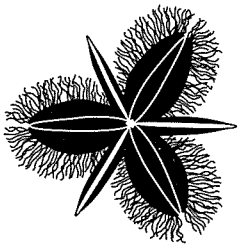

MOLECULAR SYSTEMATIC INVESTIGATIONS IN PITCAIRNIOIDEAE (BROMELIACEAE) AS A BASIS FOR UNDERSTANDING THE EVOLUTION OF CRASSULACEAN ACID METABOLISM (CAM)

Darren M. Crayn[A,D], *Randall G. Terry*[B], *J. Andrew C. Smith*[C] *and Klaus Winter*[A]

[A] Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama
[B] Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA
[C] Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK
[D] Corresponding author; email: craynd@tivoli.si.edu

Abstract

The evaluation of hypotheses regarding the evolution of crassulacean acid metabolism (CAM) in plants has been hampered by the lack of detailed phylogenetic information on families which contain CAM plants. We set out to provide this information for Bromeliaceae subfamily Pitcairnioideae, which has been well studied from an ecophysiological perspective, by subjecting nucleotide sequence data from the plastid *mat*K gene to parsimony analysis. The data show low variation among ingroup taxa and the resulting optimal trees are characterised by poor ingroup resolution and low character support. Nevertheless, there is strong support for Bromeliaceae, *Brocchinia*, and a *Dyckia* + *Encholirium* clade. *Deuterocohnia* is polyphyletic: *D. meziana* O. Kuntze ex Mez is grouped robustly with *Dyckia* and *Encholirium* species, rather than with three other *Deuterocohnia* species. We conclude that *mat*K data alone are unlikely to provide convincing resolution of relationships among CAM and C_3 taxa in Bromeliaceae, and greater taxon sampling and the use of more rapidly evolving regions will be required to shed light on the evolution of CAM in this family.

Key words: Bromeliaceae, Pitcairnioideae, crassulacean acid metabolism, evolution, molecular systematics, monocots

INTRODUCTION

Bromeliaceae are among the most conspicuous and characteristic elements of Neotropical forests, but they are by no means restricted to these environments. In fact, they are found in an extraordinary array of habitats ranging from sand dunes of the coastal deserts of Peru to tropical montane cloud forests and extremely wet, infertile savannas (Benzing 1980; Smith 1989; Givnish *et al.* 1997). Not surprisingly, members of the family exhibit remarkable ecological and physiological variability and display a concomitant diversity of life forms, ranging from terrestrial to tank-impounding and atmospheric epiphytic types (e.g. *Tillandsia usneoides* L.). Strong selection for the procurement of water and nutrients and for adaptation to extreme environments have been important in the evolutionary history of the family, and several key innovations appear tied to explosive speciation in the group (Pittendrigh 1948; Tomlinson 1969; Medina 1974; Benzing *et al.* 1985; Smith 1989; Givnish *et al.* 1997). These include the acquisition of a water-impounding capacity, the origin of absorbing trichomes, and the evolution of crassulacean acid metabolism (CAM).

CAM is one of three types of photosynthetic pathway in vascular plants and is characterised by uptake of atmospheric CO_2 at night (Winter and Smith 1996). In this pathway, phospho-*enol*pyruvate (PEP) is carboxylated by PEP carboxylase, leading to the formation of malic acid, which is stored in the vacuole at night. The malic acid is released during the following light period and decarboxylated to liberate CO_2, which is reduced in the Calvin cycle.

Because CAM plants exchange CO_2 mainly at night, their transpirational water loss per unit CO_2 fixed is typically much lower than in C_3 plants. Consequently, CAM plants often occur in environments in which water availability is low or intermittent. Many plants of arid regions, such as Cactaceae, exhibit CAM, but this photosynthetic pathway is also widespread among tropical epiphytes, such as Bromeliaceae and Orchidaceae, which may experience severe water stress periodically (Winter and Smith 1996).

It is generally accepted that CAM is polyphyletic in land plants (e.g. Teeri 1982), and it has been suggested to have arisen several times in Bromeliaceae (Smith 1989). But while the family represents an ideal system for the study of major evolutionary innovations such as CAM, and their association with adaptive radiation, phylogenetic relationships within the group are not well understood, and thus proposed evolutionary scenarios are not well founded. Apparent extensive convergence in morphological and ecological features has hampered phylogenetic reconstruction, and trees derived from molecular data have been characterised by a lack of resolution and poor character support (Ranker *et al.* 1990; Terry *et al.* 1997a, b).

Bromeliaceae is a large (c. 2800 species, Luther and Sieff 1998), Neotropical family which has been well studied taxonomically. The monophyly of the family is supported by a number of features including unique epidermal trichomes (Tomlinson 1969) and conduplicate spiral stigmas (Brown and Gilmartin 1984, 1989), and is evidenced by the recognition of the group as a monotypic order in most taxonomic treatments (Huber 1977; Cronquist 1981; Dahlgren and Rasmussen 1983; Dahlgren *et al.* 1985). Studies of monocot phylogeny using molecular data are concordant with morphology in suggesting the family is a natural group (Chase *et al.* 1993; Duvall *et al.* 1993; Chase *et al.* 1995a), although the objective of these studies was to examine interfamilial relationships and thus sample sizes within families were small.

In contrast to the apparent cohesiveness of the family, the placement of Bromeliaceae within the broader phylogenetic framework of the Monocotyledonae has been equivocal, with alternative perspectives being drawn largely along morphological/molecular lines. Cladistic analyses of traditional (i.e. mostly morphological, some cytological and palynological) data support a close relationship with Velloziaceae (Gilmartin and Brown 1987; Chase *et al.* 1995b), although the earlier paper was challenged on methodological grounds (Simpson 1988). Other morphologically based cladistic studies suggest a sister-group relationship between Bromeliaceae and a clade containing Haemodoraceae, Philydraceae, Alismatidae, and Commelinidae *sensu* Dahlgren *et al.* (1985) (Stevenson and Loconte 1995). In contrast, phylogenetic analyses of *rbc*L sequences support a sister-group relationship with Rapateaceae (Chase *et al.* 1993; Duvall *et al.* 1993; Chase *et al.* 1995a), a link first proposed by Lyman Smith (1934) on phytogeographical grounds. A Bromeliaceae–Rapateaceae sister-group relationship has been corroborated by combined morphological and molecular data sets that generally have provided the greatest resolution of relationships among families of monocots (Chase *et al.* 1995b; Linder and Kellogg 1995). On balance, these results favour Bromeliaceae and Rapateaceae as sister groups, although clearly additional study of relationships between Bromeliaceae and related monocot families is needed.

Traditionally, three subfamilies are recognised within Bromeliaceae, namely Pitcairnioideae, Tillandsioideae, and Bromelioideae, each of which is more or less well circumscribed, but relationships among which have been the source of much speculation and contention (Schimper 1888; Mez 1904; Tietze 1906; Pittendrigh 1948; Tomlinson 1969; Benzing *et al.* 1985; Smith 1989). Ecologically based arguments maintain that Pitcairnioideae are basal in the family, a perspective supported by growth habit (i.e. most pitcairnioids are obligate terrestrials) and a concomitant lack of vegetative and ecophysiological specialisation to the degree that is found in the mostly soil-independent Tillandsioideae and Bromelioideae. Cladistic analysis of morphological features supports Tillandsioideae and Bromelioideae as sister groups (Gilmartin and Brown 1987). However, restriction site and DNA sequence data from the chloroplast genome are concordant in placing Tillandsioideae near the base of the family and in suggesting a close relationship between Pitcairnioideae and Bromelioideae (Ranker *et al.* 1990; Terry *et al.* 1997a). Of those morphological features used to distinguish the subfamilies of Bromeliaceae (Smith and Downs 1974, 1977, 1979), most are autapomorphic for Bromelioideae and therefore do not suggest an obvious relationship with either of the other two subfamilies. More recent data from surveys of seed morphology and foliar scale anatomy have been cited in support of a Pitcairnioideae–Bromelioideae linkage (Varadarajan and Gilmartin 1987, 1988a), although many of the problems that have plagued the assessment of subfamily relationships using previous data are apparent in these data as well.

Comparative analysis of *ndh*F sequences supported the monophyly of both Tillandsioideae and Bromelioideae, but indicated that Pitcairnioideae *sensu* Smith and Downs (1974) is artificial, with *Brocchinia* being resolved as sister to the remainder of the family and *Puya* being sister to Bromelioideae (Terry *et al.* 1997a, Fig. 1). Based on these findings, it was suggested that *Brocchinia* be recognised as a monotypic subfamily (Terry *et al.* 1997a). Cladistic analysis of morphological features from 16 genera of Pitcairnioideae has led some authors to question the inclusion of *Brocchinia* in that subfamily (Varadarajan and Gilmartin 1988b). A subsequent revision recognised the genus as a monotypic tribe in Pitcairnioideae (Varadarajan and Gilmartin 1988c). *Brocchinia* differs from other Pitcairnioideae in a number of features including a partly to wholly inferior ovary, imbricate sepals, and an open, racemose inflorescence (Smith and Downs 1974; Smith 1986; Varadarajan and Gilmartin 1988c; see Givnish *et al.* 1997). In addition, seed morphology and ontogeny in *Brocchinia* are notably different from the remainder of Pitcairnioideae (Varadarajan and Gilmartin 1988a), and the mature tank trichomes of some *Brocchinia* are distinguished from those of other bromeliads in having living shield (cap) cells that possess an unusual labyrinthine wall organisation (Tomlinson 1969; Benzing *et al.* 1976; Owen *et al.* 1988; Owen and Thompson 1991).

Subfamily Pitcairnioideae (16 genera, c. 950 species, Luther and Sieff 1998) is delimited by taxa with spinose to serrate leaf

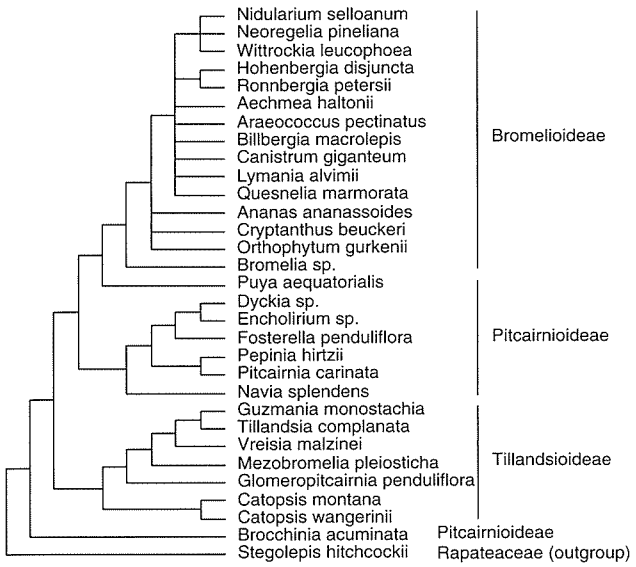

Fig. 1. Phylogenetic relationships among some bromeliad taxa as determined by parsimony analysis of *ndh*F sequences (Terry *et al.* 1997a). This tree is the strict consensus of 120 trees (length [L] = 406 steps, consistency index [CI] = 0.58, retention index [RI] = 0.78) found for the data using *Stegolepis hitchcockii* (Rapateaceae) as outgroup. Limits of suprageneric taxa are shown at right.

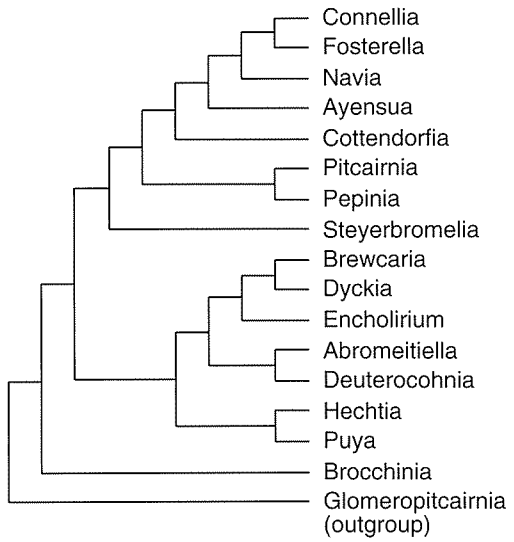

Fig. 2. Phylogenetic relationships of pitcairnioid genera based on parsimony analysis of morphological and anatomical data (Varadarajan and Gilmartin 1988b). This tree is one of two shortest trees (L = 149, CI = 0.41) found for the data using the tillandsioid genus *Glomeropitcairnia* as outgroup.

blades, entire, non-plumose and non-gelatinous outer ovular integuments, and foliar scales lacking concentric rings (Varadarajan and Gilmartin 1988c). Nearly all pitcairnioids are terrestrial plants that possess well-developed soil roots, lack absorptive foliar scales and have a limited capacity for impounding resources vegetatively. For these reasons, Pitcairnioideae are generally considered the most primitive of the three bromeliad subfamilies (Medina 1974; Benzing *et al.* 1985). Pitcairnioids typically can be found in exposed, elevated, moist and sometimes nutrient-poor habitats (McWilliams 1974; Medina 1974), although low-elevation, shade-tolerant, understorey species as well as xeromorphic taxa are also present in the group (Rauh 1981). Centres of species diversity include the Andes of Chile and Peru and the highlands of the Guayana Shield (eastern Colombia, southern Venezuela, western Guyana, and extreme northern Brazil).

Only two previous studies have addressed phylogenetic relationships in Pitcairnioideae using cladistic methods. Varadarajan and Gilmartin (1988b) examined variation in floral and vegetative features and identified three principal groups of genera (Fig. 2), although subfamily monophyly was not tested (a single outgroup, the tillandsioid genus *Glomeropitcairnia*, was used). *Brocchinia* was resolved at the base of the ingroup and was concluded to be a distinct, perhaps ancient lineage. A second clade containing four genera endemic to the Guayana Shield (*Connellia*, *Navia*, *Ayensua*, and *Steyerbromelia*) plus four additional genera (*Fosterella*, *Cottendorfia*, *Pitcairnia*, and *Pepinia*) was resolved. Synapomorphies for this clade included deciduous leaves (at least in part) and leaf anatomy with water-storage tissue restricted to the leaf periphery, although both of these characters were homoplasious (Varadarajan and Gilmartin 1988b). A third clade containing *Brewcaria*, *Dyckia*, *Encholirium*, *Abromeitiella*, *Deuterocohnia* (which now includes *Abromeitiella*, Spencer and Smith 1992), *Hechtia* and *Puya*, was resolved as the sister group to the second clade. Character states supporting this group were completely consistent and included leaf margins entire or minutely serrate, foliar scale arrangement irregular (as opposed to parallel rows), foliar scale arrangement with margins in periclinal tiers, and epidermis rough or irregular (Varadarajan and Gilmartin 1988b).

Terry *et al.* (1997a) used cladistic analysis of *ndh*F sequences to examine relationships among bromeliad subfamilies; however, only eight pitcairnioid genera were included in the analysis and thus conclusions regarding relationships within that subfamily are tentative. A weakly supported clade containing six genera (*Navia*, *Pitcairnia*, *Pepinia*, *Fosterella*, *Encholirium*, and *Dyckia*) was identified (Pitcairnioideae *sensu stricto*; Fig. 1), although few relationships within this group were well supported by *ndh*F sequences. One exception was strong support for a *Dyckia–Encholirium* clade, a finding that is largely consistent with the results of Varadarajan and Gilmartin (1988a, b). Not surprisingly, both studies (Varadarajan and Gilmartin 1988b; Terry *et al.* 1997a) resolved *Pitcairnia* as the sister group to its segregate genus *Pepinia* (see Varadarajan and Gilmartin 1988c). Sampling differences notwithstanding, the two studies conflict in the placements of both *Fosterella* and *Navia* and support for a polyphyletic Pitcairnioideae in the *ndh*F study (Figs 1 and 2). Neither study sampled within genera, so the monophyly of genera remains untested.

In this study, we examine phylogenetic relationships among 11 of the 16 traditionally recognised genera of Pitcairnioideae using DNA sequences from the *mat*K locus of the chloroplast genome. Our objectives are threefold: (1) to determine if the subfamily as traditionally circumscribed is monophyletic; (2) to assess the monophyly of individual genera; and (3) to examine relationships among genera and compare those to existing perspectives.

Results presented here are an initial attempt to produce a clearer understanding of relationships within Pitcairnioideae and, when combined with other data, will provide a clearer phylogenetic foundation for the understanding of CAM evolution and other important evolutionary innovations in Bromeliaceae.

Materials and Methods

Data Source

Previous work has demonstrated a low substitution rate among pitcairnioids in some plastid regions (*ndh*F, Terry *et al.* 1997a; *trn*L intron, R. G. Terry unpubl.). Therefore for this study we employed the *mat*K gene, which is among the most rapidly evolving plastid coding regions (Olmstead and Palmer 1994). Encoding a putative maturase (Liere and Link 1995), *mat*K comprises approximately 1520 base pairs (bp) within an intron in the *trn*K gene. We sequenced 850 nucleotides from the more rapidly evolving 5' end (Hilu and Liang 1997).

Taxa

A total of 36 species of Pitcairnioideae from 11 genera were sampled based on tissue availability and capacity to represent the diversity of the subfamily. One member (*Epidryos allenii*) of Rapateaceae, the putative sister group to Bromeliaceae, was also sampled. Three representatives of Poaceae, *Hordeum vulgare*, *Oryza sativa* and *Zea mays*, were used as the outgroup based on previous molecular and combined analyses (e.g. Chase *et al.* 1993, 1995b), which resolve Poaceae among the nearest relatives of Bromeliaceae. Furthermore, representatives of three tillandsioid genera (*Guzmania*, *Tillandsia* and *Vriesea*) and one bromelioid (*Ananas*) were included. The accession details of all these taxa are provided in the Appendix.

DNA Extraction

Total DNA was extracted from fresh or silica-gel-dried leaves using a 6× CTAB extraction protocol (modified from Hillis *et al.* 1996). Approximately 50 mg tissue (dry mass) was pre-chilled with liquid nitrogen in a mortar for up to 10 min (Givnish *et al.* 1997) and then ground with a pestle to a fine powder. The frozen powder was transferred to a pre-warmed 1.5 ml Eppendorf tube containing 500 µl 6× CTAB extraction buffer and incubated (with occasional agitation) at 65° C for 45 min. Proteins were removed with an equal volume of 24:1 chloroform:isoamyl alcohol and DNA was precipitated by the addition of an equal volume of cold 95% ethanol with overnight incubation at -20° C. The DNA was pelleted by centrifuging at 7000 *g* for 5 min, washed twice with 70% ethanol and then dried, redissolved in distilled water, and the yield assessed by agarose gel electrophoresis.

Polymerase Chain Reaction

A fragment containing the first 855 positions of the *mat*K intron was amplified for each taxon using an internal primer (*mat*K2) and one of two upstream primers (909 and *mat*K5, Table 1) in a DNA Thermal Cycler (Perkin Elmer). Reaction mixtures (100 µl containing 200 µM each dNTP, 2.5 mM $MgCl_2$, 50 pmol of each primer, 1.25 units of *Taq* DNA polymerase (Gibco BRL), 10 µl 10× PCR buffer (Gibco BRL), approximately 1 µg template DNA) were prepared in 0.5 ml tubes, incubated at 95° C for 15 min to completely denature the template and subjected to 25 cycles of the following temperature profile: 30 s at 95° C, 1 min at 42° C, 2 min at 72° C. PCR product quality and yield were assessed by agarose gel electrophoresis and the DNA purified and concentrated using QIAquick™ PCR purification columns (QIAGEN) following the manufacturer's protocol. The purified sample was quantified by agarose gel electrophoresis with reference to a size and mass standard (Low DNA Mass Ladder, Gibco BRL).

Sequencing

Primers used for sequencing are listed in Table 1. Sequence fragments were generated by cyclic amplification using AmpliTaq® FS (PE Applied Biosystems) DNA polymerase (diluted 1:1 with *half*TERM™, Genpak Ltd.) and incorporating fluorescent dye-labelled chain terminators (dRhodamine, PE Applied Biosystems) in a DNA Thermal Cycler (Perkin Elmer) set for 25 cycles of the following temperature profile: 15 s at 96° C, 1 s at 50° C, 4 min at 60° C. The sequences were determined using the ABI Prism™ 377 system (PE Applied Biosystems), and then checked, aligned, edited and a consensus for each taxon generated using AutoAssembler™ (Perkin Elmer).

Alignment

Since there appeared to be only minor length variation in the *mat*K sequences among the sampled taxa, the consensus sequences were exported to a PAUP* (version 4.0d64, Swofford 1998) database and aligned by eye. Five alignment gaps were required: the first two occurred only in Poaceae and included a 9 base pair (bp) deletion and a 3 bp insertion (relative to the ingroup) starting at positions 119 and 153, respectively; the other three varied within the ingroup and included an 18 bp insertion in *Puya laxa* beginning at position 244, which completely overlapped a 12 bp insertion in *Puya aequatorialis*, *P. humilis* and *P. wedermannii*, and a 3 bp insertion in *Pitcairnia orchidifolia* beginning at position 763. The 12 bp insertion was scored as a binary presence/absence character appended to the sequence matrix. The other four indels were not scored since they were either autapomorphic for terminal taxa in this matrix and hence not cladistically informative, or they were invariant within the ingroup. The complete alignment is available on request from the first author.

Parsimony Analyses

All phylogenetic analyses were performed using PAUP* (version 4.0d64, Swofford 1998). Optimal trees were estimated for the data using Fitch parsimony (all characters given unit weight) by heuristic search invoking tree-bisection-reconnection (TBR) branch swapping and set to retain all most-parsimonious trees (MULPARS on). Gaps were treated as missing data. To search for multiple islands of most-parsimonious trees (Maddison 1991), 100 replicate searches with random taxon entry order were initially programmed. However, from the first replicate it was clear there exists a large population of trees at or near the optimum (58,300 trees of 466 steps were found before computer memory was exhausted; over 44,000 of these had been swapped to completion). Therefore, we modified our search strategy. A strict consensus tree constructed from the initial 58,300 trees was

Table 1. Sequences and approximate annealing positions of primers used in PCR and sequencing reactions. All primers were developed as part of this study except for 909, which was developed by Gadek *et al.* (1996). r = g or a, y = c or t

Primer	Sequence 5'-3'	Approx. Annealing Position (3' base)
matK2 (R)	aacataatgcatgaaaggatcc	855
matK3 (F)	caaaatcgattcgttgrgcay	250
matK4 (R)	gagaatggaatttccacaatgaccg	340
matK5 (F)	ataccctgttctgaccatattg	62 bp upstream of *mat*K start codon
matK6 (R)	ggatataggaagtcttgttgccgag	38
909 (F)	ggggttgctaactcaacgg	3' end of upstream *trn*K exon

used to search for alternative relationships also supported at this level of optimality. Thus, 1000 random taxon entry order replicates were performed invoking the consensus tree as a reverse constraint, saving only one tree of length 467 steps per replicate. These trees were then TBR-swapped to completion (again invoking the reverse constraint), saving all the shortest trees.

Character support for the trees was assessed by parsimony jackknife (Farris *et al.* 1996) and Bremer support (Bremer 1988) analyses. Comparative analyses have shown that for large or 'dirty' matrices parsimony jackknifing is able to identify poorly supported groups better than bootstrap analyses employing extensive branch-swapping, since effort is not wasted in a search for all the shortest solutions for each data sample (Farris *et al.* 1996). We attempted bootstrap analyses on these data, but the large numbers of trees recovered rendered effective bootstrapping practically unfeasible. Jackknife values were determined using PAUP* (version 4.0.0d64, Swofford 1998) set for 100,000 replicates emulating 'JAC' resampling (37% of characters omitted per replicate) and employing 'fast' stepwise taxon addition (i.e. no branch swapping).

Bremer support values were determined using replicate reverse-constraint parsimony analyses performed in PAUP. The procedure was automated using Auto Decay ver. 2.7 (Eriksson 1995), which constructs constraint trees for each node on one of the most parsimonious global trees and writes a PAUP batch file to analyse them sequentially. The shortest tree(s) in which each node was not present was then found by heuristic search set for identical options as the primary searches. The difference in tree-length between the global minimum and the constrained minimum is the Bremer support value for that node.

One tree was chosen randomly from the set of maximally parsimonious trees and the branch lengths were calculated using accelerated transformation (ACCTRAN) optimisation. The trees from all analyses were rooted using the three grasses *Hordeum*, *Oryza* and *Zea*.

RESULTS AND DISCUSSION

Parsimony Analysis

A total of 851 characters were analysed, of which 326 (38%) were variable and 179 (21%) cladistically (parsimony) informative. Within the Bromeliaceae, 171 (20%) positions were variable and 72 (8%) cladistically informative.

Figure 3 (the tree on the right) is the strict consensus tree of the 58,300 trees (rescaled consistency index = 0.665, retention index = 0.831) found in the first phase of analysis. No shorter trees, or trees of the same length but containing relationships incompatible with the consensus tree, were found in the subsequent replicate constraint analyses. Therefore we regard Fig. 3 as an accurate summary of relationships present in the shortest trees for these data. As might be expected of a consensus of such a large population of trees, there is relatively poor resolution of relationships within the ingroup. Furthermore, Bremer support and jackknife values indicate that many of the nodes retrieved by the analysis are supported by few characters.

The branch lengths optimised for one of the most-parsimonious trees (Fig. 3, left tree) indicate relatively low divergence among the ingroup taxa: only three branches are of length ten steps or longer, one of which is a terminal branch. Indeed, the maximum pairwise divergence among Bromeliaceae was 5.45% between *Fosterella penduliflora* and *Guzmania monostachia*. Among the three grass taxa pairwise divergence exceeded 8.40% and was as high as 9.01%. A low divergence value among bromeliads has been demonstrated for other chloroplast loci (e.g. *ndh*F, maximum pairwise divergence of 2.5% among Bromeliaceae, among the lowest values demonstrated for any group of flowering plants; Terry *et al.* 1997a) and seems to be characteristic of the lineage rather than being gene-specific. Low relative divergence in Bromeliaceae may be attributable to two general phenomena: low relative substitution rate and/or a recent diversification of the family. The former may have a host of explanations including reduced mutation rate, but in the absence of experimental data such causes may be impossible to identify.

A relatively recent origin for the Bromeliaceae has been previously suggested based primarily on its New World distribution (Smith 1934). However, the age of the family is currently indeterminable due to the paucity of unambiguous bromeliad fossils. The assignment to the family of a number of leaf fossils of Upper Oligocene to Miocene age from Europe, and one from the Cretaceous Dakota Formation of Kansas, is considered highly doubtful (Smith and Downs 1974). Thus, there is no good evidence to refute a recent origin of the Bromeliaceae, but extant distributions cannot be considered a solid basis from which to estimate age. Varadarajan and Gilmartin (1988b) speculated that the Pitcairnioideae had a late Cretaceous origin in the Guayana Shield, based on a morphological phylogeny and the geological, ecological and climatic history of South America. *ndh*F data (Terry *et al.* 1997a) provided some support for this by resolving *Brocchinia acuminata* (which is largely restricted to the Guayana Shield; Holst 1997) as the first-diverging lineage in the family, but the *mat*K data resolve a tillandsioid lineage (comprising *Tillandsia*, *Vriesea* and *Guzmania*) in this position. Clearly, more

evidence is needed, particularly fossils of early bromeliads, before a consensus on this issue can emerge.

Although the strict consensus is relatively unresolved, several important relationships are supported. The Bromeliaceae (clade A, Fig. 3) is retrieved with strong support, although the power of this analysis as a test of family monophyly suffers from low outgroup representation; only a single member (*Epidryos allenii*) of the putative sister group (Rapateaceae) was available. Nevertheless, all cladistic analyses to date support a monophyletic Bromeliaceae (Gilmartin and Brown 1987; Gaut *et al.* 1992; Chase *et al.* 1993; Clark *et al.* 1993; Duvall *et al.* 1993; Chase *et al.* 1995b; Terry *et al.* 1997a).

Compared with the short branches among ingroup taxa, Bromeliaceae and its probable sister group, Rapateaceae, are separated by a relatively long, well-supported branch (Fig. 3). Other molecular studies using the plastid gene *rbc*L (e.g. Gaut *et al.* 1992; Clark *et al.* 1993) have achieved a similar result. This is consistent with the traditional view of Bromeliaceae as a rather isolated lineage.

Within the ingroup, the monophyly of the Tillandsioideae (clade B, Fig. 3) and its separation from the remaining Bromeliaceae at the deepest branch are well supported by the *mat*K data. Ranker *et al.* (1990) also retrieved this position for some tillandsioids using cpDNA RFLP data, but the limited ingroup sample (11 taxa), and the use of Velloziaceae as outgroup, engenders doubt regarding the validity of their result. The *ndh*F analysis (Terry *et al.* 1997a) also placed Tillandsioideae near the base of the family, although in that analysis *Brocchinia acuminata* was strongly supported as sister to all other Bromeliaceae.

Further interpretation of bromeliad relationships is hampered by the limited resolution on the tree. A number of groups are resolved, some corresponding to genera, but the pattern of relationship among them is obscure. *Ananas* (Bromelioideae) is most parsimoniously placed as part of a large polytomy of pitcairnioids (clade C) subtended by *Hechtia* (clade D), which is consistent with the *ndh*F data (Terry *et al.* 1997a; Fig. 1) where Bromelioideae was resolved as a derived lineage within Pitcairnioideae. However, Bremer support and jackknife values (+1 and >50%, respectively) indicate there is little support in the *mat*K data for this. In contrast, cladistic analyses of morphological data have claimed to support a monophyletic Pitcairnioideae (Gilmartin *et al.* 1987; Varadarajan and Gilmartin 1988b), but character polarities were determined using Velloziaceae (shown to be distant from Bromeliaceae based on molecular data, e.g. Duvall *et al.* 1993) as outgroup, and thus the putative synapomorphies for Pitcairnioideae may in fact be symplesiomorphies.

Of the ten genera represented by more than one species in this analysis, only *Brocchinia* (clade E), *Fosterella* (clade F), *Pepinia* (clade G) and *Puya* (clade H) are resolved as monophyletic. A recent analysis based on cp- and nrDNA restriction site data (Givnish *et al.* 1997) provided strong evidence for the polyphyly of *Brocchinia*: *B. serrata* did not group with the remaining 15 *Brocchinia* species. In our analysis, *Brocchinia* is represented by *B. acuminata* and *B. micrantha*, which form a highly robust (10; 99%) clade (clade E). This is consistent with the close alliance of these two species on the combined cp- and nrDNA tree (Givnish *et al.* 1997). The position of *B. acuminata*, however, is in conflict with the *ndh*F tree, in which it was placed as sister to all other Bromeliaceae (Terry *et al.* 1997).

Fosterella, a genus of 17 species of mesophytes with a disjunct Mesoamerican–Andean distribution, is retrieved as a clade in the present *mat*K analysis (clade F, Fig. 3), although with only moderate support (2; 67%). The three species sampled represent well the morphological diversity in the genus, including both secund (*F. elata*, *F. penduliflora*) and non-secund (*F. petiolata*) inflorescence types, although only species from the southern part of the distribution are represented. Future work will add the northern species *F. micrantha* (Lindley) L. B. Smith.

Pepinia is usually treated as a subgenus of *Pitcairnia*, but Varadarajan and Gilmartin (1988c) recognised it as a distinct genus and we will follow this treatment for the purpose of this discussion. The three *Pepinia* species are monophyletic within *Pitcairnia* (excluding *Pitcairnia heterophylla*) in the most-parsimonious *mat*K trees (clade G, Fig. 3), although the support for this is weak (1; <50%). Indeed the entire *Pitcairnia–Pepinia* clade (clade I) obtains only moderate support (2; 60%). Thus the evidence for an origin of *Pepinia* from within *Pitcairnia* is not convincing and needs confirmation from further studies. *Pepinia* was elevated based primarily on seed shape, but floral, inflorescence and vegetative characters were also considered (Varadarajan and Gilmartin 1988c). A sister relationship between *Pepinia* and *Pitcairnia* was also supported by cladistic analyses of morphological (Fig. 2; Varadarajan and Gilmartin 1988b) and *ndh*F data (Fig. 1; Terry *et al.* 1997).

Pitcairnia heterophylla, according to these data, may be distant from the remainder of the genus, although its relationships are not well resolved. It is one of the most widespread *Pitcairnia* species, being distributed from Mexico to Peru. Further work with a greatly expanded database and alternative data sources is warranted on this diverse genus of approximately 300 species.

The two species of *Navia* included in this analysis (Fig. 3) do not form a clade. *Navia phelpsiae* is grouped, albeit weakly (2; 57%), with *Cottendorfia florida* (clade J). The two sequences representing the other species, *N. igneosicola*, are robustly grouped (clade K: 5; 92%), and most parsimoniously placed as sister to the *Pitcairnia–Pepinia* group, but this is not well supported (1; 52%). *N. phelpsiae* and *N. igneosicola* are members of two morphologically and anatomically distinct groups within *Navia* (B. Holst pers. comm.), a diverse genus of some 93 species endemic to the Guayana Shield (Holst 1997). *Cottendorfia* has comprised as many as 28 species (Smith and Downs 1974; Steyermark and Maguire 1984), but is currently recognised as monotypic and endemic in the Bahia region of Brazil (Luther and Sieff 1998).

Dyckia and *Encholirium* were strongly grouped based on *ndh*F data (Fig. 1; Terry *et al.* 1997a), and in the morphological cladistic analysis (Fig. 2; Varadarajan and Gilmartin 1988b) these two formed a clade that also included *Brewcaria* (not represented in this analysis). Using *mat*K data (Fig. 3), the three representatives of each genus together form a robust clade (clade L: 7; 95%), but the relationships among them are unresolved. Broader sampling would be required to provide clarification of generic boundaries in this group. Interestingly, *Deuterocohnia meziana* is robustly

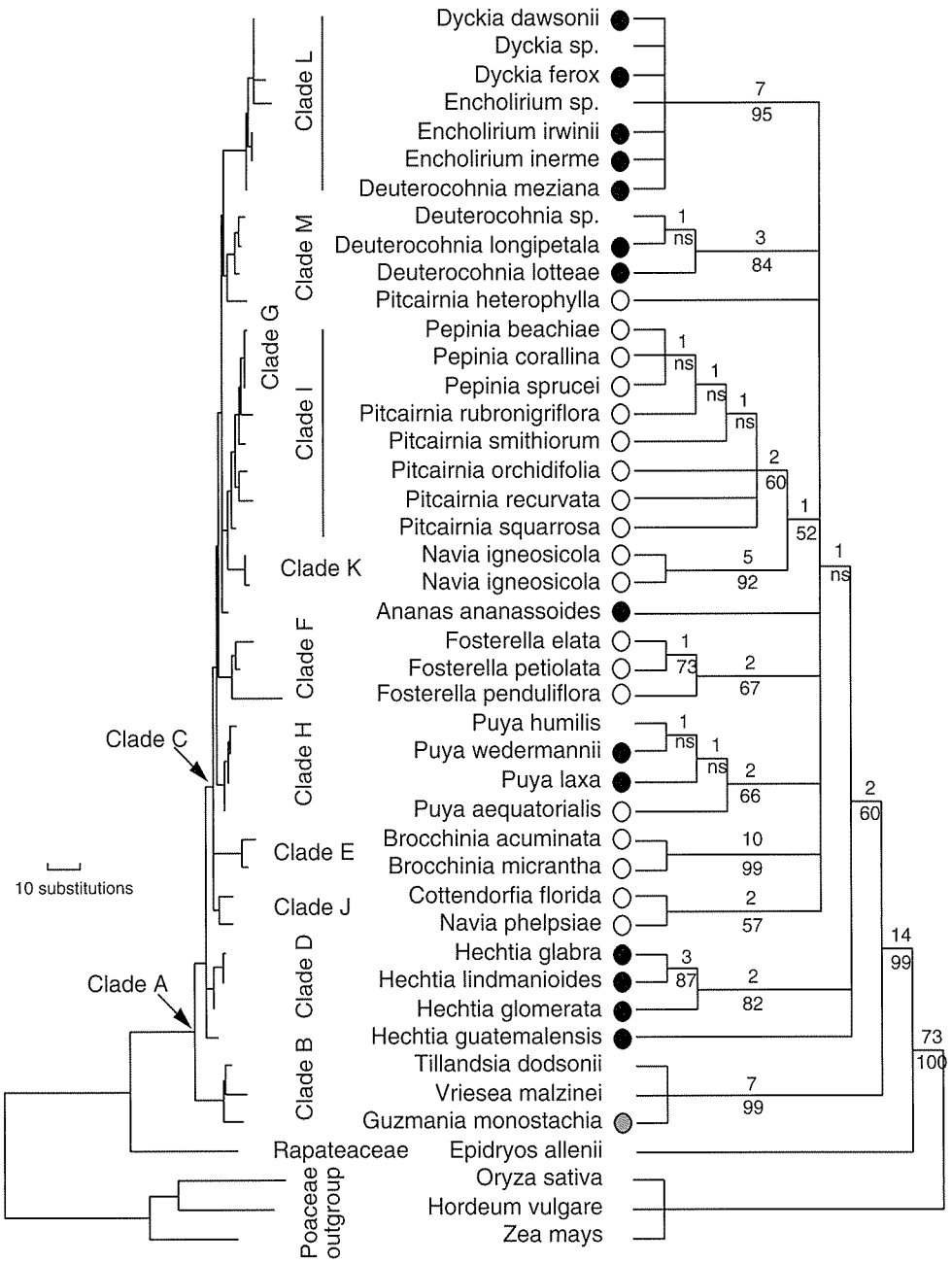

Fig. 3. Phylogenetic relationships among genera of Bromeliaceae subfamily Pitcairnioideae plus representatives of the other two subfamilies (Bromelioideae and Tillandsioideae) and Rapateaceae. The trees were constructed by parsimony analysis of the *mat*K data, and were rooted using three species of Poaceae. The tree on the right is the strict consensus of the first 58,300 trees (L = 466, RC = 0.665, RI = 0.831) found by an initial heuristic search; subsequent replicate searches with random taxon order input and invoking this consensus tree as a reverse constraint found no alternative trees supported at this level of optimality. Bremer support values are above the branches; parsimony jackknife percentage values (100,000 replicates) are below the branches (ns denotes the clade was retrieved in less than 50% of replicates). Terminal taxa marked with closed circles are known CAM species, those marked with open circles are C_3 species (see Appendix). The photosynthetic pathway in the unmarked species is unknown. *Guzmania monostachia* is a CAM/C_3 intermediate. The tree on the left was chosen randomly from the 58,300 found, and shows the branch lengths optimised under the accelerated transformation (ACCTRAN) criterion. Clades referred to in the text are labelled A – M.

placed with this group, and not with the three other *Deuterocohnia* species, which form a strong clade (clade M: 3; 84%). *D. meziana* is endemic in southeastern Bolivia, southwestern Brazil and Paraguay, and is considered highly unusual in its possession of a cambium-like layer beneath the cortex of the woody scape that allows repeated blooms on the same inflorescence (Foster 1945; Spencer and Smith 1992). *D. meziana* may indeed represent a unique lineage, but its placement among *Dyckia* and *Encholirium* species on this analysis is intriguing. If other data from the maternally inherited chloroplast genome strongly support this relationship, a possible explanation might be a hybrid ancestry for the species, with the maternal parent being a

member of the *Dyckia–Encholirium* group. Information from the nuclear genome would be required to confirm this.

Hechtia, represented by four species in this analysis, may not be monophyletic. *H. guatemalensis* is not part of the clade (clade D: 2; 82%) formed by the remaining species; its position is unresolved in a polytomy with the *Hechtia* clade and a clade containing all other pitcairnioids.

Phylogenetic Distribution of CAM

Previous work (reviewed in Martin 1994) has identified the photosynthetic pathway of 249 bromeliad species, of which 31% are C_3 whereas the remainder show varying degrees of CAM. Recently, information has been obtained on a further 400 spp. (D. M. Crayn, K. Winter and J. A. C. Smith unpubl.) using carbon isotope ratios ($\delta^{13}C$) in leaf tissue from either herbarium or fresh material. Based on these studies, some genera of Pitcairnioideae appear to be uniformly CAM (e.g. *Deuterocohnia*, *Dyckia*, *Encholirium*, *Hechtia*) or C_3 (*Ayensua*, *Brewcaria*, *Brocchinia*, *Connellia*, *Cottendorfia*, *Fosterella*, *Lindmania*, *Navia*, *Pepinia*, *Pitcairnia*, *Steyerbromelia*), whereas *Puya* contains both CAM and C_3 species. Thus, knowledge of the distribution of CAM among pitcairnioid species is relatively advanced, but phylogenetic hypotheses for the subfamily are not yet sufficiently refined to infer the evolutionary origins of CAM in this group. We are currently sequencing other rapidly evolving chloroplast (*rps*16 intron, *trn*L-F spacer) and nuclear regions (ITS1&2) in an effort to provide sufficient characters to resolve this uncertainty.

Conclusions and Prospects

Many aspects of the systematics of Bromeliaceae, particularly Pitcairnioideae, are still unclear. Only three studies (including this one) have explored relationships within this subfamily, and these have suffered from low taxon sampling (Terry *et al.* 1997a), the assumption of subfamilial and generic monophyly (Varadarajan and Gilmartin 1988b), or poor resolution (this study). Moreover, a consensus of these studies is not possible due to inconsistent circumscription of terminals: Varadarajan and Gilmartin (1988b) scored genera, whereas the other studies scored species. While it may be tempting to extrapolate generic relationships from exemplar species, this analysis, which is the first to sample more than one species of any pitcairnioid genus, highlights the dangers of assuming generic monophyly *a priori* in this subfamily.

Several pitcairnioid genera, namely *Ayensua*, *Brewcaria*, *Connellia*, *Lindmania* and *Steyerbromelia*, were not represented in this analysis due to the unavailability of tissue. These genera are all endemic to the Guayana Shield, an ancient formation consisting of dissected sandstone plateaux (tepuis) and associated uplands that marks the northern rim of the Amazon Basin. Varadarajan and Gilmartin (1988b) suggested that the earliest bromeliad radiations may have occurred in this area, and hence these taxa may represent relicts of lineages that diverged at or near the base of the family. Knowledge of their relationships is critical to understanding pitcairnioid evolution, and representatives of these taxa will be included in future analyses.

Although the monophyly of Bromeliaceae seems established, the provision of additional outgroups for future analyses is essential in order to accurately determine ancestral states for key characteristics. Molecular analyses consistently resolve Rapateaceae among the nearest outgroups and, recently, the small tropical aquatic family Mayacaceae has been suggested to be close to Rapateaceae and Bromeliaceae (Givnish *et al.* 1998). Future analyses should incorporate more taxa from these two families.

The positions of Tillandsioideae and Bromelioideae in relation to pitcairnioid taxa are among the most interesting unsolved problems of bromeliad phylogenetics. Some evidence has accumulated for the early divergence of a tillandsioid lineage from the remaining bromeliads (Ranker *et al.* 1990; this study), but whether this dichotomy is basal in the family is questionable (Terry *et al.* 1997a). The monophyly of Bromelioideae is supported by all analyses to date, but controversy exists regarding the membership of Tillandsioideae, particularly with respect to *Glomeropitcairnia*. Resolution of these arguments will hinge on obtaining data from other genomic regions and the inclusion of a broader systematic sample.

Acknowledgements

We sincerely thank Marie Selby Botanical Gardens (Sarasota, Florida, USA), particularly Bruce Holst and Harry Luther for access to their living collection of bromeliads, for encouragement and advice on this project, and, together with Randall Bayer (Canberra, Australia), for comments on the manuscript. We also thank Elton Leme (Rio de Janiero, Brazil) for providing leaf tissue from his personal collection. Various staff of Naos Marine Laboratories, Smithsonian Tropical Research Institute, provided advice and assistance with sequencing and PCR procedures. This research was supported by grants from the Andrew W. Mellon Foundation through the Smithsonian Institution to JACS and KW, and from the Bromeliad Society Inc. and the University of Montana to RGT.

References

Benzing, D. H. (1980). 'The Biology of the Bromeliads.' (Mad River Press: California).

Benzing, D. H., Givnish, T. J., and Bermudes, D. (1985). Absorptive trichomes in *Brocchinia reducta* (Bromeliaceae) and their evolutionary and systematic significance. *Systematic Botany* **10**, 81–91.

Benzing, D. H., Henderson, K., Kessel, B., and Sulak, J. (1976). The absorptive capacities of bromeliad trichomes. *American Journal of Botany* **63**, 1009–1014.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**, 795–803.

Brown, G. K., and Gilmartin, A. J. (1984). Stigma structure and variation in Bromeliaceae – neglected taxonomic characters. *Brittonia* **36**, 364–374.

Brown, G. K., and Gilmartin, A. J. (1989). Stigma types in Bromeliaceae – a systematic survey. *Systematic Botany* **14**, 110–132.

Chase, M. W., Soltis, D. E., Olmstead, R. G., Morgan, D., Les, D. H., Mishler, B. D., Duvall, M. R., Price, R. A., Hills, H. G., Qiu, Y. L., Kron, K. A., Rettig, J. H., Conti, E., Palmer, J. D., Manhart, J. R., Sytsma, K. J., Michaels, H. J., Kress, W. J., Karol, K. G., Clark, W. D., Hedrén, M., Gaut, B. S., Jansen, R. K., Kim, K.-J., Wimpee, C. F., Smith, J. F., Furnier, G. R., Strauss, S. H., Xiang, Q.-Y., Plunkett, G. M., Soltis, P. S., Swensen, S., Williams, S. E., Gadek, P. A., Quinn, C. J., Eguiarte, L. E.,

Golenberg, E., Learn, G. H. Jr., Graham, S. W., Barrett, S. C. H., Dayanandan, S., and Albert, V. A. (1993). Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. *Annals of the Missouri Botanical Garden* **80**, 528–580.

Chase, M. W., Duvall, M. R., Hillis, H. G., Conran, J. G., Cox, A. V., Eguiarte, L. E., Hartwell, J., Fay, M. F., Caddick, L. R., Cameron, K. M., and Hoot, S. (1995a). Molecular phylogenetics of Lilianae. In 'Monocotyledons: Systematics and Evolution'. (Eds P. J. Rudall, P. J. Cribb, D. F. Cutler and C. J. Humphries.) pp. 109–137. (Royal Botanic Gardens: Kew).

Chase, M. W., Stevenson, D. W., Wilkin, P., and Rudall, P. J. (1995b). Monocot systematics: a combined analysis. In 'Monocotyledons: Systematics and Evolution'. (Eds P. J. Rudall, P. J. Cribb, D. F. Cutler and C. J. Humphries.) pp. 685–730. (Royal Botanic Gardens: Kew).

Clark, W. D., Gaut, B. S., Duvall, M. R., and Clegg, M. T. (1993). Phylogenetic relationships of the Bromeliiflorae–Commeliniflorae–Zingiberiflorae complex of monocots based on *rbc*L sequence comparisons. *Annals of the Missouri Botanical Garden* **80**, 987–998.

Cronquist, A. (1981). 'An Integrated System of Classification of Flowering Plants'. (Columbia University Press: New York).

Dahlgren, R., Clifford, H. T., and Yeo, P. F. (1985). 'The Families of Monocotyledons'. (Springer-Verlag: Berlin).

Dahlgren, R., and Rasmussen, F. N. (1983). Monocotyledon evolution: characters and phylogenetic estimation. *Evolutionary Biology* **16**, 255–395.

Duvall, M. R., Clegg, M. T., Chase, M. W., Clark, W. D., Kress, W. J., Hills, H. G., Eguiarte, L. E., Smith, J. F., Gaut, B. S., Zimmer, E. A., and Learn, G. H. Jr. (1993). Phylogenetic hypotheses for the monocotyledons constructed from *rbc*L sequence data. *Annals of the Missouri Botanical Garden* **80**, 607–619.

Eriksson, T. (1995). 'Auto Decay version 2.7'. (Harvard University Herbaria: Cambridge).

Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D., and Kluge, A. (1996). Parsimony jackknifing outperforms neighbour-joining. *Cladistics* **12**, 99–124.

Foster, M. B. (1945). Lateral inflorescences in the Bromeliaceae. *The National Horticultural Magazine* **25**, 14–22.

Gadek, P. A., Wilson, P. G., and Quinn, C. J. (1996). Phylogenetic reconstruction in Myrtaceae using *mat*K, with particular reference to the position of *Psiloxylon* and *Heteropyxis*. *Australian Systematic Botany* **9**, 283–290.

Gaut, B. S., Muse, S. V., Clark, W. D., and Clegg, M. T. (1992). Relative rates of nucleotide substitution at the *rbc*L locus of monocotyledonous plants. *Journal of Molecular Evolution* **35**, 292–303.

Gilmartin, A. J., and Brown, G. K. (1987). Bromeliales, related monocots, and resolution of relationships among Bromeliaceae subfamilies. *Systematic Botany* **12**, 493–500.

Givnish, T. J., Evans, T. M., and Sytsma, K. J. (1998). Polyphyly and convergent morphological evolution in Commelinales and Commelinidae: evidence from *rbc*L sequence data. *Abstracts, Monocots II Conference, Sydney, Australia* pp. 22–23.

Givnish, T. J., Sytsma, K. J., Smith, J. F., Hahn, W. J., Benzing, D. H., and Burkhardt, E. M. (1997). Molecular evolution and adaptive radiation in *Brocchinia* (Bromeliaceae: Pitcairnioideae) atop tepuis of the Guayana Shield. In 'Molecular Evolution and Adaptive Radiation'. (Eds T. J. Givnish and K. J. Sytsma.) pp. 259–311. (Cambridge University Press: Cambridge).

Griffiths, H. (1984). Ecological distribution of bromeliads in Trinidad and their $\delta^{13}C$ values: implications for the use of δ values to indicate carboxylation pathways in plants. In 'Physiological Ecology of CAM Plants'. (Ed. E. Medina.) pp. 145–174. (CIET (IVIC-UNESCO): Caracas).

Hillis, D. M., Mable, B. K., Larson, A., Davis, S. K., and Zimmer, E. A. (1996). Nucleic acids IV: sequencing and cloning. In 'Molecular Systematics', 2nd edn. (Eds D. M. Hillis, C. Moritz and B. K. Mable.) pp. 321–381. (Sinauer Associates: Sunderland, MA).

Hilu, K. W., and Liang, H. P. (1997). The *mat*K gene – sequence variation and application in plant systematics. *American Journal of Botany* **84**, 830–839.

Holst, B. K. (1997). Bromeliaceae. In 'Flora of the Venezuelan Guayana.' (Eds P. E. Berry, B. K. Holst and K. Yatskievych.) pp. 548–676. (Missouri Botanical Garden: St. Louis).

Huber, H. (1977). The treatment of the monocotyledons in an evolutionary system of classification. In 'Evolution and Classification of Higher Categories'. (Ed. K. Kubitzki.) (Plant Systematics and Evolution Supplement 1, Springer: Vienna).

Liere, K., and Link, G. (1995). RNA-binding activity of the *mat*K protein encoded by the chloroplast *trn*K intron from mustard (*Sinapis alba* L.). *Nucleic Acids Research* **23**, 917–921.

Linder, H. P., and Kellogg, E. A. (1995). Phylogenetic patterns in the commelinoid clade. In 'Monocotyledons: Systematics and Evolution'. (Eds P. J. Rudall, P. J. Cribb, D. F. Cutler and C. J. Humphries.) pp. 473–496. (Royal Botanic Gardens: Kew).

Luther, H. E., and Sieff, E. (1998). 'An Alphabetical List of Bromeliad Binomials'. (The Bromeliad Society, Inc.: Newberg).

Lüttge, U., and Ball, E. (1987). Dark respiration of CAM plants. *Plant Physiology and Biochemistry* **25**, 3–10.

Maddison, D. R. (1991). The discovery and importance of multiple islands of most-parsimonious trees. *Systematic Zoology* **40**, 315–328.

Martin, C. E. (1994). Physiological ecology of the Bromeliaceae. *The Botanical Review* **60**, 1–82.

McWilliams, E. L. (1970). Comparative rates of dark CO_2 uptake and acidification in the Bromeliaceae, Orchidaceae, and Euphorbiaceae. *Botanical Gazette* **131**, 285–290.

McWilliams, E. L. (1974). Evolutionary ecology. In 'Pitcairnioideae (Bromeliaceae)'. (Eds L. B. Smith and R. J. Downs) Monograph No. 14, Part 1. pp. 40–55. (Hafner: New York).

Medina, E. (1974). Dark CO_2 fixation, habitat preference and evolution within Bromeliaceae. *Evolution* **28**, 677–686.

Medina, E., Popp, M., Olivares, E., Janett, H.-P., and Lüttge, U. (1993). Daily fluctuations of titratable acidity, content of organic acids (malate and citrate) and soluble sugars of varieties and wild relatives of *Ananas comosus* L. growing under natural tropical conditions. *Plant Cell Environment* **16**, 55–63.

Mez, C. (1904). Physiologische Bromeliaceen-Studien. I. Die Wasser-Ökonomie der extrem atmosphärischen Tillandsien. *Jahrbücher Wissenschaftliche Botanik* **40**, 157–229.

Olmstead, R. G., and Palmer, J. D. (1994). Chloroplast DNA systematics: a review of methods and data analysis. *American Journal of Botany* **81**, 1205–1224.

Owen, T. P., Benzing, D. H., and Thomson, W. W. (1988). Apoplastic and ultrastructural characterizations of the trichomes from the carnivorous bromeliad *Brocchinia reducta*. *Canadian Journal of Botany* **66**, 941–948.

Owen, T. P., and Thomson, W. W. (1991). Structure and function of a specialized cell wall in the trichomes of the carnivorous bromeliad *Brocchinia reducta*. *Canadian Journal of Botany* **69**, 1700–1706.

Pittendrigh, C. S. (1948). The bromeliad–*Anopheles*–malaria complex in Trinidad. I. The bromeliad flora. *Evolution* **2**, 58–89.

Ranker, T. A., Soltis, D. E., Soltis, P. S., and Gilmartin, A. J. (1990). Subfamilial phylogenetic relationships of the Bromeliaceae: evidence from chloroplast DNA restriction site variation. *Systematic Botany* **15**, 425–434.

Rauh, W. (1981). 'Bromelien: Tillandsien und Andere Kulturwürdige Bromelien. Zweite Auflage'. (Eugen Ulmer: Stuttgart.)

Schimper, A. F. W. (1888). 'Die epiphytische Vegetation Amerikas. Botanische Mitteilungen aus den Tropen, Heft 2'. (Gustav Fischer: Jena.)

Simpson, M. G. (1988). A critique of 'Bromeliales, related monocots, and resolution of relationships among Bromeliaceae subfamilies'. *Systematic Botany* **13**, 610–619.

Smith, J. A. C. (1989). Epiphytic bromeliads. In 'Vascular Plants as Epiphytes'. (Ed. U. Lüttge) pp. 109–138. (Springer-Verlag: Berlin).

Smith, L. B. (1934). Geographical evidence on the lines of evolution in the Bromeliaceae. *Botanische Jahrbücher* **66**, 446–468.

Smith, L. B. (1986). Revision of the Guayana Highland Bromeliaceae. *Annals of the Missouri Botanical Garden* **73**, 689–721.

Smith, L. B., and Downs, R. J. (1974). 'Pitcairnioideae (Bromeliaceae)'. Flora Neotropica, Monograph No. 14, Part 1. (Hafner: New York).

Smith, L. B., and Downs, R. J. (1977). 'Tillandsioideae (Bromeliaceae)'. Flora Neotropica, Monograph No. 14, Part 2. (Hafner: New York).

Smith, L. B., and Downs, R. J. (1979). 'Bromelioideae (Bromeliaceae)'. Flora Neotropica, Monograph No. 14, Part 3. (Hafner: New York).

Spencer, M. A., and Smith, L. B. (1992). A revison of the genus *Deuterocohnia* Mez (Bromeliaceae: Pitcairnioideae). *Bradea* **6**, 141–146.

Stevenson, D. W., and Loconte, H. (1995). Cladistic analysis of monocot families. In 'Monocotyledons: Systematics and Evolution'. (Eds P. J. Rudall, P. J. Cribb, D. F. Cutler and C. J. Humphries.) pp. 543–578. (Royal Botanic Gardens: Kew).

Steyermark, J. A., and Maguire, B. (1984). Nuevos taxa de la Guayana Venezolana. *Acta Botánica Venezuélica* **14**, 5–52.

Swofford, D. L. (1998). 'PAUP*, version 4.0d64.' (Computer program, commercially unavailable test version).

Teeri, J. A. (1982). Carbon isotopes and the evolution of C_4 photosynthesis and crassulacean acid metabolism. In 'Biochemical Aspects of Evolutionary Biology'. (Ed. M. H. Nitecki.) pp. 93–130. (University of Chicago Press: Chicago)

Terry, R. G., Brown, G. K., and Olmstead, R. G. (1997a). Examination of subfamilial phylogeny in Bromeliaceae using comparative sequencing of the plastid locus *ndh*F. *American Journal of Botany* **84**, 664–670.

Terry, R. G., Brown, G. K., and Olmstead, R. G. (1997b). Phylogenetic relationships in subfamily Tillandsioideae (Bromeliaceae) using *ndh*F sequences. *Systematic Botany* **22**, 333–345.

Tietze, M. (1906). Physiologische Bromeliaceen-Studien II. Die Entwickelung der wasseraufnehmenden Bromeliaceen – Trichome. *Zeitschrift für Naturwissenschaften* **78**, 1–50.

Tomlinson, P. B. (1969). III Commelinales - Zingiberales. In 'Anatomy of the Monocotyledons'. (Ed. C. R. Metcalfe.) (Oxford University Press: Oxford).

Varadarajan, G. S., and Gilmartin, A. J. (1987). Foliar scales of the subfamily Pitcairnioideae (Bromeliaceae). *Systematic Botany* **12**, 562–571.

Varadarajan, G. S., and Gilmartin, A. J. (1988a). Seed morphology of the subfamily Pitcairnioideae (Bromeliaceae) and its systematic implications. *American Journal of Botany* **75**, 808–818.

Varadarajan, G. S., and Gilmartin, A. J. (1988b). Phylogenetic relationships of groups of genera within the subfamily Pitcairnioideae (Bromeliaceae). *Systematic Botany* **13**, 283–293.

Varadarajan, G. S., and Gilmartin, A. J. (1988c). Taxonomic realignments within the subfamily Pitcairnioideae (Bromeliaceae). *Systematic Botany* **13**, 294–299.

Winter, K., and Smith, J. A. C. (1996). An introduction to crassulacean acid metabolism. Biochemical principles and ecological diversity. In 'Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution'. (Eds K. Winter and J. A. C. Smith.) pp. 1–13. (Springer-Verlag: Berlin).

Appendix. Details of taxa used in phylogenetic analyses: their voucher details, GenBank accession details of their *mat*K sequences and their photosynthetic pathway (where determined). Accessions beginning with 'MSBG' are plants held in the living collection at Marie Selby Botanical Garden, Sarasota, Florida, USA.

Taxon	Voucher or Accession No.	GenBank Accession No.	CAM or C_3, Reference
Ananas ananassoides (Baker) L.B. Smith	*G. Brown 3129* (RM)	AF162227	CAM (e.g. Medina *et al.* 1993)
Brocchinia acuminata L.B. Smith	MSBG 1981-1937	AF162228	C_3 (Medina 1974)
Brocchinia micrantha (Baker) Mez	MSBG 1986-0517	AF162229	C_3 (e.g. Medina 1974)
Cottendorfia florida Schultes f.	*E. Leme 3692* (HB)	AF162230	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Deuterocohnia longipetala (Baker) Mez	MSBG 075767	AF162231	CAM (Griffiths 1984)
Deuterocohnia lotteae (Rauh) M.A. Spencer & L.B. Smith	MSBG 1994-0142	AF162232	CAM (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Deuterocohnia meziana Kuntze ex Mez	*M.D. Remmick 96* (MSBG)	AF162233	CAM (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Deuterocohnia sp.	none	AF162226	undetermined
Dyckia dawsonii L.B. Smith	MSBG 1994-0146A	AF162234	CAM (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Dyckia ferox Mez	MSBG 1996-0211A	AF162235	CAM (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Dyckia sp.	*G. Brown 3131* (RM)	AF162236	undetermined
Encholirium inerme Rauh	MSBG 1995-0113A	AF162239	CAM (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Encholirium irwinii L.B. Smith	*E. Leme 2881* (HB)	AF162237	CAM (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Encholirium sp.	MSBG 1984-0364	AF162238	undetermined
Epidryos allenii (Steyermark) Maguire	*C. Galdames 4285* (SCZ)	AF162225	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Fosterella elata H. Luther	MSBG 1981-0059A	AF162240	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Fosterella penduliflora (C.H. Wright) L.B. Smith	MSBG 69-1976-12	AF162241	C_3 (McWilliams 1970)
Fosterella petiolata (Mez) L.B. Smith	MSBG 1995-0007A	AF162242	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)

Appendix. Details of taxa used in phylogenetic analyses: their voucher details, GenBank accession details of their *mat*K sequences and their photosynthetic pathway (where determined). Accessions beginning with 'MSBG' are plants held in the living collection at Marie Selby Botanical Garden, Sarasota, Florida, USA. *(Continued)*

Taxon	Voucher or Accession No.	GenBank Accession No.	CAM or C_3, Reference
Guzmania monostachia (L.) Rusby ex Mez	MSBG 1982-0225	AF162243	C_3/CAM (e.g. McWilliams 1970; Medina 1974)
Hechtia glabra Brandegee	*D. Crayn s.n.* (MSBG)	AF162244	CAM (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Hechtia glomerata Zuccarini	*M.D. Remmick 139* (MSBG)	AF162245	CAM (Lüttge and Ball 1987)
Hechtia guatemalensis Mez	*D. Crayn s.n.* (MSBG)	AF162246	CAM (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Hechtia lindmanioides L.B. Smith	*D. Crayn s.n.* (MSBG)	AF162247	CAM (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Hordeum vulgare L.		X64129	
Navia igneosicola L.B. Smith, Steyermark and H. Robinson	MSBG 1983-0288A	AF162248	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Navia igneosicola L.B. Smith, Steyermark and H. Robinson	MSBG 1983-0288	AF162250	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Navia phelpsiae L.B. Smith.	MSBG 1986-0523A	AF162249	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Oryza sativa L.		X15901	
Pepinia beachiae (J. Utley & Burt-Utley) H. Luther	MSBG 1986-0798A	AF162251	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Pepinia corallina (Linden & André) G.S. Varadarajan & Gilmartin	MSBG 1986-0574A	AF162252	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Pepinia sprucei (Baker) G.S. Varadarajan & Gilmartin	MSBG 1994-0307A	AF162253	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Pitcairnia heterophylla (Lindley) Beer	MSBG 1996-0452A	AF162254	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Pitcairnia orchidifolia Mez	MSBG 1994-0036A	AF162255	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Pitcairnia recurvata (Scheidweiler) K. Koch	MSBG 1986-0310A	AF162256	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Pitcairnia rubronigriflora Rauh	MSBG 1989-0114A	AF162257	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Pitcairnia smithiorum H. Luther	MSBG 1989-0004A	AF162258	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Pitcairnia squarrosa L.B. Smith	MSBG 1989-0579A	AF162259	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Puya aequatorialis André	MSBG 1993-0211	AF162260	C_3 (D. Crayn, K. Winter and J.A.C. Smith unpubl.)
Puya humilis Mez	MSBG 1994-0293A	AF162261	undetermined
Puya laxa L.B. Smith	*T. Walters s.n.* (MSBG)	AF162262	probably CAM (Griffiths 1984)
Puya wedermanii Harms	MSBG 1994-0269	AF162263	CAM (Griffiths 1984)
Tillandsia dodsonii L.B. Smith	*G. Brown 3218* (RM)	AF162264	undetermined
Vriesea malzinei E. Morren	MSBG 1978-0757	AF162265	undetermined
Zea mays L.		X86563	