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Original Article

$\delta^{13}\text{C}$ values and crassulacean acid metabolism in *Clusia* species from Panama

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Abstract The genus *Clusia* is notable in that it contains arborescent crassulacean acid metabolism (CAM) plants. As part of a study of CAM in *Clusia*, titratable acidities were measured in 25 species and $\delta^{13}\text{C}$ values were measured for 38 species from Panamá, including seven undescribed species, and 11 species from Colombia, Costa Rica and Honduras. CAM was detected in 12 species. *Clusia flava*, *C. rosea* and *C. uvitana* exhibited $\delta^{13}\text{C}$ values or diurnal fluctuations in acidity indicative of strong CAM. In *C. croatii*, *C. cylindrica*, *C. fructianguستا*, *C. lineata*, *C. odorata*, *C. pratensis*, *C. quadrangula*, *C. valerioi* and *C. sp.* D diurnal fluctuations in acidity were consistent with weak CAM but the $\delta^{13}\text{C}$ values were C_3 -like. All of the species that exhibited strong or weak CAM were in the *C. flava* or *C. minor* species groups. CAM was not detected in any member of the *C. multiflora* species group. Strong CAM species were not collected at altitudes above 680 m a. s.l. On the basis of $\delta^{13}\text{C}$ values, the expression of CAM was similar in terrestrial, hemi-epiphytic and epiphytic species and did not differ between individuals of the same species

that exhibited different life-forms. This study indicates that phylogenetic affiliation may be a predictor of an ability to exhibit CAM in *Clusia* species from the Panamanian region, and that weak CAM is probably a common photosynthetic option in many *Clusia* species. $\delta^{13}\text{C}$ value is not a particularly good indicator of a potential of *Clusia* species growing in the field to exhibit CAM because it appears that the contribution in most species of CAM to carbon gain is generally rather small when integrated over the life-time of leaves.

Keywords *Clusia* - Crassulacean acid metabolism (CAM) - Photosynthetic pathway - Stable carbon isotopes

Introduction

Arborescent plants within the genus *Clusia*, a neotropical group comprising an estimated 300 species, exhibit a remarkable diversity of life-forms that includes terrestrial shrubs and trees, epiphytes, hemi-epiphytes (including stranglers) and lianas (Hammel [1986](#); Pipoly et al. [1998](#)). Within the genus, it is not uncommon for a species to exist as an epiphyte, hemi-epiphyte or as a terrestrial form. This plasticity of life-form extends to a plasticity in the expression of photosynthetic physiology, with crassulacean acid metabolism (CAM), C_3 -CAM intermediate and C_3 species of *Clusia* having been described (Tinoco Ojanguren and Vásquez-Yanez [1983](#); Franco et al. [1990](#); Borland et al. [1992](#); Lüttge [1996](#), [1999](#)).

Recent molecular phylogenies based on comparisons of *rbcL* and ITS sequences from species of *Clusia* from Central and South America have made substantial progress towards reconciling molecular and morphological characters at both the family and genus levels (Gustafsson et al. [2002](#); Gustafsson and Bittrich [2002](#); Vaasen et al. [2002](#); Gehrig et al. [2003](#)). The Central American *Clusia* can be divided into three major clades that broadly correspond to three morphological species groups, the *C. flava* group, the *C. minor* group and the *C. multiflora* group (Hammel [1986](#); Gehrig et al. [2003](#)). CAM has been reported in at least some members of the *C. flava* and *C. minor* groups, whereas CAM has not been reported for any member of the *C. multiflora* group. Mapping of photosynthetic pathway onto the phylogenetic trees, at least for the few species for which photosynthetic pathway is known, is consistent with CAM having evolved in two of the three *Clusia* clades. However, the photosynthetic pathway is not known for the majority of the species that were used to derive the phylogenies.

Ascribing the presence or absence of CAM to a *Clusia* species can be problematic as few of

the *Clusia* species studied to date are obligate CAM or C₃ species. Many appear to be C₃-CAM intermediates in which the expression of CAM is strongly modulated by environment (Lüttge 1999). In intermediate species, individuals of the same species sampled at different sites, different times of the year, or at different developmental stages may or may not exhibit the same patterns of light–dark net CO₂ exchange and so the contributions of the CAM cycle to carbon gain need to be assessed under a range of conditions.

Surveys of the contributions of dark fixation to carbon gain are typically performed by measuring $\delta^{13}\text{C}$ values, diel variations in organic acid content or, less commonly, by measuring net CO₂ exchange (Winter et al. 1983; Earnshaw et al. 1987; Arroyo et al. 1990; Winter and Smith 1996; Crayn et al. 2001; Pierce et al. 2002a, b; Crayn et al. 2004). Measurements of $\delta^{13}\text{C}$ values are less time consuming and are most convenient for large species surveys but $\delta^{13}\text{C}$ values alone do not reveal species in which CAM makes a small contribution to total carbon gain, a characteristic common to many C₃-CAM intermediate species (Borland et al. 1993; Holtum and Winter 1999; Winter and Holtum 2002). Small contributions of dark fixation to carbon gain are best surveyed by measuring diel variations in leaf titratable acidity. Measurements of net CO₂ exchange are the most revealing but are more time-consuming and thus less amenable to large surveys.

In order to evaluate the robustness of the phylogenetic tree-based predictions about the evolutionary origins of CAM in *Clusia*, we have measured $\delta^{13}\text{C}$ values for 31 species described for Panamá and for 7 undescribed species, and for 11 species from Colombia, Costa Rica and Honduras. We have also measured titratable acidities in 25 of the species in order to test for the presence of weak CAM.

Our observations, which include the first report of strong CAM in *C. flava* and of weak CAM in 7 species of *Clusia*, support the view that phylogenetic affiliation may be a predictor of an ability to exhibit CAM in *Clusia*, and that weak CAM is a relatively common photosynthetic option, particularly in lowland species of *Clusia* from Central America.

Materials and methods

Plant material and carbon isotope determinations

Plant material was collected in 2000–2002 during field excursions to different provinces of

the Republic of Panamá. The life-form of each specimen was recorded and the location and altitude were estimated using GPS (Garmin Plus III, Garmin International, Kan.). Voucher numbers listed in Table 1 refer to the plant specimens lodged in the Plant Physiology Research Group Collection, Smithsonian Tropical Research Institute (STRI). Specimens of species not collected in the field by us were obtained from the Summit Herbarium, now located at the Smithsonian Tropical Research Institute (SCZ), or the herbarium of the Universidad de Panamá (PMA, Holmgren et al. [1990](#)).

Table 1 $\delta^{13}\text{C}$ values of leaves from *Clusia* spp. growing under natural conditions in Panamá. Specimens are lodged in the Summit Herbarium, now located at the Smithsonian Tropical Research Institute (SCZ), the herbarium of the Universidad de Panamá (PMA, Holmgren et al. [1990](#)) or in the Plant Physiology Research Group Collection, Smithsonian Tropical Research Institute (STRI). Life forms are defined as tree or shrub (*T*), hemi-epiphyte (*H*) or epiphyte (*E*). ? denotes tentative identification by B.E. Hammel (Gehrig et al. [2003](#)). *ND* not determined

Taxon	Collector, voucher number and herbarium	Location	Altitude (m)	Life-form	$\delta^{13}\text{C}$ (‰)
<i>C. amazonica</i> Planch. and Triana	Mori and Kallunki 4674 PMA	El Llano-Cartí	≈350	H	-27.1
<i>C. amazonica</i> Planch. and Triana	McPherson 11885 PMA	El Llano-Cartí	250	T	-30.0
<i>C. coclensis</i> Standl. ?	Aranda et al. 3812 STRI	Cerro Colorado	1,139	T	-24.2
<i>C. coclensis</i> Standl.	Antonio 1492 PMA	Cerro Colorado	≈1,500	T	-24.8
<i>C. coclensis</i> Standl. ?	Aranda et al. 3851 STRI	Cerro Colorado	1,137	T	-25.1

<i>C. coclensis</i> Standl. ?	Aranda et al. 3813 STRI	Cerro Colorado	1,139	T	-26.1
<i>C. congestiflora</i> Cuatrec.	Aranda et al. 3711 STRI	El Llano-Cartí	492	T	-25.8
<i>C. congestiflora</i> Cuatrec.	Aranda et al. 3858 STRI	Cana	1,454	E	-29.5
<i>C. congestiflora</i> Cuatrec.	Aranda et al. 3857 STRI	Cerro Pirre	1,374	E	-30.1
<i>C. congestiflora</i> Cuatrec.	Aranda et al. 3868 STRI	Cerro Pirre	1,276	E	-31.1
<i>C. croatii</i> D ¹ Arcy	Aranda et al. 3759 STRI	Bocas del Toro	2	E	-25.3
<i>C. croatii</i> D ¹ Arcy	Aranda et al. 3764 STRI	Bocas del Toro	40	E	-26.2
<i>C. croatii</i> D ¹ Arcy	Aranda et al. 3802 STRI	Cerro Colorado	1,502	T	-26.7
<i>C. croatii</i> D ¹ Arcy	Aranda and Virgo 3680 STRI	Bocas del Toro	52	E	-26.8
<i>C. croatii</i> D ¹ Arcy	Aranda and Virgo 3673 STRI	Fortuna	1,142	T	-28.0
<i>C. croatii</i> D ¹ Arcy	Aranda et al. 3787 STRI	Fortuna	1,143	T	-28.4

<i>C. croatii</i> D [†] Arcy	Aranda et al. 3808 STRI	Cerro Colorado	1,689	H	-28.6
<i>C. croatii</i> D [†] Arcy	Aranda et al. 3853 STRI	Cerro Colorado	1,487	T	-28.8
<i>C. croatii</i> D [†] Arcy	Aranda et al. 3758 STRI	Bocas del Toro	76	H	-29.5
<i>C. croatii</i> D [†] Arcy	Aranda et al. 3761 STRI	Bocas del Toro	65	E	-29.5
<i>C. croatii</i> D [†] Arcy	Aranda et al. 3788 STRI	Fortuna	1,156	H	-29.6
<i>C. croatii</i> D [†] Arcy	Aranda et al. 3656 STRI	El Copé	842	H	-29.7
<i>C. croatii</i> D [†] Arcy	Aranda et al. 3762 STRI	Bocas del Toro	65	E	-29.9
<i>C. croatii</i> D [†] Arcy	Aranda et al. 3770 STRI	Bocas del Toro	145	T	-30.3
<i>C. cupulata</i> (Maguire) Maguire	Aranda et al. 3712 STRI	El Llano-Cartí	250	E	-26.7
<i>C. cupulata</i> (Maguire) Maguire	Folsom 3695 PMA	Santa Rita	ND	E	-27.5
<i>C. cupulata</i> (Maguire) Maguire	Aranda et al. 3688 STRI	El Llano-Cartí	343	T	-29.3

<i>C. cylindrica</i> Hammel	Aranda and Virgo 3683 STRI	Bocas del Toro	76	H	-24.9
<i>C. cylindrica</i> Hammel	Aranda et al. 3774 STRI	Bocas del Toro	85	T	-28.3
<i>C. cylindrica</i> Hammel	Aranda et al. 3832 STRI	Santa Fé	700	E	-30.3
<i>C. cylindrica</i> Hammel	Aranda et al. 3871 STRI	Cerro Pirre	780	E	-32.9
<i>C. divaricata</i> Maguire	Aranda et al. 3622 STRI	El Valle	876	T	-25.1
<i>C. divaricata</i> Maguire	Aranda et al. 3653 STRI	El Copé	798	H	-26.1
<i>C. divaricata</i> Maguire	Aranda et al. 3624 STRI	El Valle	863	T	-27.5
<i>C. divaricata</i> Maguire	Aranda et al. 3585 STRI	Altos de Campana	860	T	-27.9
<i>C. divaricata</i> Maguire	Aranda et al. 3586 STRI	Altos de Campana	860	T	-29.0
<i>C. flavida</i> (Benth.) Pipoly	Croat 16195 SCZ	Barro Colorado Is	ND	H	-23.4
<i>C. flavida</i> (Benth.) Pipoly	Correa and Dressler 764 PMA	Santa Rita	ND	E	-28.9

<i>C. flavida</i> (Benth.) Pipoly	Foster 1767 SCZ	Barro Colorado Is	ND	E	-30.1
<i>C. fructiangusta</i> Cuatrec.	Aranda et al. 3776 STRI	Bocas del Toro	447	H	-26.0
<i>C. fructiangusta</i> Cuatrec.	Aranda et al. 3834 STRI	Santa Fé	807	E	-26.4
<i>C. fructiangusta</i> Cuatrec	Aranda et al. 3692 STRI	El Llano-Cartí	320	T	-27.2
<i>C. fructiangusta</i> Cuatrec.	Aranda et al. 3691 STRI	El Llano-Cartí	320	T	-27.3
<i>C. gracilis</i> Standl.	Nee 11158 PMA	Santa Fé	870	T	-25.1
<i>C. gracilis</i> Standl.	McPherson 9151 PMA	Bocas del Toro	≈400	E	-28.4
<i>C. latipes</i> Planch. and Triana	Correa et al. 10108 SCZ	Rio Harino	≈900	T	-25.9
<i>C. latipes</i> Planch. and Triana	Folsom 3173 PMA	El Copé	≈700	E	-29.9
<i>C. liesneri</i> Maguire	Aranda et al. 3638 STRI	Cerro Jefe	918	T	-25.5
<i>C. liesneri</i> Maguire	Aranda et al. 3755 STRI	Cerro Jefe	950	H	-26.4
<i>C. liesneri</i> Maguire	Aranda and Virgo. 3670 STRI	Altos de Pacora	870	T	-26.7

<i>C. liesneri</i> Maguire	Aranda et al. 3637 STRI	Cerro Jefe	906	E	-27.3
<i>C. liesneri</i> Maguire	Aranda and Virgo 3671 STRI	Altos de Pacora	875	T	-27.6
<i>C. liesneri</i> Maguire	Aranda et al. 3630 STRI	Altos de Pacora	850	T	-27.9
<i>C. liesneri</i> Maguire	Aranda and Virgo 3669 STRI	Altos de Pacora	870	T	-27.9
<i>C. liesneri</i> Maguire	Aranda et al. 3835 STRI	Santa Fé	807	T	-29.2
<i>C. liesneri</i> Maguire	Aranda et al. 3652 STRI	El Copé	810	T	-29.4
<i>C. lineata</i> (Benth.) Planch. and Triana	Aranda et al. 3654 STRI	El Copé	810	H	-28.8
<i>C. lineata</i> (Benth.) Planch. and Triana	Aranda et al. 3655 STRI	El Copé	814	H	-28.8
<i>C. lineata</i> (Benth.) Planch. and Triana	Knapp et al. 4590 PMA	Portobelo	≈25	T	-31.9
<i>C. longipetiolata</i> Schery	Aranda et al. 3772 STRI	Bocas del Toro	145	T	-28.3
<i>C. minor</i> L.	Tyson 3720 SCZ	Veraguas	ND	T	-24.0
<i>C. minor</i> L.	Aranda et al. 3829 STRI	Rio Sereno	1,154	T	-27.0

<i>C. minor</i> L.	McPherson 12629 PMA	El Copé	≈825	T	-29.1
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3635 STRI	Cerro Jefe	915	T	-24.0
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3714 STRI	El Llano-Cartí	492	E	-24.7
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3807 STRI	Cerro Colorado	1,744	T	-24.7
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3698 STRI	Cerro Jefe	966	E	-25.3
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3806 STRI	Cerro Colorado	1,551	T	-25.7
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3628 STRI	Cerro Jefe	911	E	-25.8
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3615 STRI	El Valle	985	E	-26.1
<i>C. aff. multiflora</i> Kunth	Lazon 3329 PMA	Cerro Campana	ND	T	-26.1
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3700 STRI	Cerro Jefe	927	T	-26.3
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3805 STRI	Cerro Colorado	1,509	T	-26.4

<i>C. aff. multiflora</i> Kunth	Aranda et al. 3702 STRI	Cerro Azul	920	T	-26.6
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3803 STRI	Cerro Colorado	1,497	T	-26.9
<i>C. aff. multiflora</i> Kunth	Aranda et al. 3731 STRI	Santa Fé	500	T	-29.0
<i>C. odorata</i> Seem.	Aranda et al. 3823a STRI	Cerro Punta	1,591	T	-24.6
<i>C. odorata</i> Seem.	Aranda et al. 3811 STRI	Cerro Colorado	1,277	T	-26.9
<i>C. odorata</i> Seem.	Aranda et al. 3823b STRI	Cerro Punta	1,591	T	-26.9
<i>C. odorata</i> Seem.	Aranda et al. 3794 STRI	Boquete	1,432	T	-27.1
<i>C. osseocarpa</i> Maguire	Aranda et al. 3785 STRI	Fortuna	1,170	T	-24.8
<i>C. osseocarpa</i> Maguire	Aranda et al. 3645 STRI	Cerro Jefe	950	T	-25.6
<i>C. osseocarpa</i> Maguire	Aranda et al. 3850 STRI	Cerro Colorado	1,518	E	-27.0
<i>C. osseocarpa</i> Maguire	Aranda et al. 3634 STRI	Cerro Jefe	915	T	-28.0

<i>C. osseocarpa</i> Maguire	Aranda et al. 3658 STRI	El Copé	737	H	-29.2
<i>C. osseocarpa</i> Maguire	Aranda et al. 3639 STRI	Cerro Jefe	950	T	-29.9
<i>C. palmana</i> Standl.	Aranda et al. 3789 STRI	Fortuna	1,264	E	-24.7
<i>C. palmana</i> Standl.	Aranda et al. 3697 STRI	Cerro Jefe	945	E	-25.6
<i>C. palmana</i> Standl.	Aranda et al. 3629 STRI	Cerro Jefe	911	T	-25.9
<i>C. palmana</i> Standl.	Aranda and Virgo 3677 STRI	Fortuna	1,142	T	-29.1
<i>C. palmana</i> Standl.	Folsom 4507 PMA	Cerro Pirre	ND	T	-29.2
<i>C. palmana</i> Standl.	Valdespino et al. 469 PMA	Fortuna	≈1,150	T	-31.3
<i>C. aff. palmana</i> Standl.	Aranda et al. 3699 STRI	Cerro Jefe	1,007	T	-26.7
<i>C. aff. palmana</i> Standl.	Aranda et al. 3861 STRI	Cerro Pirre	1,594	T	-27.0
<i>C. aff. palmana</i> Standl.	Aranda et al. 3864 STRI	Cerro Pirre	1,646	E	-28.1

<i>C. aff. palmana</i> Standl.	Aranda et al. 3703 STRI	Cerro Jefe	974	E	-28.6
<i>C. aff. palmana</i> Standl.	Aranda et al. 3836 STRI	Santa Fé	804	T	-29.4
<i>C. aff. palmana</i> Standl.	Aranda et al. 3865 STRI	Cerro Pirre	1,677	E	-30.2
<i>C. penduliflora</i> Engl.	Davidse and Ham. 23709 PMA	Cerro Moreno	≈ 190	E	-30.5
<i>C. pratensis</i> Seeman	Aranda et al. 3855 STRI	Cerro Colorado	1,098	T	-24.5
<i>C. pratensis</i> Seeman	Aranda et al. 3793 STRI	Boquete	621	T	-24.9
<i>C. pratensis</i> Seeman	Aranda et al. 3728 STRI	Santa Fé	507	E	-25.6
<i>C. pratensis</i> Seeman	Aranda et al. 3621 STRI	El Valle	742	T	-26.4
<i>C. pratensis</i> Seeman	Aranda et al. 3651 STRI	El Copé	362	T	-27.1
<i>C. pratensis</i> Seeman	Aranda et al. 3589 STRI	Altos de Campana	755	T	-28.1
<i>C. pratensis</i> Seeman	Aranda et al. 3590 STRI	Altos de Campana	755	T	-29.2

<i>C. quadrangula</i> Bartlett	Aranda and Virgo 3685 STRI	Bocas del Toro	35	E	-29.8
<i>C. quadrangula</i> Bartlett	Aranda et al. 3840 STRI	Santa Fé	271	H	-30.0
<i>C. rosea</i> Jacq.	Aranda et al. 3729 STRI	Santa Fé	510	H	-16.7
<i>C. rosea</i> Jacq.	Aranda et al. 3792 STRI	Boquete	621	T	-17.0
<i>C. rosea</i> Jacq.	Aranda et al. 3724 STRI	El Valle	612	T	-19.9
<i>C. rosea</i> Jacq.	Aranda et al. 3592 STRI	Altos de Campana	652	H	-21.0
<i>C. rosea</i> Jacq.	Aranda et al. 3716 STRI	El Llano-Cartí	370	T	-24.8
<i>C. rosea</i> Jacq.	Aranda et al. 3632 STRI	Cerro Azul	680	T	-27.5
<i>C. rotundada</i> Standl.	Mendoza et al. 283 PMA	Fortuna	≈1,150	T	-26.2
<i>C. rotundada</i> Standl.	Antonio 1456 PMA	Cerro Colorado	1,400	T	-28.7
<i>C. salvinii</i> Donn. Sm.	Aranda et al. 3821 STRI	Boquete	1,287	H	-24.8

<i>C. salvinii</i> Donn. Sm.	Aranda et al. 3739 STRI	Santa Fé	892	T	-26.0
<i>C. salvinii</i> Donn. Sm.	Aranda et al. 3809 STRI	Cerro Colorado	1,527	T	-27.5
<i>C. sipapoana</i> (Maguire) Pipoly	Aranda and Virgo 3668 STRI	Altos de Pacora	700	E	-27.8
<i>C. stenophylla</i> Standl.	Aranda et al. 3620 STRI	El Valle	876	H	-26.0
<i>C. stenophylla</i> Standl.	Aranda and Virgo 3675 STRI	Fortuna	1,142	T	-26.6
<i>C. stenophylla</i> Standl.	Aranda and Virgo 3678 STRI	Fortuna	930	T	-26.9
<i>C. stenophylla</i> Standl.	Aranda et al. 3839 STRI	Santa Fé	804	T	-27.3
<i>C. stenophylla</i> Standl.	Aranda and Virgo 3679 STRI	Fortuna	1,134	T	-27.5
<i>C. stenophylla</i> Standl.	Aranda and Virgo 3674 STRI	Fortuna	1,142	T	-27.9
<i>C. stenophylla</i> Standl.	Aranda et al. 3846 STRI	Cerro Colorado	1,710	T	-28.0
<i>C. stenophylla</i> Standl.	Aranda and Virgo 3676 STRI	Fortuna	1,142	T	-28.0

<i>C. stenophylla</i> Standl.	Aranda et al. 3779 STRI	Fortuna	886	T	-28.5
<i>C. stenophylla</i> Standl.	Aranda et al. 3747 STRI	El Llano-Cartí	492	E	-28.5
<i>C. stenophylla</i> Standl.	Aranda et al. 3738 STRI	Santa Fé	897	T	-28.7
<i>C. stenophylla</i> Standl.	Aranda et al. 3732 STRI	Santa Fé	982	T	-29.1
<i>C. stenophylla</i> Standl.	Aranda et al. 3612 STRI	El Valle	876	T	-29.1
<i>C. stenophylla</i> Standl.	Aranda et al. 3619 STRI	El Valle	778	T	-29.4
<i>C. stenophylla</i> Standl.	Aranda et al. 3591 STRI	Altos de Campana	838	T	-29.9
<i>C. stenophylla</i> Standl.	Aranda et al. 3720 STRI	El Valle	873	T	-30.4
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3823 STRI	Cerro Punta	1,599	T	-24.9
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3818 STRI	Boquete	1,186	T	-25.2
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3604 STRI	Altos de Campana	870	T	-25.7

<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3859 STRI	Cana	1,497	E	-25.8
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3601 STRI	Altos de Campana	870	T	-26.1
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3797 STRI	Boquete	1,600	T	-26.1
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3828 STRI	Volcan	1,196	T	-26.7
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3819 STRI	Boquete	1,450	T	-26.7
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3826 STRI	Volcan	1,571	T	-26.7
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3587 STRI	Altos de Campana	849	T	-26.8
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3820 STRI	Boquete	1,462	T	-27.3
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3596 STRI	Altos de Campana	868	T	-28.2
<i>C. aff. stenophylla</i> Standl.	Aranda et al. 3869 STRI	Cana	949	E	-29.9
<i>C. torresii</i> Standl.	Aranda et al. 3801 STRI	Cerro Colorado	1,504	H	-27.4

<i>C. torresii</i> Standl.	Aranda et al. 3799 STRI	Cerro Colorado	1,581	T	-27.7
<i>C. torresii</i> Standl.	Aranda et al. 3783 STRI	Fortuna	1,145	T	-27.9
<i>C. torresii</i> Standl.	Aranda et al. 3847 STRI	Cerro Colorado	1,709	T	-30.3
<i>C. triflora</i> Cuatrec. ?	Aranda et al. 3804 STRI	Cerro Colorado	1,502	T	-25.8
<i>C. triflora</i> Cuatrec.	Folsom 4531 PMA	Cerro Pirre	ND	T	-26.4
<i>C. uvitana</i> Pittier	Aranda et al. 3775 STRI	Bocas del Toro	53	H	-18.5
<i>C. uvitana</i> Pittier	Aranda and Virgo 3682 STRI	Bocas del Toro	44	E	-18.6
<i>C. uvitana</i> Pittier	Aranda and Virgo 3684 STRI	Bocas del Toro	76	H	-18.7
<i>C. uvitana</i> Pittier	Aranda et al. 3763 STRI	Bocas del Toro	81	E	-19.1
<i>C. uvitana</i> Pittier	Aranda et al. 3842 STRI	Portobelo	15	T	-19.7
<i>C. uvitana</i> Pittier	Aranda et al. 3845 STRI	Portobelo	50	H	-19.7

<i>C. uvitana</i> Pittier	Aranda et al. 3843 STRI	Portobelo	15	T	-21.4
<i>C. uvitana</i> Pittier	Aranda et al. 3730 STRI	Santa Fé	510	H	-22.1
<i>C. uvitana</i> Pittier	Aranda et al. 3602 STRI	Altos de Campana	652	E	-22.9
<i>C. uvitana</i> Pittier	Aranda and Virgo 3667 STRI	Fort Sherman	50	H	-23.1
<i>C. uvitana</i> Pittier	Aranda et al. 3771 STRI	Bocas del Toro	145	T	-24.1
<i>C. uvitana</i> Pittier	Aranda et al. 3577 STRI	Gamboia	50	E	-27.4
<i>C. valerioi</i> Standl.	Aranda et al. 3717 STRI	El Llano-Cartí	270	T	-25.3
<i>C. valerioi</i> Standl.	Aranda et al. 3844 STRI	Portobelo	41	T	-25.6
<i>C. valerioi</i> Standl.	Aranda et al. 3830 STRI	Rio Sereno	929	T	-25.8
<i>C. valerioi</i> Standl.	Aranda et al. 3713 STRI	El Llano-Cartí	250	T	-25.9
<i>C. valerioi</i> Standl.	Aranda et al. 3686 STRI	El Llano-Cartí	390	T	-26.0

<i>C. valerioi</i> Standl.	Aranda et al. 3822 STRI	Boquete	921	H	-26.2
<i>C. valerioi</i> Standl.	Aranda et al. 3687 STRI	El Llano-Cartí	329	T	-26.9
<i>C. valerioi</i> Standl.	Aranda et al. 3721 STRI	El Valle	873	H	-26.9
<i>C. valerioi</i> Standl.	Aranda et al. 3690 STRI	El Llano-Cartí	400	T	-27.1
<i>C. valerioi</i> Standl.	Aranda et al. 3689 STRI	El Llano-Cartí	400	T	-27.4
<i>C. valerioi</i> Standl.	Aranda et al. 3870 STRI	Cerro Pirre	877	E	-28.5
<i>C. valerioi</i> Standl.	Aranda and Virgo 3666 STRI	Fort Sherman	122	T	-29.1
<i>C. valerioi</i> Standl.	Aranda and Virgo 3662 STRI	Fort Sherman	280	H	-29.4
<i>C. sp. A</i>	Aranda et al. 3631 STRI	Altos de Pacora	850	H	-25.2
<i>C. sp. A</i>	Aranda et al. 3701 STRI	Cerro Jefe	909	T	-25.7
<i>C. sp. A</i>	Aranda et al. 3863 STRI	Cerro Pirre	1,600	E	-26.2

C. sp. A	Aranda et al. 3862 STRI	Cerro Pirre	1,596	E	-26.6
C. sp. A	Aranda et al. 3860 STRI	Cana	1,573	E	-26.9
C. sp. A	Aranda et al. 3636 STRI	Cerro Jefe	950	H	-27.7
C. sp. A	Aranda et al. 3780 STRI	Fortuna	1,097	T	-27.8
C. sp. B	Aranda et al. 3854 STRI	Cerro Colorado	1,533	E	-25.5
C. sp. C	Aranda et al. 3849 STRI	Cerro Colorado	1,487	H	-30.4
C. sp. C	Aranda et al. 3852 STRI	Cerro Colorado	1,487	H	-27.9
C. sp. D	Aranda et al. 3831 STRI	Rio Sereno	927	T	-24.0
C. sp. E	Aranda and Virgo 3663 STRI	Fort Sherman	286	T	-27.1
C. sp. E	Aranda et al. 3588 STRI	Altos de Campana	850	E	-27.5
C. sp. E	Aranda and Virgo 3664 STRI	Fort Sherman	280	H	-27.8

C. sp. E	Aranda et al. 3600 STRI	Altos de Campana	850	T	-28.9
C. sp. E	Aranda et al. 3599 STRI	Altos de Campana	849	T	-29.1
C. sp. E	Aranda et al. 3597 STRI	Altos de Campana	850	E	-29.6
C. sp. F	Aranda et al. 3745 STRI	El Llano-Cartí	450	H	-28.3
C. sp. F	Aranda et al. 3833 STRI	Santa Fé	726	E	-28.3
C. sp. F	Correa and Mon. 11037 PMA	Campana	≈850	T	-29.8
C. sp. G	McPherson 15908 PMA	Cerro Punta	≈2,300	T	-27.5

Carbon isotope ratios were determined for CO₂ derived from 3 mg samples of dried mature leaves (Crayn et al. [2001](#); Pierce et al. [2002b](#); Winter and Holtum [2002](#)).

¹³C/¹²C analyses were performed at the Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, Athens, Georgia, using isotope ratio mass spectrometry. Following the appropriate corrections for other isotopes, the abundance of ¹³C in each sample was calculated relative to the abundance of ¹³C in standard CO₂ that had been calibrated against Pee Dee belemnite (*Belemnitella americana*). Relative abundance was determined using the relationship $\delta^{13}\text{C} = [(\text{^{13}C/^{12}C of sample}) / (\text{^{13}C/^{12}C of standard}) - 1] \times 1,000$. The value of $\delta^{13}\text{C}$ has been expressed in ‰.

Estimation of titratable acidity

Plant material was sampled, on 2–3 July 2003 and 4–5 January 2004, from plants growing outdoors in 200 l containers at the Smithsonian Tropical Research Institute Santa Cruz Research Station in Gamboa, Panamá. At dawn and dusk at least four discs per sample, each disc of 1.4 cm², were excised from mature leaves, weighed and frozen at –196°C in liquid N. Tissue was freeze-dried and reweighed. Titratable acidity was determined subsequently from the amount of NaOH required to neutralise extracts of the discs boiled sequentially in 50% methanol and H₂O.

Results

The $\delta^{13}\text{C}$ values of leaves of 31 of the 33 species of *Clusia* which have to our knowledge been described for Panamá, and 7 species that have yet to be described, ranged from –32.9 to –16.7 ‰ (Table 1) and the values of 11 species from Colombia, Costa Rica and Honduras were between –28.7 and –16.6 ‰ (Table 2). $\delta^{13}\text{C}$ values more positive than –20 ‰, which are characteristic of pronounced CAM, were detected in three species, *C. uvitana* and *C. rosea* from Panamá and *C. flava* from Costa Rica. Among the 192 $\delta^{13}\text{C}$ values for Panamanian specimens that were more negative than –20.0 ‰, 186 exhibited C₃-like values that were more negative than –24.0 ‰. Among the six specimens that exhibited intermediate values between –20 and –24 ‰, an indicator of a potential contribution of dark CO₂ fixation to net carbon gain, five were for specimens of the strong CAM species *C. rosea* and *C. uvitana* and one was for an individual of *C. flavida* (previously *Havetiopsis flexilis*; Zotz et al. 1999).

Table 2 $\delta^{13}\text{C}$ values of *Clusia* spp. from Colombia, Costa Rica and Honduras. Specimens are deposited in the herbarium at Universidad de Panamá (PMA, Holmgren et al. 1990). Life-forms are defined as tree or shrub (T), hemi-epiphyte (H) or epiphyte (E). ND, not determined

Taxon	Collector and voucher number	Location	Altitude (m)	Life-form	$\delta^{13}\text{C}$ (‰)

<i>C. articulata</i> Vesque	MacDougal et al. 4030	Amalfi, Colombia	≈1,220	T	-28.0
<i>C. columnaris</i> Engl.	Davidse 5162	Vichada, Colombia	220	T	-28.7
<i>C. cuneifolia</i> Cuatrec.	Zarucchi et al. 6019	Salgar, Antioquia, Colombia	2,320	T	-28.1
<i>C. ducuoides</i> Engl.	McPherson 13030	San Pedro, Antioquia, Colombia	2,370	T	-26.2
<i>C. ducuoides</i> Engl.	Brant and Betancur 1589	Urrao, Antioquia, Colombia	1,860	T	-25.8
<i>C. flava</i> Jacq.	Hammel 8349	La Selva, Costa Rica	ND	E	-16.6
<i>C. aff. garciabarrigae</i>	Gentry and Keating 59721	R. N. La Planada, Nariño, Colombia	1,800	T	-28.7
<i>C. lundellii</i> Standl.	Molina R. 30611	Copán, Honduras	713	T	-23.7
<i>C. monantha</i> Cuatrec.	Betancur et al. 964	Caramanta, Colombia	≈2,380	T	-27.4
<i>C. palmiada</i> L.C. Rich	Zarucci et al. 6742	San Carlos, Antioquia, Colombia	800	H	-25.2

<i>C. aff. polyantha</i> Cuatrec.	Zarucci et al. 5615	Fromtino, Antioquia, Colombia	1,830	T	-27.7
<i>C. weberbaueri</i> Engl.	Benarides 8784	R. N. La Planada, Nariño, Colombia	1,800	T	-26.1

The presence of strong CAM in *C. rosea* and *C. uvitana* was confirmed by measurements of titratable acidity (Figs. 1, 2). Diel fluctuations in titratable acidity characteristic of weak CAM were observed in *C. croatii*, *C. cylindrica*, *C. fructiangusta*, *C. lineata*, *C. odorata*, *C. pratensis*, *C. quadrangula*, *C. valerioi* (forms A and B) and in *C. sp. D*. No evidence of CAM expression was detected in the seven members of the *C. multiflora* group that were tested viz. *C. coclenis*, *C. cupulata*, *C. aff. multiflora*, *C. palmana*, *C. salvinii*, *C. stenophylla* and *C. sp. A*. The preponderance of C₃ and weak CAM species in the species sampled resulted in a distribution of $\delta^{13}\text{C}$ values that exhibited a predominant peak with a median value of -26 to -27 ‰ (Fig. 3).

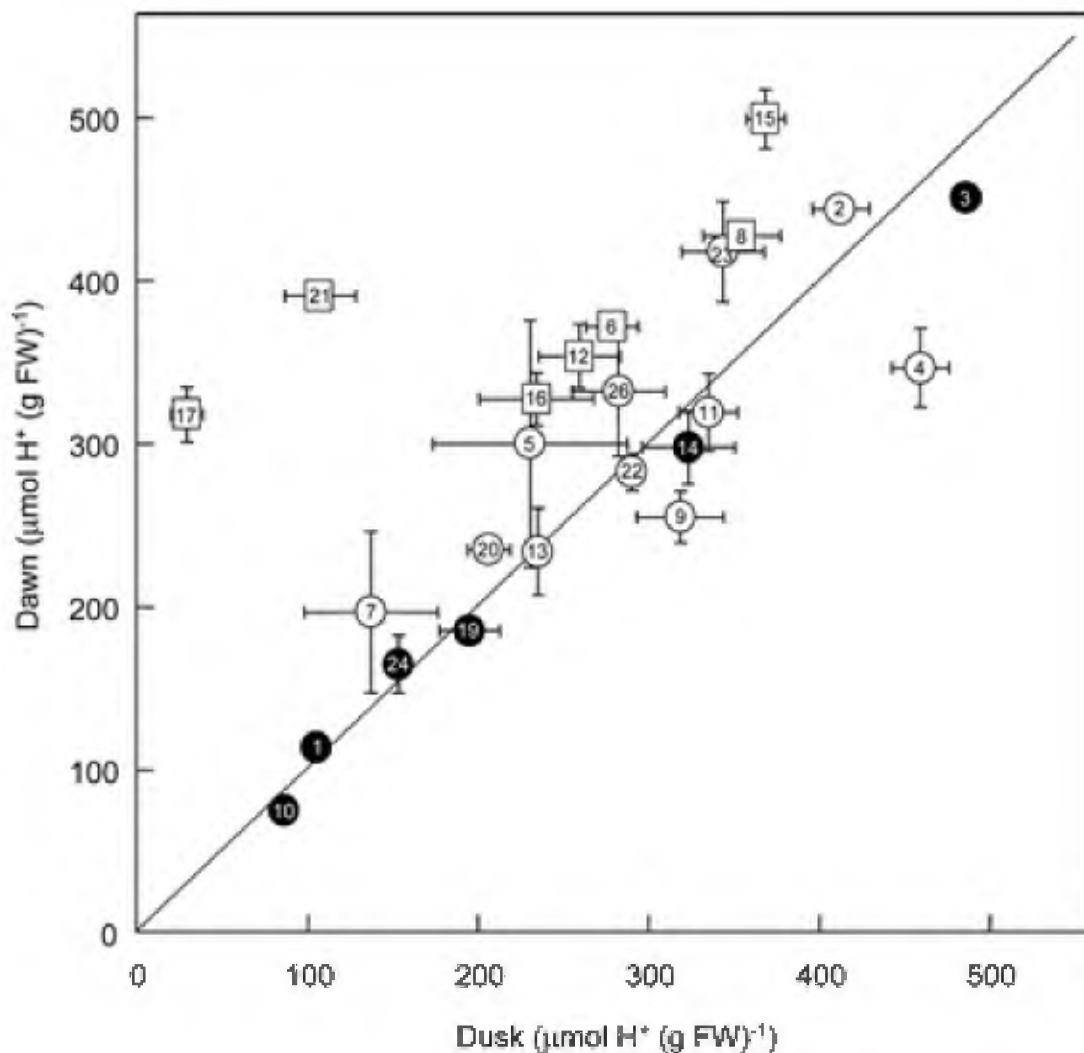


Fig. 1 Relationship between titratable acidities at dawn and dusk on 2–3 July 2003, for 23 species of well-watered *Clusia* from Panamá grown under natural conditions in 200 l containers at the Smithsonian Tropical Research Institute Santa Cruz Research Station in Gamboa, Panamá. The species tested were *C. coclensis* (1), *C. croatii* (2), *C. cupulata* (3), *C. cylindrica* (4), *C. divaricata* (5), *C. fructiangusta* (6), *C. liesneri* (7), *C. lineata* (8), *C. longipetiolata* (9), *C. aff. multiflora* (10), *C. minor* (11), *C. odorata* (12), *C. osseocarpa* (13), *C. palmana* (14), *C. pratensis* (15), *C. quadrangula* (16), *C. rosea* (17), *C. stenophylla* (19), *C. torresii* (20), *C. uvitana* (21), *C. valerioi* form A (22), *C. valerioi* form B (23), *C. sp. A* (24), and *C. sp. E* (26). Values for which the titratable acidities at dawn are significantly greater than the titratable acidities at dusk ($P \leq 0.05$, single-tailed *t*-test) are indicated by *open squares*, values for which the dawn and dusk means are not significantly different are indicated by *open circles* or *filled circles*. Species in the *C. multiflora* species group (group III) are indicated by *filled circles*. Bars indicate standard errors of the means for measurements on three leaves sampled at dawn and three leaves sampled at dusk. Where there are no bars visible, they are covered by the *symbol*

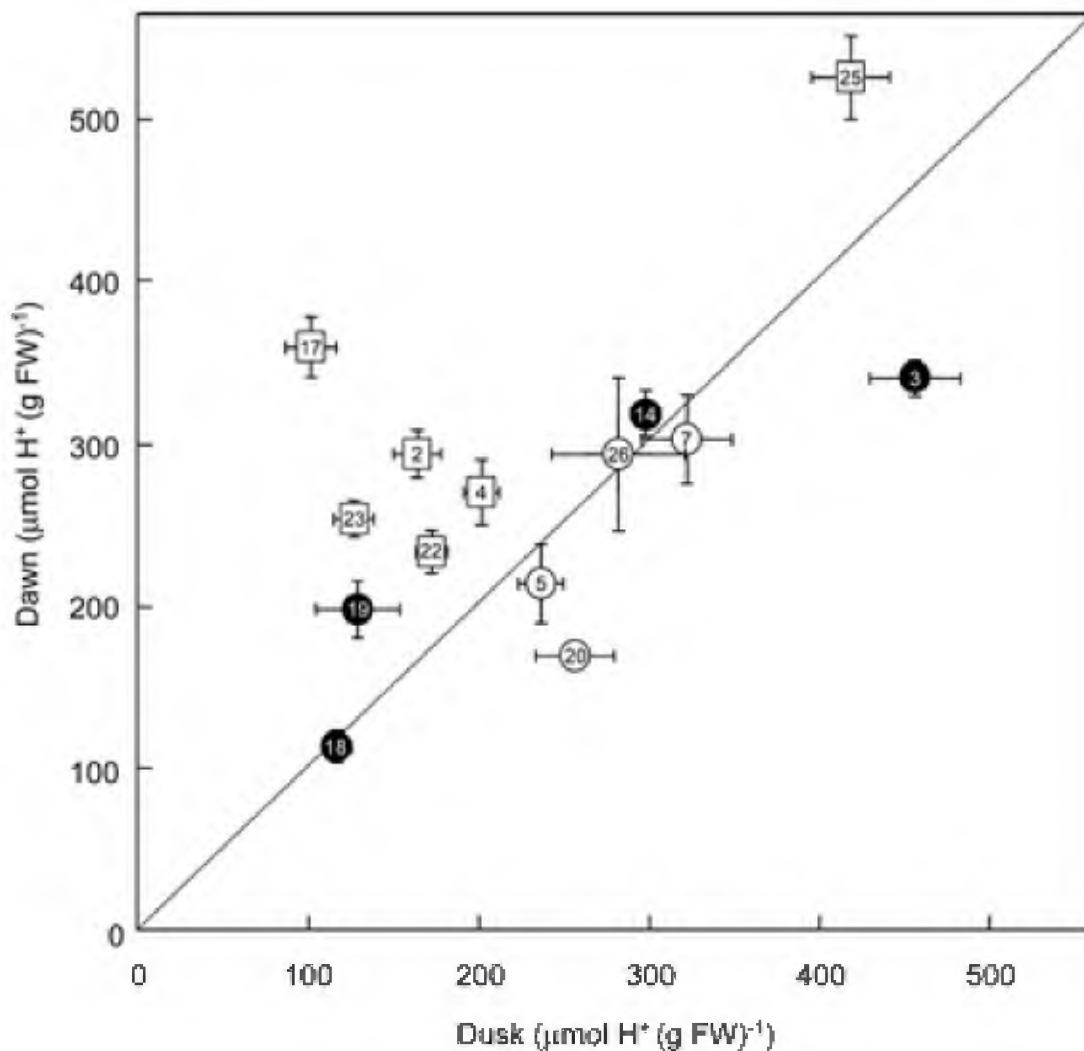


Fig. 2 Relationship between titratable acidities at dawn and dusk on 4–5 January 2004, for 13 species of well-watered *Clusia* from Panamá grown under natural conditions in 200 l containers at the Smithsonian Tropical Research Institute Santa Cruz Research Station in Gamboa, Panamá. The species tested were *C. croatii* (2), *C. cupulata* (3), *C. cylindrica* (4), *C. divaricata* (5), *C. liesneri* (7), *C. palmana* (14), *C. rosea* (17), *C. salvinii* (18), *C. stenophylla* (19), *C. torresii* (20), *C. valerioi* form A (22), *C. valerioi* form B (23), *C. sp. D* (25) and *C. sp. E* (26). Values for which the titratable acidities at dawn are significantly greater than the titratable acidities at dusk ($P \leq 0.05$, single-tailed *t*-test) are indicated by *open squares*, values for which the dawn and dusk means are not significantly different are indicated by *open circles* or *filled circles*. Species in the *C. multiflora* species group (group III) are indicated by *filled circles*. Bars indicate standard errors of the means for measurements on five leaves sampled at dawn and five leaves sampled at dusk. Where there are no bars visible, they are covered by the *symbol*

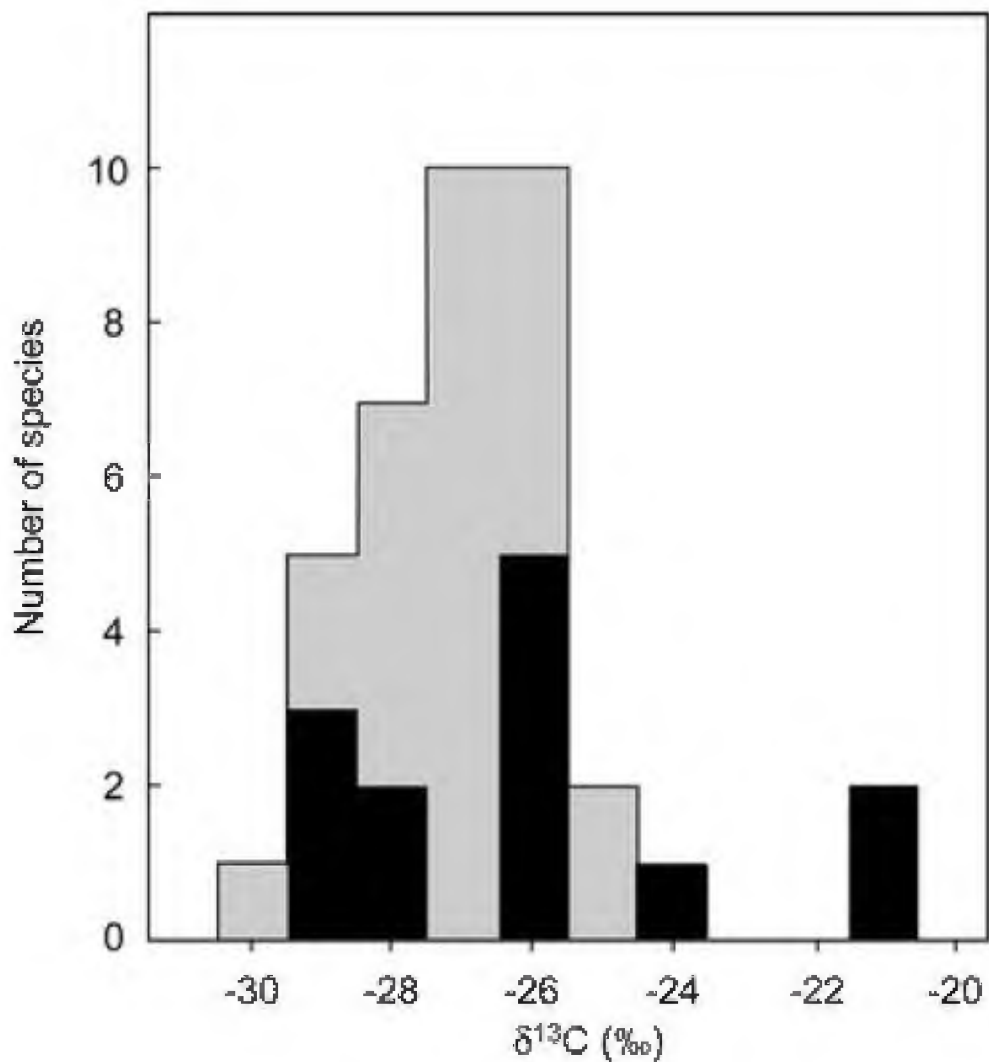


Fig. 3 Relationship between mean $\delta^{13}\text{C}$ values and the presence (solid bar) or absence (shaded bar) of CAM, derived from titratable acidity measurements, in mature leaves from species of *Clusia* growing in their natural environments in Panamá

The majority of species with individuals that exhibited strong or weak CAM were collected at altitudes of 1,100 m a.s.l. or below, whereas species with exclusively C₃-like $\delta^{13}\text{C}$ or acidity values were collected across the altitudinal sampling range (Table 1, Fig. 4). The exceptions among the species that exhibited CAM were *C. odorata*, a species with weak CAM, which was collected between 1,277 and 1,591 m a.s.l. and *C. croatii* which was collected to 1,689 m a.s.l.

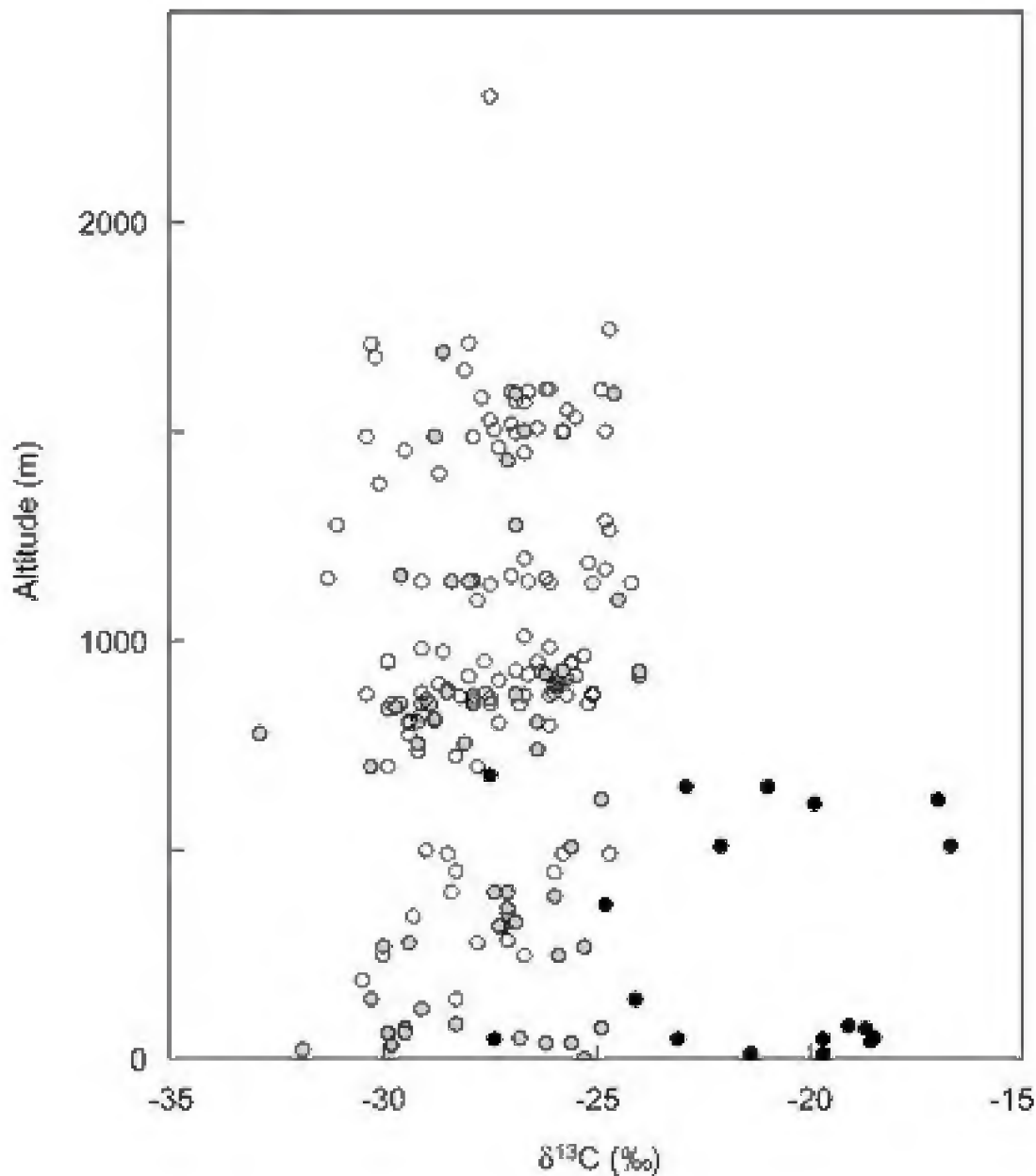


Fig. 4 Relationship between $\delta^{13}\text{C}$ values and altitude for Panamanian species of *Clusia* that exhibit strong CAM (filled circles), weak CAM (grey-shaded circles), or in which CAM has not been detected (open circles)

Neither of the Panamanian strong CAM species, *C. rosea* and *C. uvitana*, were collected above 680 m a.s.l. (Fig. 4). For *C. uvitana*, $\delta^{13}\text{C}$ values more positive than -20‰ were observed for terrestrial, epiphytic and hemi-epiphytic individuals growing within 100 m of sea level, values more negative than -20‰ were recorded for individuals growing from sea level to 650 m a.s.l.

Discussion

Of the 49 of *Clusia* species analysed on the basis of carbon isotope ratio, only three species, *C. flava*, *C. rosea* and *C. uvitana*, exhibited isotopic signals that were typical of strong CAM, the remainder had isotopic signals indistinguishable from those known for C₃ species (Tables 1, 2). Strong CAM has been reported previously for *C. rosea* and *C. uvitana* (Ting et al. 1985; Winter et al. 1992), but not for *C. flava*. For individuals with $\delta^{13}\text{C}$ values more negative than -24‰ , and perhaps even -25‰ , one cannot distinguish by isotopic methods alone, particularly for plants growing in natural environments, between plants that have solely fixed carbon in the light and those that have fixed up to $\approx 20\%$ of their carbon during the dark (Winter and Holtum 2002). Analysis of titratable acidity of Panamanian species growing in large soil containers permitted a more sensitive investigation of small contributions of dark fixation to net carbon gain, and identified weak CAM for the first time in *C. croatii*, *C. cylindrica*, *C. fructiangusta*, *C. lineata*, *C. odorata*, *C. pratensis* and *C. quadrangula*. Weak CAM was also observed in *C. sp. D* and in thick-leaf and thin-leaf forms of *C. valerioi*, both species which have previously been reported to exhibit CAM (Wanek et al. 2002).

Instead of a pronounced bimodal distribution of $\delta^{13}\text{C}$ values typically observed for plant groups that contain both C₃ and CAM members (Crayn et al. 2004), the values of *Clusia* species from Panamá exhibited a predominant C₃-type peak within which fell the weak CAM species (Fig. 3). Although a more pronounced CAM-type peak is to be expected if a wider range of species will be sampled in future studies, the distribution in Fig. 3 illustrates well the observation that, in the field, strong CAM is the exception rather than the rule in *Clusia* and that the contribution of dark CO₂ fixation to leaf life-time carbon gain is often small.

The Central American species of *Clusia* can be assigned on the basis of their morphology and ITS sequences into three groups, the *C. flava* group (group I), the *C. minor* group (group II) and the *C. multiflora* group (group III) (Hammel 1986; Gehrig et al. 2003). It was postulated, on the basis of the presence or absence of CAM in 15 of the 40 species used to create the molecular phylogeny, that CAM is present in groups I and II but is absent from group III (Gehrig et al. 2003).

Our observations on the presence or absence of CAM in 38 *Clusia* species from Panamá are

consistent with, and strengthen considerably, the predictions of the phylogenetic study. None of the plants we tested from group III, viz. *C. coclensis*, *C. cupulata*, *C. congestiflora*, *C. aff. multiflora*, *C. palmana*, *C. salvinii*, *C. stenophylla*, *C. sp. A*, *C. sp. B*, *C. sp. C* or *C. sp. F*, exhibited detectable contribution by CO₂ uptake in the dark to net carbon gain under the conditions in which the plants were growing in their natural environments or exhibited diel fluctuations of titratable acidity under the well-watered conditions in which plants were cultivated at the Santa Cruz Research Station at Gamboa (Fig. 5). In contrast, CAM was detected in six species from group I, *C. cylindrica*, *C. flava*, *C. rosea*, *C. quadrangula*, *C. valerioi* and *C. sp. D*, and in six species from group II, *C. croatii*, *C. fructiangusta*, *C. lineata*, *C. odorata*, *C. pratensis* and *C. uvitana*.

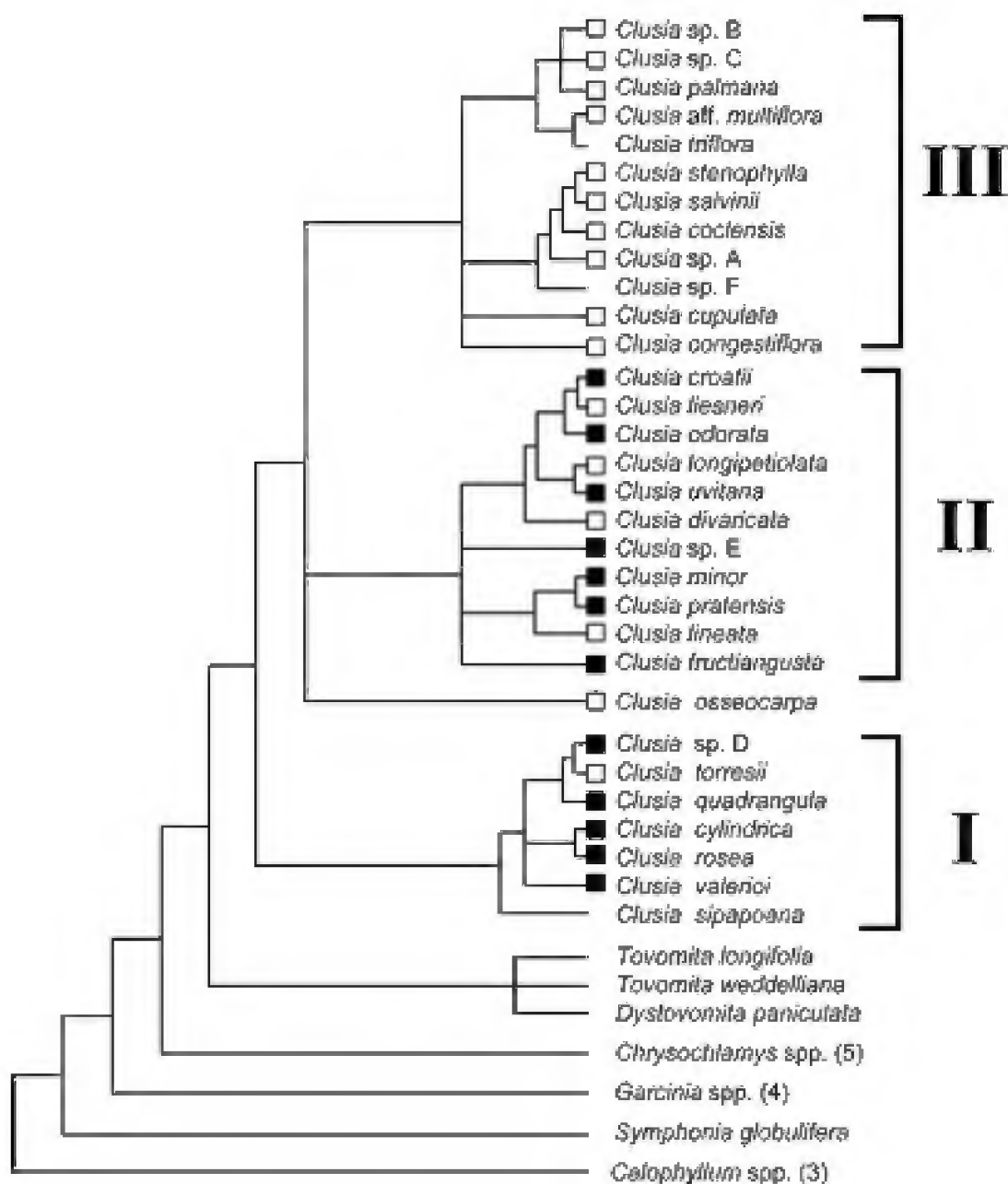


Fig. 5 The presence (*filled squares*) or absence (*open squares*) of CAM reported to date in *Clusia* species in groups I (*C. flava* group), II (*C. minor* group) and III (*C. multiflora* group). Absence of a symbol indicates that titratable acidities have not to our knowledge been reported for the species. The phylogeny, derived by Gehrig et al. (2003), is a strict consensus tree of the most parsimonious trees generated by heuristic analyses of ITS sequences of the nuclear rDNA, rooted with three species of *Calophyllum*

The proportion of all *Clusia* species that have an ability to express CAM is unknown as prior to this report fewer than 10% of species have been subjected to ecophysiological analyses. Extrapolation of the presence of CAM, measured as diel fluctuations in titratable acidity, in at least 14 [11 detected by us and in *C. minor* (Borland et al. 1992), *C. flavida* (Zotz et al. 1999) and *C. sp. E* (Wanek et al. 2002)], of the 25 of the Panamanian species tested to the ca. 300 species of *Clusia* worldwide (Hammel 1986; Pipoly et al. 1998) indicates that roughly half of all species of *Clusia* could be expected to have the capacity to perform CAM. Our estimate is most probably an under-estimate as our measurements of titratable acidity were performed under a single set of conditions. The extent to which CAM is expressed in those species of *Clusia* in which it is known may differ during the lifetime of an individual. Not only may the proportion of carbon gained during the dark vary depending upon life-form or during ontogeny, it can be modulated by nutrient regime or in response to seasonal or intra-seasonal variation in water stress, day–night temperature differences or light regimes (Sternberg et al. 1987; Schmitt et al. 1988; Franco et al. 1991; Haag-Kerwer et al. 1992; Winter et al. 1992; Borland et al. 1993; Zotz and Winter 1993, 1994, 1996). Moreover, the transition between C_3 and CAM is often rapidly reversible (Schmitt et al. 1988; Haag-Kerwer et al. 1992; Zotz and Winter 1993; de Mattos and Lüttge 2001). Indeed, it has been speculated that all *Clusia* species may have the capacity to exhibit some degree of the CAM cycle (Borland et al. 1998; Lüttge 1999) albeit CAM has not been observed in plants from the *C. multiflora* complex or *C. sp. A* subjected to water deficits (Grams et al. 1998; K. Winter, unpublished data). Even if CAM is detected in the future in species from group III that have been stressed, the general observation will probably still stand that CAM is poorly expressed in members of this clade.

Mature leaves from the same *C. cylindrica* plant did not exhibit CAM when measured in July 2003 but did when measured in January 2004, indicating that CAM is inducible in this species. The shift from C_3 to CAM was accompanied by a decrease in both the dusk and dawn levels of titratable acidity (Figs. 1, 2), the decrease in the dusk level being greater than the decrease in the dawn level. It appears that the induction of CAM was not the result of an increase in the capacity of the plant to accumulate acids in the vacuole. A similar

phenomenon has been reported for *C. uvitana* (Zotz and Winter [1993](#)).

Low levels of titratable acidity at dusk were present in the species with the greatest capacity to perform CAM, *C. rosea* and *C. uvitana* (Figs. [1](#), [2](#)). However, CAM was also observed in eight species which exhibited dusk titratable acidities that ranged between 100 and 400 $\mu\text{mol H}^+$ (g FW) $^{-1}$ and CAM was not observed in *C. coclensis*, *C. aff. multiflora* and *C. salvinii*, species which exhibited 100 or less $\mu\text{mol H}^+$ (g FW) $^{-1}$. It is most likely that capacity for acid accumulation is limited by characteristics of the tonoplast (Smith et al. [1996](#); Lüttge et al. [2000](#)). As a result, the high day–night fluctuations in acid typical of strong CAM *Clusia* species might be possible only in species with low background levels of acidity. The restrictions on dusk acidity levels in weak CAM species would be correspondingly less and might explain the greater variation in background acidities in these plants.

In order to assess the variability in the contribution of C_3 and CAM pathways to net carbon gain in situ we measured $\delta^{13}\text{C}$ values of Panamanian *Clusia* species from the range of natural vegetation assemblages and altitudes in which they are reported to occur. Terrestrial, lithophytic, epiphytic and hemi-epiphytic plants were collected from lowland and montane rainforests, tropical seasonally dry forests and from forests on the Atlantic and Pacific coasts. Both CAM-type and C_3 -type isotope ratios were detected. The largest range of $\delta^{13}\text{C}$ values, which varied from CAM-type to C_3 -type, was observed for the strong CAM species, *C. rosea* and *C. uvitana*. Despite the range in $\delta^{13}\text{C}$ values of 9–11 ‰, no pattern was detected between the expression of CAM, life-form and site of collection for these two lowland species. The isotopic compositions and range in composition of species in which weak CAM was observed did not differ from those in which CAM was not detected on the basis of diel fluctuations of titratable acidity. Similarly, no pattern was detected between the expression of CAM, life-form and site of collection.

The identities of the majority of species in this study were cross-checked with ITS sequences established by Gehrig et al. ([2003](#)) in order to reduce the taxonomic uncertainties that bedevil *Clusia* comparisons. Apart from a confusing profusion of synonyms, such as *C. major* being used for *C. rosea* (cf. Hammel [1986](#); Diaz et al. [1996](#)), many species are difficult to identify. For example, *C. multiflora* appears to be a complex of forms as does *C. minor* which contains apomictic, hermaphroditic and dioecious plants (Maguire [1976](#); Hammel [1986](#)). Our confidence in our *Clusia* identifications allows us to identify paradoxes that require further investigation. For example, in two forms of *C. valerioi* growing under similar conditions in pots at the Santa Cruz Research Station measurement of titratable

acidities in July 2003, indicated one form was C₃ but the second exhibited weak CAM (Fig. 1), whereas in January 2004, both forms exhibited CAM (Fig. 2).

No *Clusia* species known to exhibit CAM was collected above 1,689 m a.s.l. *C. rosea* and *C. uvitana*, the two strong CAM species, were not collected above 680 m (Fig. 4). Other species that contained individuals known to exhibit weak CAM were restricted to altitudes below 1,100 m a.s.l., with the exceptions of *C. croatii* which was collected between sea level and 1,689 m a.s.l., and *C. odorata* which was only collected at altitudes between 1,277 and 1,591 m a.s.l. No correlations between altitude and $\delta^{13}\text{C}$ values were observed within species, although the majority of group III species were collected above 750 m a.s.l. Our observations of decreasing expression of CAM with altitude in *Clusia* in Panamá are consistent with those of Diaz et al. (1996) who reported that CAM in *Clusia* spp. in Venezuela was restricted to altitudes below 1,500 m a.s.l. Extensive isotopic surveys of orchids in New Guinea and bromeliads also revealed a similar relationship between altitude and the expression of CAM (Earnshaw et al. 1987; D. Crayn, J.A.C. Smith, K. Winter, unpublished data). However, although uncommon, some *Clusia* species and some CAM species can grow at high altitudes. *C. arborea* and *C. ducuoides* have been collected from 3,000 to 3,200 m a.s.l. (Davidse et al. 1984; Harling and Andersson 1985), and CAM cacti, bromeliads, *Echeveria* spp., Portulacaceae spp., *Isoetes andicola* and aquatic CAM plants are known from altitudes above 3,000 m a.s.l. (Medina and Delgado 1976; Keeley et al. 1984; Keeley and Keeley 1989; Arroyo et al. 1990; Gibson and Nobel 1990; D. Crayn, J.A. C. Smith, K. Winter, unpublished data). The factors that limit the distribution of *Clusia* species with CAM at high altitudes are not known.

This study indicates that phylogenetic affiliation may be a predictor of an ability to exhibit CAM in *Clusia* that grow in Panamá. Moreover, it demonstrates that weak CAM is probably a relatively common photosynthetic option in many species of *Clusia* of the region, particularly in the lowland species. $\delta^{13}\text{C}$ value is not a good indicator of the potential of *Clusia* species to exhibit CAM in the field because it appears that the contribution in most species of CAM to net carbon gain is generally rather small when integrated over the life-time of leaves.

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