelia* in Bromelioideae was found by TERRY et al. (1997 a), CRAYN et al. (2004) and GIVNISH et al. (2004), who did not include *Greigia*, *Ochagavia, Fascicularia* and *Deinacanthon*.

Looking at the photosynthetic pathway, *Greigia* resembles *Puya* by its clearly Andean distribution and is characterised by the occurrence in more humid, often shady habitats and C3-photosynthesis. Among the comparatively few bromelioid genera with C3 photosynthesis, we find *Ochagavia* and *Fascicularia* forming another one of the principal clades, together with *Deinacanthon urbanianum*, a CAM species. The most derived "core Bromelioideae" being almost exclusively CAM and the concentration of C3 genera in other, putatively basal lineages of Bromelioideae (Fig. 2) indicate to our opionion, that C3 photosynthesis is plesiomorphic in the subfamily. We suppose this for *Puya* too, suggesting that CAM in this genus might have developed secondarily in the course of colonization of (semi-)arid habitats or climatic changes. This view contradicts the opinion of SMITH (1989), who interpreted CAM as plesiomorphic in Bromelioideae, and CRAYN et al. (2004), the latter based on a phylogenetic reconstruction that did not include *Greigia, Ochagavia* and *Fascicularia* but only representatives of CAM species of Bromelioideae.

Regarding habitats and ecology, we find terrestrial species without water-impounding phytotelmata by far dominating among the basal Bromelioideae clades (and the subfamily´s sister group). The recent representatives of these clades occur in more humid high elevation or temperate habitats (*Greigia, Fascicularia, Ochagavia* p.p.) or under semiarid, subtropical conditions (*Deinacanthon, Bromelia, Ochagavia* p.p.). A closer look at the Chilean bromeliads illustrates, that obviously closely related taxa are capable of colonizing both types of habitats. In the monospecific *Fascicularia*, we find *F. bicolor* ssp. *canaliculata* as an element of the Valdivian forest, often under shady conditions and sometimes growing epiphytic. *F. bicolor* ssp. *bicolor*, with conspicuously more succulent leaves grows terrestrial, predominantly in more open, often coastal habitats (ZIZKA et al. 1999). In *Ochagavia*, we find *O. litoralis* in fully exposed habitats, covering coastal cliffs near Valparaiso in Central Chile, while *O. carnea* is growing in shady, humid forest habitats at the bottom of ravines (ZIZKA et al. 2002, ZIZKA & NOVOA 2004). It might be added, that in Chile also *Puya* species managed to successfully colonize semiarid and even arid areas (e.g. *P. chilensis, P. alpestris, P. boliviensis*). *Deinacanthon* and *Bromelia*, both displaying CAM-metabolism, are widespread or occur in semiarid habitats in southeastern South America. The position of *Fernseea itatiaiae* basal to the largest Bromelioideae clade can help to interpret the migration of early bromelioids: The species is endemic to cool, humid high altitude habitats in southeastern Brazil and also characterised by C3 metabolism. These habitats might have been part of a "corridor" during past periods allowing migration from the Andes to southeastern Brazil. Such a corridor is postulated to explain the clear Andean affinities of a considerable amount of the South Brazilian flora (SAFFORD 1999).

The astonishingly low sequence variation in the two groups of the "core Bromelioideae" indicates a rapid radiation. Looking at life forms and habitats, we find epiphytes by far dominating among the most derived "core Bromelioideae" and almost exclusively terrestrials among the putatively basal clades. Radiation in the "core Bromelioideae" thus might have been fostered by the successful invasion into epiphytic habitats.

To present the information about distribution at hand, we recognized 7 principal types of distribution areas found within Bromelioideae (Fig. 3). It is obvious, that the subfamily is centered in southeastern South America. Aberrant distribution types can be found in *Puya* and some of the basal Bromelioideae clades (Andean, Chilean). Although in our analysis the "core Bromelioideae" are not sufficiently resolved to disclose details, the separation of the core Bromelioideae into two groups displaying different distribution patterns is noteworthy: In group I we find representatives of the genera *Aechmea, Araeococcus, Chevaliera, Lymania, Streptocalyx, Hohenbergia, Hohenbergiopsis, Ursulaea, Portea, Canistrum*, and *Quesnelia*, representing a mixture of distribution types a, b, c, and f (Fig. 2). Group II includes *Aechmea, Billbergia, Edmundoa, Quesnelia, Neoregelia, Nidularium*, and *Wittrockia*, but the members of this group almost exclusively represent distribution type f and are principally elements of the Atlantic Forest.

Among the clades of group I, one lineage comprises all species restricted to Central America, suggesting a radiation of the group in this area. In group II, representatives of *Aechmea* subgenus *Ortgiesia* form a extraordinarily well supported lineage. Other included species of *Aechmea* are scattered over the "core Bromelioideae", illustrating the well known taxonomic difficulties in this genus and its allies.

To sum up, we forward the following hypothesis on Bromelioideae evolution: after Bromeliaceae originated

in the Guayana highlands, the ancestor of the *Puya*/Bromelioideae clade spread counterclockwise to the northern Andes and from there southward along the Andean cordillera. These plants were terrestrials, adapted to ± humid conditions and C3. An early separating, Andean centered line led to the genus *Greigia*. In the southern Andes, lines of terrestrial plants — primarily adapted to more humid and shady habitats — separated and were also characterized by C3 photosynthesis and lack of water-impounding phytotelmata. Obviously, these lineages were also capable of colonizing semiarid and arid, open habitats, giving rise to the endemic Chilean genera *Fascicularia* and *Ochagavia* and also to *Deinacanthon* and *Bromelia*. From the southern Andes, Bromelioideae reached southeastern and eastern Brazil, where radiation led to the recent center of diversity of the subfamily especially in the Atlantic Forest. Possibly, the "campos de altitude", temperate, humid high elevation habitats (SAFFORD 1999) played an important role in this migration, where *Fernseea itatiaiae* can be found as a local endemic today. At present we have no hypothesis, how Bromelioideae colonized Central America, the Caribbean and northern South America and in which way and by what time distribution areas extending over almost the whole of tropical America (e.g. *Aechmea nudicaulis, Ae. bromeliifolia*) developed.

Phylogenetic reconstruction based on more sequence data is the most promising approach to obtain better resolution and more statistical support to answer these questions. Nevertheless, for genetically obviously very similar groups within the "core Bromelioideae" highly informative fingerprinting techniques (e.g. AFLPs) might prove to be most appropriate.

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