

TITLE: “The CAM lineages of planet Earth”

**Ian S. Gilman^{1*}, J. Andrew C. Smith^{2*}, Joseph A. M. Holtum³, Rowan F. Sage⁴, Katia
Silvera^{5,6}, Klaus Winter⁵, and Erika J. Edwards¹**

¹Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut,
USA

²Department of Biology, University of Oxford, Oxford, United Kingdom

³College of Science and Engineering, James Cook University, Townsville, Queensland, Australia

⁴Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada

⁵Smithsonian Tropical Research Institute, Balboa, Ancón, Panama

⁶Department of Botany & Plant Sciences, University of California, Riverside, California, USA

Running title: CAM lineages of planet Earth

*Corresponding authors: Ian S. Gilman (ian.gilman@yale.edu) and J. Andrew C. Smith
(andrew.smith@biology.ox.ac.uk)

- 1 • **Background and Scope:** The growth of experimental studies of crassulacean acid
2 metabolism (CAM) in diverse plant clades, coupled with recent advances in molecular
3 systematics, presents an opportunity to re-assess the phylogenetic distribution and diversity
4 of species capable of CAM. It has been more than two decades since the last comprehensive
5 lists of CAM taxa were published, and an updated survey of the occurrence and distribution
6 of CAM taxa is needed to facilitate and guide future CAM research. We aimed to survey the
7 phylogenetic distribution of these taxa, their diverse morphology, physiology, and ecology,
8 and the likely number of evolutionary origins of CAM based on currently known lineages.
- 9 • **Results and Conclusions:** We found direct evidence – in the form of experimental or field
10 observations of gas exchange, day–night fluctuations in organic acids, carbon isotope ratios,
11 and enzymatic activity – for CAM in 370 genera of vascular plants, representing 38 families.
12 Further assumptions about the frequency of CAM species in CAM clades, and the
13 distribution of CAM in the Cactaceae and Crassulaceae, bring the currently estimated
14 number of CAM-capable species to nearly 7% of all vascular plants. The phylogenetic
15 distribution of these taxa suggests a minimum of 66 independent origins of CAM in vascular
16 plants, with possibly dozens more. To achieve further insight into CAM origins, there is a
17 need for more extensive and systematic surveys of previously unstudied lineages, particularly
18 in living material to identify low-level CAM activity, and for denser sampling to increase
19 phylogenetic resolution in CAM-evolving clades. This should allow further progress in
20 understanding the functional significance of this pathway by integration with studies on the
21 evolution and genomics of CAM in its many forms.
- 22 **Key words:** crassulacean acid metabolism, nocturnal acidification, vascular plants, C₃
23 photosynthesis, C₃+CAM, C₄+CAM, strong CAM, photosynthetic pathway evolution

1 INTRODUCTION

2 Crassulacean acid metabolism (CAM) is now recognized as a key ecological adaptation to water
3 and CO₂ limitation. From the outset, this metabolism was noted for its distinctive association
4 with succulent plants. De Saussure (1804), in the course of extensive manometric measurements
5 of gas exchange by plants, made the seminal observation that *Opuntia* and several other
6 succulent plants were able to show net uptake of CO₂ at night. Also, Heyne (1815) detected the
7 rhythmic nocturnal acidification of *Kalanchoë* leaves by the simple expedient of comparing their
8 taste in the morning and afternoon. By the end of the 19th century, these characteristic day–night
9 changes in acidity had become recognized as a well-known phenomenon of succulent plants.
10 Since then, a full description of CAM, from biochemistry to ecophysiology, has involved
11 researchers from around the globe and continues into the genomics age.

12 CAM research has been spurred by advances in technology that have enabled precise
13 biochemical and physiological descriptions of the marked differences between succulent and
14 non-succulent plants. Mayer (1875), Kraus (1884), and Warburg (1886) appreciated that the
15 metabolism of succulents involved a specific acid, malic acid, and that carbohydrate
16 concentrations showed reciprocal, diel cycling. However, it was to be another half-century
17 before the biochemical mechanism of nocturnal CO₂ fixation was established. The experiments
18 of Thurlow and Bonner (1948), Thomas (1949), and Thomas and Beevers (1949) showed that
19 nocturnal synthesis of malic acid could be explained by direct fixation of CO₂ via the Wood–
20 Werkman reaction, first discovered in bacteria, and now known to be catalyzed (using HCO₃[−] as
21 the true substrate) by the enzyme phosphoenolpyruvate (PEP) carboxylase (PEPC). Work at the
22 Connecticut Agricultural Experiment Station and elsewhere around the same time confirmed the
23 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 analyzers 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 CO₂, the sensitivity of the gas-exchange cycle to photoperiod, and the endogenous rhythmicity of dark CO₂ fixation (Gregory *et al.*, 1954; Wilkins, 1959; Nuernbergk, 1961; Warren and Wilkins, 1961; Nishida, 1963). Following the discovery of C₄ 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-hour cycle.

Phase 1 occurs in the dark period, when stomata are open, CO₂ 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 CO₂ fixation at the beginning of the light period, initially catalyzed mainly by PEPC but later increasingly by Rubisco, while stomata remain open. Malic acid is then released from the vacuole and decarboxylated during the central part of the day in Phase III, raising intercellular CO₂ 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 CO₂ 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 may be phenotypically plastic over short time scales and throughout life history (see *The landscape of CAM phenotypes and CAM evolution*, below). Regardless of individual-, life history-, and environmentally induced-variation, CAM is most broadly defined by the cyclic diel rhythm of (1) nocturnal fixation of CO₂ into malate, catalyzed 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 CO₂, 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 Rubisco CO₂ fixation efficiency 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 C₄ and CAM plants could be distinguished from C₃ species by their lesser discrimination against ¹³C relative to ¹²C (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 fully describe the mechanisms behind carbon isotope discrimination (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 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 (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 primarily conducted 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 both experience frequent water-deficit stress, but is also present in a handful of aquatic lineages (Keeley, 1998). As isotope data accumulated, C₃ and CAM species in many clades largely separated into two distinct groups, showing a bimodal distribution of $\delta^{13}\text{C}$ 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 ($\delta^{13}\text{C}$) and gas-exchange patterns that indicated that – unlike C₄ species – CAM plants could regulate their use of CAM relative to the C₃ pathway depending on environmental conditions (Bender *et al.*, 1973; Osmond *et al.*, 1973; Burris and Black, 1976). The variation in $\delta^{13}\text{C}$ 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 from ferns and lycophytes to cycads, gnetales, and even C₄ angiosperms (see ***The phylogenetic diversity of CAM***, below). Unlike C₄ photosynthesis (Sage *et al.*, 2011), the cataloging of CAM species (e.g., Szarek and Ting, 1977; Szarek, 1979; Winter and Smith, 1996; Sayed, 2001) has not so far 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 Information 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 19th century, and multiple CAM phenotypes have since been described (recently reviewed by Winter (2019)). These phenotypes are often projected along two axes: CAM mode, the degree to which CAM is constitutive and/or facultative, and CAM “strength”, 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^+), or integrated over longer periods by $\delta^{13}\text{C}$ values (Box 1). The best-studied CAM plants tend to exhibit either facultative CAM with moderate to large ΔH^+ (e.g.,

1 *Mesembryanthemum crystallinum*), or strong and constitutive CAM (e.g., *Kalanchoë*
 2 *daigremontiana*) (Winter *et al.*, 2008). As CAM has been more broadly and deeply surveyed, a
 3 complex landscape of CAM phenotypes has emerged and experiments in the Aizoaceae (Winter,
 4 2019) and Montiaceae (Hancock *et al.*, 2019) demonstrate that both mode and strength of CAM
 5 can vary considerably among plants that primarily utilize C₃ photosynthesis (Fig. 3). CAM can
 6 be both constitutive and weak (*Calandrinia ptychosperma* F.Muell.) or facultative with strong
 7 CAM induction (*Jordaaniella cuprea* (L.Bolus) H.E.K.Hartmann). Constitutive CAM plants
 8 may have further facultative responses (*Disphyma crassifolium* (L.) L.Bolus) or reduce CAM
 9 (e.g., ΔH^+) under stress (*Titanopsis calcarea* (Marloth) Schwantes) (Winter, 2019). Although
 10 strongly and constitutively expressed CAM can be readily identified by $\delta^{13}C$ values less negative
 11 than -20‰ (in non-C₄ species), plants that obtain one-third or less of their carbon in the dark
 12 have isotopic signatures in the range of C₃ plants (Winter and Holtum, 2002; Fig. 3 inset). While
 13 many labels have been applied to various CAM phenotypes in the past, here we use “C₃+CAM”
 14 (*sensu* Edwards, 2019) to refer to species that use C₃ photosynthesis as the principal pathway of
 15 carbon gain, but also express CAM to various degrees. We use the term “strong CAM” to refer
 16 to species that use CAM as the principal pathway of carbon gain; this corresponds to the “strong
 17 CAM” category of Edwards (2019), and the “CAM plant” recommendation of Winter *et al.*
 18 (2015). Many species capable of CAM have been demonstrated to induce or upregulate CAM in
 19 response to stress (typically drought or salinity stress) in natural or laboratory experiments; this
 20 behavior is known as “facultative CAM”. These species do not, or only weakly, express CAM
 21 under favorable conditions. Although plants with intermediate $\delta^{13}C$ values have been found in
 22 many clades, and constitute the majority of species in Aizoaceae subfamily
 23 Mesembryanthemoideae (most likely 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 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 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 from C_3 to C_3 +CAM to strong CAM because strong CAM lineages are frequently subtended by earlier-branching C_3 +CAM lineages in phylogenetic studies of CAM evolution (Hancock and Edwards, 2014), though 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 C_3 +CAM, and well-resolved phylogenies to be able to infer the relationships between states with confidence. For example, Orchidaceae – which numerically contain a substantial proportion of the planet’s CAM species – and Crassulaceae – which include multiple model CAM species of *Kalanchoë* – have generally poorly resolved phylogenies at the species level and relatively few surveys of C_3 +CAM (but see Silvera *et al.* (2005, 2014)). More detailed studies have demonstrated phylogenetic patterns that support a C_3 to C_3 +CAM to strong CAM evolutionary trajectory in multiple clades, for example, 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).

Further exploration may dramatically change our understanding of CAM evolution, as in the Portulacineae (Fig. 2), where tests of C₃+CAM and new phylogenomic studies have revealed lineages with varying CAM phenotypes and very few, if any, purely C₃ 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; Silvestro *et al.*, 2014; Horn *et al.*, 2014), it has been challenging to identify well-resolved and well-sampled clades with known C₃, C₃+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 *E. pusilla* (L.) N.H. Williams & M.W. Chase (Pan *et al.*, 2012) and comparative transcriptomic research between *E. pusilla* and C₃ *E. crista-galli* (Rchb.f.) N.H. Williams & M.W. Chase (Heyduk *et al.*, 2019). The Oncidiinae may have multiple transitions from C₃ to strong CAM if C₃+CAM is indeed absent in the currently recognized “C₃” 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 over 300 species with multiple recognized C₃, C₃+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 C₃, C₃+CAM, and strong CAM species in the Agavoideae and hypothesized multiple origins of CAM over the past 25 Ma

(Heyduk *et al.*, 2022). *Yucca* also produces natural C₃+CAM hybrids (*Yucca gloriosa* L.) arising from C₃ (*Y. filamentosa* L.) and strong CAM (*Y. 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 C₃+CAM observations continue to amend the hypothesized timing and phylogenetic placement of CAM origins (Box 2), their diversity offers an unrivaled opportunity to study the evolution of convergent ecophysiology. The evolutionary trajectories from C₃ 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 C₃ plants (Bräutigam *et al.*, 2017; Schiller and Bräutigam, 2021), while 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 C₃ plants but during the nighttime in CAM plants. Others have argued that C₃+CAM can (and has) evolved readily in a diversity of C₃ 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 C₃+CAM species, and particularly those that facultatively use CAM in response to stress, are poised to shed light on C₃ to CAM transitions. Paired whole-transcriptome and physiological (e.g., gas exchange, ΔH^+ , 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.*, 2008), and *Isoëtes* (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; for example, varied peak transcription of CAM-specific isoforms of PEPC from midafternoon 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 C₃+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, Portulacineae), but it might be more fruitful to develop model systems around relatively species-poor CAM origins, which afford relevant C₃ comparisons in more closely related species. At the time of publication, publicly available genomes exist for at least 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 C₃+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 Information 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 indeed tend to have very high water-use efficiency relative to C₃ and C₄ 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 CO₂ limitation represent two sides of the same coin for terrestrial species: water limitation leads to stomatal closure, thereby restricting CO₂ 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 CO₂ concentration because CO₂ is depleted during the day by the photosynthetic activity of non-CAM vegetation, solar warming of the shallow water, and CO₂ diffusion limitations from the air; at night, heterotrophic respiration by plants and animals increases the concentration of dissolved CO₂ in the water dramatically (Raven and Spicer, 1996; Keeley, 1998). These diel trends in dissolved CO₂ create a niche for CAM plants, which can capture dissolved CO₂ when it is abundant at night and use it during the day when pools become CO₂ depleted. CAM activity decreases or ceases entirely in tissues of vernal CAM plants that become emergent because CO₂ diffusion in air is orders of magnitude higher than in water, further supporting the functional significance of CAM in CO₂-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 CO₂ directly through their extensive root systems, with diffusion through the well-developed air canals carrying CO₂ to the shoot, where it can be fixed in the dark via CAM in leaves; roots may thereby account for the majority of CO₂ uptake in species such as *I. andicola* (Amstutz) L.D.Gómez (previously *Stylites andicola* Amstutz) (Keeley *et al.*, 1984).

While most species with carbon concentrating mechanisms (CCMs) are hypothesized to have evolved and diversified following the Oligocene atmospheric CO₂ decline (Arakaki *et al.*, 2011; Edwards and Ogburn, 2012), the selective pressures to evolve aquatic CAM are less coupled to atmospheric CO₂ levels; it is possible that CAM is quite 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), as well as in *Crassula aquatica* Schönland (Crassulaceae) (Keeley and Morton, 1982) and *Crassula helmsii* (Kirk) Cockayne (Newman and Raven, 1995), *Littorella uniflora* (L.) Asch. (Plantaginaceae) (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 of their native ranges (Klavsén *et al.*, 2011), but if and how CAM may 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 may 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 (CBB) cycle (Keeley, 1999). Certain aquatic lineages use a dissolved bicarbonate based CCM in addition to fixing dissolved CO₂ directly (Keeley, 1998) and there are several known submerged C₄ lineages, a small number of which use a form of single-cell C₄ photosynthesis without Kranz anatomy (Bowes, 2011). Similar to aquatic CAM, aquatic C₄ (with or without Kranz anatomy) can be induced by low CO₂ (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, even though *Eleocharis maculosa* (Vahl) Roem. & Schult. (Cyperaceae) may show very small ΔH^+ , radiolabel pulse–chase experiments have demonstrated that carbon in nocturnally produced malate does not flow into the CBB 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-CO₂ induced C₄ and likely weak CAM (see *Terrestrial C₄+CAM*, below). Similarly, *Ottelia alismoides* (L.) Pers. (Hydrocharitaceae) has been shown to be a constitutive submerged C₄ plant with inducible CAM (Zhang *et al.*, 2014; Shao *et al.*, 2017). The diversity of photosynthetic pathways in aquatic plants, as well as their ability to transition between them and use multiple CCMs simultaneously, presents a more complicated photosynthetic landscape than in terrestrial plants, and many classifications as “CAM” should be carefully evaluated or further investigated.

Terrestrial C₄+CAM

The combination of CCMs is not restricted to aquatic plants: CAM has been clearly demonstrated in two succulent terrestrial C₄ clades (hereafter “C₄+CAM”), *Portulaca*

(Portulacaceae) and *Trianthema* (Aizoaceae). Laboratory measurements of gas exchange and ΔH^+ 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 C₃–C₄ intermediacy (Winter *et al.*, 2019), while low-level constitutive CAM has been demonstrated in C₄ *Trianthema portulacastrum* L. (Winter *et al.*, 2022). *Portulaca* is the most studied of the terrestrial C₄+CAM clades, and all research has supported the hypothesis that CAM is ancestral to *Portulaca* and likely evolved deep within (or in an ancestor of) the Portulacineae (Christin *et al.*, 2014; Goolsby *et al.*, 2018). Given the substantial variation in C₄ characters (i.e., leaf anatomy, biochemical subtypes, and species-specific gene use: Ocampo *et al.*, 2013; Voznesenskaya *et al.*, 2017; Gilman *et al.*, 2022) – as well as C₃–C₄ intermediacy in some species – it is likely that C₄ 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 C₄ and CAM cycles are integrated, with CAM-generated malate produced in the mesophyll likely being decarboxylated by the C₄ cycle in 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, or is ancestral to, the diversification of extant Aizoaceae (see **CAM by the numbers**, below). Without further surveys of CAM in the Sesuvioideae, we cannot infer whether C₄ or CAM evolved first in *Trianthema*; future comparisons of multiple C₄+CAM taxa will help resolve long-standing questions of C₄ and CAM compatibility within single tissues (Sage, 2002).

Very few C₄ species have been investigated for CAM activity and it is quite possible that there are many more examples of C₄+CAM photosynthesis yet to be discovered. C₄+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 Information Table S2]. Multiple lines of evidence point to C₄+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.) (Zalenskiï and Glagoleva, 1981). All five species exhibited small ΔH^+ in the field, and gas exchange measurements and ¹⁴C-radiolabel pulse-chase experiments showed slight dark CO₂ assimilation and formation of malate in all but *S. praecox*, which was not measured; furthermore, label from nocturnally formed malate was incorporated into sugars in the light in *Haloxylon ammodendron* (Zalenskiï 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, as well as in the C₄ grass *Spinifex littoreus*, which was recently reported to show CAM-cycling, i.e., small ΔH^+ in the absence of any net dark CO₂ uptake (Ho *et al.*, 2019). If CAM is confirmed in *Spinifex* or in any members of the Salsoleae, this would represent the first known C₄ to C₄+CAM transition.

CAM-like physiology

In addition to C₃+CAM, aquatic CAM, and C₄+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 CO₂ behind closed stomata into calcium oxalate (CaOx) crystals at night, which are then degraded to release CO₂ for photosynthesis during the day (Tooulakou *et al.*, 2016). This physiology is induced by CO₂

starvation, including that caused by drought, and has been documented in C₃, C₄, and CAM species (Tooulakou *et al.*, 2016). Further, CaOx crystals in alarm photosynthesis show less discrimination against ¹³C, which suggests that CaOx crystal biosynthesis may involve the same carboxylating enzyme used in CAM (PEPC) to form oxaloacetate (OAA), which could be converted to oxalate by OAA acetylhydrolase. Alarm photosynthesis should not be considered CAM, but the fungal-induced CAM reported in *Camellia oleifera* C.Abel (Theaceae) (Yuan *et al.*, 2012) may 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 further assess the capacity for CAM in non-infected and infected individuals, and we do not include *Camellia* as a CAM clade here [**Supplementary Information Table S2**]. But if this study is confirmed, it may help 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 phylogenetically diverse 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 publication; [**Supplementary**

Information Table S1)), Urticaceae (Winter *et al.*, 2021a), 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 – that is, 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 primarily discuss “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. We found direct evidence for CAM in 370 vascular plant genera and, assuming all cacti and Crassulaceae are capable of CAM, it is likely that CAM is present in nearly 500 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 prior to 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*, *Peperomia*), though it is possible and very likely in many large genera. Third, we use genera as the primary units of analysis only for convenience, as 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 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 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 approximately 140 species, all of which have been either demonstrated or assumed to use CAM when submerged, although may not use CAM when terrestrial (Keeley, 1983). As discussed above (see *Aquatic CAM and other photosynthetic pathways*), the CO₂ limitations of plants in aquatic habitats are less coupled to atmospheric CO₂ than terrestrial plants, which implies that CAM may 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 likely 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 CO₂ (Foster *et al.*, 2017). Gene and genome duplications have been recognized as key events facilitating the evolution of CAM and C₄ (Heyduk *et al.*, 2019), and a recent genomic and transcriptomic study of *I. 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 predates the whole-genome duplication event. Therefore, if CAM is

indeed ancestral to all extant *Isoëtes*, it likely preceded the Oligocene CO₂ 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 may be that CAM evolved in the ancestor of *Pyrrosia* and *Platynerium* in the late Eocene or early Oligocene. Unlike 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. Because 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 over 1000 years. Although CAM is an adaptation to water

1 limitation, few species with CAM tend to inhabit areas with such low and unpredictable
 2 precipitation (average annual precipitation is often less than 100 mm: Schulze *et al.*, 1976). As
 3 *Welwitschia* are among the weakest CAM plants known, their ability to survive in the Namib
 4 Desert is likely the result of multiple adaptations, including a very deep tap root and high
 5 cuticular resistances (Winter and Schramm, 1986; von Willert *et al.*, 2005). Uniquely among the
 6 gnetophytes, *Welwitschia* also shows evidence of a lineage-specific whole-genome duplication
 7 event (Li *et al.*, 2015). In contrast, *Dioon edule* inhabits seasonally dry forests of Mexico and
 8 Central America; it is the only cycad species that has been examined for CAM in detail (Vovides
 9 *et al.*, 2002). CAM has been associated with *Dioon* seedling survival throughout the long dry
 10 seasons following germination (Yáñez-Espinosa *et al.*, 2014). Other members of *Dioon* may also
 11 use CAM, as *D. edule* exhibits only very weak CAM activity that may be difficult to detect
 12 outside the dry season. With a crown age of 56 (40–75) Ma, CAM could be quite old in *Dioon* if
 13 it is present throughout the genus; conversely, most speciation events in the history of *Dioon* are
 14 within the last 20 Ma, and *D. edule* is estimated to have diverged just 5–11 Ma (Gutiérrez-Ortega
 15 *et al.*, 2017).

16 *Angiosperms*

17 *ANA grade and Magnoliideae*

18 Amongst the ANA grade (Amborella–Nymphaeales–Austrobaileyales) and Magnoliideae clade
 19 of angiosperms, CAM is only known from *Peperomia* (Piperaceae), a large genus of about 1600
 20 species. As with many extremely diverse clades, our understanding of relationships within
 21 *Peperomia* is generally poor at the species level, although CAM has been assessed using gas
 22 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 C₃+CAM, with few exhibiting strong CAM (Holthe *et al.*, 1992; Holtum and Winter, 2005). It is possible that many purported C₃ *Peperomia* species are capable of CAM because most have not been thoroughly tested for C₃+CAM. Assuming that most current C₃ species are correctly assigned and that CAM is only rarely lost, 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 C₃ do not occur. We expect the true number of origins is somewhere in between, and that some “C₃” species will be found to be C₃+CAM. It is noteworthy that the link between epiphytism and CAM does not appear especially strong in *Peperomia* given the distribution of habits in Frenzke *et al.* (2016).

Monocots

Monocots contain xeric, tropical epiphytic, and aquatic CAM species, as well as species that perform CAM in their chloroplast-containing roots (leafless orchids: Winter *et al.*, 1985) and C₄+CAM in *Ottelia* (Hydrocharitaceae: Zhang *et al.*, 2014). Within monocots, CAM species are heavily concentrated in the Orchidaceae and Bromeliaceae, which we estimate to be two of the three most CAM-rich plant families (Fig. 4; **[Supplementary Information Tables S3,S4]**). Extensive surveys of carbon isotope ratios have been conducted in these clades (e.g., Medina *et al.*, 1977; Crayn *et al.*, 2015; Silvera *et al.*, 2010; Torres-Morales *et al.*, 2020), although comparatively few studies have tested for C₃+CAM in living material (Medina and Troughton, 1974; Pierce *et al.*, 2002; Silvera *et al.*, 2005; Beltrán *et al.*, 2013). These surveys suggest that strong CAM has evolved independently in five bromeliad subfamilies, and possibly may 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 due 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 likely several times within the latter.

Despite the difficulty of precisely placing CAM origins, 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 (Silvera *et al.*, 2009), but not in bromeliads as a whole (Givnish *et al.*, 2014), since 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) 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 presence or absence of C₃+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 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 C₃+CAM and CAM genomes, as well as to C₃ 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 (likely 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 only known from 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). Similarly, 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*, as 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), while terrestrial CAM has been reported in the monotypic genus *Zamioculcas* (Araceae). Each of these four genera likely represents an independent origin of CAM based on recent phylogenies of the Alismatales (e.g., Chen *et al.*, 2022). Although CAM is only known from a single species in

Araceae (*Zamioculcas zamiifolia* (G.Lodd.) Engl.), this large family of over 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 *C₄+CAM*, above) [**Supplementary Information 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). *C₃+CAM* is likely 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 (Lösch, 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 CO₂ 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 C₃ clades and therefore representing two independent origins of CAM. Stem succulence has evolved separately in the southern African and Madagascar 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) – the latter of which form large caudices that produce succulent deciduous vines. There is only a single report of CAM in *Adenia* (Mooney *et al.* (1977) found $\delta^{13}\text{C} = -18.3\text{‰}$), so further investigations into this clade would be highly desirable.

Similarly, the only report of CAM in *Oxalis* is that of Kluge and Ting (1978), who noted apparent diurnal fluctuations in acidity in *Oxalis carnos*a (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 Information 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.*, 2021a) 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 Information Table S2]. We expect CAM to be more widespread than currently recognized in these Fabid clades, and preliminary evidence suggests the possibility of C₃+CAM for at least one additional genus: Rundel *et al.* (1999) reported a $\delta^{13}\text{C}$ value of -21.3‰ (converted from their absolute Δ isotope notation) for *Forsskaolea* (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 roughly 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 CCMs (either C₄ or CAM) was associated with increased diversification (Horn *et al.*, 2014). It appears that strong CAM has evolved over a dozen times, but the distribution of C₃+CAM is entirely unknown, meaning that *Euphorbia* could represent anywhere between one and 12 origins of CAM biochemistry (Horn *et al.*, 2014). It is noteworthy that the species-rich clade of C₃–C₄ and C₄ species is nested within *Euphorbia* subgenus *Chamaesyce* that contains multiple origins of strong CAM. To our knowledge, *Chamaesyce* C₄ species have not been tested for CAM, but we note its similarity to both *Portulaca* and *Trianthema* in being a C₄ 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 C₃+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 Information 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 likely multiple times within the latter (Jones *et al.*, 2003). In one of the earliest phylogenetic studies of C₃+CAM evolution, Jones *et al.* (2003) found a distribution of C₃+CAM and C₃ species in *Pelargonium* consistent with multiple transitions to, or reversions from, C₃+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 C₃+CAM, the Geraniaceae presents an excellent clade for future studies of CAM evolution and possibly reversion to C₃.

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 *ca.* 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 Information Tables S3,S4]). The Aizoaceae also contain C₄+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 C₃+CAM across the clade are also expanding (e.g., Winter, 2019) and suggest that CAM may 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 may truly be non-CAM plants, indicating another potential evolutionary loss of CAM (Winter, 2019; Fig. 3).

The most species-rich single origin of CAM may be the Portulacineae – a clade of over 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; L. Wang *et al.*, 2019), 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, transcriptomics) can be placed in a phylogenetic context to understand the evolution of CAM. While C₃+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 only been confirmed in a minority of cacti (representing 54 of the approximately 150 accepted genera; POWO, 2023), and strong CAM is generally assumed for all others. Within cacti, C₃+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*, *Pereskia*, and *Quiabentia* (Nobel and Hartsock, 1986; Martin and Wallace, 2000). The Montiaceae contains multiple CAM lineages, with some very weak CAM and apparently C₃ lineages nested deeply within the clade that provide the most compelling evidence to date of reversions to C₃ (e.g., *Calandrinia tumida* Syeda :Hancock *et al.*, 2019; Fig. 3). With its wide range of CAM phenotypes and possible reversions to C₃, 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 C₄ genera (*Halothamnus*, *Haloxylon*, *Horaninovia*, *Salsola*, and *Xylosalsola*) of the Amaranthaceae (see C₄+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 C₄ 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 C₄ 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 – a large clade (ca. 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 recently segregated (Ozerova *et al.*, 2017; Cicuzza *et al.*, 2018) across the genera

1 *Caputia*, *Curio*, *Delairea*, *Kleinia*, and *Senecio* (Fioretto and Alfani, 1988). Based on the most
 2 recent comprehensive phylogenetic studies of Senecioneae (Pelser *et al.*, 2007; Ozerova *et al.*,
 3 2017), CAM has likely evolved separately in the ‘Gynuroid clade’ (containing *Bacullemma*,
 4 *Caputia*, *Crassothonna*, *Curio*, *Delairea*, *Kleinia*, and *Othonna*) and in the ‘*Faujasia*–
 5 *Bethencourtia* clade’ (containing *Jacobaea aquatica* (Hill) G.Gaertn., B.Mey & Scherb
 6 (previously *Senecio aquaticus* Hill) and *Delairea odorata* Lem. (previously *Senecio scandens*
 7 DC.)), and possibly twice within the latter. Further studies are needed to confirm CAM in
 8 *Delairea odorata* and *Jacobaea aquatica*, which only exhibited small and consistent fluctuations
 9 in malic acid or net-positive dark-period CO₂ uptake in the experiments of Fioretto and Alfani
 10 (1988) [**Supplementary Information Table S2**]; however, *D. odorata* was considered ‘CAM-
 11 cycling’ by Sternberg *et al.* (1984) (reported as *Senecio mikanioides* Otto ex Walp.). Nocturnal
 12 malate accumulation has been reported for the succulent halophyte *Tripolium pannonicum*
 13 (Jacq.) Dobrocz. (previously *Aster tripolium* L.) under salt treatment, but with net CO₂ uptake
 14 occurring entirely during the light period (Ganzmann and von Willert, 1972), and this species
 15 merits re-examination in view of other reports of low-level CAM activity in certain halophytes.
 16 The possible occurrence of CAM has also been noted in the (non-succulent) scrambling vine
 17 *Mikania micrantha* Kunth (Liu *et al.*, 2020), based on the expression of genes potentially
 18 involved in the CAM pathway and small day–night changes in malate content [**Supplementary**
 19 **Information Table S2**], but measurements of gas exchange would be desirable to confirm
 20 operation of the complete CAM cycle. Information on photosynthetic metabolism only exists for
 21 ~1% of Asteraceae and therefore the extent and consequences of photosynthetic evolution in this
 22 clade have been little explored outside model clades like *Flaveria* (Siniscalchi *et al.*, 2021).

1 *Lamiids*

2 The most diverse group of CAM plants in the Lamiids are in the Apocynaceae, which we
 3 estimate to contain a minimum of four independent origins of CAM. In subfamily
 4 Apocynoideae, only one species is known to be C₃+CAM: *Pachypodium namaquanum* (Wyley
 5 ex Harv.) Welw., which exhibited low rates of nocturnal CO₂ uptake in stems after leaf-shedding
 6 and day–night fluctuations in malic acid in leaves during drought (von Willert *et al.*, 1980,
 7 1992). More detailed studies of C₃+CAM in *Pachypodium*, a genus of heavily spined trees and
 8 shrubs with water storing trunks, are warranted, as reported isotope ratios are C₃-like (Mooney *et*
 9 *al.*, 1977; Rundel *et al.*, 1999). Within subfamily Asclepiadoideae, CAM has likely arisen at least
 10 three times across three tribes containing succulent-leaved vines and stem-succulent cactiform
 11 species. In Marsdenieae, CAM is prevalent in a clade comprising two large genera of succulent
 12 epiphytic vines, *Dischidia* (ca. 80 species) and *Hoya* (350–450 species), which have diversified
 13 in tropical and subtropical Asia, New Guinea, Australia, and the western Pacific (Wanntorp *et*
 14 *al.*, 2014; Liede-Schumann *et al.*, 2022). In Ceropegieae, CAM has been reported in *Ceropegia*
 15 and in the 10 genera of stem-succulent stapeliads (core Stapeliinae) tested to date (*Apteranthes*,
 16 *Boucerosia*, *Caralluma*, *Caudanthera*, *Duvalia*, *Huernia*, *Hoodia*, *Orbea*, *Quaqua*, and
 17 *Stapelia*). Based on comparative morphology and life-form (Endress *et al.*, 2018), it seems likely
 18 that most if not all of the remaining 26 genera traditionally recognized in this clade will also
 19 possess CAM. Recent systematic studies, however, have shown the large genus *Ceropegia* to be
 20 highly polyphyletic, with *Brachystelma* and the core Stapeliinae nested within (Bruyns *et al.*,
 21 2017). More extensive sampling for CAM activity among the semi-succulent, thick-stemmed
 22 species of *Ceropegia sensu stricto* would be desirable. Finally, in tribe Asclepiadeae, the CAM
 23 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 comprising this clade, CAM has so far been reported in thicker-leaved species of *Hydnophytum* (1 species), *Myrmecodia* (1 species) and *Squamellaria* (2 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*, *Columnea*, and *Streptocarpus*) but shown to be C₃ (Guralnick *et al.*, 1986), although further investigations in this relatively large family (*ca.* 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.*, 2021b). 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 CO₂ 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 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 C₃ 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 C₃ taxa, one exclusively CAM taxa, and five contained mixed photosynthetic types (including the largest, Micropiper, with *ca.* 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 Information Tables S3,S4]**). We stress that the absolute species diversity implied by these proportions differs considerably by authority, and therefore should be treated with caution. For example, POWO (2023) and Akeroyd and Synge (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 Information Tables 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 Information Tables S4]**) is consistent with their results but skews higher due to our consideration of C₃+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, 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. While 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 v4.1.2 (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) are likely 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

1 evolution and place CAM origins in geographical, chronological, and therefore ecological
2 contexts. Like C₄, CAM has evolved in dozens of lineages convergently as a response to lower
3 atmospheric CO₂ concentrations established after the Oligocene (Edwards and Ogburn, 2012). C₄
4 has a minimum of 61 independent origins across angiosperms, resulting in roughly 8,100 extant
5 C₄ species (Sage *et al.* 2011; Sage 2016). However, CAM is also understood as an adaptation to
6 water limitation (Winter *et al.*, 2005), which has been a major stressor since plants colonized
7 terrestrial ecosystems. Most C₄ clades date to the Oligocene or later (Christin *et al.*, 2011), and
8 while many of the most species-rich CAM lineages have diversified in the past 30 Ma,
9 phylogenetic reconstructions of some CAM origins appear to predate the Oligocene CO₂ decline.
10 For example, in addition to possible ancient CAM origins in the non-seed plant clades, the
11 Portulacineae have a crown age estimated between 40–50 Ma (Arakaki *et al.*, 2011), which
12 would imply that C₃+CAM evolved while atmospheric CO₂ concentrations were roughly 1000
13 ppm (Rae *et al.*, 2021). And although the Epidendroideae represent a recent radiation of CAM
14 orchids, *Vanilla* has an estimated crown age of ~42 Ma (Givnish *et al.*, 2015); if CAM is found
15 in other members of Vanilleae (crown age ~60 Ma) or Vanilloideae (crown age ~78 Ma), these
16 clades may be among the oldest CAM lineages. CAM probably also evolved close to the base of
17 the Crassulaceae phylogeny, with an origin in the Cretaceous (Sage *et al.*, 2023). Low
18 atmospheric CO₂ is likely a major promoter of CAM evolution (Sage *et al.*, 2023), but because
19 CAM is inherently flexible – allowing less energetically costly C₃ photosynthesis to be used
20 when water and CO₂ are plentiful – and used in highly dissimilar environments, it is plausible
21 that CAM could have evolved despite high atmospheric CO₂, especially if the increased
22 succulence significantly reduces mesophyll CO₂ conductance (Nelson and Sage, 2008; Edwards,
23 2019). Furthering our understanding of CAM evolution requires continued systematic surveys of

1 CAM (along with phylogenies of these groups), which should seek to both fill gaps around
2 transitions in CAM phenotypes and expand sampling into new areas of plant diversity to find
3 new CAM lineages and confirm (or reject) assumptions of lack of CAM activity.

4 The most obvious places to start searching for new CAM taxa are in clades closely
5 related to known CAM lineages. Establishing the true placement of C_3 to CAM transitions on
6 phylogenies has been difficult thus far, but will be essential in understanding the earliest steps in
7 CAM evolution. Strong candidates include succulent, xeric, and epiphytic species, which have
8 driven many past CAM surveys. Halophytic and succulent C_4 species have been largely
9 neglected by CAM researchers, but CAM reports in Amaranthaceae (Zalenskiĭ and Glagoleva,
10 1981), Poaceae (Ho *et al.*, 2019), and Aizoaceae (Winter *et al.*, 2020) warrant reevaluation and
11 greater scrutiny of these clades. Proceeding along these routes would continue recent trends in
12 CAM exploration, but we also suggest that the CAM community cast a much wider net. A
13 growing body of research suggests that major morphological shifts (e.g., increased leaf thickness,
14 succulence) may not be needed for C_3 +CAM (Edwards, 2019), and therefore the occurrence of
15 C_3 +CAM and low-level CAM activity may be more common throughout vascular plants than
16 previously assumed.

17 Finally, while we have emphasized how much more there is to discover about the
18 distribution and evolution of CAM, we want to end by highlighting how much knowledge,
19 insight, and opportunity this current “draft” list contains. The last attempts at comprehensive lists
20 of taxa with CAM were over two decades ago, and there has since been enormous progress on
21 multiple fronts: further resolution of the phylogeny of land plants, greater taxonomic stability,
22 and increasingly sophisticated methods for analyzing character evolution; additional surveys for
23 CAM in previously unexplored clades; and a new ability, via the revolution in sequencing

1 technology, to probe genetic/regulatory elements of CAM expression. We are hopeful that this
2 updated list will inspire a new era of comparative studies of CAM biology. The repeated
3 evolution of CAM photosynthesis has played a critical role in the diversification and ecological
4 success of land plants, and presents an unparalleled opportunity to investigate questions of
5 convergence, parallelism, adaptation, and the evolutionary assembly of complex traits.

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12 Institute.

14 **Data accessibility statement**

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

1 **Figures**

2 FIG. 1. Simplified overview of (A) biochemistry and (B) four phases of CAM; phases in (B)
 3 adapted from Osmond (1978). During Phase I of CAM, atmospheric CO₂ is captured in a series
 4 of steps involving phospho*enol*pyruvate (PEP) carboxylase (PEPC) to form malate, which is
 5 stored as malic acid in the vacuole overnight. In Phase II, stomata remain open and both Rubisco
 6 and PEPC fix atmospheric CO₂. Stomatal closure marks the beginning of Phase III, in which
 7 malate is released from the vacuole and decarboxylated by malic enzyme (NADP- or NAD-ME)
 8 as shown (or PEP carboxykinase, not shown), releasing CO₂ to be refixed by Rubisco. Finally, if
 9 Phase IV occurs, stomata reopen and atmospheric CO₂ is fixed predominantly by Rubisco. If
 10 Phase IV does not occur, stomatal opening is delayed until Phase I begins again. 3PGA, 3-
 11 phosphoglycerate; BCA, β -carbonic anhydrase; CBB, Calvin-Benson-Bassham cycle; MDH,
 12 malate dehydrogenase; OAA, oxaloacetate.

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

22 FIG. 3. Mean diel ΔH^+ under control (well-watered) and stress (drought or drought + salt stress)
 23 conditions of 45 Aizoaceae species (circles) (Winter, 2019) and 22 Montiaceae species

(triangles) (Hancock *et al.*, 2019). Purple points represent species with no significant ΔH^+ under any conditions. Green points represent species with significant ΔH^+ only under stress conditions. Yellow points represent species with significant ΔH^+ under both control and stress conditions. The inset shows the maximum ΔH^+ and mean $\delta^{13}C$ values (Hancock *et al.*, 2019) of the same Montiaceae species in the main plot. Note that ΔH^+ for Aizoaceae species was calculated by multiplying changes in tissue malate concentrations in Winter (2019) by a factor of 2, assuming 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 labeled.

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 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.

Boxes

Box 1. Diagnosing CAM in the field and lab

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

- Stable carbon isotope ratios ($\delta^{13}\text{C}$, expressed in parts per thousand (‰)) reflect the ratio of $^{13}\text{C}:^{12}\text{C}$ in plant tissues relative to Pee Dee belemnite limestone or a secondary standard. Rubisco discriminates against ^{13}C more strongly than does PEPC; thus, the more 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 CO_2 fixed in the dark: purely light and dark 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 CO_2 in the dark have $\delta^{13}\text{C}$ values indistinguishable from C_3 species; therefore, $\delta^{13}\text{C}$ values can be used as positive evidence for strong CAM, but are at best suggestive of C_3+CAM . Given the bimodality of $\delta^{13}\text{C}$ values across most clades containing C_3 and CAM species (see *The landscape of CAM phenotypes and CAM evolution*), $\delta^{13}\text{C} > -20\text{‰}$ is typically indicative of strong or constitutive CAM. Two strengths of isotopic analysis are the small amount of tissue needed (~ 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 (ΔH^+ , typically expressed in $\text{mmol H}^+ \text{kg}^{-1}$ fresh mass (FM)) measure the change in acid content in photosynthetic tissues and can confirm the presence of CAM. Calculating ΔH^+ requires measuring 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 ΔH^+ greater than zero indicates CAM activity; strong CAM species can have $\Delta\text{H}^+ > 200 \text{ mmol H}^+ \text{kg}^{-1} \text{ FM}$, while most C_3+CAM and C_4+CAM species have $\Delta\text{H}^+ < 200 \text{ mmol H}^+ \text{kg}^{-1} \text{ FM}$ (Holtum *et al.*, 2017; Winter and Smith, 2022) and can be $< 10 \text{ mmol H}^+ \text{kg}^{-1}$

FM in species with very low CAM activity (Fig. 3; Hancock *et al.*, 2019); the lowest differences in ΔH^+ that can currently be experimentally resolved are $\sim 1 \text{ mmol H}^+ \text{ kg}^{-1} \text{ FM}$. A thorough test for CAM requires measuring ΔH^+ under stressful conditions to assess a plant's capacity for facultative CAM, including in 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 CO_2 fixation and precluding the evaluation of CAM behavior.

- Gas exchange curves show net CO_2 exchange over 24-hour periods, with dark-period net CO_2 uptake providing evidence of CAM. However, many C_3 +CAM (and C_4 +CAM) species exhibit net negative CO_2 exchange during the dark-period. In these taxa, dark-period CO_2 loss is reduced towards the middle of the night while CO_2 fixation by PEPC occurs (Phase I) (e.g., *Pilea peperomioides*: Winter *et al.* (2021a)). In contrast, C_3 (and C_4) species show relatively constant dark-period CO_2 loss rates, representing background respiration. As in measurements of ΔH^+ 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 24 hours or more 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 diagnose carboxylation and decarboxylation enzyme subtypes used in CAM. Increased abundance and activity of key enzymes (e.g., PEPC) – in combination with other physiological data — can provide

evidence for CAM (and rule out C₄ photosynthesis) in biochemical assays. 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 must be used in conjunction with other CAM assays. Measuring protein amount/activity and transcript abundance is still mostly restricted to the lab to avoid sample degradation.

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

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 (Horn *et al.*, 2014; Givnish *et al.*, 2014, 2015); 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 CO₂ 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

C₄ pattern of post-Oligocene evolution followed by extensive diversification (Sage *et al.*, 2023). Some large CAM clades, however, such as the Portulacineae and Crassulaceae may have ancient origins predating the late-Oligocene CO₂ decline (L. Wang *et al.*, 2019; Messerschmid *et al.*, 2020), implying CAM evolution in higher CO₂ settings than C₄ evolution. These hypotheses will be re-evaluated as phylogenomic data, paired with new phylogenetic comparative analyses, produce 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 has sharpened our understanding that CAM evolution likely involves both discrete and continuous changes (Bräutigam *et al.*, 2017; Edwards, 2019, 2023; Winter and Smith, 2022). However, we still 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 relations have not been described. Similarly, we lack quantitative analyses of CAM-environment associations at evolutionary scales. The development of climate-dependent trait evolution models (Clavel and Morlon, 2017) may facilitate such investigations and the creation of CAM physiological models (Shameer *et al.*, 2018; Töpfer *et al.*, 2020) may help identify potential hotspots of CAM evolution in deep time, as has been done for C₄ (Zhou *et al.*, 2018). Physiological models may also shed light on the functional significance of C₃+CAM. Many C₃+CAM species do not assimilate

substantial amounts of carbon via CAM (Herrera, 2008; Winter, 2019), but other benefits, such as recapture of respiratory CO₂, and energetic 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.

Tables

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 following 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 Information Table S1]**.

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

Supplementary Information

SUPPLEMENTARY INFORMATION TABLE S1. List of genera containing species capable of CAM photosynthesis. Major clades are ordered following the linear classification system of APG IV (2016). References are provided for the initial reports substantiating CAM activity (as defined in Box 1) in each taxon, with additional references are for reports of species sampled in their natural habitat (including carbon-isotope analyses) where appropriate. For species names that have been synonymized since first publication, the nomenclature used in the original report is

1 indicated together with the currently accepted name in bold. Other reports of possible CAM
2 activity that remain to be substantiated are listed in Supplementary Data Table S2.

3

4 SUPPLEMENTARY INFORMATION TABLE S2. Lineages reported or suspected to use CAM, but for
5 which further corroborative evidence is required.

6

1 Table 1.

Major clade	Genus	Putative CAM origins
ISOËTALES		
Isoëtaceae	<i>Isoëtes</i> (including <i>Stylites</i>)	1
POLYPODIALES		
Polypodiaceae		
Microsorioideae	<i>Lecanopteris</i> ; <i>Microsorium</i>	2; 1 each in <i>Lecanopteris</i> and <i>Microsorium</i> (Chen <i>et al.</i> , 2020)
Loxogrammoideae	<i>Dictymia</i>	1
Polypodioideae	<i>Niphidium</i>	1
Platycterioideae	<i>Platycterium</i> ; <i>Pyrrosia</i> (including <i>Drymoglossum</i>)	1-2; 1 in the ancestor of <i>Platycterium</i> and <i>Pyrrosia</i> or 1 in each clade (Wei <i>et al.</i> , 2017)
Pteridaceae		
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> (Schuettpehl <i>et al.</i> , 2016)
CYCADALES		
Zamiaceae	<i>Dioon</i>	1
WELWITSCHIALES		
Welwitschiaceae	<i>Welwitschia</i>	1
PIPERALES		
Piperaceae		
Piperoideae	<i>Peperomia</i>	5-12 (Frenzke <i>et al.</i> , 2016; Lim <i>et al.</i> , 2019)
ALISMATALES		
Alismataceae	<i>Sagittaria</i>	1
Araceae	<i>Zamioculcas</i>	1
Hydrocharitaceae	<i>Ottelia</i> ; <i>Vallisneria</i>	2; 1 each in <i>Ottelia</i> and <i>Vallisneria</i> (Chen <i>et al.</i> , 2022)
ASPARAGALES		
Orchidaceae		

Epidendroideae	<i>Acianthera; Aerangis; Aeranthes; Anathallis; Angraecum; Arachnis; Aspasia; Barkeria; Bogoria; Brassavola; Brassia; Bryobium; Bulbophyllum; Campylocentrum; Cattleya (including Sophronitis); Caularthron; Caluera; Capanemia; Chiloschista; Cischweinfia; Coelogyne (including Pholidota); Comparettia (including Scelochilus); Coryanthes; Cymbidium; Cyrtopodium; Dendrobium (including Cadetia, Dockrillia, Flickingeria, and Grastidium); Dendrophylax; Didymoplexis; Domingoa; Dimerandra; Echinosepala (including Brenesia); Elleanthus; Encyclia; Epidendrum (including Lanium and Oerstedella); Eriopsis; Erycina (including Psygmorchis); Eulophia (including Acrolophia, Lissochilus, Oeceoclades, and Orthochilus); Gomesa; Gongora; Guarianthe; Hintonella; Ionopsis; Jacquiniella; Laelia (including Schomburgkia); Leochilus; Lockhartia; Luisia; Lycaste; Macradenia; Macroclinium; Maxillaria (including Camaridium, Heterotaxis, Ornithidium, and Trigonidium); Meiracyllium; Microcoelia (including Gussonea); Micropera; Mobilabium; Mormodes; Myoxanthus; Myrmecophila; Notylia; Oberonia; Oeonia; Oncidium; Ornithocephalus; Pabstiella; Peristeria; Phalaenopsis (including Sedirea); Platyrrhiza; Plectorrhiza; Plectrophora; Pleurothallis; Pomatocalpa; Prosthechea; Psychilis; Psychopsis; Pterostemma; Quekettia; Rhinerrhiza; Robiquetia; Rodriguezia; Rossioglossum (including Chelyorchis); Saccolabiopsis; Saccolabium; Sarcochilus; Scaphyglottis; Schoenorchis; Sobralia; Solenidium; Stanhopea; Stelis; Taeniophyllum; Tetramicra; Thrixspermum; Tolumnia; Trachoma; Trichocentrum (including Cohniella and Lophiaris); Trichoglottis; Trichopilia; Trichotosia; Trizeuxis; Vanda (including Ascocentrum); Warmingia; Zygostates</i> <i>Vanilla</i>	5-9 (Silvera <i>et al.</i> , 2009)
Vanilloideae		1
Asphodelaceae		1; in the ancestor of Aloodeae and <i>Bulbine</i> .
Aloodeae	<i>Aloe; Aloidendron; Aristaloe; Astroloba (including Poellnitzia); Gasteria; Gonialoe; Haworthia; Haworthiopsis; Tulista</i> <i>Bulbine</i>	
“Asphodeloideae” ¹		
Asparagaceae		
Agavoideae	<i>Agave (including Manfreda and Polianthes); Beschorneria; Furcraea; Hesperaloe; 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 <i>et al.</i> , 2022)
Nolinoideae	<i>Beaucarnea; Sansevieria</i> ²	2; 1 each in <i>Beaucarnea</i> and <i>Sansevieria</i> (Meng <i>et al.</i> , 2021)

COMMELINALES		
Commelinaceae		
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 <i>et al.</i> , 2022)
POALES		
Bromeliaceae		
Bromelioideae	<i>Acanthostachys</i> ; <i>Aechmea</i> (including <i>Streptocaylx</i>); <i>Ananas</i> ; <i>Androlepis</i> ; <i>Araecoccus</i> ; <i>Billbergia</i> ; <i>Bromelia</i> ; <i>Canistropsis</i> ; <i>Canistrum</i> ; <i>Cryptanthus</i> ; <i>Deinacanthos</i> ; <i>Disteganthus</i> ; <i>Edmundoa</i> ; <i>Eduandrea</i> ; <i>Forzzaea</i> ; <i>Hohenbergia</i> ; <i>Hohenbergiopsis</i> ; <i>Hylaeaicum</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>Pseudaraecoccus</i> ; <i>Quesnelia</i> ; <i>Ronnbergia</i> ; <i>Sincoraea</i> ; <i>Ursulaea</i> ; <i>Wittrockia</i>	2-5 in Bromelioideae (Givnish <i>et al.</i> , 2014)
Hechtioideae	<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 <i>et al.</i> , 2014)
Tillandsioideae	<i>Guzmania</i> ; <i>Josemania</i> ; <i>Lemeltonia</i> ; <i>Tillandsia</i> ; <i>Werauhia</i>	1-5 (Barfuss <i>et al.</i> , 2016)
SAXIFRAGALES		
Crassulaceae		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		
Vitaceae		
Vitoideae	<i>Cissus</i> ; <i>Cyphostemma</i>	2; 1 each in <i>Cissus</i> and <i>Cyphostemma</i> (Wen <i>et al.</i> , 2018)
ZYGOPHYLLALES		
Zygophyllaceae		
Larreoideae	<i>Bulnesia</i>	1
CUCURBITALES		
Cucurbitaceae	<i>Seyrigia</i> ; <i>Xerosicyos</i>	2; 1 each in <i>Seyrigia</i> and <i>Xerosicyos</i> (Guo <i>et al.</i> , 2020)
ROSALES		
Urticaceae	<i>Pilea</i>	1
MALPIGHIALES		
Clusiaceae	<i>Clusia</i>	1-4 within <i>Clusia</i> (Luján <i>et al.</i> , 2022)
Passifloraceae	<i>Adenia</i>	1
Euphorbiaceae		

Euphorbiodeae	<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
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GERANIALES		
Geraniaceae	<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; Kerke <i>et al.</i> , 2019)
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CARYOPHYLLALES		
Aizoaceae		1-4; CAM may be ancestral to Aizoaceae or has evolved independently in Aizooideae, Mesembryanthemoideae, Ruschioideae, and Sesuvioideae, perhaps twice in the latter (Klak <i>et al.</i> , 2004, 2017a; Valente <i>et al.</i> , 2014)
Aizooideae	<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>Sarcozona</i> ; <i>Schlechteranthus</i> ; <i>Stoeberia</i> ; <i>Titanopsis</i> ; <i>Trichodiadema</i> ; <i>Vanheerdea</i>	
Sesuvioideae	<i>Sesuvium</i> ; <i>Trianthema</i>	
PORTULACINEAE		
Montiaceae	Australian <i>Calandrinia</i> ³ ; <i>Calyptridium</i> ; <i>Cistanthe</i> ; <i>Claytonia</i> ; <i>Lewisia</i> ; <i>Phemeranthus</i>	1; CAM likely ancestral to the Portulacineae (Goolsby <i>et al.</i> , 2018)
Didiereaceae		
Didiereoideae	<i>Alluaudia</i> ; <i>Alluaudiopsis</i> ; <i>Decarya</i> ; <i>Didierea</i>	
Portulacarioideae	<i>Portulacaria</i> (including <i>Ceraria</i>)	
Basellaceae	<i>Anredera</i> ; <i>Basella</i>	
Halophytaceae	<i>Halophytum</i>	
Talinaceae	<i>Talinum</i> (including <i>Talinella</i>)	
Portulacaceae	<i>Portulaca</i>	
Anacampserotaceae	<i>Anacampseros</i> ; <i>Grahamia</i> ; <i>Talinopsis</i>	
Cactaceae ⁴		

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>Cochemia</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>Eriosyce</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>Pelecyphora</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>Turbinicarpus</i>	
Opuntioideae	<i>Austrocylindropuntia</i> ; <i>Cylindropuntia</i> ; <i>Grusonia</i> ; <i>Maihueniopsis</i> ; <i>Opuntia</i> (including <i>Nopalea</i>); <i>Pereskioopsis</i> ; <i>Pterocactus</i> ; <i>Quiabentia</i> ; <i>Tephrocactus</i>	
Pereskioideae	<i>Maihuenia</i> ; <i>Pereskia</i> ⁵	
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GENTIANALES		
Rubiaceae		
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)
Apocynaceae		
Apocynoideae	<i>Pachypodium</i>	1
Asclepiadoideae	<i>Apteranthes</i> ; <i>Boucerosia</i> (including <i>Frerea</i>); <i>Caralluma</i> ; <i>Caudanthera</i> ; <i>Ceropegia</i> ⁶ ; <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 and Asclepiadeae, and Ceropegieae (Wanntorp <i>et al.</i> , 2014; Bruyns <i>et al.</i> , 2017; Liede-Schumann <i>et al.</i> , 2022)
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LAMIALES		
Plantaginaceae	<i>Littorella</i>	1
Gesneriaceae		
Didymocarpoideae	<i>Haberlea</i> ; <i>Ramonda</i>	1
Gesnerioideae	<i>Codonanthopsis</i>	1
Lamiaceae		
Lamioideae	<i>Marrubium</i>	1
Nepetoideae	<i>Coleus</i> (including some members of <i>Plectranthus</i>)	1
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ASTERALES		
Asteraceae		
Asteroideae	<i>Baculellum</i> ; <i>Caputia</i> ; <i>Crassothonna</i> ; <i>Curio</i> ; <i>Kleinia</i> ; <i>Othonna</i> ; <i>Senecio</i>	2-3; 1 in the Gynuroid clade and 1-2 in the <i>Faujasia-Bethencourtia</i> clade (Pelser <i>et al.</i> , 2007; Ozerova <i>et al.</i> , 2017)
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Total known origins: 66-114+		

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

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

³*Calandrinia* is nonmonophyletic (Hancock *et al.*, 2019) and CAM has only been observed in the monophyletic clade inclusive of all Australian members of *Calandrinia s.l.*

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

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

⁶*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 recircumscribed *Ceropegia* (Bruyns *et al.*, 2017), but are still recognized by Kew (POWO, 2023) and other authorities (Endress *et al.*, 2018), .

Table 2.

Taxon*	CAM type [†]	Genome Size	Scaffold N50	Scaffold L50	Complete Embryophyta BUSCOs
Isoëtaceae					
<i>Isoëtes taiwanensis</i> De Vol (Wickell <i>et al.</i> , 2021)	aquatic strong CAM	1.65 Gb	17.4 Mb	Not reported	94.5% [‡]
Asphodelaceae					
<i>Aloe vera</i> (L.) Burm.F. (Jaiswal <i>et al.</i> , 2021)	strong CAM (Winter and Holtum, 2002)	12.93 Gb	14.6 kb	Not reported	74.6%
Orchidaceae					
<i>Cymbidium crassifolium</i> Herb. (Fan <i>et al.</i> , 2023; reported as <i>Cymbidium mannii</i> Rchb.f.)	strong CAM	2.88 Gb	22.7 Mb	Not reported	97.0%
<i>Dendrobium nobile</i> Lindl. (Xu <i>et al.</i> , 2022)	C ₃ +CAM (Qui <i>et al.</i> , 2015)	1.16 Gb	64.5 Mb	8	96.2%
<i>Dendrobium officinale</i> Kimura & Migo (Yan <i>et al.</i> , 2021)	C ₃ +CAM (Zou <i>et al.</i> , 2018; reported as <i>D. catenum</i> Lindl.)	1.35 Gb	76.5 kb	4,697	Not reported
<i>Dendrobium chrysotoxum</i> Lindl. (Zhang <i>et al.</i> , 2021)	C ₃ +CAM (Qui <i>et al.</i> , 2015)	1.37 Gb	67.8 Mb	8	90.3%
<i>Phalaenopsis equestris</i> (Schauer) Rchb.f. (Cai <i>et al.</i> , 2015)	strong CAM (Zhang <i>et al.</i> , 2016)	1.16 Gb	359 kb	523	Not reported
<i>Vanilla planifolia</i> Andrews (Hasing <i>et al.</i> , 2020)	strong CAM (Silvera <i>et al.</i> , 2010)	1.48 Gb	42.0 Mb	13	93.9%
Bromeliaceae					
<i>Aechmea fasciata</i> (Lindl.) Baker (Li <i>et al.</i> , 2022)	strong CAM (Crayn <i>et al.</i> , 2015)	359 Mb	4.68 Mb	38	93.4%
<i>Ananas comosus</i> (L.) Merr. V3 (Ming <i>et al.</i> , 2015)	strong CAM	526 Mb	11.8 Mb	13	89.3%
Crassulaceae					
<i>Kalanchoë fedtschenkoi</i> Raym.-Hamet & H.Perrier v1.1 (Yang <i>et al.</i> , 2017)	strong CAM	256.35 Mb	2.5 Mb	28	89.5%
<i>Kalanchoë laxiflora</i> Baker v3.1 (Yang <i>et al.</i> , 2017)	strong CAM	262 Mb	12.5 Mb	8	93.2%
<i>Sedum album</i> L. (Wai <i>et al.</i> , 2019)	C ₃ +CAM	302 Mb	93 kb	868	97%
Vitaceae					
<i>Cissus rotundifolia</i> Vahl (Xin <i>et al.</i> , 2022)	strong CAM (Nelson and Sage, 2008)	350.69 Mb	27.6 Mb	5	92.4%

Euphorbiaceae

<i>Jatropha curcas</i> L. (Ha <i>et al.</i> , 2019)	C ₃ +CAM (Winter and Holtum, 2015)	414 Mb	1.5 Mb	Not reported	82.5%
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Aizoaceae

<i>Mesembryanthemum crystallinum</i> L. (Shen <i>et al.</i> , 2022)	facultative CAM	377.97 Mb	40.5 Mb	5	98.0%
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Cactaceae

<i>Carnegiea gigantea</i> (Engelm.) Britton & Rose (Copetti <i>et al.</i> , 2017)	strong CAM (Nobel and Hartsock, 1986)	1.30 Gb	61.5 kb	4,575	91%
<i>Lophocereus schottii</i> (Engelm.) Britton & Rose (Copetti <i>et al.</i> , 2017)	strong CAM (Mooney <i>et al.</i> , 1974)	1.47 Gb	9.3 kb	21,671	74%
<i>Pachycereus pringlei</i> (S.Watson) Britton & Rose (Copetti <i>et al.</i> , 2017)	strong CAM (Mooney <i>et al.</i> , 1974)	1.41 Gb	5.4 kb	32,562	61%
<i>Pereskia horrida</i> DC. (Copetti <i>et al.</i> , 2017; reported as <i>P. humboldtii</i> Britton & Rose)	C ₃ +CAM (Martin and Wallace, 2000)	980 Mb	4.4 kb	28,266	52%
<i>Stenocereus thurberi</i> (Engelm.) Buxb. (Copetti <i>et al.</i> , 2017)	strong CAM (Dettmen <i>et al.</i> , 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 <i>et al.</i> , 2021)	strong CAM (N. Wang <i>et al.</i> , 2019)	1.41 Gb	127.2 Mb	670	93.8% [‡]
Portulacaceae					
<i>Portulaca amilis</i> Speg. (Gilman <i>et al.</i> , 2022)	C ₄ +CAM	403.89 Mb	42.6 Mb	5	96.9%
<i>Portulaca oleracea</i> L. (Wang <i>et al.</i> , 2023)	C ₄ +CAM	1.13 Gb	35.1 Mb	11	98.0%

*Genome assembly version 1.0 unless otherwise noted.

[†]Evidence of CAM provided under the Taxon column unless provided here.

[‡]Eukaryota BUSCOs were used to benchmark *I. taiwanensis* (Wickell *et al.*, 2021) and *H. undatus* (Chen *et al.*, 2021).

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