Macroclimatic niche limits and the evolution of C_4 photosynthesis in Gomphrenoideae (Amaranthaceae)

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During the evolution of vascular plants, C_4 photosynthesis has developed > 60 times, but most studies concerning the evolution and the ecophysiological advantages of the C_4 syndrome have been carried out in grasses. Among eudicots, C_4 photosynthesis has developed in lineages that are morphologically more diverse than the relatively homogeneous grasses, and conclusions about C_4 evolution based on grasses may or may not apply to eudicots. Little is known about the origin of C_4 photosynthesis in Amaranthaceae *s.s.*, one of the most C_4 species-rich lineages of eudicots. Among subfamily Gomphrenoideae, C_3 and C_4 species are found in arid regions, but high Andean species display the C_4 pathway. In this study, we aimed to compare the climatic extremes between C_3 and C_4 species in Gomphrenoideae. In particular, we evaluated the upper and lower extremes of three climatic variables among closely related C_3 and C_4 lineages, using phylogenetic generalized least squares (PGLS), applied on four topologies: maximum parsimony, Bayesian and maximum likelihood (ML) topologies including species with available DNA sequences and a ML topology that included unsequenced species with available climate data. We tested whether the evolution of C_4 photosynthesis correlates with a niche specialization where both the upper and the lower extremes change position or a niche expansion where only a single extreme changes position. PGLS results showed that C_4 species of Gomphrenoideae have specialized to dryer regions and expanded into colder ones than their C_3 relatives. However, the niche expansion into colder climates only includes areas with low temperatures during winter, not during the growing period. Finally, we found no evidence for a niche change into warmer climates. Therefore, the model for the evolution of C_4 photosynthesis in Gomphrenoideae may differ from the one supported in Chenopodioideae and seems to be more similar to that found in grasses, where the C_4 pathway appears to have evolved in humid habitats, but facilitated an expansion into arid ones. However, we found no support for the common expectation that C_4 species reach warmer climates than their C_3 relatives, contrasting with previous results on grasses.

ADDITIONAL KEYWORDS: C_4 photosynthesis – climatic extremes – eudicots – Gomphrenoideae – macroclimatic niche evolution – macroecology.

INTRODUCTION

The origin of C_4 photosynthesis and its potential for inducing niche shifts is currently receiving much attention in the literature, as are studies on diversification of plant species in relation to climate (Yesson & Culham, 2006; Evans *et al.*, 2009). Several studies have quantified macroclimatic niches among diverse C_4 lineages and compared these with the niches of related C_3 taxa to evaluate if there is a niche shift among C_4 species. It has been proposed that C_4 photosynthesis requires open environments, but the high occurrence of C_4 lineages in dry habitats is also thought to have arisen as a pre-adaptation to xeric conditions (Osborne & Freckleton, 2009). Edwards & Smith (2010) proposed that C_4 origins in grasses coincided with ecological shifts into drier environments. Among angiosperms, the C_4 pathway has evolved > 60 times independently (Sage, 2016), but most attention has been given to its occurrence in Poaceae (Sage, Sage & Kocacinar, 2012; Still *et al.*, 2003). C_4 grasses and sedges dominate nearly all grasslands in the tropics, subtropics and warm temperate zones, and are major representatives of arid landscapes from the temperate zones to the tropics. They are also capable of growing

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in habitats that may be too harsh for their C$_3$ relatives, such as outcrops and hypersaline or arid soils at low elevations (Sage, 2004).

Rather than a single adaptation, C$_4$ photosynthesis is a syndrome of complex genetic, biochemical and anatomical modifications (Sage, 2004; Christin & Osborne, 2013). Due to enhanced water and nutrient use efficiency, C$_4$ plants are capable of growing in habitats that are unfavourable to C$_3$ species. The partial closure of stomata to conserve water in arid and saline soils or dry atmospheric conditions has been hypothesized to select for the C$_4$ pathway via indirect effects on photosynthetic efficiency. Reduced stomatal opening restricts the CO$_2$ supply for photosynthesis and decreases transpiration. Both effects increase photorespiration, favouring the C$_4$ type in high-light, warm and water-stressed habitats (Sage & Kubien, 2011; Osborne & Sack, 2012). Photorespiration decreases with falling temperatures, probably explaining why species with the more energetically expensive C$_4$ pathway are absent from polar biomes and rare in cool temperate to boreal biomes, at alpine and montane elevations and in forest understorey (Sage, Wedin, & Li, 1999; Sage & Kubien, 2011). There is much discussion in the literature concerning whether the C$_4$ syndrome is an adaptation to water-stressed environments or whether it just improves the conditions for adapting to these (Long, 1999; Osborne & Freckleton, 2009). For example, in grasses it has been shown that selection for C$_4$ photosynthesis occurred in open habitats, but once the pathway evolved, C$_4$ lineages adapted to saline or arid conditions at a faster rate than C$_3$ lineages (Osborne & Freckleton, 2009). Therefore, in grasses the C$_4$ pathway would be a pre-adaptation to arid environments. On the other hand, the available data among eudicots suggests that in Chenopodioideae (Chenopodiaceae *s.s.*) drought tolerance, achieved by physiological and morphological adaptations, was a pre-adaptation that enhanced the evolution of the C$_4$ pathway (Kadereit, Ackerly & Pirie, 2012). In this case, conclusions about C$_4$ evolution based on grasses are not applicable to eudicots, where C$_4$ photosynthesis has developed in lineages that are morphologically much more diverse than the relatively homogeneous grasses (Muhaidat, Sage & Dengler, 2007; Kadereit *et al.*, 2012; Sánchez del Pino, Motley & Borsch, 2012; Ocampo *et al.*, 2013).

Amaranthaceae (including Chenopodioideae) comprises the largest number of C$_4$ species and lineages among eudicot families (Sage *et al.*, 2007). In Chenopodioideae, C$_4$ photosynthesis has evolved in nine lineages (Sage, 2016) and 15 anatomically different C$_4$ leaf types have been distinguished. Moreover, two C$_4$ biochemical subtypes have evolved independently in this subfamily (Kadereit *et al.*, 2003).

Little is known about the origin of C$_4$ photosynthesis in Amaranthaceae *s.s.*, which comprises > 200 C$_4$ species (Sage *et al.*, 2007). Most C$_4$ taxa in Amaranthaceae *s.s.* are found in the mainly Neotropical subfamily Gomphrenoideae, in which the C$_4$ syndrome arose independently at least three times (Sage *et al.*, 2007; Sánchez del Pino, Borsch & Motley, 2009; Sánchez del Pino *et al.*, 2012). Most of the species diversity in this subfamily is dominated by the large genera *Gomphrena* L. (*c.* 120 species) and *Alternanthera* Forssk (*c.* 100 species). Both genera contain C$_3$ and C$_4$ species and *Alternanthera* also includes C$_3$–C$_4$ intermediate species (Sánchez del Pino *et al.*, 2009, 2012). Unlike Chenopodioideae, Amaranthaceae *s.s.* are not generally salt-resistant (Menzel & Lieth, 2013) and leaf anatomy is more uniform among the C$_4$ species (Carolin, Jacobs & Vesk, 1975). Gomphrenoideae are distributed in a variety of environments such as tropical savannas, forests, deserts and the high Andes. Although C$_3$ and C$_4$ members of Gomphrenoideae may be found in arid regions, high Andean species display the C$_4$ pathway, and are frequently mentioned among the C$_4$ taxa that occur in tropical highlands (Sage, Christin & Edwards, 2011)

Recent macro-ecological studies have aimed to quantify niche evolution in C$_4$ grasses by comparing macro-climatic distribution data between C$_3$ and C$_4$ species in a phylogenetic context (Edwards & Smith, 2010; Liu, Peterson, & Ge, 2011; Pau, Edwards & Still, 2013; Aagesen *et al.*, 2016). However, most of these studies only examined mean values for species and not climatic extremes. The niche concept and wide biogeographical patterns are based on distributional limits (Wiens, 2011). Niches can expand, contract or shift (Pearman *et al.*, 2008) and these changes bear different implications on the evolutionary and ecological advantages of the C$_4$ syndrome. The evolution of C$_4$ photosynthesis may correlate with a niche specialization in which both climatic extremes (upper and lower) change position, or an expansion in which C$_4$ species reach new environments without withdrawal from the inherited niche. When distributional limits were analysed within Poaceae tribe Paniceae, a niche expansion of the C$_4$ lineages into warmer climates was supported (Aagesen *et al.*, 2016). The same study also found a niche change of the C$_4$ species into more arid climates.

In the present study we explored whether C$_4$ evolution in Gomphrenoideae correlates with a macro-climatic niche evolution among C$_4$ taxa. Our approach was to compare the climatic extremes between C$_3$ and C$_4$ species in the subfamily. We explored if C$_3$ → C$_4$ transitions correlate with a general pattern of macro-ecological niche evolution. In particular, we evaluated the upper and lower extremes of the variables mean temperature of the warmest quarter (MTWQ), mean

temperature of the coldest quarter (MTCQ) and arid-ity (AI) among closely related C$_3$ and C$_4$ species, in a phylogenetic context.

Our main hypothesis is that the C$_4$ photosynthetic pathway provides an advantage over C$_3$ lineages in arid and/or high Andean regions. Overall, this work will allow us to suggest whether the evolution of C$_4$ photosynthesis in Gomphrenoideae correlates with a niche specialization or a niche expansion and to address the attributes of the macro-climatic C$_4$ niche in Gomphrenoideae.

MATERIAL AND METHODS

DISTRIBUTION AND CLIMATE DATA

We downloaded all georeferenced species of Gomphrenoideae through the Global Biodiversity Information Facility (www.gbif.org/, accessed between April 2012 and December 2013) and complemented the dataset with georeferences from the literature (Pedersen, 2000; McCauley, 2004; Marchioretto, Sfoggia Miotto & De Siqueira, 2010). All species names were validated or synonymized according to the Catalogue of the vascular plants of the southern cone (Zuloaga, Morrone & Belgrano, 2008) or the Plant List (www.theplantlist.org, last accessed in 2015).

Temperature variables were extracted from BioClim (Hijmans *et al.*, 2005) and data of aridity from CGIAR-CSI (www.cgiar-csi.org, last accessed in March 2014), both with a spatial resolution of 30 arc seconds. We only included distribution data from native regions and verified the georeferences, to avoid distortion from outliers (misidentified species, spatially imprecise coordinates, errors caused during digitalization of the herbarium specimens, etc.) (Maldonado *et al.*, 2015). Among the verified georeferences, we used trimmed observations, excluding 5% of the records at each extreme (5th and 95th percentile observations), which led to a more sensitive analysis in relation to the sampling efforts. We compiled > 16,000 georeferences for 158 species of Gomphrenoideae (76 C$_3$ and 82 C$_4$). We only considered those species with at least three local-ity records available (all verified and trimmed obser-vations). Among the 169 taxa compiled in the complete dataset (158 Gomphrenoideae, 11 outgroup species), only 13 species had three to six georeferences avail-able; the others had more than six georeferences.

There has been much discussion in the literature as to whether the C$_4$ syndrome is an adaptation to arid environments or merely improves the condi-tions for adapting to these, because it has an inher-ited higher water-use efficiency (Long, 1999; Osborne & Freckleton, 2009). We compared climatic extremes among C$_3$ and C$_4$ species using the Aridity Index (AI = mean annual precipitation/mean annual potential
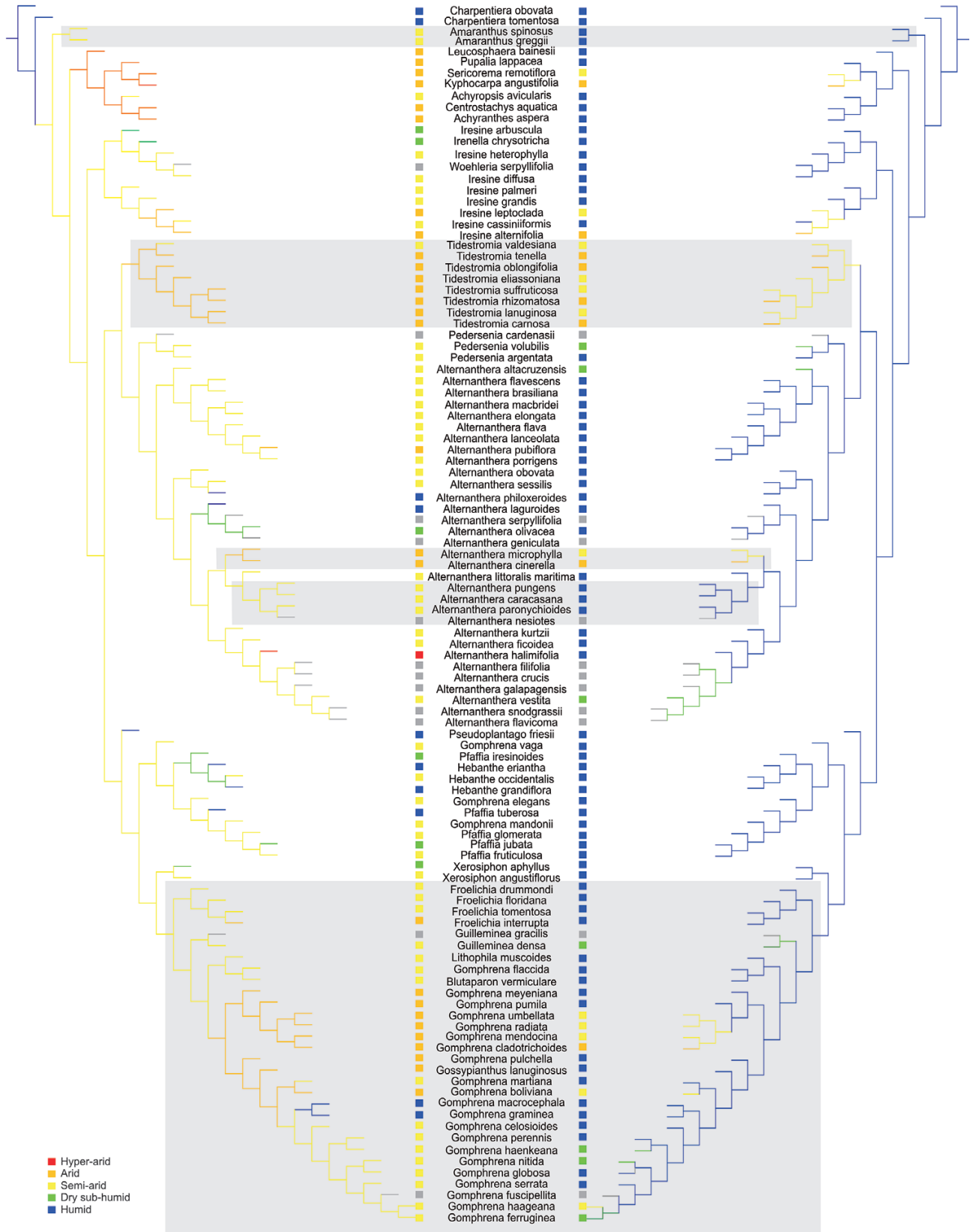
evapo-transpiration) from the CGIARCSI database (Trabucco & Zomer, 2009). We based all quantitative analyses on continuous values but used the following categories for graphic and descriptive purposes: AI < 0.03 hyper-arid, 0.03–0.2 arid, 0.2–0.5 semi-arid, 0.5–0.65 dry sub-humid, > 0.65 humid (Trabucco & Zomer, 2009).

In warm conditions, the C$_4$ pathway shows higher rates of photosynthesis than C$_3$, but at lower tem-peratures it loses competitiveness (Kubien *et al.*, 2003; Sage & Kubien, 2007). That may be the rea-son why C$_4$ species are rare in cold climates. To test this common hypothesis, we compared MTCQ and MTWQ to explore if C$_4$ species occupy warmer regions than their closest C$_3$ relatives. We based all quantitative analyses on continuous values, but used bins of 5 °C for graphic and descriptive purposes. According to the Köppen-Geiger climate classification (Peel, Finlayson & McMahon, 2007), the limit for tropical climate coincides with a mean temperature of the coldest month of 18 °C or higher and in the present study the limit for tropical cli-mate therefore falls in the 15–20 °C bin. Subtropical climate is defined by different summer and winter temperatures, with the limit between subtropical and temperate climate falling approximately in the 5–10 °C bin.

In a previous phylogenetic analysis, Edwards & Smith (2010) found a shift towards an increased pre-cipitation seasonality in C$_4$ species, which was consist-ent with a shift from a closed-canopy tropical moist forest into an open tropical savanna biome. However, among Gomphrenoideae there are no apparent shifts from closed to open biomes, as both C$_3$ and C$_4$ spe-cies are mainly found in open regions. Thus, we did not consider precipitation seasonality as a potentially important parameter. Notwithstanding, phyloge-netic generalized least squares (PGLS) comparisons on precipitation seasonality (PS) are included in the Supporting Information, where it is shown that PS does not differ between C$_3$ and C$_4$ members of Gomphrenoideae (Table S1).

TAXON SAMPLING AND MOLECULAR STUDIES

We searched GenBank for all available sequences of *trnL-F*, *rpl16*, *rbcL* and *matK* regions for species of Gomphrenoideae (Appendix 1). In addition, the plas-tid tRNA-Leu (*trnL*) gene and the *trnL-trnF* inter-genic spacer (hereafter *trnL-F*) were amplified and sequenced for ten additional species of Gomphrenoideae (Appendix 2). The *trnL-F* marker was selected because it has successfully been used for addressing phyloge-netic relationships in this subfamily (Sánchez del Pino *et al.*, 2009). These newly sequenced species were cho-sen to represent the macro-climatic variability found in

Charpentiera obovata
Charpentiera tomentosa
Amaranthus spinosus
Amaranthus greggii
Leucosphaera bainesii
Pupalia lappacea
Sericorema remotiflora
Kyphocarpa angustifolia
Achyropsis avicularis
Centrostachys aquatica
Achyranthes aspera
Iresine arbuscula
Irenella chrysotricha
Iresine heteryphylla
Woehleria serpyllifolia
Iresine diffusa
Iresine palmeri
Iresine grandis
Iresine leptoclada
Iresine cassiniiformis
Iresine alternifolia
Tidestromia valdesiana
Tidestromia tenella
Tidestromia oblongifolia
Tidestromia eliassoniana
Tidestromia suffruticosa
Tidestromia rhizomatosa
Tidestromia lanuginosa
Tidestromia carnosa
Pedersenia cardenasii
Pedersenia volubilis
Pedersenia argentata
Alternanthera altacruzensis
Alternanthera flavescens
Alternanthera brasiliana
Alternanthera macbridei
Alternanthera elongata
Alternanthera flava
Alternanthera lanceolata
Alternanthera pubiflora
Alternanthera porrigens
Alternanthera obovata
Alternanthera sessilis
Alternanthera philoxeroides
Alternanthera laguroides
Alternanthera serpyllifolia
Alternanthera olivacea
Alternanthera geniculata
Alternanthera microphylla
Alternanthera cinerella
Alternanthera littoralis maritima
Alternanthera pungens
Alternanthera caracasana
Alternanthera paronychioides
Alternanthera nesiotes
Alternanthera kurtzii
Alternanthera ficoidea
Alternanthera halimifolia
Alternanthera filifolia
Alternanthera crucis
Alternanthera galapagensis
Alternanthera vestita
Alternanthera snodgrassii
Alternanthera flavicoma
Pseudoplantago friesii
Gomphrena vaga
Pfaffia iresinoides
Hebanthe eriantha
Hebanthe occidentalis
Hebanthe grandiflora
Gomphrena elegans
Pfaffia tuberosa
Gomphrena mandonii
Pfaffia glomerata
Pfaffia jubata
Pfaffia fruticulosa
Xerosiphon aphyllus
Xerosiphon angustiflorus
Froelichia drummondi
Froelichia floridana
Froelichia tomentosa
Froelichia interrupta
Guilleminea gracilis
Guilleminea densa
Lithophila muscoides
Gomphrena flaccida
Blutaparon vermiculare
Gomphrena meyeniana
Gomphrena pumila
Gomphrena umbellata
Gomphrena radiata
Gomphrena mendocina
Gomphrena cladotrichoides
Gomphrena pulchella
Gossypianthus lanuginosus
Gomphrena martiana
Gomphrena boliviana
Gomphrena macrocephala
Gomphrena graminea
Gomphrena celosioides
Gomphrena perennis
Gomphrena haenkeana
Gomphrena nitida
Gomphrena globosa
Gomphrena serrata
Gomphrena fuscipellita
Gomphrena haageana
Gomphrena ferruginea

Hyper-arid
Arid
Semi-arid
Dry sub-humid
Humid

Gomphrena and *Alternanthera*. The primers employed were those from Taberlet *et al.* (1991). Total DNA was isolated from fresh tissue dried in silica gel, following the protocol of Doyle & Doyle (1987). PCR was performed in 25 µL containing 1 µL DNA template, and a final concentration of 1× PCR buffer minus Mg, 5 mM MgCl$_2$, 0.025 mM each dNTP, 0.2 mM each primer and 1.25–3 U Taq polymerase (Invitrogen, Buenos Aires, Argentina). The PCR amplifications were set at one cycle of 95 °C for 4 min; 39 cycles of 95 °C for 30 s, 54 °C for 1 min and 72 °C for 90 s; and a final extension cycle of 72 °C for 7 min. For the species that failed this protocol, variations in the annealing temperature (48–58 °C) were followed. Sequencing reactions were performed by Macrogen, Inc. (Seoul, Korea). All the sequences were integrated in a single matrix comprising 108 species (including outgroup species) and 6677 characters (Table 1).

PHYLOGENETIC ANALYSES

Sequences were initially aligned with ClustalX v.2.0 (Larkin *et al.*, 2007) under the default settings, and then adjusted manually using Bioedit v.7.0.9.0 (Hall, 1999). The phylogenetic analyses were done using maximum parsimony (MP), maximum likelihood (ML) and Bayesian approaches.

For MP, we used the program TNT v.1.1, treating gaps as missing data (Goloboff, Farris & Nixon, 2008). We performed heuristic searches with 10 000 random addition sequences, TBR swapping and holding ten trees per replicate. All optimal trees were submitted to a new round of TBR branch swapping to completion. Since tree space in memory overflowed, we also realized a Driven search, finding the minimum length 100 times with default settings for Sectorial searches and Tree fusing (Goloboff, 1999). The resulting trees were submitted to 100 cycles of Ratchet (Nixon, 1999) and Drift (both default settings). The strict consensus trees obtained from both heuristic and new technologies searches were identical

and consequently we did not continue the searches. Support values for nodes were estimated using jackknife (JC) analysis with 10 000 replicates of ten random addition sequences, holding ten trees per replicate and using 0.36 as removal probability.

In the ML and Bayesian analyses, the best-fit substitution model for *trnL-F* was the general time reversible with a gamma-shape parameter (GTR + G) and the general time reversible with a gamma-shape parameter and a proportion of invariant sites (GTR + G + I) for the remaining plastid markers (*rpl16*, *matK*, *rbcL*). Models were selected by the Akaike information criterion (AIC) as implemented in jModelTest 2.0 (Darriba & Posada, 2012).

We performed the ML analysis in RAxML v.8.0.0 (Stamatakis, 2014) on the concatenated dataset. RAxML was used to conduct non-parametric bootstrap (BS) analysis and searches for the best-scoring ML tree in a single run (Stamatakis, Hoover & Rougemont, 2008). We executed 1000 rapid bootstrap inferences and, thereafter, a thorough ML search.

Bayesian inferences of phylogenetic trees were conducted with MrBayes 3.2 (Ronquist, Huelsenbeck & Teslenko, 2012) through the CIPRES portal (Miller, Pfeiffer & Schwartz, 2010). Two analyses, each of four parallel chains, were run for 5 000 000 generations, sampling a tree every 1000 generations and with a burn-in period of 250 000. The convergence of the Markov chain Monte Carlo (MCMC) run and the adequacy of the burn-in length were confirmed using the program Tracer v.1.6.0 (Rambaut *et al.*, 2014).

ANALYSES OF THE CLIMATE DATASET

We aimed to determine whether the evolution of the C$_4$ pathway correlates with changes in the macroclimatic niches. For this, we tested if the upper and lower climatic extremes of three variables (AI, MTCQ and MTWQ) differed among C$_3$ and C$_4$ species. We used PGLS regression as implemented in the R packages

Table 1. Features of the DNA regions included in the phylogenetic analyses

DNA region	Number of terminals	Number of characters	Parsimony uninformative characters	Potentially parsimony informative characters	% informative characters
trnL-F	98	1242	860	382	31
rpl16	88	1517	1065	452	30
matK	30	2628	2278	350	13
rbcL	23	1290	1230	60	5
Combined dataset	108	6677	5433	1244	19

Figure 1. Optimal tree obtained from the maximum parsimony analyses showing 'aridity' (AI) extremes. Left tree shows minimum AI extremes and right tree shows maximum AI extremes. Shaded sections indicate the C$_4$ lineages. All quantitative analyses treated AI as a continuous character, but for illustrative purposes the values have been binned.
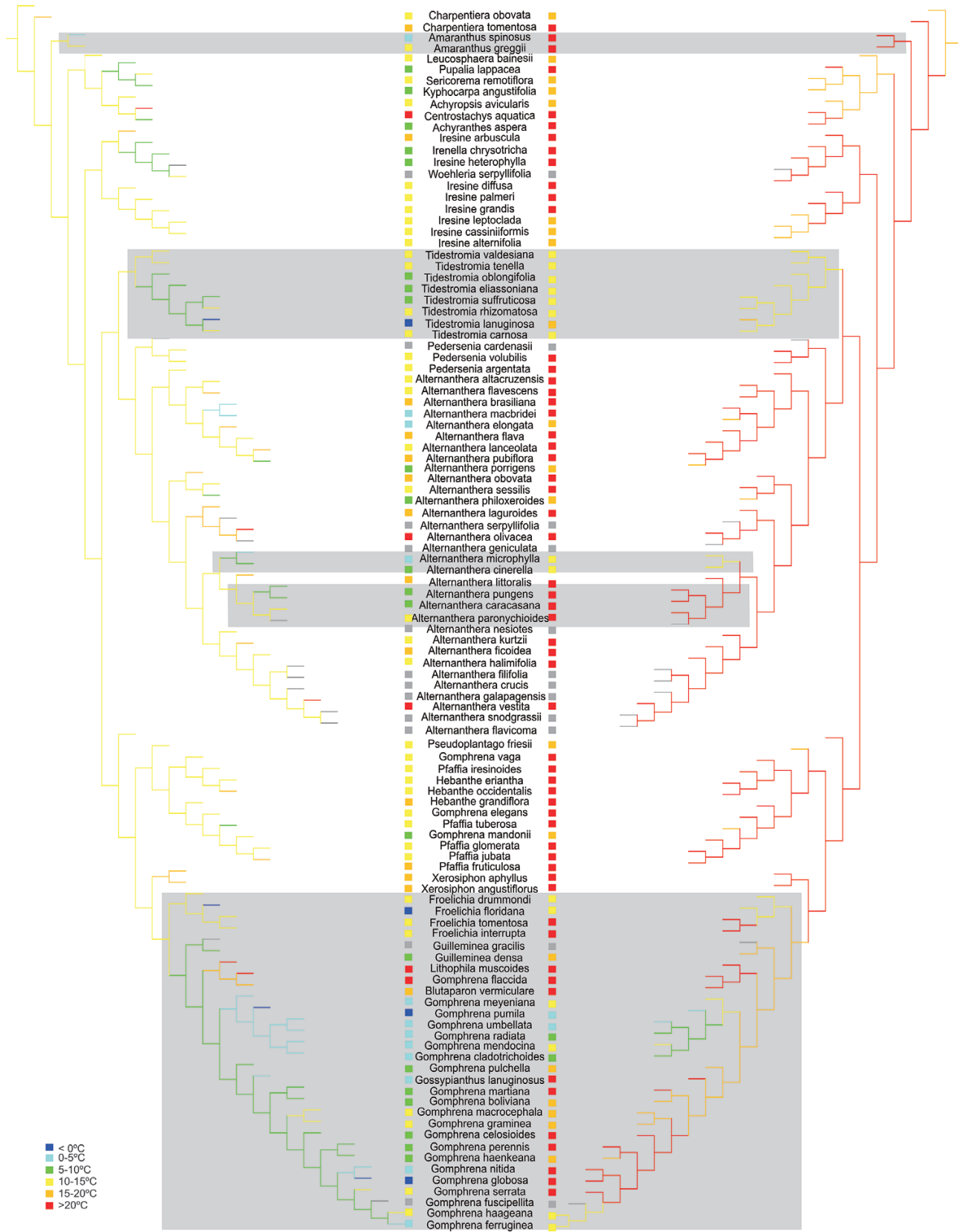
nlme (Pinheiro *et al.*, 2015) and ape (Paradis, Claude & Strimmer, 2004) (R Core Team, 2014). We evaluated five evolutionary models with variations in the phylogenetic correlation structure: Brownian motion (Felsenstein, 1985), Pagel's λ (Pagel, 1999), Martins' α (Martins & Hansen, 1997), Blomberg's γ (Blomberg, Garland & Ives, 2003) and Grafen's ρ (Grafen, 1989) and selected the model with lowest AIC (Johnson & Omland, 2004).

The evolutionary models were evaluated on the MP, Bayesian and two ML trees, one including species for which DNA sequence data were available and another including all species that have available climate data. The complete climate dataset included 158 species of Gomphrenoideae (82 C$_4$, 76 C$_3$), of which DNA sequences were available for only 79 (37 C$_4$, 42 C$_3$). Thus, we added to the ML phylogenetic analysis all the species that have available climate data (but lack DNA sequences), assuming genera to be monophyletic, unless non-monophyly had been previously demonstrated, as is the case for *Gomphrena*, which has been shown to be polyphyletic, with C$_3$ and C$_4$ species gathered in different clades (Kadereit *et al.*, 2003; Müller & Borsch, 2005; Sánchez del Pino *et al.*, 2009). Consequently, C$_3$ species were added to the C$_3$ *Gomphrena* clade and C$_4$ species to the C$_4$ *Gomphrena* clade. In the C$_4$ *Gomphrena* clade, species endemic to Australia were grouped with the Australian species already present in the phylogenetic tree, as these form a clade (Palmer, 1998; Sánchez del Pino *et al.*, 2009). To add species with no available DNA sequences, we created a polytomy at the node of insertion and assigned the added species the average branch length in the clade. Consequently, we used as input tree both the trees for which DNA sequences were available and the tree with the unsequenced species that have available climate data.

We optimized the climatic variables that showed significant differences among C$_3$ and C$_4$ species in the PGLS (AI, MTCQ) as quantitative continuous characters using Fitch optimization in TNT (Goloboff, Mattoni & Quinteros, 2006), but only for graphic and descriptive purposes.

RESULTS

Trees obtained from the combined analyses

Figures 1–3 show the topologies obtained from MP and ML approaches, performed with available DNA sequences. Monophyly of the gomphrenoid, alternantheroid and iresinoid clades was obtained by all the approaches, but only well supported in the ML and the Bayesian analyses (Figs 1–3). All the topologies showed three C$_3$→C$_4$ transitions in Gomphrenoideae. The major C$_4$ clade is the one that comprises *Froelichia* Moench, *Guilleminea* Kunth and *Gomphrena s.s.*

Analyses of the climate dataset

In most cases, the AIC pointed to Grafen or Pagel as the best fitting models, except for the variable MTWQ when analysed with the complete dataset, for which the best fitting model was Martins (Table 2). In all the cases, none of the alternative models contradicted the results shown in Table 2. Nearly all PGLS results were consistent among the four analysed topologies.

Aridity

Most Gomphrenoideae, both C$_3$ and C$_4$ species, are found in semi-arid to humid regions (AI > 0.2). However, there are many more C$_4$ taxa (*c.* 24% C$_4$ vs. *c.* 4% C$_3$) that occupy habitats which range from arid to semi-arid climates (Fig. 4). When we analysed the distribution in a phylogenetic context we found significant differences between the AI extremes of C$_3$ and C$_4$ species. Both the upper and the lower extremes of the C$_4$ species were more arid than the climatic extremes of the C$_3$ species (Table 2). This result was consistent among the four topologies analysed.

Mean temperature of the coldest quarter

Both photosynthetic types in Gomphrenoideae have a similar percentage of species (*c.* 24%) restricted to regions with winter temperatures above 15 °C. The C$_3$ species are mostly found in regions where the MTCQ is > 10 °C, whereas C$_4$ Gomphrenoideae reach colder regions either by occupying wide climatic ranges or by being restricted to regions with low MTCQ (Fig. 5).

The PGLS results for this variable showed that the lowest extreme of the MTCQ was lower among the C$_4$ species than among their closest C$_3$ relatives, but the upper extreme did not show significant differences between the two photosynthetic types (Table 2). When we used the ML tree with the complete climate dataset as input, the maximum extreme of MTCQ also showed significant differences between C$_3$ and C$_4$ species (Table 2).

Figure 2. Optimal tree obtained from the maximum parsimony analyses showing 'mean temperature of the coldest quarter' (MTCQ) extremes. Left tree shows minimum MTCQ extremes and right tree shows maximum MTCQ extremes. Shaded sections indicate the C$_4$ lineages. All quantitative analyses treated MTCQ as a continuous character, but for illustrative purposes the values have been binned.

Charpentiera obovata
Charpentiera tomentosa

100
99 — *Amaranthus greggii*
— *Amaranthus spinosus*

Leucosphaera bainesii
Pupalia lappacea
97 *Sericorema remotiflora*
96 *Kyphocarpa angustifolia*
Achyranthes aspera
100 67 *Centrostachys aquatica*
64 *Achyropsis avicularis*
98

Iresine leptoclada
Iresine grandis
Iresine cassiniiformis
Iresine alternifolia
Iresine palmeri
Iresine arbuscula
100 *Irenella chrysotricha*
66 100 *Iresine diffusa*
70 56 *Woehleria serpyllifolia*
52 *Iresine heterophylla*

Pseudoplantago friesii
Pfaffia iresinoides
60 *Hebanthe occidentalis*
57 96 *Hebanthe eriantha*
63 *Hebanthe grandiflora*
Gomphrena vaga
Pfaffia jubata
Pfaffia tuberosa
Pfaffia dunaliana
Gomphrena elegans
Gomphrena mandonii
Pfaffia fruticulosa
100 *Xerosiphon aphyllus*
84 *Xerosiphon angustiflorus*
Froelichia floridana
99 *Froelichia drummondi*
58 *Froelichia interrupta*
Froelichia tomentosa
100 *Guilleminea gracilis*
91 *Guilleminea densa*
Gomphrena meyeniana
95 *Blutaparon vermiculare*
60 *Lithophila muscoides*
Gomphrena flaccida
Gomphrena umbellata
Gomphrena radiata
Gomphrena cladotrichoides
Gomphrena mendocina
Gomphrena pumila
Gossypianthus lanuginosus
Gomphrena pulchella
94 *Gomphrena boliviana*
64 *Gomphrena martiana*
90 *Gomphrena macrocephala*
58 *Gomphrena graminea*
Gomphrena colosioides
Gomphrena globosa
Gomphrena serrata
Gomphrena nitida
98 *Gomphrena perennis*
59 *Gomphrena haenkeana*
Gomphrena haageana
Gomphrena ferruginea
Gomphrena fuscipellita

Tidestromia tenella
Tidestromia valdesiana
Tidestromia eliassoniana
100 96 *Tidestromia carnosa*
74 81 *Tidestromia lanuginosa*
80 *Tidestromia rhizomatosa*
69 86 *Tidestromia suffruticosa*
80 *Tidestromia oblongifolia*

100 *Pedersenia cardenasii*
81 *Pedersenia argentata*
Pedersenia volubilis
Alternanthera altacruzensis
100 *Alternanthera flavescens*
80 100 *Alternanthera brasiliana*
99 *Alternanthera flava*
100 *Alternanthera pubiflora*
80 *Alternanthera porrigens*
Alternanthera lanceolata
55 *Alternanthera elongata*
55 *Alternanthera macbridei*
Alternanthera laguroides
100 *Alternanthera serpyllifolia*
69 90 *Alternanthera olivacea*
66 100 *Alternanthera geniculata*
79
100 *Alternanthera obovata*
67 *Alternanthera philoxeroides*
Alternanthera sessilis
60 *Alternanthera littoralis*
50 85 *Alternanthera nesiotes*
69 98 *Alternanthera chacoensis*
84 87 *Alternanthera paronychioides*
60 57 *Alternanthera pungens*
82 *Alternanthera caracasana*
99 *Alternanthera cinerella*
87 75 *Alternanthera microphylla*
100 *Alternanthera kurtzii*
63 91 *Alternanthera ficoidea*
64 *Alternanthera halimifolia*
100 *Alternanthera crucis*
65 *Alternanthera galapagensis*
55 *Alternanthera vestita*
62 *Alternanthera flavicoma*
Alternanthera snodgrassii
Alternanthera filifolia

100
97

100
96

93
51

97
51

98
92

99
81

100
92

0.02

Mean temperature of the warmest quarter

Nearly 70% of the C$_4$ Gomphrenoideae and almost 50% of C$_3$ species are restricted to regions with MTWQ > 20 °C. When analysed in a phylogenetic context, and consistently among the four topologies, MTWQ showed no significant differences between the climatic extremes of C$_3$ and C$_4$ species (Table 2).

DISCUSSION

C$_4$ photosynthesis is predicted to provide an advantage in any environmental condition that promotes photorespiration (Sage *et al.*, 2012). Thus, low CO$_2$, elevated temperature, high light, drought and salinity are factors that contribute to the development of this photosynthetic pathway.

The present study supports a niche change among C$_4$ Gomphrenoideae into drier regions than those occupied by their C$_3$ relatives, whereas we found no evidence for a niche expansion into warmer climates (Table 2). C$_4$ Gomphrenoideae also reach colder regions, but this niche expansion only includes areas with low temperatures during winter, as we found no changes in MTWQ among C$_3$ and C$_4$ species (Table 2).

Previous phylogeny-based analyses in the closely related Chenopodioideae (= Chenopodiaceae *s.s.*) have shown that the C$_4$ species derived from C$_3$ lineages that were already adapted to drought. Physiological adaptations to drought such as salt tolerance and morphological/anatomical adaptations such as succulence enhanced the evolution of C$_4$ photosynthesis in several chenopod lineages (Kadereit *et al.*, 2012). Currently, there are no quantitative analyses that explore macroclimatic niche differences among C$_3$ and C$_4$ species in Chenopodioideae. However, most C$_4$ taxa in chenopods surpassed the xerophytic properties of their C$_3$ ancestors and were able not only to replace them almost completely in all suitable habitats, but also to colonize niches not accessible to C$_3$ xerophytes (Kadereit *et al.*, 2003). Thus, the presence of C$_4$ chenopods in extremely arid environments (e.g. *Atriplex* L.; *Salsola* L.; *Suaeda* Forssk. ex J.F.Gmel.) suggests that the C$_4$ species in Chenopodioideae also expanded their niches into even more arid climate than that occupied by their closest C$_3$ relatives.

In contrast, in Amaranthaceae *s.s.*, Gomphrenoideae do not include as many salt-tolerant and succulent species as Chenopodioideae and the C$_3$ species mainly occupy humid and sub-humid habitats (Figs 1, 2–4, 5, Table 2). However, C$_4$ Gomphrenoideae have clearly shifted their niches into more arid climate, as we found significant differences in both minimum and maximum AI values, supporting an expansion into more arid regions and withdrawal from humid regions. Therefore, the model for the evolution of C$_4$ photosynthesis in Gomphrenoideae may differ from that supported in Chenopodioideae and seems to be more similar to the one found in grasses, in which the C$_4$ pathway appears to have evolved in humid habitats but facilitated an expansion into arid ones (Osborne & Freckleton, 2009). Several of the C$_4$ Gomphrenoideae that inhabit arid regions are annual herbs [e.g. *Gomphrena umbellata* Remy, *G. radiata* Pedersen, *G. mendocina* (Phil.) R.E.Fr., *G. cladotrichoides* Suess.] that may complete their life cycles under short periods of rain. On the other hand, many C$_4$ Gomphrenoideae are perennial herbs that occupy arid regions, although it is unknown whether these species are drought-resistant or simply efficient microhabitat specialists. In most species of *Tidestromia* Standl., which are all C$_4$ and mainly perennial, the production of woody growth or storage organs is a common adaptive strategy for longer life spans in dry areas (Sánchez-del Pino & Motley, 2010).

Unlike the niche change into drier climates discussed above, we found no support for the common expectation that C$_4$ species reach warmer climates than their C$_3$ relatives. Both the minimum and the maximum MTWQ were undistinguishable between C$_3$ and C$_4$ Gomphrenoideae in the PGLS comparisons. These results contrast with the evidence for a niche expansion into warmer environments found in C$_4$ grasses (Aagesen *et al.*, 2016). Comparative analyses in grasses have shown that transitions to C$_4$ physiology occurred in species from open habitats in warm regions (Osborne & Freckleton, 2009; Edwards & Smith, 2010). Nevertheless, studies in Poaceae subfamily Panicoideae suggest that when comparing climatic extremes between C$_3$ and C$_4$ panicoids (as opposed to the species mean values) one of the main responses to a change from C$_3$ to C$_4$ photosynthesis is an expansion of the C$_4$ species into hotter climates (Aagesen *et al.*, 2016). We did not find support for this pattern in Gomphrenoideae, even though this subfamily has a distribution pattern similar to the Neotropical panicoid tribe Paspaleae. However, C$_3$ Gomphrenoideae are already present in warm environments, close to the maximum MTWQ values in the Neotropics (Table 2) (Hijmans *et al.*, 2005). The lack of response among the C$_4$ species in Gomphrenoideae may, therefore, simply reflect a lack of available niche space in the macro-climatic

Figure 3. Topology obtained from the maximum likelihood (ML) analysis. Numbers above branches are bootstrap support from the ML tree. Numbers below branches are jackknife support from the MP analyses.

Table 2. Results obtained from the PGLS statistics

| | MPT | | | | Bayesian | | | | MLT | | | | MLT complete dataset | | | |
| | Mean ± SE | | Model | P | Mean ± SE | | Model | P | Mean ± SE | | Model | P | Mean ± SE | | Model | P |
	C₃	C₄			C₃	C₄			C₃	C₄			C₃	C₄		
MIN AI	0.520 ± 0.103	0.288 ± 0.192	Grafen	**0.011**	0.482 ± 0.113	0.260 ± 0.203	Grafen	**0.016**	0.521 ± 0.102	0.288 ± 0.190	Grafen	**0.010**	0.471 ± 0.071	0.241 ± 0.141	Grafen	**0.001**
MAX AI	1.412 ± 0.170	0.861 ± 0.370	Grafen	**0.007**	1.813 ± 0.520	1.274 ± 0.743	Pagel	**0.018**	1.790 ± 0.423	1.260 ± 0.636	Pagel	**0.015**	1.640 ± 0.359	1.111 ± 0.514	Pagel	**0.001**
MIN MTCQ	13.081 ± 1.484	7.566 ± 2.507	Pagel	**0.003**	13.009 ± 1.960	6.343 ± 3.977	Grafen	**0.001**	12.886 ± 1.337	6.809 ± 2.964	Grafen	**0.000**	13.620 ± 1.780	7.443 ± 3.485	Grafen	**0.000**
MAX MTCQ	22.003 ± 2.324	17.779 ± 4.492	Grafen	0.054	22.112 ± 1.939	18.230 ± 3.970	Grafen	0.059	21.987 ± 2.263	18.062 ± 4.502	Grafen	0.083	21.742 ± 1.893	18.578 ± 3.374	Grafen	**0.034**
MIN MTWQ	19.799 ± 1.697	18.734 ± 3.499	Grafen	0.556	19.634 ± 1.707	18.700 ± 3.518	Grafen	0.607	19.604 ± 1.550	18.868 ± 3.284	Grafen	0.672	19.783 ± 0.883	18.097 ± 2.216	Martins	0.208
MAX MTWQ	27.028 ± 1.154	26.728 ± 2.367	Grafen	0.805	27.293 ± 1.938	26.497 ± 3.510	Grafen	0.614	27.007 ± 1.424	26.571 ± 2.820	Grafen	0.755	26.766 ± 1.298	26.541 ± 2.167	Grafen	0.796

For each topology and the three variables analysed we show: mean ± standard error (SE) for C₃ and C₄ species, the best fitting model selected by the AIC criterion and P values obtained (significant values in bold, P < 0.05). MPT, maximum parsimony tree; Bayesian, topology from the Bayesian inference; MLT, maximum likelihood tree.

variable used in the present study. In all, our results do not preclude high temperature as a potential factor in driving the evolution of C_4 photosynthesis at a micro-climatic scale, but this needs to be studied further.

Although C_3 photosynthesis is not physiologically constrained by low temperatures (Sage & Kubien, 2011), we found no evidence that C_3 Gomphrenoideae are more successful than C_4 species at lower temperatures. To the contrary, as mentioned above, we found no differences in MTWQ values among C_3 and C_4 species, whereas the minimum MTCQ values differed between the two photosynthetic types, showing a niche expansion of C_4 species into regions with colder winter climates. When analysing the entire data set, we also obtained significantly lower maximum MTCQ values among the C_4 species, suggesting that these may be even specializing in regions with low winter temperatures. Most of the C_4 species of *Gomphrena s.s* found under low MTCQ occur in the high Andes region, whereas the North American C_4 genera *Tidestromia* and *Froelichia* have many species in regions with winter temperatures that fall below 10 °C but, in this case, they have a continental distribution. In *Alternanthera*, some of the C_4 species reach high elevations, but they are also present at sea level (Sánchez del Pino *et al.*, 2012).

There are a large number of C_4 species in a wide range of taxonomic groups that occur in regions with low winter temperatures and some of them tolerate occasional subzero temperatures during the growing season (Sage & Kubien, 2011). In the American grass tribe Paspaleae, including several high Andean species, there is also evidence for an expansion of C_4 species into cold winter regions (Aagesen *et al.*, 2016) as appears to be the case in Gomphrenoideae. In this study, we found that the minimum MTCQ values of the C_3 Gomphrenoideae remain at *c.* 13 °C, whereas the mean value for the C_4 species is well below 10 °C (Fig. 2, Table 2). The C_4 syndrome requires high daytime temperatures to be competitive during the growing season (Sage & Kubien, 2011), and field studies have shown that C_4 species from cold regions are restricted to warm micro-sites (Sage & Sage, 2002). In the case of Gomphrenoideae it is clear from the MTWQ values that cold regions occupied by C_4 species have high macro-climatic temperatures during the growing season (Table 2).

Lundgren *et al.* (2015) documented that C_4 photosynthesis appears to act as a niche opener, initially facilitating an expansion of the inherited niche, whereas specialization to the new environment may be a delayed process that requires speciation in the new habitat. Overall, we show here that the macro-climatic distribution patterns of the C_4 Gomphrenoideae correspond, in general, to the common global biogeographical patterns for C_4 species
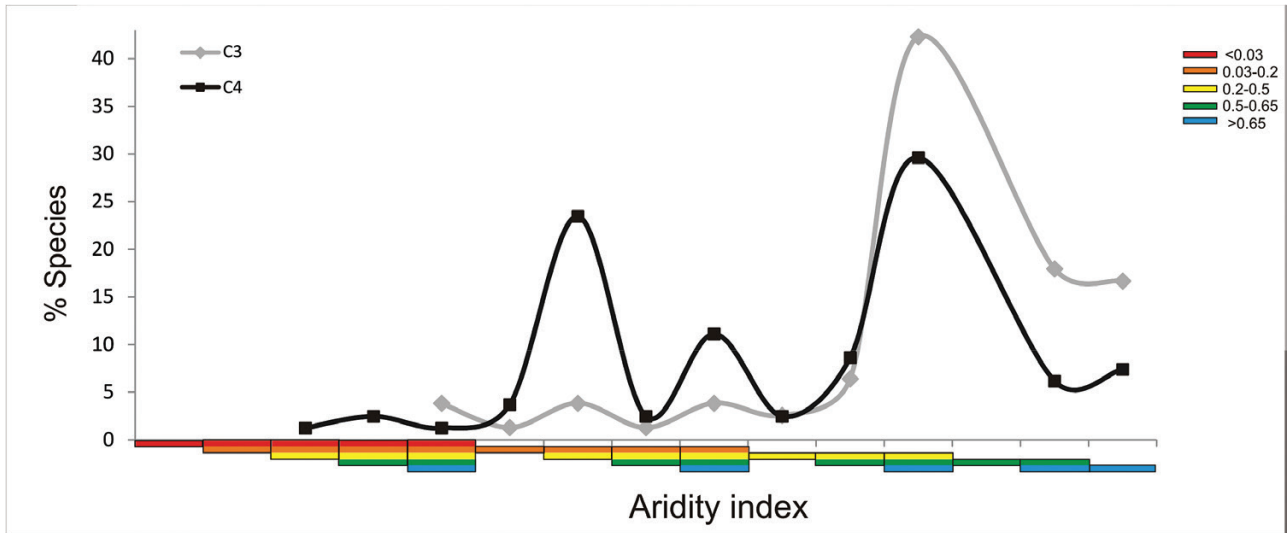
Figure 4. Percentage of species of Gomphrenoideae present throughout the different aridity categories. Vertical axis indicates percentage of species and horizontal axis shows coloured bars that represent the aridity categories occupied: AI < 0.03 hyper arid, 0.03–0.2 arid, 0.2–0.5 semi-arid, 0.5–0.65 dry sub-humid, > 0.65 humid (Trabucco & Zomer, 2009). Species with wide distribution ranges may occupy multiple categories (coloured bars). All bars have the same width.
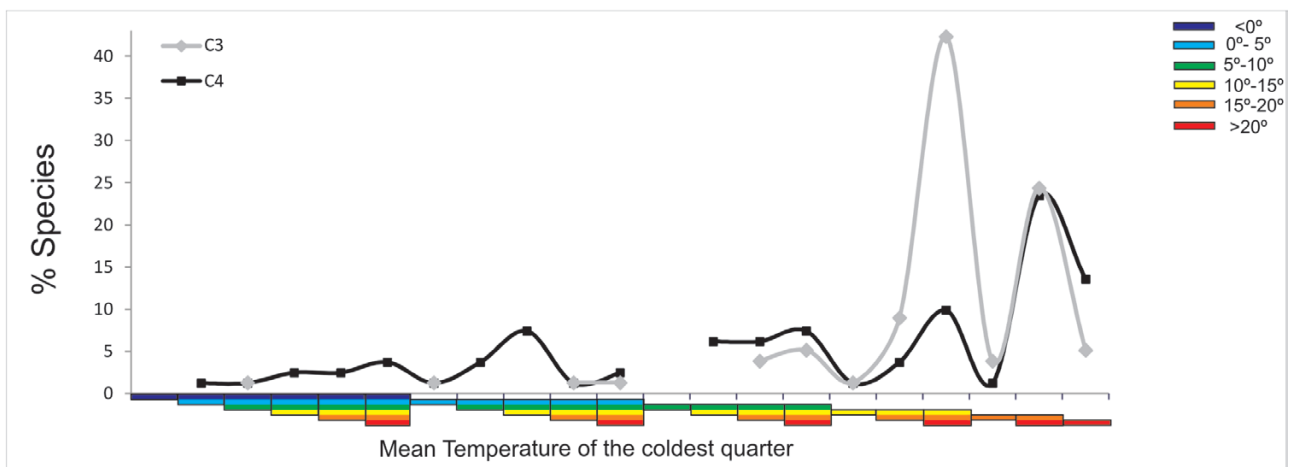
Figure 5. Percentage of species of Gomphrenoideae present throughout the different MTCQ categories. Vertical axis indicates percentage of species and horizontal axis shows coloured bars that represent the MTCQ categories occupied. We used bins of 5 °C for illustrative purposes. Species with wide distribution ranges may occupy multiple categories (coloured bars). All bars have the same width.

and that these patterns have been achieved by niche expansion, niche shifts and niche stasis, all processes that modified or maintained the inherited macroclimatic niches. By comparing the climatic extremes rather than mean values, we found evidence for a niche change of C$_4$ Gomphrenoideae into more arid environments than those occupied by theirs C$_3$ sister lineages, but not into warmer regions as observed in grasses. Also, we have found that C$_4$ Gomphrenoideae have expanded their niches into regions with colder winter climates.

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REFERENCES

Aagesen L, Biganzoli F, Bena J, Godoy-Bürki AC, Reinheimer R, Zuloaga FO. 2016. Macro-climatic distribution limits show both niche expansion and niche specialization among C_4 panicoids. *PLoS One* **11:** e0151075.

Blomberg SP, Garland T Jr, Ives AR. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution; International Journal of Organic Evolution* **57:** 717–745.

Carolin RC, Jacobs SWL, Vesk M. 1975. Leaf structure in Chenopodiaceae. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **95:** 226–255.

Christin PA, Osborne CP. 2013. The recurrent assembly of C_4 photosynthesis, an evolutionary tale. *Photosynthesis Research* **117:** 163–175.

Darriba D, Posada D. 2012. jModelTest 2.0 Manual. *Nature Methods* **9:** 772.

Doyle J, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, Botanical Society of America* **19:** 11–15.

Edwards EJ, Smith SA. 2010. Phylogenetic analyses reveal the shady history of C_4 grasses. *Proceedings of the National Academy of Sciences of the United States of America* **107:** 2532–2537.

Evans ME, Smith SA, Flynn RS, Donoghue MJ. 2009. Climate, niche evolution, and diversification of the "birdcage" evening primroses (*Oenothera*, sections *Anogra* and *Kleinia*). *The American Naturalist* **173:** 225–240.

Felsenstein J. 1985. Phylogenies and the comparative method. *The American Naturalist* **125:** 1–15.

Goloboff PA. 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* **15:** 415–428.

Goloboff PA, Mattoni CI, Quinteros AS. 2006. Continuous characters analyzed as such. *Cladistics* **22:** 589–601.

Goloboff P, Farris J, Nixon K. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* **24:** 1–13.

Grafen A. 1989. The phylogenetic regression. *Philosophical Transactions of the Royal Society B: Biological Sciences* **326:** 119–157.

Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95–98.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25:** 1965–1978.

Johnson JB, Omland KS. 2004. Model selection in ecology and evolution. *Trends in Ecology & Evolution* **19:** 101–108.

Kadereit G, Ackerly D, Pirie MD. 2012. A broader model for C_4 photosynthesis evolution in plants inferred from the goosefoot family (Chenopodiaceae *s.s.*). *Proceedings of the Royal Society B: Biological Sciences* **279:** 3304–3311.

Kadereit G, Borsch T, Weising K, Freitag H. 2003. Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C_4 photosynthesis. *International Journal of Plant Sciences* **164:** 959–986.

Kubien DS, von Caemmerer S, Furbank RT, Sage RF. 2003. C_4 photosynthesis at low temperature. A study using transgenic plants with reduced amounts of Rubisco. *Plant Physiology* **132:** 1577–1585.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics (Oxford, England)* **23:** 2947–2948.

Liu Q, Peterson PM, Ge X. 2011. Phylogenetic signals in the realized climate niches of Chinese grasses (Poaceae). *Plant Ecology* **212:** 1733–1746.

Long SP. 1999. Environmental responses. In: Sage RF, Monson RK, eds. *C_4 plant biology.* San Diego: Academic Press, 215–249.

Lundgren MR, Besnard G, Ripley BS, Lehmann CE, Chatelet DS, Kynast RG, Namaganda M, Vorontsova MS, Hall RC, Elia J, Osborne CP, Christin PA, Pannell J. 2015. Photosynthetic innovation broadens the niche within a single species. *Ecology Letters* **18:** 1021–1029.

Maldonado C, Molina CI, Zizka A, Persson C, Taylor CM, Albán J, Chilquillo E, Rønsted N, Antonelli A. 2015. Estimating species diversity and distribution in the era of big data: to what extent can we trust public databases? *Global Ecology and Biogeography* **24:** 973–984.

Marchioretto MS, Sfoggia Miotto ST, De Siqueira JC. 2010. O gênero *Pfaffia* Mart. (Amaranthaceae) no Brasil. *Hoehnea* **37:** 461–511.

Martins EP, Hansen TF. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *The American Naturalist* **149:** 646–667.

McCauley RA. 2004. New taxa and a new combination in the North American species of *Froelichia* (Amaranthaceae). *Systematic Botany* **29:** 64–76.

Menzel U, Lieth H. 2013. Halophyte Database Vers. 2.0 update. *Cashcrop Halophytes* **38.**

Miller, MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, 1–8.

Muhaidat R, Sage RF, Dengler NG. 2007. Diversity of Kranz anatomy and biochemistry in C_4 eudicots. *American Journal of Botany* **94:** 362–381.

Müller K, Borsch T. 2005. Phylogenetics of Amaranthaceae based on *matK/trnK* sequence data: evidence from parsimony, likelihood, and Bayesian analyses. *Annals of the Missouri Botanical Garden* **92:** 66–102.

Nixon KC. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* **15:** 407–414.

Ocampo G, Koteyeva NK, Voznesenskaya EV, Edwards GE, Sage TL, Sage RF, Columbus JT. 2013. Evolution of

leaf anatomy and photosynthetic pathways in Portulacaceae. *American Journal of Botany* **100:** 2388–2402.

Osborne CP, Freckleton RP. 2009. Ecological selection pressures for C$_4$ photosynthesis in the grasses. *Proceedings of the Royal Society B: Biological Sciences* **276:** 1753–1760.

Osborne CP, Sack L. 2012. Evolution of C$_4$ plants: a new hypothesis for an interaction of CO$_2$ and water relations mediated by plant hydraulics. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences* **367:** 583–600.

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401:** 877–884.

Palmer J. 1998. A taxonomic revision of *Gomphrena* (Amaranthaceae) in Australia. *Australian Systematic Botany* **11:** 73–151.

Paradis E, Claude J, Strimmer K. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics (Oxford, England)* **20:** 289–290.

Pau S, Edwards EJ, Still CJ. 2013. Improving our understanding of environmental controls on the distribution of C$_3$ and C$_4$ grasses. *Global Change Biology* **19:** 184–196.

Pearman PB, Guisan A, Broennimann O, Randin CF. 2008. Niche dynamics in space and time. *Trends in Ecology & Evolution* **23:** 149–158.

Pedersen TM. 2000. Studies in South American Amaranthaceae V. *Bonplandia* **10:** 83–112.

Peel MC, Finlayson BL, McMahon TA. 2007. Updated world map of the Koppen-Geiger climate classification. *Hydrology and Earth System Sciences* **4:** 439–473.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2015. *nlme: linear and nonlinear mixed effects models.* R Package Version 3.1, *122.* http://cran.r-project.org/package=nlme

R Core Team. 2014. *R: a language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing. http://www.r-project.org/

Rambaut A, Suchard M, Xie D, Drummond A. 2014. *Tracer v1. 6.* Institute of Evolutionary Biology, University of Edinburgh. Retrieved from website http://beast. bio. ed. ac. uk/Tracer [accessed 27 July 2014].

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61:** 539–542.

Sage RF. 2004. The evolution of C$_4$ photosynthesis. *New Phytologist* **161:** 341–370.

Sage RF. 2016. A portrait of the C$_4$ photosynthetic family on the 50th anniversary of its discovery: species number, evolutionary lineages, and Hall of Fame. *Journal of Experimental Botany* **67:** 4039–4056.

Sage RF, Christin PA, Edwards EJ. 2011. The C$_{(4)}$ plant lineages of planet Earth. *Journal of Experimental Botany* **62:** 3155–3169.

Sage RF, Kubien DS. 2007. The temperature response of C$_{(3)}$ and C$_{(4)}$ photosynthesis. *Plant, Cell & Environment* **30:** 1086–1106.

Sage RF, Kubien DS. 2011. C$_4$ photosynthesis and temperature. In: Raghavendra AS, Sage RF, eds. *C$_4$ photosynthesis and related CO$_2$ concentrating mechanisms.* Berlin: Springer Science, 161–195.

Sage RF, Sage TL, Kocacinar F. 2012. Photorespiration and the evolution of C$_4$ photosynthesis. *Annual Review of Plant Biology* **63:** 19–47.

Sage RF, Sage TL, Pearcy RW, Borsch T. 2007. The taxonomic distribution of C$_4$ photosynthesis in Amaranthaceae *sensu stricto. American Journal of Botany* **94:** 1992–2003.

Sage RF, Wedin DA, Li M. 1999. The biogeography of C$_4$ photosynthesis: patterns and controlling factors. *C$_4$ Plant Biology* 313–373.

Sage T, Sage R. 2002. Microsite characteristics of *Muhlenbergia richardsonis* (Trin.) Rydb., an alpine C$_4$ grass from the White Mountains, California. *Oecologia* **132:** 501–508.

Sánchez del Pino I, Borsch T, Motley TJ. 2009. *trnL-F* and *rpl16* sequence data and dense taxon sampling reveal monophyly of unilocular anthered Gomphrenoideae (Amaranthaceae) and an improved picture of their internal relationships. *Systematic Botany* **34:** 57–67.

Sánchez del Pino I, Motley TJ, Borsch T. 2012. Molecular phylogenetics of *Alternanthera* (Gomphrenoideae, Amaranthaceae): resolving a complex taxonomic history caused by different interpretations of morphological characters in a lineage with C$_4$ and C$_3$–C$_4$ intermediate species. *Botanical Journal of the Linnean Society* **169:** 493–517.

Sánchez-del Pino I, Motley TJ. 2010. Evolution of *Tidestromia* (Amaranthaceae) in the deserts of the southwestern United States and Mexico. *Taxon* **59:** 38–48.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England)* **30:** 1312–1313.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* **57:** 758–771.

Still CJ, Berry JA, Collatz GJ, DeFries RS. 2003. Global distribution of C$_3$ and C vegetation: carbon cycle implications. *Global Biogeochemical Cycles* **17:** 6-1–6-14.

Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17:** 1105–1109.

Trabucco A, Zomer RJ. 2009. *Global aridity index (global-aridity) and global potential evapo-transpiration (global-PET) geospatial database.* http://www.cgiar-csi.org

Wiens JJ. 2011. The niche, biogeography and species interactions. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **366:** 2336–2350.

Yesson C, Culham A. 2006. Phyloclimatic modeling: combining phylogenetics and bioclimatic modeling. *Systematic Biology* **55:** 785–802.

Zuloaga F, Morrone O, Belgrano M, eds. 2008. *Catálogo de las plantas vasclares del Cono Sur. Dicotyledoneae, Vol. 2.* St Louis: Monographs in Systematic Botany from the Missouri Botanical Garden.

APPENDICES

APPENDIX 1: GENBANK ACCESSION NUMBERS FOR SPECIES INCLUDED IN THE PHYLOGENETIC ANALYSIS (*TRNL-F/RPL16/MATK/RBCL*)

Ingroup: *Alternanthera altacruzensis*: EF688732/ EF688659/AM887483; *Alternanthera brasiliana*: JQ315137/ JQ403544/-/-; *Alternanthera caracasana*: EF688733/ EF688662/ AF542595/ HM849761; *Alternanthera chacoensis*: JQ315138/ JQ403550/-/-; *Alternanthera crucis*: EF688735/ EF688663/-/-; *Alternanthera elongata*: EF688736/ EF688664/-/-; *Alternanthera ficoidea*: EF688747/ EF688675/ FR775271/ FR775289; *Alternanthera filifolia*: JQ315140/ JQ403539/-/-; *Alternanthera flava*: EF688737/ EF688665/-/-; *Alternanthera flavescens*: EF688738/ EF688666/ AM887484/-; *Alternanthera flavicoma*: JQ315141/ JQ403540/-/-; *Alternanthera galapagensis*: EF688739/ EF688667/-/-; *Alternanthera geniculata*: JQ315142/ JQ403537/-/-; *Alternanthera halimifolia*: EF688740/ EF688668/-/-; *Alternanthera kurtzii*: JQ315143/ JQ403533/-/-; *Alternanthera laguroides*: EF688741/ EF688669/-/-; *Alternanthera lanceolata*: JQ315144/ JQ403545/-/-; *Alternanthera littoralis*: JQ315145/ JQ403548/-/-; *Alternanthera macbridei*: JQ315146/ JQ403552/-/-; *Alternanthera microphylla*: JQ315147/ JQ403535/ AM887485/-; *Alternanthera nesiotes*: JQ315148/ JQ403541/-/-; *Alternanthera obovata*: JQ315149/ JQ403549/-/-; *Alternanthera olivácea*: EF688744/ EF688672/-/-; *Alternanthera paronychioides*: JQ315150/ JQ403551/- /-; *Alternanthera philoxeroides*: EF688745/ EF688673/ GU135030/ GU135193; *Alternanthera porrigens*: JQ315151/ JQ403546/-/-; *Alternanthera pubiflora*: JQ315152/ JQ403547/-/-; *Alternanthera pungens*: EF688746/ EF688674/ AY514795/ HQ237458; *Alternanthera serpyllifolia*: JQ315153/ JQ403538/-/-; *Alternanthera sessilis*: -/-/FR775270/ FR775288; *Alternanthera snodgrassii*: JQ315154/ JQ403542/-/-; *Alternanthera vestita*: JQ315156/ JQ403543/-/-; *Blutaparon vermiculare*: EF688748/ EF688676/ AY514798/ AY270067; *Froelichia drummondii*: -/-/-/ FR775294; *Froelichia floridana*: EF688751/ EF688680/ AY514799/ AF132089; *Froelichia interrupta*: EF688752/ EF688681/-/-; *Froelichia tomentosa*: EF688753/ EF688682/-/-; *Gomphrena boliviana*: EF688754/ EF688683/-/-; *Gomphrena elegans*: EF688755/ EF688684/ -/AY270088; *Gomphrena ferruginea*: -/-/ AM887524/-; *Gomphrena fláccida*: EF688756/ EF688685/-/-; *Gomphrena fuscipellita*: -/-/ AM887525/-; *Gomphrena globosa*: EF688758/ EF688687/-/-; *Gomphrena graminea*: EF688757/ EF688686/-/-; *Gomphrena haageana*: -/-/ AY514800/ AY270089; *Gomphrena haenkeana*: EF688759/ EF688688/-/-; *Gomphrena macrocephala*: EF688760/ EF688689/-/-; *Gomphrena mandonii*: -/-/ AY514801/-; *Gomphrena nítida*: EF688761/ EF688690/-/-; *Gomphrena pulchella*: -/-/AY514802/-; *Gomphrena serrata*: EF688763/ EF688692/-/ AY270090; *Gomphrena vaga*: EF688766/ EF688695/-/-; *Gossypianthus lanuginosus*: EF688762/ EF688691/-/-; *Guilleminea densa*: EF688764/ EF688693/ AY514803/ AY270091; *Guilleminea gracilis*: EF688765/ EF688694/-/-; *Hebanthe grandiflora*: EF676098/ EF688696/ FR870370/-; *Hebanthe occidentalis*: EF688767/ EF688697/ AY514821/ AY270097; *Hebanthe eriantha*: EF688768/ EF688698/- /-; *Irenella chrysotricha*: EF688776/EF688706/-/-; *Iresine alternifolia*: EF688770/EF688700/AM887490/-; *Iresine arbuscula*: EF688769/EF688699/-/-; *Iresine cassiniiformis*: - /-/AM887489/-; *Iresine diffusa*: EF688771/ EF688701/-/JQ590121; *Iresine grandis*: EF688772/ EF688702/-/-; *Iresine heterophylla*: EF688773/ EF688703/-/-; *Iresine leptoclada*: EF688774/ EF688704/-/-; *Iresine palmeri*: EF688775/EF688705/ AY514804/AY270101; *Lithophila muscoides*: EF688780/EF688710/-/-; *Pedersenia argentata*: EF688781/EF688711/-/FR775298; *Pedersenia cardenasii*: EF688782/EF688712/AM887491/-; *Pedersenia volubilis*: -/-/FR870371/JQ693467; *Pfaffia fruticulosa*: -/-/AM887492/-; *Pfaffia glomerata*: -/-/-/ JQ693468; *Pfaffia iresinoides*: EF676099/EF688715/-/-; *Pfaffia jubata*: EF688785/EF688716/-/-; *Pfaffia tuberosa*: EF688786/EF688717/-/-; *Pseudoplantago friesii*: EF688784/EF688714/AY514820/AY270120; *Tidestromia carnosa*: EF688789/ EF688720/-/-; *Tidestromia eliassoniana*: EF688790/EF688721/-/-; *Tidestromia lanuginosa*: EF688791/EF688722/-/-; *Tidestromia oblongifolia*: EF688793/EF688724/-/-; *Tidestromia rhizomatosa*: EF688792/EF688723/-/-; *Tidestromia suffruticosa*: EF688794/EF688730/-/-; *Tidestromia tenella*: EF688795/EF688725/-/-; *Tidestromia valdesiana*: EF688796/EF688726/-/-; *Xerosiphon angustiflorus*: EF688798/EF688728/-/-; *Xerosiphon aphyllus*: EF676100/EF688729/ AM887523/JQ693470.

APPENDIX 2: TAXON SAMPLING AND VOUCHER INFORMATION FOR TAXA USED IN THIS STUDY

Table shows species name, voucher information including: locality and specimen (*collector*; *collection number*; and herbarium acronym where the specimens are housed) and GenBank accession numbers.

Taxon	Locality	Voucher specimen	GenBank accession
Alternanthera cinerella Suess.	Argentina. Salta. Cafayate. Route between Cafayate and Tolombón.	*Zuloaga, F. O. 11858.* (SI)	MF039679
Gomphrena celosioides Mart.	Argentina. Jujuy. San Pedro. National route 34, 3 km North from San Pedro.	*Zuloaga, F. O. 11472.* (SI)	MF039680
Gomphrena cladotrichoides Suess.	Argentina. San Juan. Iglesia. National route 150, from Rodeo to Jáchal, "Cuesta del Viento".	*Zuloaga, F. O. 12804.* (SI)	MF039681
Gomphrena martiana Gillies ex Moq.	Argentina. Jujuy. Santa Bárbara. Route from Palma Sola to Ojo de Agua, 3 km from Palma Sola. Chaco Serrano.	*Zuloaga, F. O. 11530.* (SI)	MF039682
Gomphrena mendocina (Phil.) R.E. Fr.	Argentina. San Juan. Valle Fértil. Provincial route 510, from Ischigualasto to Valle Fértil.	*Zuloaga, F. O. 12113.* (SI)	MF039683
Gomphrena meyeniana Walp.	Argentina. Jujuy. Manuel Belgrano. Ovejería. In southern hillside.	*Zanotti, C. A. 183.* (SI)	MF039684
Gomphrena perennis L.	Argentina. Buenos Aires. Tandil. Sierra del Tigre.	*Zuloaga, F. O. 11362.* (SI)	MF039685
Gomphrena pumila Gillies ex Moquin	Argentina. San Juan. Ullum. Provincial route 149. Portal Los Colorados.	*Zuloaga, F. O. 12744.* (SI)	MF039686
Gomphrena radiata Pedersen	Argentina. Catamarca. Andalgalá. Mina Capillitas, road to Mina Santa Rita.	*Zuloaga, F. O. 14564.* (SI)	MF039687
Gomphrena umbellata Remy	Argentina. Jujuy. Santa Catalina. Riverside.	*Zuloaga, F. O. 13112.* (SI)	MF039688

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Results obtained from the PGLS statistics for the variable 'precipitation seasonality' (PS). For each topology analysed we show: mean ± SE of C₃ and C₄ species, the best fitting model selected by the AIC and *P* values obtained. MPT, maximum parsimony tree; BAYESIAN, topology from the Bayesian inference; MLT, maximum likelihood tree.