

## The CAM lineages of planet Earth

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• **Background and Scope** The growth of experimental studies of crassulacean acid metabolism (CAM) in diverse plant clades, coupled with recent advances in molecular systematics, presents an opportunity to re-assess the phylogenetic distribution and diversity of species capable of CAM. It has been more than two decades since the last comprehensive lists of CAM taxa were published, and an updated survey of the occurrence and distribution of CAM taxa is needed to facilitate and guide future CAM research. We aimed to survey the phylogenetic distribution of these taxa, their diverse morphology, physiology and ecology, and the likely number of evolutionary origins of CAM based on currently known lineages.

• **Results and Conclusions** We found direct evidence (in the form of experimental or field observations of gas exchange, day–night fluctuations in organic acids, carbon isotope ratios and enzymatic activity) for CAM in 370 genera of vascular plants, representing 38 families. Further assumptions about the frequency of CAM species in CAM clades and the distribution of CAM in the Cactaceae and Crassulaceae bring the currently estimated number of CAM-capable species to nearly 7 % of all vascular plants. The phylogenetic distribution of these taxa suggests a minimum of 66 independent origins of CAM in vascular plants, possibly with dozens more. To achieve further insight into CAM origins, there is a need for more extensive and systematic surveys of previously unstudied lineages, particularly in living material to identify low-level CAM activity, and for denser sampling to increase phylogenetic resolution in CAM-evolving clades. This should allow further progress in understanding the functional significance of this pathway by integration with studies on the evolution and genomics of CAM in its many forms.

**Key words:** crassulacean acid metabolism, nocturnal acidification, vascular plants, C<sub>3</sub> photosynthesis, C<sub>3</sub> + CAM, C<sub>4</sub> + CAM, strong CAM, photosynthetic pathway evolution.

### INTRODUCTION

Crassulacean acid metabolism (CAM) is now recognized as a key ecological adaptation to water and CO<sub>2</sub> limitation. From the outset, this metabolism was noted for its distinctive association with succulent plants. De Saussure (1804), in the course of extensive manometric measurements of gas exchange by plants, made the seminal observation that *Opuntia* and several other succulent plants were able to show net uptake of CO<sub>2</sub> at night. Also, Heyne (1815) detected the rhythmic nocturnal acidification of *Kalanchoë* leaves by the simple expedient of comparing their taste in the morning and afternoon. By the end of the 19<sup>th</sup> century, these characteristic day–night changes in acidity had become recognized as a well-known phenomenon of succulent plants. Since then, a full description of CAM, from biochemistry to ecophysiology, has involved researchers from around the globe and continues into the genomics age.

Research into CAM has been spurred by advances in technology that have enabled precise biochemical and physiological descriptions of the marked differences between succulent and non-succulent plants. Mayer (1875), Kraus (1883) and Warburg

(1886) appreciated that the metabolism of succulents involved a specific acid, malic acid, and that carbohydrate concentrations showed reciprocal, diel cycling. However, it was to be another half-century before the biochemical mechanism of nocturnal CO<sub>2</sub> fixation was established. The experiments of Thurlow and Bonner (1948), Thomas (1949) and Thomas and Beevers (1949) showed that nocturnal synthesis of malic acid could be explained by direct fixation of CO<sub>2</sub> via the Wood–Werkman reaction, first discovered in bacteria and now known to be catalysed (using HCO<sub>3</sub><sup>-</sup> as the true substrate) by the enzyme phosphoenolpyruvate (PEP) carboxylase (PEPC). Work at the Connecticut Agricultural Experiment Station and elsewhere around the same time confirmed the primary role of malic acid and the relationship between organic acids and the major carbohydrates involved in the CAM pathway (Pucher and Vickery, 1942; Pucher *et al.*, 1947). The development of diffusion resistance analysis and infrared gas analysers allowed precise quantification of stomatal conductance and gas exchange in natural and experimental settings, leading to the direct demonstration of nocturnal opening of stomata associated with uptake of atmospheric CO<sub>2</sub>, the sensitivity of the gas-exchange

cycle to photoperiod, and the endogenous rhythmicity of dark  $\text{CO}_2$  fixation (Gregory *et al.*, 1954; Wilkins, 1959; Nuernbergk, 1961; Warren and Wilkins, 1961; Nishida, 1963). Following the discovery of  $\text{C}_4$  photosynthesis (Hatch and Slack, 1966), the advances in biochemistry and other aspects of photosynthesis became canonized in a four-stage model of CAM (Osmond, 1978) (Fig. 1), which provides a useful framework for considering the characteristics of carbon metabolism in these plants over the 24 h cycle.

Phase I occurs in the dark period, when stomata are open,  $\text{CO}_2$  is taken up from the atmosphere, and PEP supplied by glycolysis is carboxylated by PEPC to produce malate, which is then stored overnight as malic acid in the central vacuole of chlorenchymatous mesophyll cells. Phase II is characterized by a short burst of  $\text{CO}_2$  fixation at the beginning of the light period, initially catalysed mainly by PEPC but later increasingly by Rubisco, while stomata remain open. Malic acid is then released from the vacuole and decarboxylated during the

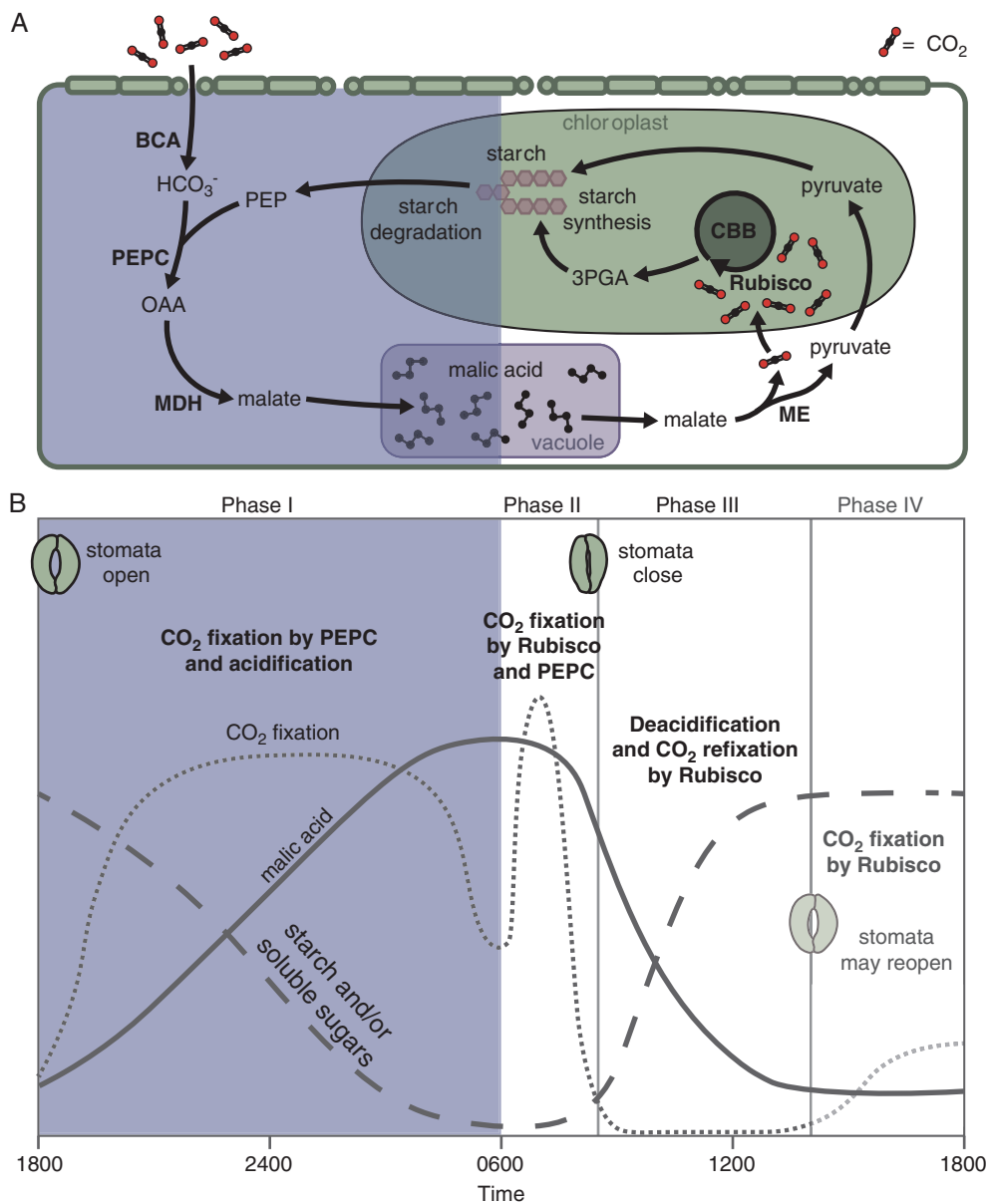


FIG. 1. Simplified overview of biochemistry (A) and four phases of CAM (B); phases in (B) are adapted from Osmond (1978). During phase I of CAM, atmospheric  $\text{CO}_2$  is captured in a series of steps involving phosphoenolpyruvate (PEP) carboxylase (PEPC) to form malate, which is stored as malic acid in the vacuole overnight. In phase II, stomata remain open, and both Rubisco and PEPC fix atmospheric  $\text{CO}_2$ . Stomatal closure marks the beginning of phase III, in which malate is released from the vacuole and decarboxylated by malic enzyme (NADP- or NAD-ME) as shown (or PEP carboxykinase, not shown), releasing  $\text{CO}_2$  to be re-fixed by Rubisco. Finally, if phase IV occurs, stomata reopen, and atmospheric  $\text{CO}_2$  is fixed predominantly by Rubisco. If phase IV does not occur, stomatal opening is delayed until phase I begins again. Abbreviations: 3PGA, 3-phosphoglycerate; BCA,  $\beta$ -carbonic anhydrase; CBB, Calvin-Benson-Bassham cycle; MDH, malate dehydrogenase; OAA, oxaloacetate.

central part of the day in phase III, raising intercellular CO<sub>2</sub> dramatically to supply Rubisco and causing stomatal closure, and yielding a three-carbon moiety (pyruvate or PEP) that is recycled via gluconeogenesis back to storage carbohydrate. Finally, when the deacidification phase is completed, stomata may reopen in phase IV in the late afternoon (environmental conditions permitting), and CO<sub>2</sub> is fixed directly from the atmosphere by Rubisco.

Species such as *Kalanchoë daigremontiana* Raym.-Hamet & H.Perrier are generally well described by this canonical four-phase model of CAM, but in many species the magnitude of the CAM cycle (and its individual phases) is variously expressed and can be phenotypically plastic over short time scales and throughout the life history (see ‘*The landscape of CAM phenotypes and CAM evolution*’, below). Regardless of individual variation, life history variation and environmentally induced variation, CAM is most broadly defined by the cyclic diel rhythm of: (1) nocturnal fixation of CO<sub>2</sub> into malate, catalysed by PEPc; (2) storage of malate as malic acid overnight in the vacuole; and (3) efflux from the vacuole and decarboxylation of the malate during the daytime to release CO<sub>2</sub>, which is re-fixed by Rubisco. The formalization of this framework coincided with the recognition of the adaptive significance of CAM: fixing carbon at night while closing stomata for much of the day reduces transpirational water loss, and the two-stage carbon-concentrating mechanism increases the efficiency of Rubisco CO<sub>2</sub> fixation when stomata are closed (Neales *et al.*, 1968; Kluge and Ting, 1978; Osmond, 1978; Cockburn *et al.*, 1979).

Concurrent with this synthesis of CAM, it was recognized that C<sub>4</sub> and CAM plants could be distinguished from C<sub>3</sub> species by their lesser discrimination against <sup>13</sup>C relative to <sup>12</sup>C (Vogel and Lerman, 1969; Bender, 1971; Smith and Epstein, 1971; Bender *et al.*, 1973; Osmond *et al.*, 1973) (Box 1). Although it took almost another decade to describe the mechanisms behind carbon isotope discrimination fully (O’Leary, 1981; Farquhar *et al.*, 1982), the realization that photosynthetic types could be discerned with relative ease from small samples of plant tissue (including desiccated and dead tissues, such as those in herbarium collections) was an impetus for broad ecological and taxonomic surveys using stable-isotope analysis. Great strides were made in major CAM clades, including the Bromeliaceae (Coutinho, 1969; Medina and Troughton, 1974; Medina *et al.*, 1977; Griffiths and Smith, 1983; Crayn *et al.*, 2004, 2015), Orchidaceae (Coutinho, 1969; Winter *et al.*, 1983; Kluge *et al.*, 1995; Silvera *et al.*, 2005, 2010a; Torres-Morales *et al.*, 2020), Clusiaceae (Holtum *et al.*, 2004; Lüttge, 2007; Pachon *et al.*, 2022), Crassulaceae (Osmond *et al.*, 1975; Rundel *et al.*, 1979; Teeri *et al.*, 1981; Tenhunen *et al.*, 1982; Pilon-Smits *et al.*, 1991, 1992; Kluge *et al.*, 1993) and Aizoaceae (Mooney *et al.*, 1977; Rundel *et al.*, 1999; Messerschmid *et al.*, 2021). Ecologically, carbon isotope surveys were conducted primarily in semi-arid regions, such as Baja California and Chile (Mooney *et al.*, 1974; Arroyo *et al.*, 1990), Southern Africa and Madagascar (Mooney *et al.*, 1977; Winter, 1979; Rundel *et al.*, 1999), North Africa and the Middle East (Winter, 1981; Ziegler *et al.*, 1981) and Mexico (Mooney *et al.*, 1989), and in tropical ecosystems rich in epiphytes, including South America (Medina and Troughton, 1974; Medina *et al.*, 1977), the Caribbean and Central America (Griffiths and Smith, 1983; Zotz and Ziegler,

1997), Papua New Guinea (Earnshaw *et al.*, 1987), Australia (Winter *et al.*, 1983) and Madagascar (Kluge *et al.*, 1998).

These broad surveys and subsequent physiological studies revealed a diversity of CAM physiology, associated morphology and ecological contexts throughout vascular plants (Fig. 2). CAM is not only common in succulent xeromorphic terrestrial and epiphytic plants, which experience frequent water-deficit stress, but is also present in a handful of aquatic lineages (Keeley, 1998). As isotope data accumulated, C<sub>3</sub> and CAM species in many clades separated largely into two distinct groups, showing a bimodal distribution of δ<sup>13</sup>C values, with a gap or minimum around −20 ‰ (Medina *et al.*, 1977; Griffiths and Smith, 1983; Winter *et al.*, 1983; Winter and Holtum, 2002; Crayn *et al.*, 2015; Messerschmid *et al.*, 2021; Orlov *et al.*, 2022). However, multiple species showed substantial intraspecific variation in carbon isotopic ratios (δ<sup>13</sup>C) and gas-exchange patterns indicating that, unlike C<sub>4</sub> species, CAM plants could regulate their use of CAM relative to the C<sub>3</sub> pathway depending on environmental conditions (Bender *et al.*, 1973; Osmond *et al.*, 1973; Black and Williams, 1976).

The variation in δ<sup>13</sup>C also reflects different degrees of CAM throughout ontogeny and seasonally. It was a landmark experiment by Winter and von Willert (1972) on *Mesembryanthemum crystallinum* L. that first demonstrated rapid induction of CAM in response to environmental stress (high salinity in this case). Since their discovery, subsequent research has uncovered diverse CAM phenotypes in dozens of lineages, ranging from ferns and lycophytes to cycads, gnetales and even C<sub>4</sub> angiosperms (see ‘*The phylogenetic diversity of CAM plants*’, below). Unlike C<sub>4</sub> photosynthesis (Sage *et al.*, 2011), the cataloguing of CAM species (e.g. Szarek and Ting, 1977; Szarek, 1979; Winter and Smith, 1996; Sayed, 2001) has not yet been coupled with an attempt to estimate the number of evolutionary origins of CAM across the plant tree of life. Here, leveraging advances in molecular phylogenetics and expanded surveys for CAM, we provide an updated occurrence record of the genera of vascular plants in which CAM activity has been detected (Table 1; a fully referenced list is presented in Supplementary Data Table S1), speculate about the number of independent evolutionary origins of CAM, discuss the challenges of this type of evolutionary accounting, and highlight outstanding questions in CAM evolution that we hope this review will facilitate in addressing (Box 2).

## THE LANDSCAPE OF CAM PHENOTYPES AND CAM EVOLUTION

The variability of CAM expression was appreciated by the late 19<sup>th</sup> century, and multiple CAM phenotypes have since been described (recently reviewed by Winter, 2019). These phenotypes are often projected along two axes: CAM mode, i.e. the degree to which CAM is constitutive and/or facultative, and CAM ‘strength’, i.e. the fraction of carbon fixed at night. CAM strength can be measured over short time scales (hours to days) by monitoring gas exchange or changes in titratable acidity (ΔH<sup>+</sup>) or it can be integrated over longer periods by δ<sup>13</sup>C values (Box 1). The best-studied CAM plants tend to exhibit either facultative CAM, with moderate to large ΔH<sup>+</sup> (e.g. *Mesembryanthemum crystallinum*), or strong and constitutive CAM (e.g. *Kalanchoë daigremontiana*) (Winter *et al.*, 2008).

## BOX 1. DIAGNOSING CAM IN THE FIELD AND LABORATORY

There are various methods for identifying CAM, with trade-offs between ease of sampling and what types of CAM expression they can capture (for a detailed review of experimental methods, see Osmond *et al.*, 1989).

- Stable carbon isotope ratios [ $\delta^{13}\text{C}$ , expressed in parts per thousand (‰)] reflect the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  in plant tissues relative to Pee Dee belemnite limestone or a secondary standard. Rubisco discriminates against  $^{13}\text{C}$  more strongly than does PEPC; thus, the more  $\text{CO}_2$  initially fixed by PEPC (during CAM) relative to Rubisco, the less negative the resulting  $\delta^{13}\text{C}$  value. Experiments demonstrate a linear relationship between  $\delta^{13}\text{C}$  and the proportion of  $\text{CO}_2$  fixed in the dark: purely light and dark  $\text{CO}_2$  assimilation translate to  $\delta^{13}\text{C}$  values of about  $-27$  and  $-8$  ‰, respectively, with  $-18$  ‰ reflecting roughly equal light and dark assimilation (Winter and Holtum, 2002). Plants that assimilate less than one-third of their  $\text{CO}_2$  in the dark have  $\delta^{13}\text{C}$  values indistinguishable from  $\text{C}_3$  species; therefore,  $\delta^{13}\text{C}$  values can be used as positive evidence for strong CAM but are, at best, suggestive of  $\text{C}_3$  + CAM. Given the bimodality of  $\delta^{13}\text{C}$  values across most clades containing  $\text{C}_3$  and CAM species (see ‘The landscape of CAM phenotypes and CAM evolution’),  $\delta^{13}\text{C} > -20$  ‰ is typically indicative of strong CAM. Two strengths of isotopic analysis are the small amount of tissue needed ( $\sim 1$  mg) and that it does not require living tissue and can therefore be assessed from herbarium and other non-living historical specimens, including fossils.
- Diel changes in titratable acidity [ $\Delta\text{H}^+$  typically expressed in millimoles of  $\text{H}^+$  per kilogram of fresh mass (FM)] measure the change in acid content in photosynthetic tissues and can confirm the presence of CAM. Calculating  $\Delta\text{H}^+$  requires measurement of titratable acidity or malate concentration at dusk (phase IV), when malic acid storage is expected to be at a minimum, and subtracting that value from the expected maximum at dawn (phase I). Statistically significant  $\Delta\text{H}^+$  greater than zero indicates CAM activity; strong-CAM species can have  $\Delta\text{H}^+ > 200$  mmol  $\text{H}^+$   $\text{kg}^{-1}$  FM, whereas most  $\text{C}_3$  + CAM and  $\text{C}_4$  + CAM species have  $\Delta\text{H}^+ < 200$  mmol  $\text{H}^+$   $\text{kg}^{-1}$  FM (Holtum *et al.*, 2017; Winter and Smith, 2022), and  $\Delta\text{H}^+$  can be  $< 10$  mmol  $\text{H}^+$   $\text{kg}^{-1}$  FM in species with very low CAM activity (Fig. 3; Hancock *et al.*, 2019); the lowest differences in  $\Delta\text{H}^+$  that can currently be resolved experimentally are  $\sim 1$  mmol  $\text{H}^+$   $\text{kg}^{-1}$  FM. A thorough test for CAM requires measurement of  $\Delta\text{H}^+$  in stressful conditions to assess the capacity of a plant for facultative CAM, including both stem and leaf tissues. Appropriate degrees of stress must be ascertained for each species. Too much stress can lead to the physiology of the tissues shutting down, resulting in little acid shift or nighttime  $\text{CO}_2$  fixation and precluding the evaluation of CAM behaviour.
- Gas-exchange curves show net  $\text{CO}_2$  exchange over 24 h periods, with dark-period net  $\text{CO}_2$  uptake providing evidence of CAM. However, many  $\text{C}_3$  + CAM (and  $\text{C}_4$  + CAM) species exhibit net negative  $\text{CO}_2$  exchange during the dark period. In these taxa, dark-period  $\text{CO}_2$  loss is typically reduced towards the middle of the night while  $\text{CO}_2$  fixation by PEPC occurs (phase I) (e.g. *Pilea peperomioides*; Winter *et al.*, 2021a). In contrast,  $\text{C}_3$  (and  $\text{C}_4$ ) species show relatively constant dark-period  $\text{CO}_2$  loss rates, representing background respiration. As in measurements of  $\Delta\text{H}^+$  or malate, facultative-CAM species require observations of gas exchange during normal and stressful conditions. Portable equipment to monitor photosynthesis has facilitated field use and enhanced screening potential; however, challenges to be addressed in gas-exchange assessments are sufficiently sized chambers for succulent leaf and/or stem tissue and sufficient battery power for lengthy gas-exchange runs over  $\geq 24$  h in remote field sites.
- Enzymatic activity and transcript and protein abundance can be used to measure the presence and expression status of key CAM enzymes, such as PEPC, and to diagnose carboxylation and decarboxylation enzyme subtypes used in CAM. Increased abundance and activity of key enzymes (e.g. PEPC) in biochemical assays, in combination with physiological data, can provide supportive evidence for CAM (and rule out  $\text{C}_4$  photosynthesis). Both whole-transcriptome sequencing and quantitative PCR allow powerful and high-throughput analysis of hundreds to thousands of genes at once, but as proxies for enzymatic activity they must be used in conjunction with other CAM assays. Measurement of protein amount/activity and transcript abundance is still mostly restricted to the laboratory to avoid sample degradation.

When combined, the CAM-identification methods discussed here [along with ancillary methods, such as  $^{14}\text{C}$  pulse–chase experiments, online stable-isotope discrimination techniques (Griffiths *et al.*, 2007; Barbour, 2017) and metabolic flux analysis following stable-isotope labelling (Szecowka *et al.*, 2013)] can illustrate how CAM is used day-to-day and throughout the life history.

As CAM has been surveyed more broadly and deeply, a complex landscape of CAM phenotypes has emerged, and experiments in the Aizoaceae (Winter, 2019) and Montiaceae (Hancock *et al.*, 2019) demonstrate that both the mode and the strength of CAM can vary considerably among plants that primarily utilize  $\text{C}_3$  photosynthesis (Fig. 3). CAM can be either constitutive and weak (*Calandrinia ptychosperma* F.Muell.) or facultative with strong CAM induction [*Jordaaniella cuprea* (L.Bolus) H.E.K.Hartmann]. Constitutive CAM plants may have further facultative responses [*Disphyma crassifolium* (L.)

L.Bolus] or reduce CAM (e.g.  $\Delta\text{H}^+$ ) under stress [*Titanopsis calcarea* (Marloth) Schwantes] (Winter, 2019). Although strongly and constitutively expressed CAM can be identified readily by  $\delta^{13}\text{C}$  values less negative than  $-20$  ‰ (in non- $\text{C}_4$  species), plants that obtain one-third or less of their carbon in the dark have isotopic signatures in the range of  $\text{C}_3$  plants (Winter and Holtum, 2002; Fig. 3 inset).

Although many labels have been applied to various CAM phenotypes in the past, here we use ‘ $\text{C}_3$  + CAM’ (*sensu* Edwards, 2019) to refer to species that use  $\text{C}_3$  photosynthesis

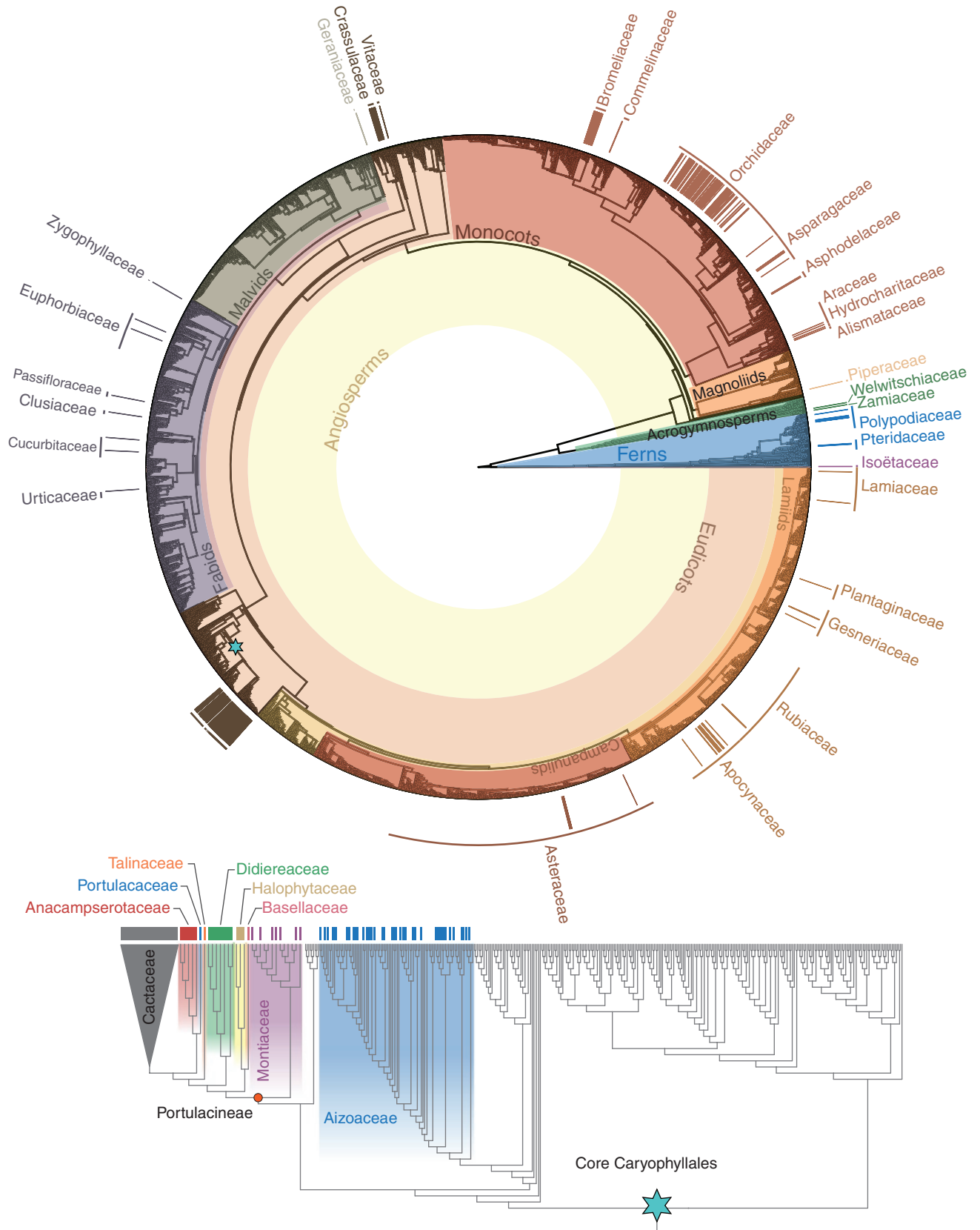


FIG. 2. The distribution of crassulacean acid metabolism among vascular plant genera and families. The topology is adapted from Hinchliff and Smith (2014), with an updated topology of some lineages in the core Caryophyllales following Moore et al. (2018); the core Caryophyllales (blue star) are expanded below, with the Cactaceae collapsed because all genera are assumed to be CAM. Only families with CAM genera are labelled, and bars above each tip indicate genera known to have one or more species capable of CAM, regardless of CAM phenotype. Branches are not to scale and have been adjusted for visualization.

TABLE 1. List of genera containing species capable of CAM photosynthesis; major clades are ordered following the linear classification system of APG IV (2016). Where species names have subsequently been synonymized or segregated into other genera, the original taxonomy is indicated in parentheses after the currently accepted name. Relevant phylogenetic studies are cited for clades with multiple putative origins of CAM. A fully referenced list with citations to initial and subsequent reports of CAM activity in these taxa, together with details of names reduced to synonymy, is provided as Supplementary Data (Table S1).

Major clade	Genus	Putative CAM origins
ISOËTALES		
<b>Isoëtaceae</b>	<i>Isoëtes</i> (including <i>Stylites</i> )	1
POLYPODIALES		
<b>Polypodiaceae</b>		
Microsoroideae	<i>Lecanopteris</i> ; <i>Microsorium</i>	2; 1 each in <i>Lecanopteris</i> and <i>Microsorium</i> (Chen et al., 2020)
Loxogrammoideae	<i>Dictymia</i>	1
Polypodioideae	<i>Niphidium</i>	1
Platynerioideae	<i>Platynerium</i> ; <i>Pyrrosia</i> (including <i>Drymoglossum</i> )	1 or 2; 1 in the ancestor of <i>Platynerium</i> and <i>Pyrrosia</i> or 1 in each clade (Wei et al., 2017)
<b>Pteridaceae</b>		
Vittarioideae	<i>Haplopteris</i> ; <i>Anetium</i> ; <i>Vittaria</i>	2; 1 in the ancestor of <i>Anetium</i> and <i>Vittaria</i> and 1 in <i>Haplopteris</i> (Schuettpepelz et al., 2016)
CYCADALES		
<b>Zamiaceae</b>	<i>Dioon</i>	1
WELWITSCHIALES		
<b>Welwitschiaceae</b>	<i>Welwitschia</i>	1
PIPERALES		
<b>Piperaceae</b>		
Piperoideae	<i>Peperomia</i>	5–12 (Frenzke et al., 2016; Lim et al., 2019)
ALISMATALES		
<b>Alismataceae</b>	<i>Sagittaria</i>	1
<b>Araceae</b>	<i>Zamioculcas</i>	1
<b>Hydrocharitaceae</b>	<i>Ottelia</i> ; <i>Vallisneria</i>	2; 1 each in <i>Ottelia</i> and <i>Vallisneria</i> (Chen et al., 2022)
ASPARAGALES		
<b>Orchidaceae</b>		
Epidendroideae	<i>Acianthera</i> ; <i>Aerangis</i> ; <i>Aeranthes</i> ; <i>Anathallis</i> ; <i>Angraecum</i> ; <i>Arachnis</i> ; <i>Aspasia</i> ; <i>Barkeria</i> ; <i>Bogoria</i> ; <i>Brassavola</i> ; <i>Brassia</i> ; <i>Bryobium</i> ; <i>Bulbophyllum</i> ; <i>Campylocentrum</i> ; <i>Cattleya</i> (including <i>Sophranitis</i> ); <i>Caularthron</i> ; <i>Caluera</i> ; <i>Capanemia</i> ; <i>Chiloschista</i> ; <i>Cischoewinfia</i> ; <i>Coelogyne</i> (including <i>Pholidota</i> ); <i>Comparettia</i> (including <i>Scelochilus</i> ); <i>Coryanthes</i> ; <i>Cymbidium</i> ; <i>Cyrtopodium</i> ; <i>Dendrobium</i> (including <i>Cadetia</i> , <i>Dockrillia</i> , <i>Flickingeria</i> and <i>Grastidium</i> ); <i>Dendrophylax</i> ; <i>Didymoplexis</i> ; <i>Domingoa</i> ; <i>Dimerandra</i> ; <i>Echinosepala</i> (including <i>Brenesia</i> ); <i>Elleanthus</i> ; <i>Encyclia</i> ; <i>Epidendrum</i> (including <i>Lanium</i> and <i>Oerstedella</i> ); <i>Eriopsis</i> ; <i>Erycina</i> (including <i>Psychmorchis</i> ); <i>Eulophia</i> (including <i>Acrolophia</i> , <i>Lissochilus</i> , <i>Oeceoclades</i> and <i>Orthochilus</i> ); <i>Gomesa</i> ; <i>Gongora</i> ; <i>Guarianthe</i> ; <i>Hintonella</i> ; <i>Ionopsis</i> ; <i>Jacquinilla</i> ; <i>Laelia</i> (including <i>Schomburgkia</i> ); <i>Leochilus</i> ; <i>Lockhartia</i> ; <i>Luisia</i> ; <i>Lycaste</i> ; <i>Macradenia</i> ; <i>Macroclinium</i> ; <i>Maxillaria</i> (including <i>Camaridium</i> , <i>Heterotaxis</i> , <i>Ornithidium</i> and <i>Trigonidium</i> ); <i>Meiracyllium</i> ; <i>Microcoelia</i> (including <i>Gussonea</i> ); <i>Micropera</i> ; <i>Mobilabium</i> ; <i>Mormodes</i> ; <i>Myoxanthus</i> ; <i>Myrmecophila</i> ; <i>Notylia</i> ; <i>Oberonia</i> ; <i>Oeonia</i> ; <i>Oncidium</i> ; <i>Ornithocephalus</i> ; <i>Pabstiella</i> ; <i>Peristeria</i> ; <i>Phalaenopsis</i> (including <i>Sedirea</i> ); <i>Platyrhiza</i> ; <i>Plectorrhiza</i> ; <i>Plectrophora</i> ; <i>Pleurothallis</i> ; <i>Pomatocalpa</i> ; <i>Prosthechea</i> ; <i>Psychilis</i> ; <i>Psychopsis</i> ; <i>Pterostemma</i> ; <i>Quekettia</i> ; <i>Rhinerrhiza</i> ; <i>Robiquetia</i> ; <i>Rodriguezia</i> ; <i>Rossioglossum</i> (including <i>Chelyorchis</i> ); <i>Saccolabiopsis</i> ; <i>Saccolabium</i> ; <i>Sarcochilus</i> ; <i>Scaphyglottis</i> ; <i>Schoenorchis</i> ; <i>Sobralia</i> ; <i>Solenidium</i> ; <i>Stanhopea</i> ; <i>Stelis</i> ; <i>Taeniophyllum</i> ; <i>Tetramicra</i> ; <i>Thrixspermum</i> ; <i>Tolumnia</i> ; <i>Trachoma</i> ; <i>Trichocentrum</i> (including <i>Cohniella</i> and <i>Lophiaris</i> ); <i>Trichoglottis</i> ; <i>Trichopilia</i> ; <i>Trichotostia</i> ; <i>Trizeuxis</i> ; <i>Vanda</i> (including <i>Ascocentrum</i> ); <i>Warmingia</i> ; <i>Zygostates</i>	5–9 (Silvera et al., 2009)

TABLE 1. *Continued*

Major clade	Genus	Putative CAM origins
Vanilloideae	<i>Vanilla</i>	1
<b>Asphodelaceae</b>		1; in the ancestor of Alooideae and <i>Bulbine</i>
Alooideae	<i>Aloe</i> ; <i>Aloidendron</i> ; <i>Aristaloe</i> ; <i>Astroloba</i> (including <i>Poellnitzia</i> ); <i>Gasteria</i> ; <i>Gonialoe</i> ; <i>Haworthia</i> ; <i>Haworthiopsis</i> ; <i>Tulista</i>	
'Asphodeloideae' <sup>1</sup>	<i>Bulbine</i>	
<b>Asparagaceae</b>		
Agavoideae	<i>Agave</i> (including <i>Manfreda</i> and <i>Polianthes</i> ); <i>Beschorneria</i> ; <i>Furcraea</i> ; <i>Hesperaloe</i> ; <i>Yucca</i>	3; 1 in the ancestor of <i>Agave</i> , <i>Beschorneria</i> and <i>Furcraea</i> , 1 in <i>Yucca</i> sect. <i>sarcocarpa</i> , and 1 in <i>Hesperaloe</i> (Heyduk et al., 2022)
Nolinoideae	<i>Beaucarnea</i> , <i>Sansevieria</i> <sup>2</sup>	2; 1 each in <i>Beaucarnea</i> and <i>Sansevieria</i> (Meng et al., 2021)
COMMELINALES		
<b>Commelinaceae</b>		
Commelinoideae	<i>Callisia</i> ; <i>Cyanotis</i> ; <i>Tradescantia</i> ; <i>Tripogandra</i>	2; 1 in the ancestor of <i>Callisia</i> , <i>Tradescantia</i> and <i>Tripogandra</i> ; and 1 in <i>Cyanotis</i> (Lee et al., 2021)
POALES		
<b>Bromeliaceae</b>		
Bromelioideae	<i>Acanthostachys</i> ; <i>Aechmea</i> (including <i>Streptocaylx</i> ); <i>Ananas</i> ; <i>Androlepis</i> ; <i>Araeococcus</i> ; <i>Billbergia</i> ; <i>Bromelia</i> ; <i>Canistropsis</i> ; <i>Canistrum</i> ; <i>Cryptanthus</i> ; <i>Deinacanthon</i> ; <i>Disteganthus</i> ; <i>Edmundoa</i> ; <i>Eduandrea</i> ; <i>Forzraea</i> ; <i>Hohenbergia</i> ; <i>Hohenbergiopsis</i> ; <i>Hylaeicum</i> ; <i>Karawata</i> ; <i>Lymania</i> ; <i>Neoglaziovia</i> ; <i>Neoregelia</i> ; <i>Nidularium</i> ; <i>Ochagavia</i> ; <i>Orthophytum</i> ; <i>Portea</i> ; <i>Pseudananas</i> ; <i>Pseudaraeococcus</i> ; <i>Quesnelia</i> ; <i>Ronnbergia</i> ; <i>Sincoraea</i> ; <i>Ursulaea</i> ; <i>Wittrockia</i>	2–5 in Bromelioideae (Givnish et al., 2014)
Hectioideae	<i>Hechtia</i>	1
Pitcairnioideae	<i>Deuterocohnia</i> ; <i>Dyckia</i> ; <i>Encholirium</i>	1
Puyoideae	<i>Puya</i>	1–3 within <i>Puya</i> (Givnish et al., 2014)
Tillandsioideae	<i>Guzmania</i> ; <i>Josemania</i> ; <i>Lemeltonia</i> ; <i>Tillandsia</i> ; <i>Werauhia</i>	1–5 (Barfuss et al., 2016)
SAXIFRAGALES		
<b>Crassulaceae</b>		1; CAM assumed ancestral to the Crassulaceae
Crassuloideae	<i>Crassula</i> (including <i>Rochea</i> )	
Kalanchoideae	<i>Adromischus</i> ; <i>Cotyledon</i> ; <i>Kalanchoë</i> ; <i>Tylecodon</i>	
Sempervivoideae	<i>Aeonium</i> (including <i>Greenovia</i> ); <i>Aichryson</i> ; <i>Cremnophila</i> ; <i>Dudleya</i> ; <i>Echeveria</i> ; <i>Graptopetalum</i> ; <i>Hylotelephium</i> ; <i>Lenophyllum</i> ; <i>Monanthes</i> ; <i>Orostachys</i> ; <i>Pachyphytum</i> ; <i>Rosularia</i> ; <i>Sedum</i> (including <i>Diamorpha</i> ); <i>Sempervivum</i> ; <i>Umbilicus</i> ; <i>Villadia</i>	
VITALES		
<b>Vitaceae</b>		
Vitaceae	<i>Cissus</i> ; <i>Cyphostemma</i>	2; 1 each in <i>Cissus</i> and <i>Cyphostemma</i> (Wen et al., 2018)
ZYGOPHYLLALES		
<b>Zygophyllaceae</b>		
Larreoideae	<i>Bulnesia</i>	1
CUCURBITALES		
<b>Cucurbitaceae</b>	<i>Seyrigia</i> ; <i>Xerosicyos</i>	2; 1 each in <i>Seyrigia</i> and <i>Xerosicyos</i> (Guo et al., 2020)
ROSALES		
<b>Urticaceae</b>	<i>Pilea</i>	1
MALPIGHIALES		
<b>Clusiaceae</b>	<i>Clusia</i>	1–4 within <i>Clusia</i> (Luján et al., 2022)
<b>Passifloraceae</b>	<i>Adenia</i>	1

TABLE 1. *Continued*

Major clade	Genus	Putative CAM origins
<b>Euphorbiaceae</b>		
Euphorbioideae	<i>Euphorbia</i> (including <i>Monadenium</i> , <i>Pedilanthus</i> and <i>Synadenium</i> )	1–13 within <i>Euphorbia</i> (Horn <i>et al.</i> , 2014)
Crotonoideae	<i>Jatropha</i>	1
GERANIALES		
<b>Geraniaceae</b>	<i>Monsonia</i> (including some members of <i>Sarcocaulon</i> ); <i>Pelargonium</i>	2–8; 1 in <i>Monsonia</i> and 1–7 in <i>Pelargonium</i> (Jones <i>et al.</i> , 2003; García-Aloy <i>et al.</i> , 2017; van de Kerke <i>et al.</i> , 2019)
CARYOPHYLLALES		
<b>Aizoaceae</b>		1–4; CAM might be ancestral to Aizoaceae or has evolved independently in Aizoioideae, Mesembryanthemoideae, Ruschioideae and Sesuvioideae, perhaps twice in Sesuvioideae (Klak <i>et al.</i> , 2004, 2017a; Valente <i>et al.</i> , 2014)
Aizoioideae	<i>Tetragonia</i>	
Mesembryanthemoideae	<i>Mesembryanthemum</i> (including <i>Aptenia</i> , <i>Aridaria</i> , <i>Aspazoma</i> , <i>Brownanthus</i> , <i>Opophytum</i> , <i>Phyllobolus</i> , <i>Prenia</i> , <i>Psilocaulon</i> , <i>Sceletium</i> , <i>Sphalmanthus</i> and <i>Synaptophyllum</i> )	
Ruschioideae	<i>Antimima</i> ; <i>Argyroderma</i> ; <i>Astridia</i> ; <i>Bergeranthus</i> ; <i>Carpobrotus</i> ; <i>Carruanthus</i> ; <i>Cephalophyllum</i> ; <i>Chasmatophyllum</i> ; <i>Cheiridopsis</i> ; <i>Conophytum</i> ; <i>Delosperma</i> ; <i>Disphyma</i> ; <i>Dracophilus</i> ; <i>Drosanthemopsis</i> (including <i>Anisocalyx</i> ); <i>Drosanthemum</i> ; <i>Eberlanzia</i> ; <i>Erepsia</i> ; <i>Faucaria</i> ; <i>Fenestraria</i> ; <i>Glottiphyllum</i> ; <i>Hartmanthus</i> ; <i>Hereroa</i> ; <i>Jacobsenia</i> ; <i>Jordaaniella</i> ; <i>Lampranthus</i> ; <i>Lithops</i> ; <i>Malephora</i> ; <i>Meyerophytum</i> ; <i>Mitrophyllum</i> ; <i>Monilaria</i> ; <i>Pleiospilos</i> ; <i>Prepodesma</i> ; <i>Psammophora</i> ; <i>Rabiea</i> ; <i>Rhinephyllum</i> ; <i>Ruschia</i> ; <i>Sarcosoma</i> ; <i>Schlechteranthus</i> ; <i>Stoebria</i> ; <i>Titanopsis</i> ; <i>Trichodiadema</i> ; <i>Vanheerdea</i>	
Sesuvioideae	<i>Sesuvium</i> ; <i>Trianthema</i>	
PORTULACINEAE		1; CAM likely to be ancestral to the Portulacineae (Goolsby <i>et al.</i> , 2018)
<b>Montiaceae</b>	Australian <i>Calandrinia</i> <sup>3</sup> ; <i>Calyptidium</i> ; <i>Cistanthe</i> ; <i>Claytonia</i> ; <i>Lewisia</i> ; <i>PheMERanthus</i>	
<b>Didiereaceae</b>		
Didiereoideae	<i>Alluaudia</i> ; <i>Alluaudiopsis</i> ; <i>Decarya</i> ; <i>Didierea</i>	
Portulacarioideae	<i>Portulacaria</i> (including <i>Ceraria</i> )	
<b>Basellaceae</b>	<i>Anredera</i> ; <i>Basella</i>	
<b>Halophytaceae</b>	<i>Halophytum</i>	
<b>Talinaceae</b>	<i>Talinum</i> (including <i>Talinella</i> )	
<b>Portulacaceae</b>	<i>Portulaca</i>	
<b>Anacampserotaceae</b>	<i>Anacampseros</i> ; <i>Grahamia</i> ; <i>Talinopsis</i>	
<b>Cactaceae</b> <sup>4</sup>		
Cactoideae	<i>Acanthocereus</i> (including some members of <i>Peniocereus</i> ); <i>Bergerocactus</i> ; <i>Carnegiea</i> ; <i>Cephalocereus</i> (including <i>Neobuxbaumia</i> ) <i>Cereus</i> (including <i>Subpilocereus</i> ); <i>Chamaecereus</i> ; <i>Cleistocactus</i> ; <i>Cochemiea</i> (including some members of <i>Mammillaria</i> ); <i>Consolea</i> ; <i>Copiapoa</i> (including <i>Pilocopiapoa</i> ); <i>Disocactus</i> ; <i>Echinocactus</i> ; <i>Echinocereus</i> ; <i>Echinopsis</i> ; <i>Epiphyllum</i> ; <i>Eriogyne</i> ; <i>Eulychnia</i> ; <i>Ferocactus</i> ; <i>Haageocereus</i> ; <i>Hattoria</i> ; <i>Leucostele</i> ; <i>Lobivia</i> ; <i>Lophocereus</i> ; <i>Lophophora</i> ; <i>Mammillaria</i> ; <i>Melocactus</i> ; <i>Myrtillocactus</i> ; <i>Oreocereus</i> ; <i>Oroya</i> ; <i>Pachycereus</i> ; <i>Parodia</i> ; <i>Pelecypora</i> ; <i>Pilosocereus</i> ; <i>Polaskia</i> ; <i>Rhipsalis</i> ; <i>Schlumbergera</i> (including <i>Zygocactus</i> ); <i>Sclerocactus</i> ; <i>Selenicereus</i> (including <i>Hylocereus</i> ); <i>Stenocereus</i> (including <i>Ritterocereus</i> ); <i>Stetsonia</i> ; <i>Trichocereus</i> ; <i>Turbiniacarpus</i>	
Opuntioideae	<i>Austrocylindropuntia</i> ; <i>Cylindropuntia</i> ; <i>Grusonia</i> ; <i>Maihueniopsis</i> ; <i>Opuntia</i> (including <i>Nopalea</i> ); <i>Peresklopsis</i> ; <i>Pterocactus</i> ; <i>Quiabentia</i> ; <i>Tephrocactus</i>	
Pereskioideae	<i>Maihuenia</i> ; <i>Pereskia</i> <sup>5</sup>	
GENTIANALES		
<b>Rubiaceae</b>		
Rubioideae	<i>Hydnophytum</i> ; <i>Myrmecodia</i> ; <i>Squamellaria</i>	1–3; either 1 in the ancestor of Hydnophytineae or 1 in each genus (Chomicki and Renner, 2016)
<b>Apocynaceae</b>		
Apocynoideae	<i>Pachypodium</i>	1



TABLE 1. *Continued*

Major clade	Genus	Putative CAM origins
Asclepiadoideae	<i>Apteranthes</i> ; <i>Boucerosia</i> (including <i>Frerea</i> ); <i>Caralluma</i> ; <i>Caudanthera</i> ; <i>Ceropegia</i> <sup>6</sup> ; <i>Cynanchum</i> (including <i>Folotsia</i> and <i>Sarcostemma</i> ); <i>Desmidorchis</i> ; <i>Dischidia</i> ; <i>Duvalia</i> ; <i>Hoodia</i> (including <i>Trichocaulon</i> ); <i>Hoya</i> ; <i>Huernia</i> ; <i>Orbea</i> ; <i>Quaqua</i> ; <i>Stapelia</i>	3; 1 each in Marsdenieae, Asclepiadeae and Ceropegieae (Wanntorp et al., 2014; Bruyns et al., 2017; Liede-Schumann et al., 2022)
LAMIALES		
<b>Plantaginaceae</b>	<i>Littorella</i>	1
<b>Gesneriaceae</b>		
Didymocarpoideae	<i>Haberlea</i> ; <i>Ramonda</i>	1
Gesnerioideae	<i>Codonanthis</i>	1
<b>Lamiaceae</b>		
Lamioideae	<i>Marrubium</i>	1
Nepetoideae	<i>Coleus</i> (including some members of <i>Plectranthus</i> )	1
ASTERALES		
<b>Asteraceae</b>		
Asteroideae	<i>Baculellum</i> ; <i>Caputia</i> ; <i>Crassothonna</i> ; <i>Curio</i> ; <i>Kleinia</i> ; <i>Othonna</i> ; <i>Senecio</i>	2 or 3; 1 in the Gynuroid clade and 1 or 2 in the <i>Faujasia</i> – <i>Bethencourtia</i> clade (Pelser et al., 2007; Ozerova et al., 2017)
		Total known origins: 66–114+

<sup>1</sup>Asphodeloideae is polyphyletic, with *Bulbine* generally sister to Alooideae, as summarized by Smith and Figueiredo (2020).

<sup>2</sup>*Sansevieria* has been proposed to be subsumed within *Dracaena* (Takawira-Nyenya et al., 2018).

<sup>3</sup>*Calandrinia* is not monophyletic (Hancock et al., 2019), and CAM has been observed only in the monophyletic clade inclusive of all Australian members of *Calandrinia sensu lato*.

<sup>4</sup>All cacti are assumed CAM; the genera presented here are those with published data confirming CAM activity.

<sup>5</sup>We do not include *Leuenbergeria* but recognize that *Pereskia* is non-monophyletic.

<sup>6</sup>*Ceropegia* has recently been shown to be polyphyletic, with *Brachystelma* and the stem-succulent stapeliads (including *Apteranthes*, *Caralluma*, *Caudanthera*, *Boucerosia*, *Duvalia*, *Hoodia*, *Huernia*, *Orbea*, *Quaqua* and *Stapelia*) nested within it. These genera have been proposed to be subsumed within an expanded and circumscribed *Ceropegia* (Bruyns et al., 2017) but are still recognized by Kew (POWO, 2023) and other authorities (Endress et al., 2018).

as the principal pathway of carbon gain but also express CAM to various degrees. We use the term ‘strong CAM’ to refer to species that use CAM as the principal pathway of carbon gain; this corresponds to the ‘strong CAM’ category of Edwards (2019), and the ‘CAM plant’ recommendation of Winter et al. (2015). Many species capable of CAM have been demonstrated to induce or upregulate CAM in response to stress (typically drought or salinity stress) in natural or laboratory experiments; this behaviour is known as ‘facultative CAM’. These species do not express CAM in favourable conditions or do so only weakly. Although plants with intermediate  $\delta^{13}\text{C}$  values have been found in many clades and constitute the majority of species in Aizoaceae subfamily Mesembryanthemoideae (most probably owing to intraspecific plasticity in carbon isotopic composition because of seasonal shifts in  $\delta^{13}\text{C}$ ) (Winter et al., 1976; Messerschmid et al., 2021; Winter and Smith, 2022), the strong bimodality in  $\delta^{13}\text{C}$  values seen in the majority of clades in which CAM occurs implies that carbon is either mostly fixed with  $\text{C}_3$  photosynthesis or mostly fixed with CAM (Winter and Holtum, 2002; Winter et al., 2015; Edwards, 2019; Messerschmid et al., 2021; Orlov et al., 2022). The development of a holistic CAM evolutionary model should explain the observations that most clades do not contain a uniform distribution of  $\delta^{13}\text{C}$  values and that  $\text{C}_3$  + CAM species can occupy

distinct regions of the  $\delta^{13}\text{C}$  distribution over long periods of evolutionary time.

It is believed that these phenotypes represent ordered character states, ranging from  $\text{C}_3$  to  $\text{C}_3$  + CAM to strong CAM, because strong-CAM lineages are frequently subtended by earlier-branching  $\text{C}_3$  + CAM lineages in phylogenetic studies of CAM evolution (Hancock and Edwards, 2014), although some have hypothesized that the evolutionary trajectories to facultative and constitutive CAM are different (Yang et al., 2019). However, there are relatively few clades for which we have both sufficiently widespread CAM surveys, including tests of  $\text{C}_3$  + CAM, and well-resolved phylogenies to be able to infer the relationships between states with confidence. For example, Orchidaceae, which contain numerically a substantial proportion of the planet’s CAM species, and Crassulaceae, which include multiple model CAM species of *Kalanchoë*, generally have poorly resolved phylogenies at the species level and relatively few surveys of  $\text{C}_3$  + CAM (but see Silvera et al., 2005, 2014). More detailed studies have demonstrated phylogenetic patterns that support an evolutionary trajectory from  $\text{C}_3$  to  $\text{C}_3$  + CAM to strong CAM in multiple clades, e.g. within subtribe Oncidiinae of Orchidaceae (Silvera et al., 2014), Cactaceae (Edwards and Donoghue, 2006), *Clusia* (Luján et al., 2022) and Agavoideae (Heyduk et al., 2022).

## BOX 2. OUTSTANDING QUESTIONS IN CAM EVOLUTION

Although CAM evolution has long been an area of CAM research, phylogenetic analyses of CAM have been published largely within the last two decades. These include the reconstruction of ancestral character states (Crayn *et al.*, 2004; Edwards and Donoghue, 2006; Bone *et al.*, 2015; Heyduk *et al.*, 2016; Hancock *et al.*, 2019; Luján *et al.*, 2022) and tests of the association between CAM and net diversification rates (Givnish *et al.*, 2014, 2015; Horn *et al.*, 2014); but many open questions remain, ripe for investigation with recently developed models of trait evolution, denser and broad phylogenies, and increased CAM-associated trait data.

## THE TIMING OF CAM EVOLUTION

Carbon-concentrating mechanisms are generally believed to have evolved in terrestrial species post-Oligocene, following a reduction in atmospheric CO<sub>2</sub> to near current values (Edwards and Ogburn, 2012), but few clades have been evaluated for the timing of CAM origins. Those that have been examined are generally consistent with the C<sub>4</sub> pattern of post-Oligocene evolution followed by extensive diversification (Sage *et al.*, 2023). Some large CAM clades, however, such as the Portulacineae and Crassulaceae, might have ancient origins, pre-dating the late-Oligocene CO<sub>2</sub> decline (Wang *et al.*, 2019a; Messerschmid *et al.*, 2020), implying CAM evolution in higher-CO<sub>2</sub> settings than C<sub>4</sub> evolution. These hypotheses will be re-evaluated as phylogenomic data, paired with new phylogenetic comparative analyses, produce the more detailed, CAM-specific phylogenies needed to identify CAM origins on time-calibrated phylogenies.

## THE EARLIEST STEPS IN CAM EVOLUTION

Research efforts towards the engineering of CAM have sharpened our understanding that CAM evolution is likely to involve both discrete and continuous changes (Borland *et al.*, 2014; Bräutigam *et al.*, 2017; Edwards, 2019, 2023; Winter and Smith, 2022). However, we lack the comparative genomic studies between very recently diverged CAM and non-CAM species or populations needed to identify protein and regulatory changes that enabled the emergence of CAM in a non-CAM ancestor.

## CAM-ASSOCIATED TRAITS

The evolution of CAM has been associated with many traits (recently reviewed by Niechayev *et al.*, 2019), but quantitative or statistical tests of these relationships are generally lacking. The link between CAM and succulence has been apparent from the earliest studies of CAM, but explicit relationships have not been described. Likewise, we lack quantitative analyses of CAM–environment associations at evolutionary scales. The development of climate-dependent trait evolution models (Clavel and Morlon, 2017) might facilitate such investigations, and the creation of CAM physiological models (Shameer *et al.*, 2018; Töpfer *et al.*, 2020) might help to identify potential hotspots of CAM evolution in deep time, as has been done for C<sub>4</sub> (Zhou *et al.*, 2018). Physiological models might also shed light on the functional significance of C<sub>3</sub> + CAM. Many C<sub>3</sub> + CAM species do not assimilate substantial amounts of carbon via CAM (Herrera, 2008; Winter, 2019), but other benefits, such as recapture of respiratory CO<sub>2</sub>, and energy and water savings (Martin, 1996; Shameer *et al.*, 2018; Töpfer *et al.*, 2020) or photoprotection (Osmond, 1982; Pieters *et al.*, 2003) under water deficit have been proposed.

Further exploration might change our understanding of CAM evolution dramatically, as in the Portulacineae (Fig. 2), where tests of C<sub>3</sub> + CAM and new phylogenomic studies have revealed lineages with varying CAM phenotypes and very few, if any, purely C<sub>3</sub> Portulacineae species outside Montiaceae. Given the young age of many CAM clades [e.g. Ruschioideae, ~7 (3.4–12.6) Ma; Klak *et al.*, 2017a] and that the evolution of CAM has been associated with increased diversification rates in multiple clades (Givnish *et al.*, 2014, 2015; Horn *et al.*, 2014; Silvestro *et al.*, 2014), it has been challenging to identify well-resolved and well-sampled clades with known C<sub>3</sub>, C<sub>3</sub> + CAM and strong-CAM species. *Erycina* (Oncidiinae: Orchidaceae) has emerged as a candidate model for CAM evolution with the publication of a whole-chloroplast genome of CAM *Erycina pusilla* (L.) N.H. Williams & M.W. Chase (Pan *et al.*, 2012) and comparative transcriptomic research between *E. pusilla* and C<sub>3</sub> *Erycina crista-galli* (Rchb.f.) N.H. Williams

& M.W. Chase (Heyduk *et al.*, 2019a). The Oncidiinae might have multiple transitions from C<sub>3</sub> to strong CAM if C<sub>3</sub> + CAM is indeed absent in the currently recognized ‘C<sub>3</sub>’ members, but the clades perhaps currently best situated for studying transitions to and between CAM phenotypes are *Clusia* (Clusiaceae) and the Agavoideae (Asparagaceae). The Neotropical woody genus *Clusia* contains >300 species with multiple recognized C<sub>3</sub>, C<sub>3</sub> + CAM and strong-CAM taxa (Lüttge, 2007; Pachon *et al.*, 2022). A recent study of the evolution of CAM physiology and morphology in *Clusia* found correlations between CAM activity and both leaf morphology and dry season severity (Luján *et al.*, 2022). The authors reconstructed a phylogeny of dozens of *Clusia* taxa with multiple photosynthetic phenotypes and, depending on model choice, ancestral state reconstructions supported either one origin of strong CAM and several reversions or multiple origins of strong CAM (Luján *et al.*, 2022). Research has confirmed C<sub>3</sub>, C<sub>3</sub> + CAM and strong-CAM

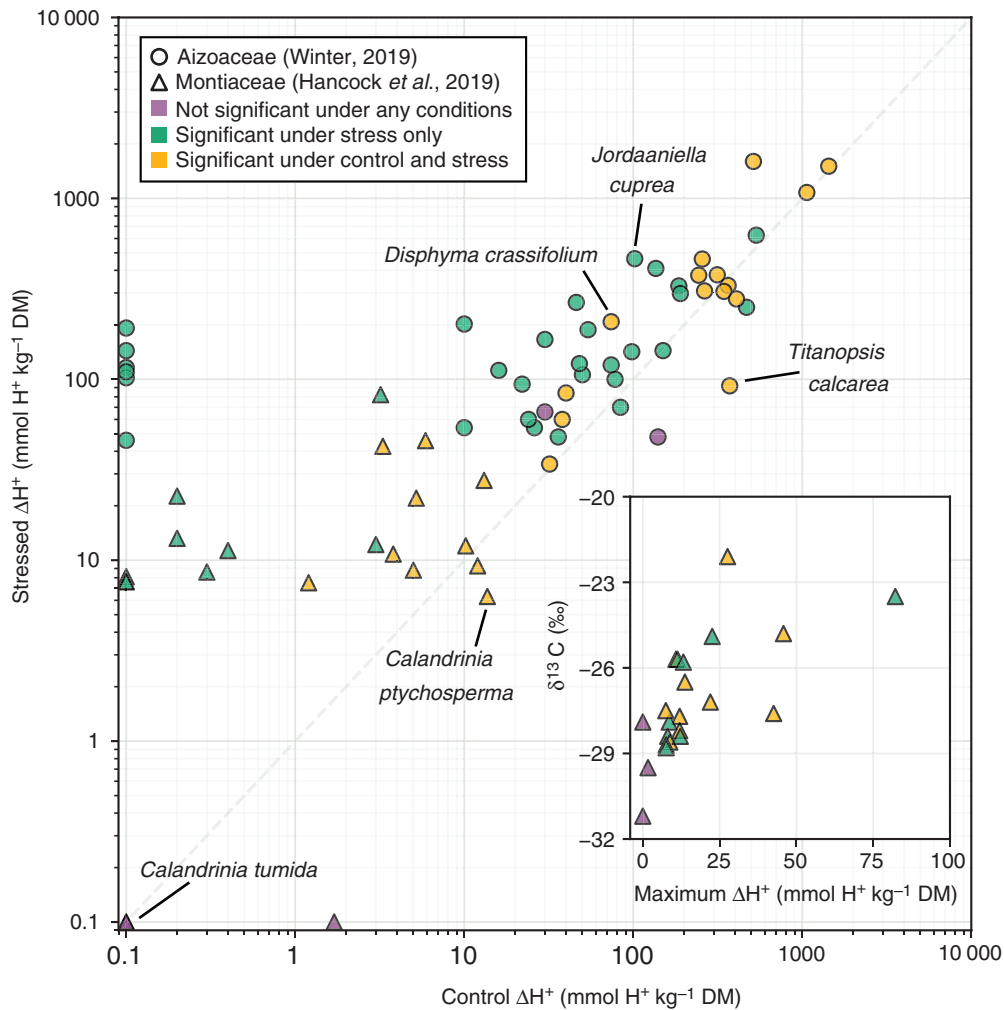


FIG. 3. Mean diel  $\Delta H^+$  in control (well-watered) and stress (drought or drought + salt stress) conditions of 45 Aizoaceae species (circles) (Winter, 2019) and 22 Montiaceae species (triangles) (Hancock et al., 2019). Purple points represent species with no significant  $\Delta H^+$  in any conditions. Green points represent species with significant  $\Delta H^+$  only in stress conditions. Yellow points represent species with significant  $\Delta H^+$  in both control and stress conditions. The inset shows the maximum  $\Delta H^+$  and mean  $\delta^{13}C$  values (Hancock et al., 2019) of the same Montiaceae species in the main plot. Note that  $\Delta H^+$  for Aizoaceae species was calculated by multiplying changes in tissue malate concentrations given by Winter (2019) by a factor of two, assuming that 1 mol malate corresponds to 2 mol  $H^+$ . Points with  $\Delta H^+ < 0.1$  were plotted at 0.1 for visualization. Five individual species discussed in the main text are labelled.

species in the Agavoideae and hypothesized multiple origins of CAM over the past 25 Ma (Heyduk et al., 2022). *Yucca* also produces natural  $C_3$  + CAM hybrids (*Yucca gloriosa* L.) arising from  $C_3$  (*Yucca filamentosa* L.) and strong-CAM (*Yucca aloifolia* L.) populations (Rentsch and Leebens-Mack, 2012; Heyduk et al., 2021) that offer a unique means to study the genetic components of CAM.

Although the growing number of  $C_3$  + CAM observations continue to amend the hypothesized timing and phylogenetic placement of CAM origins (Box 2), their diversity offers an unrivalled opportunity to study the evolution of convergent ecophysiology. The evolutionary trajectories from  $C_3$  to CAM have been debated over recent years. Some have argued that CAM requires relatively few evolutionary changes because all enzymes and biochemical pathways used in CAM exist in  $C_3$  plants (Bräutigam et al., 2017; Schiller and Bräutigam, 2021), whereas others have argued that substantial metabolic reprogramming is required to evolve CAM (Winter and

Smith, 2022), not least because malate typically accumulates during the daytime in  $C_3$  plants but during the nighttime in CAM plants. Others have argued that  $C_3$  + CAM can (and has) evolved readily in a diversity of  $C_3$  lineages, but that strong CAM requires more anatomical specialization and is less evolutionarily labile (Edwards, 2019). Whatever the path(s) to CAM, comparative studies of  $C_3$  + CAM species, and particularly those that use CAM facultatively in response to stress, are poised to shed light on  $C_3$ -to-CAM transitions. Paired whole-transcriptome and physiological (e.g. gas exchange,  $\Delta H^+$  and metabolomes) datasets from CAM-induction experiments are available for *Talinum* (Talinaceae) (Brilhaus et al., 2016), *Beschorneria*, *Agave* (including *Manfreda* and *Polianthes*) and *Yucca* (Agavoideae) (Heyduk et al., 2022), *Dendrobium* (Orchidaceae) (Zou et al., 2018), *Sedum* (Crassulaceae) (Wai et al., 2019), *Portulaca* (Portulacaceae) (Ferrari et al., 2020; Gilman et al., 2022; Moreno-Villena et al., 2022), *Mesembryanthemum* (Aizoaceae) (Cushman et al.,

TABLE 2. CAM species with publicly available whole-genome sequences and associated assembly statistics; BUSCO, benchmarking universal single-copy orthologue (Manni et al., 2021).

Taxon <sup>1</sup>	CAM type <sup>2</sup>	Genome size	Scaffold N50	Scaffold L50	Complete Embryophyta BUSCOs (%)
<b>Isoëtaceae</b> <i>Isoëtes taiwanensis</i> De Vol (Wickell et al., 2021)	Aquatic strong CAM	1.65 Gb	17.4 Mb	Not reported	94.5 <sup>3</sup>
<b>Asphodelaceae</b> <i>Aloe vera</i> (L.) Burm.F. (Jaiswal et al., 2021)	Strong CAM (Winter and Holtum, 2002)	12.93 Gb	14.6 kb	Not reported	74.6
<b>Orchidaceae</b> <i>Cymbidium crassifolium</i> Herb. (Fan et al., 2023; reported as <i>Cymbidium manni</i> Rehb.f.)	Strong CAM	2.88 Gb	22.7 Mb	Not reported	97.0
<i>Dendrobium nobile</i> Lindl. (Xu et al., 2022)	C <sub>3</sub> + CAM (Qiu et al., 2015)	1.16 Gb	64.5 Mb	8	96.2
<i>Dendrobium officinale</i> Kimura & Migo (Yan et al., 2015)	C <sub>3</sub> + CAM (Zou et al., 2018; reported as <i>D. catenatum</i> Lindl.)	1.35 Gb	76.5 kb	4697	Not reported
<i>Dendrobium chrysotoxum</i> Lindl. (Zhang et al., 2021)	C <sub>3</sub> + CAM (Qiu et al., 2015)	1.37 Gb	67.8 Mb	8	90.3
<i>Phalaenopsis equestris</i> (Schauer) Rehb.f. (Cai et al., 2015)	Strong CAM (Zhang et al., 2016)	1.16 Gb	359 kb	523	Not reported
<i>Vanilla planifolia</i> Andrews (Hasing et al., 2020)	Strong CAM (Silvera et al., 2010a)	1.48 Gb	42.0 Mb	13	93.9
<b>Bromeliaceae</b> <i>Aechmea fasciata</i> (Lindl.) Baker (Li et al., 2022)	Strong CAM (Crayn et al., 2015)	359 Mb	4.68 Mb	38	93.4
<i>Ananas comosus</i> (L.) Merr. V3 (Ming et al., 2015)	Strong CAM	526 Mb	11.8 Mb	13	89.3
<b>Crassulaceae</b> <i>Kalanchoë fedtschenkoi</i> Raym.-Hamet & H.Perrier v1.1 (Yang et al., 2017)	Strong CAM	256.35 Mb	2.5 Mb	28	89.5
<i>Kalanchoë laxiflora</i> Baker v3.1 (Yang et al., 2017)	Strong CAM	262 Mb	12.5 Mb	8	93.2
<i>Sedum album</i> L. (Wai et al., 2019)	C <sub>3</sub> + CAM	302 Mb	93 kb	868	97
<b>Vitaceae</b> <i>Cissus rotundifolia</i> Vahl (Xin et al., 2022)	Strong CAM (Nelson and Sage, 2008)	350.69 Mb	27.6 Mb	5	92.4
<b>Euphorbiaceae</b> <i>Jatropha curcas</i> L. (Ha et al., 2019)	C <sub>3</sub> + CAM (Winter and Holtum, 2015)	414 Mb	1.5 Mb	Not reported	82.5
<b>Aizoaceae</b> <i>Mesembryanthemum crystallinum</i> L. (Shen et al., 2022)	Facultative CAM	377.97 Mb	40.5 Mb	5	98.0
<b>Cactaceae</b> <i>Carnegiea gigantea</i> (Engelm.) Britton & Rose (Copetti et al., 2017)	Strong CAM (Nobel and Hartssock, 1986)	1.30 Gb	61.5 kb	4575	91
<i>Lophocereus schottii</i> (Engelm.) Britton & Rose (Copetti et al., 2017)	Strong CAM (Mooney et al., 1974)	1.47 Gb	9.3 kb	21 671	74
<i>Pachycereus pringlei</i> (S.Watson) Britton & Rose (Copetti et al., 2017)	Strong CAM (Mooney et al., 1974)	1.41 Gb	5.4 kb	32 562	61
<i>Pereskia horrida</i> DC. (Copetti et al., 2017; reported as <i>P. humboldtii</i> Britton & Rose)	C <sub>3</sub> + CAM (Martin and Wallace, 2000)	980 Mb	4.4 kb	28 266	52
<i>Stenocereus thurberi</i> (Engelm.) Buxb. (Copetti et al., 2017)	Strong CAM (Huber et al., 2018)	1.42 Gb	10.5 kb	20 352	71
<i>Selenicereus undatus</i> (Haw.) D.R.Hunt (reported as <i>Hylocereus undatus</i> (Haw.) D.R.Hunt) (Chen et al., 2021)	Strong CAM (Wang et al., 2019b)	1.41 Gb	127.2 Mb	670	93.8 <sup>3</sup>

TABLE 2. Continued

Taxon <sup>1</sup>	CAM type <sup>2</sup>	Genome size	Scaffold N50	Scaffold L50	Complete Embryophyta BUSCOs (%)
<b>Portulacaceae</b>					
<i>Portulaca amilis</i> Speg. (Gilman et al., 2022)	C <sub>4</sub> + CAM	403.89 Mb	42.6 Mb	5	96.9
<i>Portulaca oleracea</i> L. (Wang et al., 2023)	C <sub>4</sub> + CAM	1.13 Gb	35.1 Mb	11	98.0

<sup>1</sup>Genome assembly version 1.0 unless otherwise noted.

<sup>2</sup>Evidence of CAM is provided under the Taxon column unless provided here.

<sup>3</sup>Eukaryota BUSCOs were used to benchmark *Isoetes taiwanensis* (Wickell et al., 2021) and *Hyllocereus undatus* (Chen et al., 2021).

2008) and *Isoetes* (Yang and Liu, 2015). These studies have revealed substantial similarities between CAM induction across clades and regardless of life history (annual or perennial) or habit (epiphytic or terrestrial), but also key differences, e.g. varied peak transcription of CAM-specific isoforms of PEPC from mid-afternoon to late dark period. These CAM-induction experiments of the past two decades have been key for identifying and profiling core CAM elements (Winter and Holtum, 2014), and future multi-species comparisons will be essential in describing the regulation of CAM and how CAM is induced. No direct comparisons of regulatory elements (e.g. transcription factors or *cis*-elements) have been made across C<sub>3</sub> + CAM species that could explain how CAM is incorporated into stress responses or how facultative CAM becomes canalized. Most -omics research has focused on large, diverse CAM clades (e.g. Agavoideae, Crassulaceae, Orchidaceae and Portulacaceae), but it might be more fruitful to develop model systems around relatively species-poor CAM origins, which afford relevant C<sub>3</sub> comparisons in more closely related species. At the time of publication, publicly available genomes exist for ≥23 species capable of CAM, most of reference quality (Table 2); two orchids from genera that contain species with CAM have whole-genome sequences but have not been assessed for C<sub>3</sub> + CAM [*Cymbidium sinense* (Andrews) Willd. (Yang et al., 2021) and *Dendrobium huoshanense* Z.Z.Tang & S.J.Cheng (Han et al., 2020)], and a genome sequence is available for *Mikania micrantha* Kunth (Asteraceae), which has been suggested to be CAM, based on gene expression (Liu et al., 2020) (Supplementary Data Table S2).

#### ATYPICAL CAM

Most of our understanding of CAM comes from terrestrial xeric and epiphytic plants, which experience regular water limitation, and CAM species tend to have very high water-use efficiency relative to C<sub>3</sub> and C<sub>4</sub> species (Winter et al., 2005). However, CAM is also found in dozens of aquatic plant species (Keeley, 1998) and has been observed in more diverse forms and unexpected lineages. These examples, which fall outside the common CAM phenotypes, further highlight the diversity of species capable of CAM and its functional significance.

#### *Aquatic CAM and other photosynthetic pathways*

The apparent paradox of xeric adaptations in submerged and aquatic plants can be resolved by recognition that the pressures of water loss and CO<sub>2</sub> limitation represent two sides of the same coin for terrestrial species: water limitation leads to stomatal closure, thereby restricting CO<sub>2</sub> uptake. Seasonal, or vernal, pools may contain numerous aquatic CAM species from genera including *Isoetes*, *Crassula* and *Sagittaria*. Vernal pools often exhibit large fluctuations in CO<sub>2</sub> concentration because CO<sub>2</sub> is depleted during the day by the photosynthetic activity of non-CAM vegetation, solar warming of the shallow water, and CO<sub>2</sub> diffusion limitations from the air; at night, heterotrophic respiration by plants and animals increases the concentration of dissolved CO<sub>2</sub> in the water dramatically (Raven and Spicer, 1996; Keeley, 1998). These diel trends in dissolved CO<sub>2</sub> create a niche for CAM plants, which can capture dissolved CO<sub>2</sub> when

it is abundant at night and use it during the day when pools become CO<sub>2</sub> depleted. CAM activity decreases or ceases entirely in tissues of vernal CAM plants that become emergent because CO<sub>2</sub> diffusion in air is orders of magnitude higher than in water, further supporting the functional significance of CAM in CO<sub>2</sub>-limited conditions (Keeley, 1998).

In addition to vernal pools, aquatic CAM plants can be found in high-elevation oligotrophic lakes. In such nutrient-poor systems, *Isoetes* can absorb CO<sub>2</sub> directly through their extensive root systems, with diffusion through the well-developed air canals carrying CO<sub>2</sub> to the shoot, where it can be fixed in the dark via CAM in leaves; roots may thereby account for the majority of CO<sub>2</sub> uptake in species such as *Isoetes andicola* (Amstutz) L.D.Gómez (previously *Stylites andicola* Amstutz) (Keeley et al., 1984).

Although most species with carbon-concentrating mechanisms (CCMs) are hypothesized to have evolved and diversified following the Oligocene atmospheric CO<sub>2</sub> decline (Arakaki et al., 2011; Edwards and Ogburn, 2012), the selective pressures to evolve aquatic CAM are less coupled to atmospheric CO<sub>2</sub> levels; it is possible that CAM is ancient in lineages such as in the lycophyte *Isoetes*, an early vascular plant clade with a stem age estimated to be 370 Ma (Wood et al., 2020). Aquatic and terrestrial CAM species have mostly been treated separately, but the recent publication of an *Isoetes* genome (Table 2) and transcriptomic data (Wickell et al., 2021) will facilitate a fuller understanding of CAM regulation using modern comparative methods.

Since the near-simultaneous reports of CAM in the submerged plants *Isoetes howellii* Engelm. (Keeley, 1981) and *Schoenoplectus subterminalis* (Torr.) Soják (previously *Scirpus subterminalis* Torr.) (Beer and Wetzel, 1981) (the latter has not been revisited or considered a CAM plant since), aquatic CAM has been confirmed in all aquatic species of *Isoetes* tested (Keeley, 1998) and in *Crassula aquatica* Schönland (Crassulaceae) (Keeley and Morton, 1982) and *Crassula helmsii* (Kirk) Cockayne (Newman and Raven, 1995), *Littorella uniflora* (L.) Asch. (Plantaginaceae) (Madsen, 1987; Keeley and Morton, 1982), *Sagittaria* (Alismataceae) (Keeley, 1996, 1998), and *Vallisneria* and *Ottelia* (both Hydrocharitaceae) (Helder and van Harmelen, 1982; Webb et al., 1988; Zhang et al., 2014). Many aquatic CAM macrophytes are considered highly invasive outside their native ranges (Klavnsen et al., 2011), but whether and how CAM might influence invasiveness has not been studied in detail.

CAM-like phenomena (particularly nocturnal acid or malate accumulation) have been reported for a wider diversity of aquatic or submerged species, including algae (reviewed by Keeley, 1998), but many of these designations have been questioned (Supplementary Data Table S2). Submerged plants might use multiple CCMs or accumulate acid that is not maintained as malic acid through the dark period or does not ultimately enter the Calvin–Benson–Bassham cycle (Keeley, 1999). Certain aquatic lineages use a dissolved bicarbonate-based CCM in addition to fixing dissolved CO<sub>2</sub> directly (Keeley, 1998), and there are several known submerged C<sub>4</sub> lineages, a small number of which use a form of single-cell C<sub>4</sub> photosynthesis without Kranz anatomy (Bowes, 2011). Similar to aquatic CAM, aquatic C<sub>4</sub> (with or without Kranz anatomy) can be induced by low CO<sub>2</sub> (or bicarbonate) (Keeley, 1998), meaning that

detection and delineation between CCMs requires extensive field sampling and laboratory experiments that document gas exchange and diurnal changes in malic acid (Box 1). For example, although *Eleocharis maculosa* (Vahl) Roem. & Schult. (Cyperaceae) can show very small ΔH<sup>+</sup>, radiolabel pulse–chase experiments have demonstrated that carbon in nocturnally produced malate does not flow into the Calvin–Benson–Bassham cycle in the following light period, as it does in *bona fide* aquatic CAM species (Keeley, 1999). However, careful experiments with *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) (Holaday and Bowes, 1990; Rao et al., 2006) demonstrated that *H. verticillata* has low-CO<sub>2</sub>-induced C<sub>4</sub> and probably weak CAM (see ‘Terrestrial C<sub>4</sub> + CAM’, below). Likewise, *Ottelia alismoides* (L.) Pers. (Hydrocharitaceae) has been shown to be a constitutive submerged C<sub>4</sub> plant with inducible CAM (Zhang et al., 2014; Shao et al., 2017). The diversity of photosynthetic pathways in aquatic plants, in addition to their ability to transition between them and to use multiple CCMs simultaneously, presents a more complicated photosynthetic landscape than in terrestrial plants, and many classifications as ‘CAM’ should be evaluated carefully or investigated further.

#### Terrestrial C<sub>4</sub> + CAM

The combination of CCMs is not restricted to aquatic plants. CAM has been demonstrated clearly in two succulent terrestrial C<sub>4</sub> clades (hereafter ‘C<sub>4</sub> + CAM’): *Portulaca* (Portulacaceae) and *Trianthema* (Aizoaceae). Laboratory measurements of gas exchange and ΔH<sup>+</sup> revealed facultative CAM in all studied species of *Portulaca* (Koch and Kennedy, 1980; Guralnick et al., 2002; Holtum et al., 2017; Gilman et al., 2022), including those with C<sub>3</sub>–C<sub>4</sub> intermediacy (Winter et al., 2019), and low-level constitutive CAM has been demonstrated in C<sub>4</sub> *Trianthema portulacastrum* L. (Winter et al., 2021a).

*Portulaca* is the most studied of the terrestrial C<sub>4</sub> + CAM clades, and all research has supported the hypothesis that CAM is ancestral to *Portulaca* and probably evolved deep within (or in an ancestor of) the Portulacineae (Christin et al., 2014; Goolsby et al., 2018). Given the substantial variation in C<sub>4</sub> characters (i.e. leaf anatomy, biochemical subtypes and species-specific gene use; Ocampo et al., 2013; Voznesenskaya et al., 2017; Gilman et al., 2022), in addition to C<sub>3</sub>–C<sub>4</sub> intermediacy in some species, it is likely that C<sub>4</sub> evolved convergently in three facultative CAM *Portulaca* lineages: the Pilosa + Umbraticola, Oleracea + Cryptopetala and opposite-leaved clades. Spatially explicit analyses of gene expression in *Portulaca oleracea* L. demonstrated that C<sub>4</sub> and CAM cycles are integrated, with CAM-generated malate produced in the mesophyll probably being decarboxylated by the C<sub>4</sub> cycle in the bundle sheath during the day (Moreno-Villena et al., 2022).

The precise origins (or perhaps single origin) of CAM in Aizoaceae are not known, but the presence of CAM in most subfamilies suggests that CAM evolved very early during the diversification of extant Aizoaceae or is ancestral to it (see ‘The phylogenetic diversity of CAM’, below). Without further surveys of CAM in the Sesuvioideae, we cannot infer whether C<sub>4</sub> or CAM evolved first in *Trianthema*; future comparisons of multiple C<sub>4</sub> + CAM taxa will help to resolve long-standing

questions of  $C_4$  and CAM compatibility within single tissues (Sage, 2002).

Very few  $C_4$  species have been investigated for CAM activity, and it is possible that there are many more examples of  $C_4$  + CAM photosynthesis yet to be discovered.  $C_4$  + CAM has also been reported in five succulent members of Amaranthaceae and the succulent grass *Spinifex littoreus* (Burm.f.) Merr. (Poaceae), all of which are halophytes (Supplementary Data Table S2). Multiple lines of evidence point to  $C_4$  + CAM in five species of the tribe Salsoleae (Amaranthaceae): *Halothamnus subaphyllus* (C.A.Mey.) Botsch. [previously *Aellenia subaphylla* (C.A.Mey.) Aellen], *Haloxylon ammodendron* (C.A.Mey.) Bunge ex Fenzl [previously *Haloxylon aphyllum* (Minkw.) Iljin], *Horaninovia ulicina* Fisch. & C.A.Mey., *Salsola praecox* (Litv.) Litv. and *Xylosalsola richteri* (Moq.) Akhani & Roalson [previously *Salsola richteri* (Moq.) Karel ex Litv.] (Zalenskii and Glagoleva, 1981). All five species exhibited small  $\Delta H^+$  in the field, and gas-exchange measurements and  $^{14}\text{C}$ -radiolabel pulse–chase experiments showed slight dark  $\text{CO}_2$  assimilation and formation of malate in all but *Salsola praecox*, which was not measured; furthermore, label from nocturnally formed malate was incorporated into sugars in the light in *Haloxylon ammodendron* (Zalenskii and Glagoleva, 1981). However, these taxa have not been revisited for CAM-specific research; further laboratory studies are needed to confirm CAM in these taxa and in the  $C_4$  grass *Spinifex littoreus*, which was recently reported to show CAM-cycling, i.e. small  $\Delta H^+$  in the absence of any net dark  $\text{CO}_2$  uptake (Ho et al., 2019). If CAM is confirmed in *Spinifex* or in any members of the Salsoleae, this would represent the first known evolution of CAM in a  $C_4$  lineage, because all *Portulaca* are hypothesized to have evolved  $C_4$  + CAM from  $C_3$  + CAM ancestors.

#### CAM-like physiology

In addition to  $C_3$  + CAM, aquatic CAM and  $C_4$  + CAM, photosynthetic physiology that mimics CAM and unexpected CAM-inducing stimuli have been discovered. In a process reminiscent of CAM-cycling, plants with ‘alarm photosynthesis’ recapture respired  $\text{CO}_2$  behind closed stomata into calcium oxalate crystals at night, which are then degraded to release  $\text{CO}_2$  for photosynthesis during the day (Tooulakou et al., 2016). This physiology is induced by  $\text{CO}_2$  starvation, including that caused by drought, and has been documented in  $C_3$ ,  $C_4$  and CAM species (Tooulakou et al., 2016). Furthermore, calcium oxalate crystals in alarm photosynthesis show less discrimination against  $^{13}\text{C}$ , which suggests that calcium oxalate crystal biosynthesis might involve the same carboxylating enzyme used in CAM (PEPC) to form oxaloacetate, which could be converted to oxalate by oxaloacetate acetylhydrolase.

Alarm photosynthesis should not be considered CAM, but the fungal-induced CAM reported in *Camellia oleifera* C. Abel (Theaceae) (Yuan et al., 2012) might change our definition of a ‘CAM plant’. Following up on the observation of rare succulent small leaves (microphylls) in natural populations of *C. oleifera*, Yuan et al. (2012) demonstrated that infection by the fungus *Exobasidium vexans* Masee (Exobasidiaceae) caused the development of succulent tissue and appeared to be correlated

with the induction of a CAM cycle. More research is needed to assess the capacity for CAM further in non-infected and infected individuals, and we do not include *Camellia* as a CAM clade here (Supplementary Data Table S2). But if this study is confirmed, it might help to identify direct mechanistic links between succulent development and CAM expression that have interested botanists for centuries.

#### THE PHYLOGENETIC DIVERSITY OF CAM PLANTS

CAM plants can be found from hot semi-deserts to rainforest canopies and high-elevation lakes and are as diverse phylogenetically as they are ecologically. The growing list of CAM taxa can both expand and reduce the number of evolutionary origins of CAM. Since the last survey list of genera containing species capable of CAM (Smith and Winter, 1996), CAM has been demonstrated in an additional 134 genera from 18 families. Five of these families are new and were the subjects of detailed studies of at most a few taxa: Araceae (Holtum et al., 2007), Basellaceae (Holtum et al., 2018), Halophytaceae (this study; Supplementary Data Table S1), Urticaceae (Winter et al., 2021b) and Zygophyllaceae (Mok et al., 2023). Many of the other new genera are the result of extensive surveys to fill in gaps in photosynthetic types in species-rich clades, including Aizoaceae, Bromeliaceae, Crassulaceae and Orchidaceae.

Below, we update the estimated number of taxa with CAM and hypothesize the number of CAM origins (i.e. the evolution of the ability to perform CAM) through an extensive assessment of CAM reports and recent phylogenies of groups with CAM. We want to emphasize several limitations of this analysis. First, we discuss primarily ‘CAM genera’, but there are few genera with exhaustive searches for CAM; more often, evidence of CAM has been documented in one or perhaps a handful of species within a CAM genus, and we found direct evidence for CAM in 370 vascular plant genera scattered across 38 families (Fig. 2; Table 1). Second, in our estimates of origins, we treat each CAM genus as containing or representing a single origin of CAM unless its phylogenetic relationship to other CAM genera within a clade suggests that CAM evolved before the origin of the genus. We generally do not assume that a CAM genus has more than one origin of CAM unless evidence shows multiple origins (e.g. *Euphorbia* and *Peperomia*), although it is possible and very likely in many large genera. Third, we use genera as the primary units of analysis only for convenience, because they are typically the most specific clade name available to refer to a region of the phylogeny in the absence of an established phylogenetic nomenclature. But we do not consider genera to represent equivalent units across the broad history of vascular plants, and the finding that there are currently 370 reported CAM genera conveys limited information about the diversity, phylogenetic distribution and evolutionary history of CAM; rather, it is a consequence of how various taxonomists have delineated taxa over many years. For example, the numbers of CAM genera in Mesembryanthemoideae (Aizoaceae) and Euphorbiaceae have decreased as a result of taxonomic revision, but the number of species reported to use CAM has increased in both taxa as a result of additional experimental surveys.

### Non-seed plants

CAM has been found in the lycophyte genus *Isoetes* (Isoëtaceae) and the fern order Polypodiales. The genus *Isoetes* contains ~140 species, all of which have been either demonstrated or assumed to use CAM when submerged, although they may not use CAM when terrestrial (Keeley, 1983). As discussed above (see ‘Aquatic CAM and other photosynthetic pathways’), the CO<sub>2</sub> limitations of plants in aquatic habitats are less coupled to atmospheric CO<sub>2</sub> than terrestrial plants, which implies that CAM might have been adaptive for aquatic plants long before plants in terrestrial habitats. At 45–60 Ma, the *Isoetes* crown age is much younger than the stem age of ~370 Ma (Wood et al., 2020), but probably still represents one of the earliest origins of CAM. *Isoetes* has been considered a ‘living fossil’ because of morphological similarities between some extant and extinct species dating back to the Carboniferous (Pigg, 2001), a period estimated to have had relatively low atmospheric CO<sub>2</sub> (Foster et al., 2017). Gene and genome duplications have been recognized as key events facilitating the evolution of CAM and C<sub>4</sub> (Heyduk et al., 2019b), and a recent genomic and transcriptomic study of *Isoetes taiwanensis* De Vol found evidence of a whole-genome duplication event ~200 Ma in *Isoetes* (Wickell et al., 2021). *Isoetes taiwanensis* also uses the bacterial-type PEPC for CAM (Wickell et al., 2021), and the lateral transfer of this gene pre-dates the whole-genome duplication event. Therefore, if CAM is indeed ancestral to all extant *Isoetes*, it is likely to have preceded the Oligocene CO<sub>2</sub> decline and could conceivably be of Carboniferous origin if large-scale duplication events were not needed.

All CAM-exhibiting ferns are epiphytic and belong to the species-rich families Polypodiaceae and Pteridaceae (Table 1). As in other diverse groups, many clades lack either broad CAM surveys or robust phylogenies (or both) at the resolution needed to infer CAM origins with confidence. CAM has been studied in detail in multiple species of Australasian *Pyrrosia* (including *Drymoglossum*) (Wong and Hew, 1976; Winter et al., 1983; Griffiths et al., 1989), but not in species from Africa or mainland Asia. Given recent phylogeographic hypotheses (Wei et al., 2017), CAM appears to have evolved once in *Pyrrosia* as the lineage spread from mainland Asia throughout the Pacific islands, Australia and New Zealand. However, if CAM is found to be more widespread in *Pyrrosia*, it might be that CAM evolved in the ancestor of *Pyrrosia* and *Platyserium* in the late Eocene or early Oligocene. Unlike the situation in other diverse clades of CAM epiphytes, such as the orchids or bromeliads, strong CAM appears to have evolved only once in ferns, in the genus *Pyrrosia*, and most investigated ferns show only very low levels of CAM activity. Given that such low-level CAM is difficult to detect in the field, it is likely that more CAM fern lineages will be found, particularly in the mostly epiphytic Polypodiaceae. To our knowledge, no transcriptomic or genomic data are available for CAM ferns.

### Acrogymnosperms

Just two acrogymnosperm lineages are known to use CAM: *Welwitschia mirabilis* Hook.f. (Welwitschiaceae) and *Dioon edule* Lindl. (Cycadaceae).

*Welwitschia mirabilis* is the only extant species in the order Welwitschiales and is native to the extremely harsh Namib Desert, where individuals can live for >1000 years. Although CAM is an adaptation to water limitation, few species with CAM tend to inhabit areas with such low and unpredictable precipitation (average annual precipitation is often <100 mm: Schulze et al., 1976). Given that *Welwitschia* are among the weakest CAM plants known, their ability to survive in the Namib Desert is likely to be the result of multiple adaptations, including a very deep tap root and high cuticular resistances (Winter and Schramm, 1986; von Willert et al., 2005). Uniquely among the gnetophytes, *Welwitschia* also shows evidence of a lineage-specific whole-genome duplication event (Li et al., 2015).

In contrast, *Dioon edule* inhabits seasonally dry forests of Mexico and Central America; it is the only cycad species that has been examined for CAM in detail (Vovides et al., 2002). CAM has been associated with *Dioon* seedling survival throughout the long dry seasons following germination (Yáñez-Espinosa et al., 2014). Other members of *Dioon* might also use CAM, because *D. edule* exhibits only very weak CAM activity that might be difficult to detect outside the dry season. With a crown age of 56 (40–75) Ma, CAM could be old in *Dioon* if it is present throughout the genus; conversely, most speciation events in the history of *Dioon* are within the last 20 Ma, and *D. edule* is estimated to have diverged only 5–11 Ma (Gutiérrez-Ortega et al., 2017).

### Angiosperms

*Amborella–Nymphaeales–Austrobaileya* grade and *Magnoliideae* Amongst the *Amborella–Nymphaeales–Austrobaileya* grade and *Magnoliideae* clade of angiosperms, CAM is known only from *Peperomia* (Piperaceae), a large genus of ~1600 species. As with many extremely diverse clades, our understanding of relationships within *Peperomia* is generally poor at the species level, although CAM has been assessed using gas exchange, titratable acidity, enzyme activity and carbon isotopes (or a combination of these data) for dozens of species across the major subgenera (Holthe et al., 1992). Most *Peperomia* species with CAM have been demonstrated to be C<sub>3</sub> + CAM, with few exhibiting strong CAM (Holthe et al., 1992; Holtum and Winter, 2005). It is possible that many purported C<sub>3</sub> *Peperomia* species are capable of CAM, because most have not been tested thoroughly for C<sub>3</sub> + CAM. Assuming that most current C<sub>3</sub> species are correctly assigned and that CAM is lost only rarely, based on recent *Peperomia* phylogenies (Frenzke et al., 2016; Lim et al., 2019), we estimate that CAM has evolved at least five times in *Peperomia* and perhaps a dozen times if reversions from CAM to C<sub>3</sub> do not occur. We expect the true number of origins to be somewhere in between and that some ‘C<sub>3</sub>’ species will be found to be C<sub>3</sub> + CAM. It is noteworthy that the link between epiphytism and CAM does not appear especially strong in *Peperomia*, given the distribution of habits described by Frenzke et al. (2016).

**Monocots** Monocots contain xeric, tropical epiphytic and aquatic CAM species, in addition to species that perform CAM in their chloroplast-containing roots (leafless orchids; Winter et al., 1985) and C<sub>4</sub> + CAM in *Ottelia* (Hydrocharitaceae; Zhang et al., 2014). Within monocots, CAM species are heavily concentrated in the Orchidaceae and Bromeliaceae, which we estimate



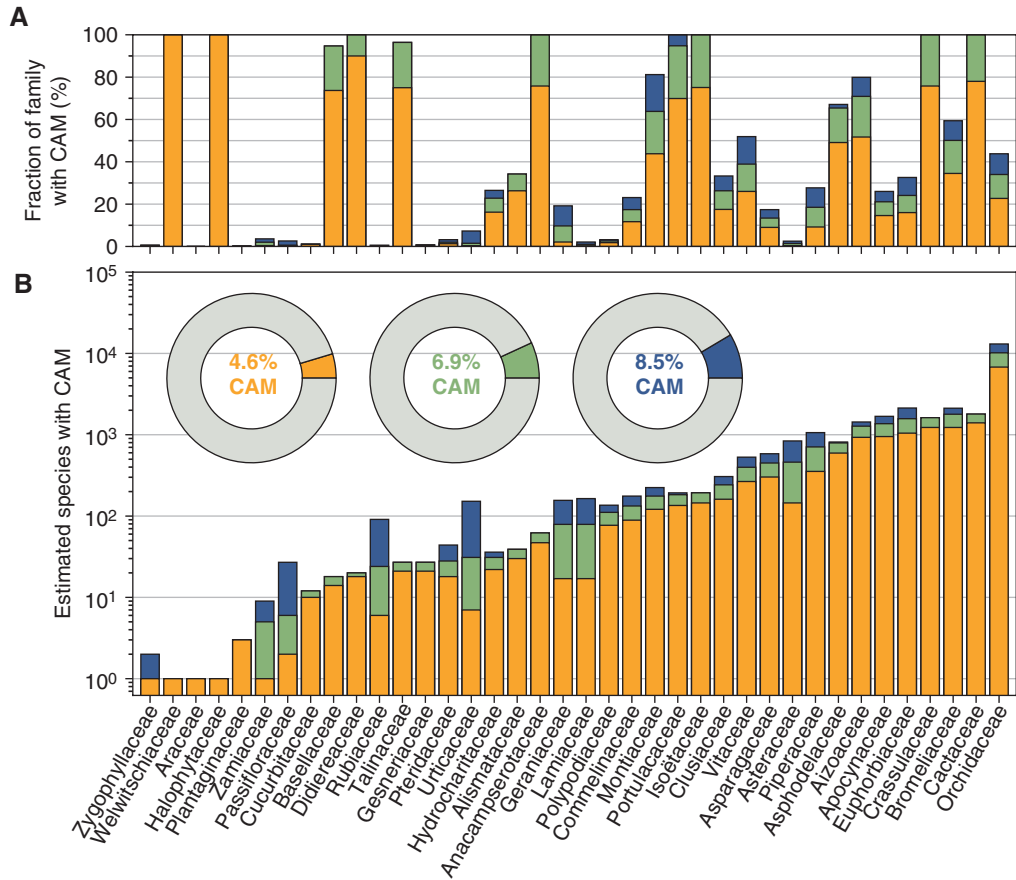


FIG. 4. Estimated CAM species diversity in vascular plants by family. The proportional (A) and absolute (B) species diversity capable of CAM is shown in each family with known CAM lineages. Orange, green and blue bars and pie charts show lower bounds, expected and upper bounds of CAM species diversity, respectively, as defined in the main text. CAM species diversity was calculated using the list of CAM genera and assumptions about the phylogenetic placement of CAM origins (Table 1), extent of CAM surveys, and links between CAM and other plant traits. Numbers of species in each genus were taken from POWO (2023), which lists 349 036 accepted names for non-hybrid vascular plant species.

to be two of the three most CAM-rich plant families (Fig. 4; Supplementary Data Tables S3 and S4). Extensive surveys of carbon isotope ratios have been conducted in these clades (e.g. Medina *et al.*, 1977; Silveira *et al.*, 2010a; Crayn *et al.*, 2015; Torres-Morales *et al.*, 2020), although comparatively few studies have tested for C<sub>3</sub> + CAM in living material (Medina and Troughton, 1974; Pierce *et al.*, 2002; Silveira *et al.*, 2005; Beltrán *et al.*, 2013). These surveys suggest that strong CAM has evolved independently in five bromeliad subfamilies and, possibly, might have arisen more than once within subfamilies Bromelioideae, Puyoideae and Tillandsioideae (Crayn *et al.*, 2004, 2015; Givnish *et al.*, 2014). The specific number of origins of strong CAM in Orchidaceae is less clear owing to poor resolution along the backbone of subfamily Epidendroideae, but it is almost certain that CAM has evolved independently in the Vanilloideae and the Epidendroideae, and probably several times within the latter.

Despite the difficulty of placing CAM origins precisely, comparative phylogenetic studies in Orchidaceae and Bromeliaceae have uncovered correlations between CAM, diversification, and plant morphology and habit. Mapping the occurrence of strong CAM onto recent phylogenies has revealed significant correlations between strong CAM and epiphytism in orchids (Silveira *et al.*,

2009), but not in bromeliads as a whole (Givnish *et al.*, 2014), given that three of the five bromeliad clades in which CAM is found are exclusively terrestrial (Hechtioideae, the xeric clade of Pitcairnioideae, and Puyoideae), and in a fourth (Bromelioideae) the CAM terrestrial species were resolved as ancestral to CAM epiphytes (Crayn *et al.*, 2004, 2015). Under some assumptions, strong CAM was found to be associated with higher diversification rates in both families (Givnish *et al.*, 2014, 2015; Silvestro *et al.*, 2014), but these conclusions should be regarded as tentative, pending denser taxon sampling to improve phylogenetic resolution and studies of living material to confirm the presence or absence of C<sub>3</sub> + CAM. The role of CAM as a ‘key innovation’ in the diversification of epiphytic orchids has also been challenged recently in studies where strong CAM was associated with lineages with a higher extinction rate (Hu *et al.*, 2022; see also Zotz *et al.*, 2023). The horticultural and traditional medicinal value of orchids has spurred the generation of many orchid genome assemblies (Table 2). Comparisons between C<sub>3</sub> + CAM and CAM genomes, and with C<sub>3</sub> orchid genomes (e.g. *Apostasia*; Zhang *et al.*, 2017), should be fruitful for understanding transitions to and between CAM phenotypes.

In addition to the orchids, CAM is present in arid-adapted lineages of the Asparagales: the Agavoideae and Nolinoideae

of Asparagaceae (multiple origins) and Asphodeloideae of Asphodelaceae (probable single origin). As noted above, detailed studies have recently updated our understanding of the evolution of CAM in the Agavoideae; it is now believed that CAM evolved separately in: (1) the ancestor of *Agave* (including *Manfreda* and *Polianthes*), *Beschorneria* and *Furcraea*; (2) in *Hesperaloe*; and (3) in *Yucca* section *sarcocarpa* (Heyduk et al., 2022). Like *Isoëtes*, the Agavoideae are noteworthy for their use of the bacterial type of PEPC in CAM (Heyduk et al., 2022). In contrast to the dense transcriptomic, anatomical and physiological data now available for many Agavoideae subclades, the Nolinoideae and Asphodelaceae have received less attention. CAM is known from only two of 23 genera of the Nolinoideae (*Beaucarnea* and *Sansevieria*), which is a diverse clade inhabiting both New and Old World semi-arid ecosystems. Taxonomy of the Nolinoideae has been unstable, but will be aided by recent molecular phylogenies (Meng et al., 2021; Ji et al., 2023). Likewise, Asphodelaceae systematics have improved over the last decade (Manning et al., 2014), supporting a single origin of CAM in the ancestor of subfamily Alooideae and its sister clade *Bulbine*, because CAM has been found throughout the clade (Table 1). However, CAM-related -omics and physiological data are generally lacking across the clade, although the only genome (*Aloe vera* L.; Table 2) has been studied in the context of drought tolerance (Jaiswal et al., 2021).

Both aquatic and terrestrial CAM are found in the order Alismatales: aquatic CAM occurs in the genera *Sagittaria* (Alismataceae), *Ottelia* and *Vallisneria* (both Hydrocharitaceae), whereas terrestrial CAM has been reported in the monotypic genus *Zamioculcas* (Araceae). Each of these four genera is likely to represent an independent origin of CAM, based on recent phylogenies of the Alismatales (e.g. Chen et al., 2022). Although CAM is known from only a single species in Araceae [*Zamioculcas zamiifolia* (G.Lodd.) Engl.], this large family of >4000 recognized species (POWO, 2023), including many tropical climbers and epiphytes, merits screening for other taxa capable of CAM. Three genera with CAM have been identified in the Commelinaceae: *Callisia* (including *Tripogandra*), *Cyanotis* and *Tradescantia*. These taxa form a monophyletic group with *Gibasis* (Jung et al., 2021), which has not been surveyed for CAM to our knowledge. Finally, we note the recent report of small CAM-type acid fluctuations in natural populations of *Spinifex littoreus* (Ho et al., 2019), the first report of a CAM feature in grasses (Poaceae) (see ' $C_4 + CAM$ ', above) (Supplementary Data Table S2); however, further studies are needed to confirm CAM in this species.

*Saxifragales and Vitales* The early-diverging eudicot order Saxifragales contains the Crassulaceae, for which crassulacean acid metabolism is named. CAM has been found in every genus surveyed, spanning all three subfamilies, and it has been assumed that all members of Crassulaceae are capable of CAM (e.g. Pilon-Smits et al., 1996).  $C_3 + CAM$  is likely to be ancestral in Crassulaceae, with at least one independent evolution of strong CAM in each subfamily, and very weak CAM is found in multiple lineages that have radiated in more temperate environments (Löscher, 1984). Phylogenomic data have not been applied to resolve the large and diverse clades within Crassulaceae, but a recent phylogeny of subfamily Sempervivoideae has

dated crown Crassulaceae to 65–100 Ma (Messerschmid et al., 2020), considerably before  $CO_2$  began to decrease to near-modern levels. Although there are few phylogenomic studies in the Crassulaceae, multiple whole-genome sequences have been produced (Table 2), and the genus *Kalanchoë* has become a model for CAM genomics and functional genetics (e.g. Yang et al., 2017; Boxall et al., 2020). Similar to the Agavoideae, hybrids between Crassulaceae species with different CAM phenotypes have been found to exhibit intermediate photosynthetic physiology and leaf thickness (Teeri and Gurevitch, 1984).

In the Vitales, CAM has been found in *Cissus* and *Cyphostemma*, succulent vines and lianas of subfamily Vitoideae, each nested within  $C_3$  clades and therefore representing two independent origins of CAM. Stem succulence has evolved separately in the southern African and Madagascan lineages of *Cyphostemma* (Hearn et al., 2018), but only the southern African clade has been assessed for CAM.

*Fabids* CAM is currently known from eight genera distributed throughout the Cucurbitales, Zygophyllales and Malpighiales. Many are caudiciform [e.g. *Adenia* (Passifloraceae), *Seyrigia* and *Xerosicyos* (both Cucurbitaceae)], with *Xerosicyos* forming large caudices that produce succulent deciduous vines. There is only a single report of CAM in *Adenia* [Mooney et al. (1977) found  $\delta^{13}C = -18.3\text{‰}$ ], hence further investigations into this clade would be highly desirable. Likewise, the only report of CAM in *Oxalis* is that of Kluge and Ting (1978), who noted apparent diurnal fluctuations in acidity in *Oxalis carnosa* (no authority), but it is not clear which of three possible currently recognized taxa this sample represents, and more recent studies of *Oxalis* have not been able to confirm CAM activity (Supplementary Data Table S2). These *Oxalis* species are part of a very diverse South American radiation that includes other leaf and stem succulent species from xeric habitats, which would warrant re-evaluation for CAM. In Urticaceae, *Pilea peperomioides* Diels was recently discovered to be capable of CAM (Winter et al., 2021b) and belongs to a species-rich genus (>600 species) containing other succulents and epiphytes. CAM was recently observed in the succulent, photosynthetic stems of *Bulnesia retama* (Gillies ex Hook. & Arn.) Griseb. (Zygophyllaceae), a shrub native to semi-arid habitats of South America (Mok et al., 2023). This represents the first confirmed species capable of CAM in Zygophyllaceae, although other succulent shrubs in the family have been suggested to be, or discussed in the context of, CAM (Supplementary Data Table S2). We expect CAM to be more widespread than currently recognized in these fabid clades, and preliminary evidence suggests the possibility of  $C_3 + CAM$  for at least one additional genus, in that Rundel et al. (1999) reported a  $\delta^{13}C$  value of  $-21.3\text{‰}$  (converted from their absolute  $\Delta$  isotope notation) for *Forsydia* (Urticaceae), a small genus of fleshy-leaved shrubs that inhabit arid lands from the Mediterranean to India and South Africa.

Strong CAM has also been found in each of the four subgenera of *Euphorbia* (Euphorbiaceae), a genus of ~2000 species exhibiting extreme diversity in growth form, from thin-leaved perennials to cactiform and large tree species; nearly half of all *Euphorbia* are succulent xeric species. Phylogenetic studies within *Euphorbia* have shown that the evolution of CCM (either  $C_4$  or CAM) was associated with increased diversification

(Horn *et al.*, 2014). It appears that strong CAM has evolved more than a dozen times, but the distribution of  $C_3 + CAM$  is entirely unknown, meaning that *Euphorbia* could represent anywhere between 1 and 12 origins of CAM biochemistry (Horn *et al.*, 2014). It is noteworthy that the species-rich clade of  $C_3-C_4$  and  $C_4$  species is nested within *Euphorbia* subgenus *Chamaesyce*, which contains multiple origins of strong CAM. To our knowledge, *Chamaesyce*  $C_4$  species have not been tested for CAM, but we note its similarity to both *Portulaca* and *Trianthema* in being a  $C_4$  clade that is phylogenetically sister to or nested within a diverse, CAM-evolving lineage.

**Malvids** Two genera of the Geraniaceae, *Monsonia* (originally reported as *Sarcocaulon*) and *Pelargonium*, contain  $C_3 + CAM$  species. *Geranium pratense* L. was included in earlier lists of CAM species (Bennet-Clark, 1933; Szarek and Ting, 1977; Kluge and Ting, 1978) based on reports from Kraus (1883), but CAM activity in this taxon could not be confirmed by Thomas and Beevers (1949), who noted the leaves were high in acidity but did not show day–night fluctuations (Supplementary Data Table S2). Based on the relatively distant relationship between *Monsonia* and *Pelargonium* (García-Aloy *et al.*, 2017), CAM presumably evolved independently in each lineage, and probably multiple times within the latter (Jones *et al.*, 2003). In one of the earliest phylogenetic studies of  $C_3 + CAM$  evolution, Jones *et al.* (2003) found a distribution of  $C_3 + CAM$  and  $C_3$  species in *Pelargonium* consistent with multiple transitions to, or reversions from,  $C_3 + CAM$ . Changes in life form, dispersal type and the evolution of CAM in *Monsonia* are hypothesized to be key innovations that facilitated their diversity throughout the succulent Karoo and Cape region of South Africa (García-Aloy *et al.*, 2017). With their diverse life forms and distribution of  $C_3 + CAM$ , the Geraniaceae presents an excellent clade for future studies of CAM evolution and, possibly, reversion to  $C_3$ .

**Caryophyllales** The Caryophyllales contains an extraordinary diversity of CAM plants distributed across xeric ecosystems of the Americas, southern Africa and Australia, and extending into the Middle East and central Asian steppe. With ~1880 species, the majority of which are assumed to use CAM to varying degrees, the Aizoaceae are one of the largest CAM families (Fig. 4B; Supplementary Data Tables S3 and S4). The Aizoaceae also contain  $C_4 + CAM$  (i.e. *Trianthema portulacastrum*) and the model facultative CAM plant *Mesembryanthemum crystallinum* that was the subject of many of the earliest molecular genetic studies of CAM (Cushman *et al.*, 1989, 2008). The Aizoaceae are one of the fastest-radiating lineages of eukaryotes (Klak *et al.*, 2004, 2017a; Valente *et al.*, 2014), and resolution within the hyperdiverse subfamily Ruschioideae remains particularly poor, but the phylogenetic structure between and within the other four subfamilies is stable (Klak *et al.*, 2017b). Tests for  $C_3 + CAM$  across the clade are also expanding (e.g. Winter, 2019) and suggest that CAM might be ancestral, because it is found in the Sesuvioideae, the sister clade to all other Aizoaceae. CAM has been found in all but one of five subfamilies, although a recent broad investigation of facultative CAM found that several species might truly be non-CAM plants, indicating another potential evolutionary loss of CAM (Winter, 2019; Fig. 3).

The most species-rich single origin of CAM might be the Portulacineae, a clade of >2200 species (the majority of

which are cacti) distributed among seven families: Cactaceae, Portulacaceae, Anacampserotaceae, Talinaceae, Didiereaceae, Halophytaceae, Basellaceae and Montiaceae. Increased phylogenetic resolution from a variety of data types (Moore *et al.*, 2018; Wang *et al.*, 2019b), systematic CAM surveys (e.g. Hancock *et al.*, 2019) and an ancestral gene duplication event resulting in a shared CAM-specific PEPC (Christin *et al.*, 2014) have led to hypotheses that CAM is ancestral in the Portulacineae (Goolsby *et al.*, 2018). Similar to the Agavoideae, the Portulacineae demonstrate how decades of traditional and modern ecophysiology data (gas exchange, titratable acidity and transcriptomics) can be placed in a phylogenetic context to understand the evolution of CAM. Although  $C_3 + CAM$  is inferred to be ancestral to the clade, strong CAM has evolved repeatedly: in Didiereaceae (Winter, 1979), *Anacampseros* (Guralnick *et al.*, 2008) and multiple times in cacti (Edwards and Donoghue, 2006). Although Cactaceae are one of the more well-studied CAM clades, CAM has been confirmed in only a minority of cacti (representing 54 of the ~150 accepted genera; POWO, 2023), and strong CAM is generally assumed for all others. Within cacti,  $C_3 + CAM$  is not restricted to *Pereskia sensu lato* (the leafy clades that form a grade leading to the core cacti) (Edwards and Donoghue, 2006) and can be found in genera including *Maihuenia*, *Peresklopsis* and *Quiabentia* (Nobel and Hartsock, 1986; Martin and Wallace, 2000). The Montiaceae contains multiple CAM lineages, with some very weak CAM and apparently  $C_3$  lineages nested deeply within the clade that provide the most compelling evidence to date of reversions to  $C_3$  (e.g. *Calandrinia tumida* Syeda; Hancock *et al.*, 2019; Fig. 3). With its wide range of CAM phenotypes and possible reversions to  $C_3$ , the Portulacineae will continue to be an important clade for the study of CAM evolution.

Aspects of CAM activity have also been shown in five  $C_4$  genera (*Halothammus*, *Haloxylon*, *Horaninovia*, *Salsola* and *Xylosalsola*) of the Amaranthaceae (see ' $C_4 + CAM$ ', above), but further study is needed to confirm CAM in these taxa. The phylogenetic backbone of the Salsoleae tribe, which encompasses these genera, is not resolved, and *Salsola* is currently polyphyletic; furthermore, tests for CAM have been rare because this clade was already known to possess  $C_4$  photosynthesis. The Salsoleae share many features with *Euphorbia* and the Portulacineae, including halophytism, multiple evolutions of stem photosynthesis, and highly modified leaf morphologies that combine Kranz anatomy and hydrenchyma in succulent  $C_4$  leaves and branches.

**Campanulids** Given the species richness and ecological diversity of clades such as Asteraceae, CAM has been reported from relatively few campanulid lineages. All Asteraceae genera with confirmed CAM species are restricted to the tribe Senecioneae, which is a large clade (~3000 species) with only partially resolved relationships that contains succulent, halophytic and stem-photosynthetic species. CAM has been studied in most detail in succulent members of *Senecio sensu lato* that have been segregated recently (Ozerova *et al.*, 2017; Cicuzza *et al.*, 2018) across the genera *Caputia*, *Curio*, *Delairea*, *Kleinia* and *Senecio* (Fioretto and Alfani, 1988). Based on the most recent comprehensive phylogenetic studies of Senecioneae (Pelsner *et al.*, 2007; Ozerova *et al.*, 2017), CAM has probably evolved

separately in the ‘Gynuroid clade’ (containing *Baculellum*, *Caputia*, *Crassothonna*, *Curio*, *Delairea*, *Kleinia* and *Othonna*) and in the ‘*Faujasia*–*Bethencourtia* clade’ [containing *Jacobaea aquatica* (Hill) G.Gaertn., B.Mey & Scherb (previously *Senecio aquaticus* Hill) and *Delairea odorata* Lem. (previously *Senecio scandens* DC.)], and possibly twice within the latter. Further studies are needed to confirm CAM in *Delairea odorata* and *Jacobaea aquatica*, which only exhibited small and consistent fluctuations in malic acid or net-positive dark-period CO<sub>2</sub> uptake in the experiments of [Fioretto and Alfani \(1988\)](#) (Supplementary Data Table S2); however, *Delairea odorata* was considered ‘CAM-cycling’ by [Sternberg et al. \(1984\)](#) (reported as *Senecio mikanioides* Otto ex Walp.). Nocturnal malate accumulation has been reported for the succulent halophyte *Tripolium pannonicum* (Jacq.) Dobrocz. (previously *Aster tripolium* L.) under salt treatment, but with net CO<sub>2</sub> uptake occurring entirely during the light period ([Ganzmann and von Willert, 1972](#)), and this species merits re-examination in view of other reports of low-level CAM activity in certain halophytes. The possible occurrence of CAM has also been noted in the (non-succulent) scrambling vine *Mikania micrantha* Kunth ([Liu et al., 2020](#)), based on the expression of genes potentially involved in the CAM pathway and small day–night changes in malate content (Supplementary Data Table S2), but measurements of gas exchange would be desirable to confirm operation of the complete CAM cycle. Information on photosynthetic metabolism exists for only ~1 % of Asteraceae, and therefore the extent and consequences of photosynthetic evolution in this clade have been little explored outside model clades, such as *Flaveria* ([Siniscalchi et al., 2021](#)).

**Lamiids** The most diverse group of CAM plants in the Lamiids are in the Apocynaceae, which we estimate to contain a minimum of four independent origins of CAM. In subfamily Apocynoideae, only one species is known to be C<sub>3</sub> + CAM: *Pachypodium namaquanum* (Wyley ex Harv.) Welw., which exhibited low rates of nocturnal CO<sub>2</sub> uptake in stems after leaf-shedding and day–night fluctuations in malic acid in leaves during drought ([von Willert et al., 1980, 1992](#)). More detailed studies of C<sub>3</sub> + CAM in *Pachypodium*, a genus of heavily spined trees and shrubs with water-storing trunks, are warranted, because reported isotope ratios are C<sub>3</sub>-like ([Mooney et al., 1977](#); [Rundel et al., 1999](#)). Within subfamily Asclepiadoideae, CAM has probably arisen at least three times across three tribes containing succulent-leaved vines and stem-succulent cactiform species. In Marsdenieae, CAM is prevalent in a clade comprising two large genera of succulent epiphytic vines, *Dischidia* (~80 species) and *Hoya* (350–450 species), which have diversified in tropical and subtropical Asia, New Guinea, Australia and the western Pacific ([Wanntorp et al., 2014](#); [Liede-Schumann et al., 2022](#)). In Ceropegieae, CAM has been reported in *Ceropegia* and in the ten genera of stem-succulent stapeliads (core Stapeliinae) tested to date (*Apteranthes*, *Boucerosia*, *Caralluma*, *Caudanthera*, *Duvalia*, *Huernia*, *Hoodia*, *Orbea*, *Quaqua* and *Stapelia*). Based on comparative morphology and life form ([Endress et al., 2018](#)), it seems likely that most, if not all, of the remaining 26 genera traditionally recognized in this clade will also possess CAM. Recent systematic studies, however, have shown the large genus *Ceropegia* to be highly polyphyletic, with *Brachystelma* and the core Stapeliinae nested within ([Bruyns et al., 2017](#)). More extensive sampling for

CAM activity among the semi-succulent, thick-stemmed species of *Ceropegia sensu stricto* would be desirable. Finally, in tribe Asclepiadeae, the CAM genera *Folotsia* and *Sarcostemma* have now been subsumed within an expanded *Cynanchum* ([Khanum et al., 2016](#)), which includes the dominant succulent vine *Cynanchum viminale* (L.) L. [previously *Sarcostemma viminale* (L.) R.Br.] that spreads rampantly in the absence of browsing by megaherbivores ([Coverdale et al., 2021](#)).

Within the Gentianales, CAM has also been found in several species of epiphytic ‘ant-plants’ in the Rubiaceae. These distinctive plants form the subtribe Hydnophytinae within tribe Psychotrieae and are united by the synapomorphy of enlarged woody tubers derived from the hypocotyl that become hollow throughout development and house ant colonies in mutualistic relationships ([Huxley, 1980](#); [Chomicki and Renner, 2016](#)). Among the five genera composing this clade, CAM has so far been reported in thicker-leaved species of *Hydnophytum* (one species), *Myrmecodia* (one species) and *Squamellaria* (two species) ([Winter et al., 1983](#); [Tsen and Holtum, 2012](#); [Chomicki and Renner, 2016](#)), but the full extent of CAM occurrence within this subtribe has not yet been explored.

Finally, CAM has been observed in three families of the Lamiales: Gesneriaceae, Lamiaceae and Plantaginaceae. In the Gesneriaceae, CAM probably evolved twice: once in a small clade of resurrection plants (*Haberlea* and *Ramonda*) and separately in the succulent epiphytic lineage *Codonanthopsis*. Other fleshy-leaved and epiphytic lineages of Gesneriaceae have been investigated for CAM (i.e. *Aeschynanthus*, *Columnnea* and *Streptocarpus*) but shown to be C<sub>3</sub> ([Guralnick et al., 1986](#)), although further investigations in this relatively large family (~3800 spp.) would be worthwhile. Two genera from different subfamilies of the Lamiaceae contain CAM lineages: *Marrubium* (subfamily Lamioideae) and *Coleus* (subfamily Nepetoideae). The genus *Marrubium* is nested within the paraphyletic *Ballota*, which has not been assessed for CAM. Taxonomic uncertainty has recently been resolved in *Coleus* and *Plectranthus* ([Paton et al., 2019](#)), with all CAM species of *Plectranthus* ([Herppich and Herppich, 1996](#)) now treated under *Coleus* ([Winter et al., 2021c](#)). The aquatic lineage *Littorella* (Plantaginaceae) also expresses CAM ([Keeley and Morton, 1982](#)) and has an ‘isoëtoid’ growth form (stiff rosette of leaves, large root biomass and continuous root-to-leaf air channels) that facilitates CO<sub>2</sub> diffusion directly from soils.

#### SPECIES DIVERSITY, CAM ORIGINS AND IMPLICATIONS, AND WHERE TO FIND NEW CAM LINEAGES

Using our list of CAM genera ([Table 1](#)), lists of species from Kew’s Plants of the World Online ([POWO \(2023\)](#)), and by placing known CAM and non-CAM species in their phylogenetic context, we estimated the number of species capable of CAM on a genus-by-genus basis (Supplementary Data Table S3). Genera with multiple taxa investigated for CAM were binned into all (100 %), most (75 %), half (50 %), some (25 %), few (5 %) or rare (1 %) species estimated to be capable of CAM based on the proportions of CAM and non-CAM taxa reported and their phylogenetic distribution. For example, all species in all genera of Cactaceae were considered to use CAM,

because every species studied thus far has been reported to use CAM to some degree; furthermore, these taxa are well distributed throughout the Cactaceae phylogeny. Combining the list of  $C_3$  and CAM *Peperomia* from Holthe et al. (1992) and a recent phylogeny by Frenzke et al. (2016) showed that two major subclades have not been surveyed for CAM, one contained exclusively  $C_3$  taxa, one exclusively CAM taxa, and five contained mixed photosynthetic types (including the largest, Micropiper, with ~800 species). Given this phylogenetic distribution of photosynthetic types, we estimated that CAM evolved several times in *Peperomia* and that roughly half of *Peperomia* species are capable of CAM. We generally did not assume that CAM evolved along a tip branch (except in monotypic genera, e.g. *Welwitschia*); that is, despite only single records of CAM so far, we categorized genera including *Jatropha* and *Pilea* as having few CAM species (5%). Finally, to estimate lower and upper bounds on these counts, we moved every genus either down or up a bin, respectively; genera binned as ‘few’ were recategorized as ‘rare’ (1%) when estimating lower bounds, and genera binned as ‘all’ were not altered when estimating upper bounds. The upper bounds on Australian *Calandrinia* and on *Clusia* were reduced to 95% (rather than 100%), because multiple taxa have been demonstrated not to express detectable CAM (Hancock et al., 2019; Pachon et al., 2022).

After removing synonymous and hybrid species and using the above assumptions, we estimated the total number of plant species capable of CAM to be 6.9% (4.6–8.5%) of all vascular plants (Fig. 4B; Supplementary Data Tables S3 and S4). We stress that the absolute species diversity implied by these proportions differs considerably by authority and should therefore be treated with caution. For example, POWO (2023) and Akeroyd and Syngé (1992), the latter of which was used by Winter and Smith (1996), differ in their estimates of vascular plant diversity by nearly 100 000 species, and some upper estimates of seed plant diversity alone are ~450 000 species (Govaerts, 2003). Our proportional estimates, however, are consistent with previous estimates of total CAM species diversity (e.g. 6% of vascular plant species by Winter and Smith, 1996) and of specific clades. Our estimate of 34% (23–43%) of orchids being capable of CAM (Fig. 4A; Supplementary Data Table S4), taking into account the detection of low-level CAM activity in many species, is congruent with estimates by Winter and Smith (1996) (36%) and Silvera et al. (2010b) (30%). In an isotopic survey of nearly 60% of all bromeliad species, Crayn et al. (2015) found 43% to be CAM; our estimated 50% (35–59%) (Fig. 4A; Supplementary Data Table S4) is consistent with their results but skews higher owing to our consideration of  $C_3$  + CAM taxa.

By placing reports of CAM species in their phylogenetic contexts and with the caveats listed above, we estimate that the ability to perform CAM has evolved a minimum of 66 times and, possibly, as many as 114 times (Table 1). Although it is probable that some clades will see multiple CAM origins collapsed into fewer origins after further sampling for  $C_3$  + CAM, large swaths of the vascular plant phylogeny have not been investigated for CAM activity beyond isotopic surveys. Although it is unlikely that many more strong (constitutive)-CAM lineages will be discovered, the prevalence of  $C_3$  + CAM, even among CAM clades that have received substantial attention,

could be much higher. It is tempting to use lists of known CAM species, as presented in Table 1, in large-scale evolutionary or ecological studies, but we caution against the treatment of this list as complete for most clades. As an exercise to show the often poor overlap of phylogenetic trees and CAM phenotypic data, we attempted to estimate the number of CAM origins in the Caryophyllales using the topology from Hinchliff and Smith (2014); we coded species not confirmed to be CAM as ‘non-CAM’ (i.e. species from genera not included in Table 1) and assumed an all rates different (ARD) model of trait evolution. Results from 100 stochastic character maps created using the package ‘phytools’ (Revell, 2012) in R v.4.1.2 (R Core Team, 2021) found an average of 9.43 CAM gains and 64.13 CAM losses in the Caryophyllales alone. This number of CAM origins is high but not implausible based on our assessment of the Caryophyllales; however, the extreme number of reversions (and underlying transition rates) is likely to be inflated by lack of CAM information in many genera. In our example, the paucity of information in the core Ruschioideae of Aizoaceae forces reversions on very short branches; a problem likely to affect estimates of CAM transitions in other recently diversifying clades, such as the Epidendroideae (Orchidaceae), Bromeliaceae and *Peperomia* (Piperaceae).

Estimating the number and phylogenetic placement of CAM origins can inform the ecological pressures leading to CAM evolution (Box 2). Phylogenetically informed studies of CAM genomics and physiology will generate and provide support for hypotheses of CAM evolution and place CAM origins in geographical, chronological and therefore ecological contexts. Like  $C_4$ , CAM has evolved in dozens of lineages convergently, as a response to lower atmospheric  $CO_2$  concentrations established after the Oligocene (Edwards and Ogburn, 2012; Sage et al., 2012).  $C_4$  has a minimum of 61 independent origins across angiosperms, resulting in ~8100 extant  $C_4$  species (Sage et al., 2011; Sage 2016). However, CAM is also understood as an adaptation to water limitation (Winter et al., 2005), which has been a major stressor since plants colonized terrestrial ecosystems. Most  $C_4$  clades date to the Oligocene or later (Christin et al., 2011), and although many of the most species-rich CAM lineages have diversified in the past 30 Myr, phylogenetic reconstructions of some CAM origins appear to pre-date the Oligocene  $CO_2$  decline. For example, in addition to possible ancient CAM origins in the non-seed plant clades, the Portulacaceae have a crown age estimated between 40 and 50 Ma (Arakaki et al., 2011), which would imply that  $C_3$  + CAM evolved while atmospheric  $CO_2$  concentrations were ~1000 ppm (Rae et al., 2021). And although the Epidendroideae represent a recent radiation of CAM orchids, *Vanilla* has an estimated crown age of ~42 Ma (Givnish et al., 2015); if CAM is found in other members of Vanilloideae (crown age ~60 Ma) or Vanilloideae (crown age ~78 Ma), these clades might be among the oldest CAM lineages. CAM probably also evolved close to the base of the Crassulaceae phylogeny, with an origin in the Cretaceous (Sage et al., 2023). Low atmospheric  $CO_2$  is likely to be a major promoter of CAM evolution (Sage et al., 2023), but because CAM is inherently flexible (allowing less energetically costly  $C_3$  photosynthesis to be used when water and  $CO_2$  are plentiful) and used in highly dissimilar environments, it is plausible that CAM could have evolved despite

high atmospheric CO<sub>2</sub>, especially if the increased succulence significantly reduces mesophyll CO<sub>2</sub> conductance (Nelson and Sage, 2008; Edwards, 2019). Furthering our understanding of CAM evolution requires continued systematic surveys of CAM (along with phylogenies of these groups), which should seek both to fill gaps around transitions in CAM phenotypes and to expand sampling into new areas of plant diversity to find new CAM lineages and confirm (or reject) assumptions of lack of CAM activity.

The most obvious places to start searching for new CAM taxa are in clades closely related to known CAM lineages. Establishing the true placement of C<sub>3</sub>-to-CAM transitions on phylogenies has been difficult thus far, but will be essential in understanding the earliest steps in CAM evolution. Strong candidates include succulent, xeric and epiphytic species, which have driven many past CAM surveys. Halophytic and succulent C<sub>4</sub> species have been largely neglected by CAM researchers, but reports of CAM in Amaranthaceae (Zalenskii and Glagoleva, 1981), Poaceae (Ho et al., 2019) and Aizoaceae (Winter et al., 2021a) warrant re-evaluation and greater scrutiny of these clades. Proceeding along these routes would continue recent trends in CAM exploration, but we also suggest that the CAM community should cast a much wider net. A growing body of research suggests that major morphological shifts (e.g. increased leaf thickness and succulence) might not be needed for C<sub>3</sub> + CAM (Edwards, 2019), and therefore the occurrence of C<sub>3</sub> + CAM and low-level CAM activity might be more common throughout vascular plants than previously assumed.

Finally, although we have emphasized how much more there is to discover about the distribution and evolution of CAM, we want to end by highlighting how much knowledge, insight and opportunity this current ‘draft’ list contains. The last attempts at comprehensive lists of taxa with CAM were more than two decades ago, and there has since been enormous progress on multiple fronts: further resolution of the phylogeny of land plants, greater taxonomic stability, and increasingly sophisticated methods for analysing character evolution; additional surveys for CAM in previously unexplored clades; and a new ability, via the revolution in sequencing technology, to probe genetic/regulatory elements of CAM expression. We are hopeful that this updated list will inspire a new era of comparative studies of CAM biology. The repeated evolution of CAM photosynthesis has played a crucial role in the diversification and ecological success of land plants, and it presents an unparalleled opportunity to investigate questions of convergence, parallelism, adaptation and the evolutionary assembly of complex traits.

#### SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Table S1: list of genera containing species capable of CAM photosynthesis. Table S2: lineages reported or suspected to use CAM, but for which further corroborative evidence is required. Table S3: estimated CAM species diversity in each genus containing CAM species. Table S4: estimated CAM species diversity in each family containing CAM species.

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#### DATA AVAILABILITY

The data used to infer family-level statistics of CAM species diversity are available online at: <https://github.com/sgilman/CAM-diversity>

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