Carbon-Isotope Ratios and Photosynthetic Pathways in the Neotropical Family Rapateaceae

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Abstract: **The Rapateaceae is a small, mainly Neotropical family of terrestrial or occasionally epiphytic herbs that grow on mesic, nutrient-poor sites. Some recent studies suggest that the Rapateaceae may be closely related to the Bromeliaceae, one of the major families containing CAM plants. To investigate the photosynthetic pathway in Rapateaceae, the plant carbon-isotope ratio ($\delta^{13}C$) was determined for samples from dried herbarium specimens for 85 of the approximately 100 species in the family. The $\delta^{13}C$ values ranged from – 37.7 to – 19.8 ‰. Most Rapateaceae showed $\delta^{13}C$ values typical of C_3 plants. However, six species (*Kunhardtia rhodantha* Maguire, *Marahuacaea schomburgkii* (Maguire) Maguire, *Saxofridericia compressa* Maguire, *Stegolepis grandis* Maguire, *St. guianensis* Klotzsch ex Körn. and *St. squarrosa* Maguire) showed $\delta^{13}C$ values less negative than – 23 ‰, i.e., at the higher end of the range for C_3 plants and at the lower end of the distribution for plants exhibiting CAM. The $\delta^{13}C$ values became significantly less negative with increasing altitude (regression analysis indicating a change from about – 30.7 ‰ at sea level to – 22.5 ‰ at 2500 m). Although other environmental factors and the type of tissue analysed may also influence $\delta^{13}C$ values, these results suggest that some Rapateaceae may be capable of performing CAM. Further studies, including measurements of diel gas exchange patterns and leaf organic-acid fluctuations, would be needed to demonstrate CAM in Rapateaceae unequivocally, but living material of many of these enigmatic plants is difficult to obtain.**

Key words: **Carbon-isotope ratio, photosynthetic pathway, crassulacean acid metabolism, Rapateaceae, monocotyledons.**

Abbreviation:
CAM: crassulacean acid metabolism

Introduction

The Rapateaceae Dumort. is a small family of monocotyledonous herbs with a primarily Neotropical distribution (Fig. **1 a**). The only genus occurring outside the Neotropics is the monotypic *Maschalocephalus* in West Africa (Smith, 1934[40]; Stevenson et al., 1998[41]). No recent monograph exists for the family, but it is estimated to comprise 17 genera and approximately 100 species (Givnish et al., 2000[20]). Rapateaceae are mostly plants of moist, partly open habitats, often growing on sandy infertile soils, although *Rapatea* spp. are forest understory herbs, and a few other species grow epiphytically (*Epidryos* spp.) or lithophytically (*Stegolepis* spp.). The family is particularly well-represented in the meadows of the Venezuelan Guayana, a region of southern Venezuela dominated by an extensive system of sheer-sided tabletop massifs (tepuis) formed by the erosion and dissection of uplifted Roraima sandstone (Huber, 1988[25]; Huber, 1995[26]). In this region, rapateads are characteristic and often dominant floristic elements both in lowland meadows (e.g., *Schoenocephalium*), which are characterized by deep, sandy, acidic, poorly drained and extremely nutrient-poor soils, and in the upland (e.g., *Stegolepis*) and highland (tepui) meadows *(Amphiphyllum, Kunhardtia, Marahuacaea, Phelpsiella, Stegolepis)* (Huber, 1995[26]; Lüttge, 1997[32]; Michelangeli, 2000[37]). The early evolution of Rapateaceae may have taken place in inundated areas followed by diversification in lowland Amazonian savannas and elevated wet savannas (meadows) on tepui summits, with subsequent re-invasion of lowland habitats (Givnish et al., 2000[20]).

Rapateaceae have been commonly allied with Xyridaceae (e.g., Cronquist, 1981[10]; Dahlgren et al., 1985[11]), but links with Commelinaceae (Venturelli and Bouman, 1988[42]) and Bromeliaceae (Mez, 1896[36]; Smith, 1934[40]) have also been proposed. An affinity with the latter family has found support in molecular systematic studies based on the plastid gene *rbc*L, which consistently place Rapateaceae near Bromeliaceae within a commelinoid clade (Chase et al., 1993[5], 1995[6]; Duvall et al., 1993[13]; see also Horres et al., 2000[24]). Recently, a more detailed *rbc*L analysis resolved Rapateaceae as sister to a clade comprising the Bromeliaceae and the small Neotropical aquatic family Mayacaceae (see Fig. **1 b**; Givnish et al., 1999[19]). However, this relationship was supported only weakly, and a broader analysis incorporating data from one nuclear (18S rDNA) and two plastid (*atp*B, *rbc*L) regions suggested an alternative arrangement, with the Rapateaceae placed sister to the remaining Poales, within which the Bromeliaceae and the Mayacaceae are embedded and widely separated (Chase et al., 2000[7]). This phylogenetic arrangement is also only weakly supported.

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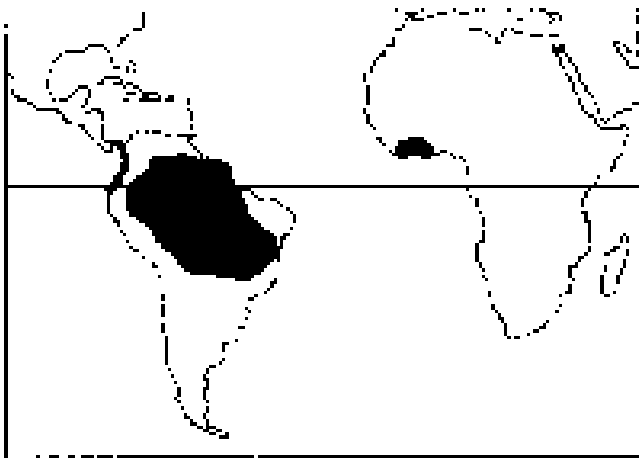

Fig. 1 a Approximate geographical distribution of Rapateaceae (after P. Berry, personal communication).

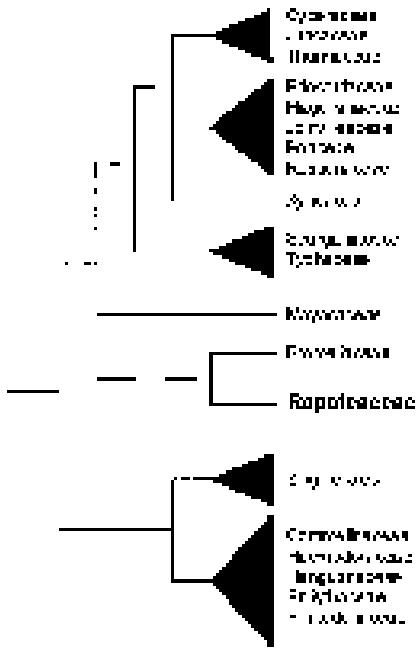

Fig. 1 b A summary of possible relationship of Rapateaceae based on cladistic analyses of nucleotide sequence data (Chase et al., 1995 [6]; Givnish et al., 1999[19]; Chase et al., 2000[7]). Relationship denoted by dotted lines lack strong character support and should be considered tentative.

We are currently conducting a survey of the large Neotropical family Bromeliaceae using the plant tissue carbon-isotope ratio ($\delta^{13}C$) to determine the taxonomic distribution of photosynthetic pathways (crassulacean acid metabolism [CAM] and C_3). If future work confirms the possible sister relationship of Rapateaceae and Bromeliaceae (Givnish et al., 1999[19]), knowledge of the photosynthetic pathway in the former would be of great interest for two reasons: (1) their phylogenetic proximity to the Bromeliaceae (one of the major families containing CAM plants) would raise the possibility of CAM being present in rapateads, some of which are remarkably similar in life form to certain CAM bromeliads; and (2) knowledge of the photosynthetic pathway in Rapateaceae may aid in determining the ancestral photosynthetic pathway for the Bromeliaceae by mapping the character states "CAM" and "C_3" onto cladograms under the parsimony criterion. To our knowledge, the photosynthetic pathway has not been determined for any Rapateaceae.

The relative abundance (measured in parts per thousand, ‰) of the stable carbon isotopes ^{12}C and ^{13}C in plant tissue ($\delta^{13}C$ value) has been used extensively as an index of the relative contribution of CAM to photosynthetic carbon gain (e.g., Osmond et al., 1973[39]; Griffiths and Smith, 1983[21]; Winter et al., 1983[45]; Winter and Smith, 1996[44]). In the CAM and C_4 pathways, the primary CO_2-fixing enzyme is phospho*enol*-pyruvate (PEP) carboxylase, which discriminates less strongly against the heavier isotope, ^{13}C, than does ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), the primary CO_2-fixing enzyme in C_3 plants. C_3 plants typically show $\delta^{13}C$ values in the range – 35‰ to – 23‰, and CAM plants typically show $\delta^{13}C$ values in the range – 19‰ to – 10‰ (Osmond et al., 1973[39]; Griffiths and Smith, 1983[21]; Winter et al., 1983[45]). In the present work, we have investigated photosynthetic pathways within Rapateaceae by determining the $\delta^{13}C$ values for herbarium material of 85 out of the approximately 100 species in the family. The significance of these values is discussed in terms of the ecological characteristics of the species in this relatively little-studied group of monocotyledons.

Materials and Methods

Samples of dried tissue of species of Rapateaceae were collected from various herbaria (Missouri Botanical Garden, St. Louis, Missouri, USA; Marie Selby Botanical Gardens, Sarasota, Florida, USA; US National Herbarium, Smithsonian Institution, Washington, DC, USA) during 1998 and 1999. Where available, samples were taken from the leaf lamina. However, some species are represented by one or very few (and hence valuable) collections. Therefore, in some cases samples were taken from a less taxonomically important part of the specimen, such as the leaf base or the inflorescence axis. Collection information was recorded where available from the herbarium sheets and ecological information from the sheets or from field knowledge (P. Berry, personal communication).

Natural abundance of ^{12}C and ^{13}C was measured for each sample at the Duke University Phytotron (Durham, North Carolina, USA) using a SIRA Series II isotope ratio mass spectrometer (Micromass, Manchester, UK) operated in automatic trapping mode after combustion under oxygen (DUMAS combustion) of samples (about 5 mg) in an elemental analyser (NA 1500 Series 1, Carlo Erba Instrumentazione, Milan, Italy). The reference CO_2, calibrated against standard Pee Dee belemnite, was obtained from Oztech (Dallas, Texas, USA). A system check of analysis of combustion and mass spectrometer measurement was performed after every ten samples. This was achieved with two working standards of cellulose (Sigma, St. Louis, Missouri, USA), which had $\delta^{13}C$ values of -24.10 ± 0.03‰ and -23.55 ± 0.06‰, respectively. The ^{12}C and ^{13}C values were corrected for oxygen isotope contribution using the measured $\delta^{18}O$ and the method of Craig (1957[9]). The $\delta^{13}C$ value was determined from the following formula:

$$\delta^{13}C\ (‰) = \left[\frac{^{13}C_{sample} / {}^{12}C_{sample}}{^{13}C_{PDB} / {}^{12}C_{PDB}} - 1 \right] \times 1000$$

where PDB refers to Pee Dee belemnite.

Results

A total of 91 samples was collected and analysed, representing 16 genera and 85 species of Rapateaceae; *Duckea cyperaceoidea, D. flava, D. junciformis, D. squarrosa* and *Rapatea longipes* were each sampled more than once. This represents 85% of species in the family based on a current estimate of 100 species (Givnish et al., 2000[20]). Only one genus, the monotypic *Phelpsiella* Maguire, was not sampled.

The $\delta^{13}C$ values for all 91 samples are listed in Table **1**. They ranged from – 37.7‰ for both *Rapatea paludosa* and *Saxofridericia subcordata* to – 19.8‰ for *Saxofridericia compressa.* Five species were represented by samples obtained from more than one collection. The $\delta^{13}C$ values for different samples for one of these, *Duckea cyperaceoidea,* were very similar (– 28.2‰, – 28.2‰, – 29.1‰). The difference between values obtained for the replicate samples of four other species was somewhat greater: *Rapatea longipes,* 1.5‰; *Duckea flava,* 1.9‰; *D. junciformis,* 1.9‰; *D. squarrosa,* 4.0‰. The $\delta^{13}C$ values for the 85 species sampled were approximately normally distributed, with a mean and standard deviation of – 28.5‰ and 4.1‰, respectively (Fig. **2**). Where more than one sample was analysed for a species, the mean $\delta^{13}C$ value of the replicates was used.

The great majority of samples analysed showed $\delta^{13}C$ values characteristic of C_3 plants. Six species *(Kunhardtia rhodantha, Marahuacaea schomburgkii, Saxofridericia compressa, Stegolepis grandis, St. guianensis, St. squarrosa),* however, showed $\delta^{13}C$ values ranging from – 23.0‰ to – 19.8‰, which are less negative than the values typically observed for C_3 plants, but are intermediate between those typically observed for C_3 plants and those of plants with pronounced CAM. Although many Rapateaceae occur at low altitudes, all of the plants that showed $\delta^{13}C$ values less negative than – 23‰ were collected at altitudes above 1200 m (Table **1**).

Other species showed $\delta^{13}C$ values that are unusually low for C_3 plants. For example, *Rapatea paludosa* and *Saxofridericia subcordata* showed $\delta^{13}C$ values of – 37.7‰. A further 14 species showed $\delta^{13}C$ values more negative than – 32‰ (Table **1**).

The $\delta^{13}C$ values were plotted against altitude for the 73 samples for which altitudinal data were available. Where an altitudinal range was recorded, the median value was used. The linear regression indicates a highly significant correlation ($r^2 = 0.420$, $p < 0.001$), with specimens showing less negative $\delta^{13}C$ values with increasing altitude (Fig. **3**). The fitted linear regression suggested that the $\delta^{13}C$ value increased by 8.2‰ from – 30.7 to – 22.5‰ over an altitudinal range from sea level to 2500 m, or by an average of 3.3‰ per 1000 m.

Discussion

Based on the $\delta^{13}C$ values obtained in this study, it appears that most, if not all, Rapateaceae are C_3 plants. Six of the 85 species studied, however, showed $\delta^{13}C$ values between – 23.0‰ and – 19.8‰ (Table **1**), which is less negative than the great majority of values recorded for C_3 plants. For example, Körner et al. (1988[31]) sampled 100 C_3 species from a diverse range of altitudes, habitats and taxonomic groups, and the least negative $\delta^{13}C$ value recorded was – 22.7‰. CAM plants usually show $\delta^{13}C$ values less negative than – 19.0‰, although there are some notable exceptions. For example, the confirmed CAM plants *Tillandsia usneoides* (L.) L. (Bromeliaceae), *Didierea madagascariensis* Baill. (Didiereaceae) and *Microsorum punctatum* (L.) Copel. (Polypodiaceae) show $\delta^{13}C$ values of – 19.8‰ (Griffiths and Smith, 1983[21]), – 21.2‰ (Winter, 1979[43]) and – 22.6‰ (Holtum and Winter, 1999[23]), respectively. Thus, the discovery of $\delta^{13}C$ values less negative than – 23.0‰ in Rapateaceae raises the interesting possibility of CAM in this family of mostly terrestrial mesophytes. These species are unlikely to be C_4 plants: Kranz anatomy is not known to occur in Rapateaceae (Carlquist, 1969[3]), and these specimens show $\delta^{13}C$ values that are much more negative than those usually exhibited by C_4 plants (i.e., – 10 to – 14‰; Cerling, 1999[4]).

Factors other than photosynthetic pathway that may affect $\delta^{13}C$ values in plants include altitude, drought stress, relative humidity, salinity, tissue type and the isotope composition of the source CO_2. Previous work has demonstrated a trend toward less negative $\delta^{13}C$ values with increasing altitude in C_3 plants (Körner et al., 1988[31]; Marshall and Zhang, 1993[34]; Cordell et al., 1999[8]) associated with lower intercellular CO_2 concentrations. Körner et al. (1988[31]), in their study of 100 species of C_3 plants, found that the mean $\delta^{13}C$ value differed by 4‰ over an altitudinal range of 5600 m. Within a species, the difference may be as great as 6‰ over a range of 2500 m (*Metrosideros polymorpha* Gaudich.; Cordell et al., 1999[8]). Our data show a difference of 8.2‰ over a range of 2500 m according to the linear regression analysis (Fig. **3**), one of the steepest relationships yet reported.

Increasing salinity and drought stress may also lead to less negative $\delta^{13}C$ values in C_3 plants due to decreased stomatal conductance and hence increased diffusional limitation of CO_2 uptake. For example, in the C_3 mangrove *Avicennia marina* (Forssk.) Vierh. var. *australasica* (Walp.) Moldenke, $\delta^{13}C$ values as high as – 19.6‰ (with a range of 4.4‰) have been reported (Farquhar et al., 1982[15]). However, Rapateaceae are very unlikely to suffer salt stress in their natural environments. Nonetheless, although most Rapateaceae, including those that show the highest $\delta^{13}C$ values, grow in generally mesic sites, (Maguire, 1982[33]), they may experience drought stress during the dry season (January to March; P. Berry, personal communication).

The determinations for *Saxofridericia compressa* (– 19.8‰) and *Stegolepis grandis* (– 21.4‰) were made on sclerenchyma from the inflorescence axis, whereas most other determinations were made on leaf lamina tissue. Tissues that are lipid-rich, such as those comprised mainly of living cells, are relatively depleted in ^{13}C and may therefore show more negative $\delta^{13}C$ values, whereas tissues composed largely of dead cells, such as sclerenchyma, may show less negative $\delta^{13}C$ values (Ziegler, 1996[46]). However, Winter (1979[43]) found little variation in the $\delta^{13}C$ value between sclerenchymatous and non-sclerenchymatous organs in CAM plants from Madagascar.

The $\delta^{13}C$ value is dependent on the isotope composition of the source CO_2 (Farquhar et al., 1989[16]), which, in the case of atmospheric CO_2, may vary geographically and chronologically (Keeling, 1958[27], 1961[28]; Keeling et al., 1979[29], 1989[30]). For example, over the period 1956 to 1982, the atmospheric ^{13}C composition decreased from – 6.7‰ (at 314 ppm) to – 7.9‰

Table 1 List of carbon-isotope ratios for species of Rapateaceae, together with information on the source of the material and ecology of the species

Taxon	Collector and herbarium[a]	Date[b]	Altitude[b] (m)	Life form[c]	Material[d]	$\delta^{13}C$ (‰)
Amphiphyllum						
A. rigidum Gleason	O. Huber 13225 (MO)		1520	T*	I.I.	– 25.7
Cephalostemon						
C. affinis Körn.	B. Maguire 36606 (US)	20.11.1953	125	T	I.I.	– 28.4
C. angustatus Malme	H. Irwin et al. 13859 (US)	9.3.1966	1200	T	I.I.	– 27.5
C. gracilis (Poepp. and Endl.) R. H. Schomb.	S. Hill et al. 12978 (US)	7.7.1983		T	I.I.	– 27.7
C. microglochin Sandwith	O. Huber 4620 (US)	10.10.1979	600	T	I.I.	– 29.2
C. riedelianus Körn.	W. Anderson et al. 36230 (US)	18.2.1972	1125	T	I.I.	– 26.3
Duckea						
D. cyperaceoidea (Ducke) Maguire	B. Maguire 37573 (US)	7.2.1954	110	T	I.I.	– 28.2
D. cyperaceoidea (Ducke) Maguire	P. Berry and E. Melgueiro 5386 (SEL)	10.11.1992	100	T	I.I.	– 29.1
D. cyperaceoidea (Ducke) Maguire	P. Berry 6269 et al. (MO)	26.5.1996	110	T	I.b.	– 28.2
D. flava (Link) Maguire	G. Davidse 17346 (US)	8.5.1979	120	T	I.I.	– 29.1
D. flava (Link) Maguire	B. Stergios 16365 (US)	9.11.1994		T*	I.I.	– 31.0
D. junciformis Maguire	B. Maguire et al. 41866 (US)		130	T	I.I.	– 26.6
D. junciformis Maguire	P. Berry and I. Sanchez 5052 (US)	3.7.1991	125	T	I.I.	– 28.5
D. squarrosa (Willd. ex Link) Maguire	G. Davidse et al. 17200 (US)	6.5.1979		T	I.I.	– 29.7
D. squarrosa (Willd. ex Link) Maguire	P. Berry 6131 et al. (MO)	9.3.1996	110	T	I.I.	– 33.7
Epidryos						
E. guayanensis Maguire	T. Henkel et al. 4296 (US)	11.11.1993	1150 – 1200	E	I.I.	– 25.6
E. micrantherus (Maguire) Maguire	A. Gentry 65549 et al. (MO)	7.2.1989	50	E	I.I.	– 31.0
Guacamaya						
G. superba Maguire	B. Maguire and J. Wurdack 35619 (US)	14.4.1953	140	T*	I.I.	– 27.7
Kunhardtia						
K. radiata Maguire and Steyerm.	A. Gentry and P. Berry 14551 (MO)	29.6.1975	150	T	I.I.	– 28.9
K. rhodantha Maguire	Steyermark 105114 (US)	20. – 22.9.1971	1230 – 1240	T	I.b.	– 21.9
Marahuacaea						
M. schomburgkii (Maguire) Maguire	Steyermark et al. 126019 (US)	2.2.1982	2480 – 2500	T*	I.I.	– 22.6
Maschalocephalus						
M. dinklagei Gilg and K. Schum.	J. Baldwin Jr. 11364 (MO)	11.3.1948		T*	I.I.	– 37.3
Monotrema						
M. aemulans Körn.	M. Jansen-Jacobs et al. 1429 (US)	3.9.1989	240 – 260	T	I.t.	– 28.2
M. affine Maguire	B. Maguire et al. 41500 (US)	9/1957	125	T	I.t.	– 26.0
M. arthrophyllum (Seub.) Maguire	R. Schultes and I. Cabrera s.n. (US)		274 – 305	T	I.I.	– 28.4
M. bracteatum Maguire	B. Maguire et al. 36605 (US)	11/1953	150	T	I.I.	– 29.9
M. xyridoides Gleason	G. Prance et al. 28884 (US)	7.2.1984		T	I.I.	– 29.7
Potarophytum						
P. riparium Sandwith	J. Pipoly 9999 (US)	26.1.1987	400	T	I.I.	– 31.2
Rapatea						
R. angustifolia Spruce ex Körn.	B. Maguire et al. 36646 (US)		120	T	I.I.	– 31.9
R. aracamuniana Steyerm.	R. Liesner and F. Delascio 22197 (MO)	20.10.1987	600	T*	I.I.	– 30.6
R. chimantensis Steyerm.	J. Steyermark 75584 (MO)	5/1953	1000	T*	I.I.	– 33.5
R. circasiana García-Barr. and Mora	J. Zarucchi and M. Balick 1807 (US)	1.7.1976		T*	I.I.	– 33.8
R. elongata G. K. Schulze	B. Maguire et al. 56536 (US)	3.9.1963		T	I.I.	– 34.2
R. fanshawei Maguire	J. Pipoly 7645 (US)	12.6.1986	550	T	I.I.	– 31.8
R. linearis Gleason	J. Pipoly 9513 (US)	21.12.1986	25	T	I.I.	– 27.6
R. longipes Spruce ex Körn.	C. Berg et al. 19498 (US)	13.11.1993		T	I.I.	– 35.5
R. longipes Spruce ex Körn.	R. Schultes and I. Cabrera 17500 (US)	18.9.1952		T	I.I.	– 34.0
R. membranacea Maguire	S. Tillet et al. 43972 (US)	3.7.1960	1140	T	I.b.	– 28.6
R. muaju García-Barr. and Mora	R. Schultes and I. Cabrera 16621 (US)	5.6.1952	213	T	I.I.	– 34.8
R. paludosa Aubl.	B. Hoffman 1412 (US)	22.4.1992	60 – 70	T	I.I.	– 37.7
R. pycnocephala Seub.	G. Prance et al. 24960 (US)	6.11.1977		T*	I.I.	– 30.1
R. saülensis B. M. Boom	T. Croat 74180 (MO)	10.2.1993	350	T	I.I.	– 34.3
R. spectabilis Pilg.	W. Anderson 11830 (US)	24.1.1978		T	I.I.	– 34.7

continued next page

Table 1 continued

Taxon	Collector and herbarium[a]	Date[b]	Altitude[b] (m)	Life form[c]	Material[d]	$\delta^{13}C$ (‰)
R. spruceana Körn.	G. Davidse et al. 16887 (US)	30.4.–1.5.1979	100	T	I.I.	– 28.8
R. steyermarkii Maguire	T. McDowell 2812 (US)	24.5.1990	900–975	T*	I.I.	– 35.1
R. ulei Pilg.	T. McDowell 3934 and A. Stobey (MO)		396	T	I.I.	– 32.2
R. undulata Ducke	G. Prance et al. 23867 (US)	17.10.1976		T*	I.I.	– 37.5
R. wettsteinii Suess. vel. sp. aff.	J. Zarucchi 1970 (MO)	7.9.1976		T	I.I.	– 30.3
R. xiphoides Sandwith	L. Kvist et al. 110A (US)	7.10.1987	550	T*	I.I.	– 32.9
R. yapacana Maguire	R. Kral and O. Huber 70715 (MO)	10.8.1983	100	T*	I.b.	– 29.3
Saxofridericia						
S. aculeata Körn.	G. Prance et al. 4982 (US)	5.6.1968		T*	I.b.	– 31.1
S. compressa Maguire	T. Croat 59543 (US)	1.12.1984	2200	T	i.a.	– 19.8
S. duidae Maguire	Steyermark et al. 126416 (US)	10.2.1982	1230	T*	I.I.	– 25.4
S. grandis Maguire	O. Huber 4347 (US)	4.10.1979	1100	T*	I.I.	– 29.7
S. inermis Ducke	B. Maguire et al. 42624 (US)	11.1.1958	110	T	I.I.	– 30.1
S. regalis R. H. Schomb.	W. Hahn et al. 4491 (US)	13.4.1988	500	T	i.a.	– 24.7
S. spongiosa Maguire	B. Maguire 42621 (US)	10.1.1958	150–200	T	I.I.	– 25.3
S. subcordata Körn.	W. Kress et al. 94-3635 (US)		50	T	I.I.	– 37.7
Schoenocephalium						
S. cucullatum Maguire	C. Calderon 2747 (US)	2.7.1979		T	I.I.	– 26.1
S. martianum Seub.	A. Gentry and M. Sanchez 65169 (MO)	25.1.1989	250	T	I.b.	– 28.3
S. schultesii Maguire	R. Schultes and I. Cabrera 14506 (MO)	29.10.1951		T*	I.I.	– 31.2
S. teretifolium Maguire	O. Huber and E. Medina 5854 (US)	8.2.1981	125	T	I.I.	– 29.4
Spathanthus						
S. bicolor Ducke	G. Davidse et al. 17440 (US)	8.5.1979	120	T	I.I.	– 25.0
S. unilateralis (Rudge) Desv.	P. Mutchnik 738 (US)	14.2.1995	50	T*	I.I.	– 36.1
Stegolepis						
S. albiflora Steyerm.	O. Huber 13019 (MO)		1750–1800	T	I.I.	– 25.2
S. angustata Gleason	L. Gillespie and H. Persaud 900 (US)	29.3.1989	450	T	I.b.	– 25.8
S. cardonae Maguire	O. Huber and M. Colella 8929 (US)	10.–12.2.1984	2250	L	I.b.	– 23.4
S. celiae Maguire	G. Davidse and J. Miller 27365 (US)	9.7.1984	400–700	L	I.I.	– 27.6
S. choripetala Maguire	O. Huber 12724 (US)	28.3.1988		T	I.I.	– 25.6
S. ferruginea Baker	S. Tillet et al. 43943 (US)	3.7.1960	824	E	I.I.	– 30.0
S. grandis Maguire	Steyermark et al. 109303 (US)	22.2.1974	1750–1800	T*	i.a.	– 21.4
S. guianensis Klotzsch ex Körn.	B. Hoffman and T. Henkel 3217 (US)	3.11.1992	1800–2000	T	I.I.	– 22.3
S. hitchcockii Maguire	R. Cowan and J. Wurdack 31194 (US)	2.2.1951	2000	T	I.b.	– 24.7
S. huberi Steyerm.	O. Huber 9224 (MO)		1200	T	I.b.	– 27.3
S. humilis Steyerm.	R. Liesner 21075 et al. (MO)	26.5.1986	2135	T*	I.b.	– 24.3
S. jauaensis Maguire	Steyermark et al. 109249 (US)	2–3/1974	1800	T*	i.a.	– 23.6
S. ligulata Maguire	J. Luteyn et al. 9480 (US)	14.2.1984	1920	T	I.b.	– 23.4
S. linearis Gleason	S. Tillet et al. 751-75 (US)	1–2/1975	1350	L	I.b.	– 24.6
S. maguireana Steyerm.	O. Huber 12468 (US)	28.1.1988	2150	T	I.I.	– 23.0
S. membranacea Maguire	G. Prance and J. Guedes 29545 (US)	15.7.1985	800–900	L	I.I.	– 28.3
S. microcephala Maguire	O. Huber 13053 (MO)		1750–1800	T*	I.I.	– 26.8
S. neblinensis Maguire	B. Boom and A. Weitzman 5776 (US)	12.2.1985	1670–1690	T	I.b.	– 24.3
S. parvipetala Steyerm.	Steyermark et al. 132044 (US)	22.–24.5.1986	1800–1825	T*	I.b.	– 27.0
S. pauciflora Gleason	Steyermark 58330 (US)	4.9.1944	1820–2075	T*	I.I.	– 24.2
S. ptaritepuiensis Steyerm.	L. Gillespie and D. Smart 2782 (US)	19.12.1989	550	T	I.b.	– 25.6
S. pulchella Maguire	B. Maguire et al. 31744 (US)	2.2.1951	1800	L	I.b.	– 24.3
S. pungens Gleason	B. Maguire 29669 (US)	23.11.1950	2000–2300	T	I.I.	– 27.2
S. squarrosa Maguire	O. Huber 10395 (US)	27.3.1985	1400	T*	I.b.	– 22.5
S. steyermarkii Maguire	T. Henkel et al. 1533 (US)	20.2.1993	1530	T (E)*	I.I.	– 27.2
S. wurdackii Maguire	J. Steyermark 75463 (MO)	16.5.1953	1000–1700	T*	I.I.	– 24.9
Windsorina						
W. guianensis Gleason	B. Maguire 32143 (MO)	17.10.1951		T	I.I.	– 31.2

[a] Herbaria are denoted by their acronyms (Holmgren et al., 1990[22]): MO: Missouri Botanical Garden, St. Louis, Missouri, USA; SEL: Herbarium, Marie Selby Botanical Gardens, Sarasota, Florida, USA; US: United States National Herbarium, Smithsonian Institution, Washington, DC, USA.

[b] Refers to the date and the altitude at which the herbarium specimen was collected. Blank cells indicate the information was not available.

[c] The herbarium specimens were obtained from plants growing terrestrially (T), epiphytically (E), or lithophytically (L). This information was obtained from the specimen label or where the habit is marked with an asterisk, from published treatments (Maguire, 1982[33]) and field knowledge (P. Berry, personal communication).

[d] The material analysed was taken from either: leaf base (l.b.), leaf lamina (l.l.), leaf tip (l.t.), or the inflorescence axis (i.a.).

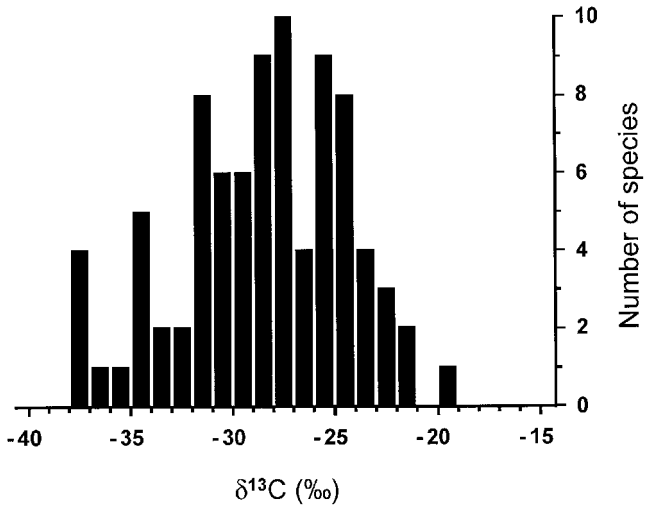

Fig. 2 Frequency histogram of $\delta^{13}C$ values of 85 species of Rapateaceae in class intervals of 1.0‰. For those species for which more than one sample was analysed, the mean $\delta^{13}C$ value of the replicates was used.

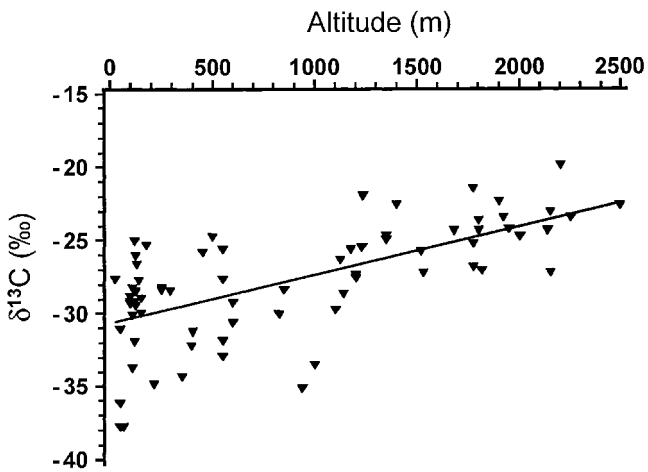

Fig. 3 Linear regression of $\delta^{13}C$ value against altitude for 73 samples of Rapateaceae ($y = 0.00330\,x - 30.7$, $r^2 = 0.420$, $p < 0.001$).

(at 342 ppm) (Keeling et al., 1979[29]; Mook et al., 1983[38]) due to anthropogenic fossil fuel emissions. As a result, plants collected many years ago may contain relatively more ^{13}C (and hence show less negative $\delta^{13}C$ values) compared with those collected more recently. The specimens sampled in this study were collected between the years 1944 and 1996, whereas the six species identified as showing CAM-like $\delta^{13}C$ values were collected between the years 1971 and 1992. Thus, it is unlikely that chronological variation in atmospheric carbon isotope composition can account for the relatively high $\delta^{13}C$ values in these species.

Of interest is the observation that the rapateads with the least negative $\delta^{13}C$ values *(Kunhardtia rhodantha, Marahuacaea schomburgkii, Saxofridericia compressa, Stegolepis grandis, St. guianensis, St. squarrosa),* indicating the possibility of CAM, were all plants growing at middle to high altitudes (Table **1**). While CAM plants are certainly known from high altitudes (e.g., cacti in the Andes of Chile and Peru; Gibson and Nobel, 1986[18]), field studies have shown that the occurrence of CAM tends to decrease with altitude. In Papua New Guinea, CAM epiphytes are absent from the highest altitudes (Earnshaw et al., 1987[14]), and in northern Venezuela *Clusia* L. species do not perform CAM above about 1500 m (Diaz et al., 1996[12]). The discovery of the restriction of CAM to relatively high altitudes in a family of mesophytes would be of great interest. It should be noted, however, that these rapateads conform to the altitudinal trend in $\delta^{13}C$ values discussed above (Fig. **3**), and thus perhaps more likely represent values towards the upper limit of those occurring in C_3 plants.

Most Rapateaceae grow in mesic conditions in which CAM would seem to confer little or no ecological advantage. Some species grow epiphytically (*Epidryos* spp.) or lithophytically (*Stegolepis* spp.), but in habitats where the water supply is at least seasonally plentiful, such as cloud forests or seepage or splash zones. In these conditions, it is not expected that there would be strong selection for improved water economy and indeed, these species show $\delta^{13}C$ values typical of C_3 plants (Table **1**).

Although $\delta^{13}C$ values can provide unequivocal evidence for the presence of the CAM pathway, the converse is not necessarily true: some taxa that can perform CAM under natural conditions show $\delta^{13}C$ values in the range typical of C_3 plants. Griffiths and Smith (1983[21]) detected significant nocturnal increases in titratable acidity in two species of Bromeliaceae in Trinidad, *Tillandsia elongata* Kunth var. *subimbricata* (Baker) L. B. Sm. and *Guzmania monostachia* (L.) Rusby ex Mez var. *monostachia,* that had leaf $\delta^{13}C$ values of – 26.4‰ and – 26.5‰, respectively. Therefore it is possible that other Rapateaceae with $\delta^{13}C$ values more negative than – 23.0‰ might be capable of performing CAM, but the identification of such plants would require systematic investigation of living material and is beyond the scope of this study. Future work to identify such species should perhaps initially be focused on the taxa most closely related to those reported herein to show CAM-like carbon isotope ratios.

A recent molecular phylogeny of Rapateaceae (Givnish et al., 2000[20]) resolved species of *Kunhardtia* and *Saxofridericia* within a derived clade that was sister to a clade containing, *inter alia,* species of *Marahuacaea* and *Stegolepis.* This suggests that CAM, if confirmed in those species with the least negative $\delta^{13}C$ values (viz. *Kunhardtia rhodantha, Marahuacaea schomburgkii, Saxofridericia compressa, Stegolepis grandis, St. guianensis, St. squarrosa*), is a derived condition in Rapateaceae, and may have arisen more than once in the family. However, the relationships suggested by the molecular analysis need to be corroborated by further analyses with other data and more complete taxon sampling.

Very negative $\delta^{13}C$ values have been reported for plants growing in moist, shaded conditions (Flanagan et al., 1997[17]), in which high intercellular CO_2 concentrations during photosynthetic CO_2 uptake allow increased isotope discrimination by Rubisco. However, more negative plant $\delta^{13}C$ values can also result from ^{13}C-depleted source air, as can occur in the understory of forests (Medina and Minchin, 1980[35]; Broadmeadow and Griffiths, 1993[1]; Buchmann et al., 1998[2]). The rapateads with the most negative $\delta^{13}C$ values (e.g., *Rapatea paludosa* and

Saxofridericia subcordata) are generally restricted to wet or seasonally inundated forest understories (P. Berry, personal communication) at the low end of the altitudinal range for the family, where the combination of ^{13}C-depleted source air and shady, mesic conditions may have resulted in some of the most negative $\delta^{13}C$ values yet reported for terrestrial plants growing under natural conditions (Körner et al., 1988[31]; Farquhar et al., 1989[16]; Flanagan et al., 1997[17]).

This study is consistent with previous work demonstrating a general increase in $\delta^{13}C$ of plant tissue with increasing altitude (Körner et al., 1988[31]; Marshall and Zhang, 1993[34]; Cordell et al., 1999[8]), but the possibility that CAM may contribute to carbon gain in some Rapateaceae cannot be excluded. This needs to be followed up by studies of day-night gas exchange patterns and tissue organic-acid fluctuations in this family. However, most Rapateaceae grow in the relatively inaccessible Guayana Shield and we know of very few species in cultivation. Even if further investigations confirmed CAM in those Rapateaceae showing high $\delta^{13}C$ values, this pathway would still constitute a relatively minor evolutionary theme in this family. However, it would be of considerable interest since to date CAM has not been detected in Rapateaceae.

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