Supplementary Material for:

“Key changes in gene expression identified for different stages of C_4 evolution in *Alloteropsis semialata*”

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This supplementary material contains eight tables and one figure:

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Figure S1: Phylogenetic patterns of changes in gene expression in (A) *Alloteropsis angusta,* and (B) *Alloteropsis cimicina*.

Table S1: List of enzymes considered as core C_4 enzymes.

Protein	**Enzyme**	**Gene ID**	**Number of genes in reference genome**
ALA-AT	alanine aminotransferase	*alaat*	3
ASP-AT	aspartate aminotransferase	*aspat*	4
NAD-MDH	NAD malate dehydrogenase	*nadmdh*	11
NAD-ME	NAD malic enzyme	*nadme*	2
NADP-MDH	NADP malate dehydrogenase	*nadpmdh*	6
NADP-ME	NADP malic enzyme	*nadpme*	3
PCK	phosphoenolpyruvate carboxykinase	*pck*	2
PEPC	phosphoenolpyruvate carboxylase	*ppc*	8
PPDK	pyruvate orthophosphate dikinase	*ppdk*	2
CA	carbonic anhydrase	*ßca*	5

Table S2: Information for populations sampled in triplicates.

Species	Photo.	Population	Country	Lat/long	Sampling
Entolasia marginata	C_3	EMAR1	Australia	-26.57,150.55	3 x clones of a single plant grown from seed
Alloteropsis cimicina	C_4	ACIM2	Madagascar	-	3 x different plants grown from seed
Alloteropsis angusta	C_4	AANG1	Uganda	0.34,31.89	1 x plant collected as a live cuttings + 2 x clones of a single plant collected as a live cutting.
Alloteropsis semialata	C_3	ZIM2	Zimbabwe	-18.42,32.77	3 x different plants collected as live cuttings
		RSA6[1]	South Africa	-33.32,26.53	3 x different plants collected as live cuttings
	C_3+C_4	TAN1602[1]	Tanzania	-8.35,31.28	3 x different plants collected as live cuttings
		ZAM3[1]	Zambia	-10.23,29.83	3 x different plants collected as live cuttings
	C_4	CMR1[1]	Cameroon	5.93,10.62	3 x different plants collected as live cuttings
		TAN4[1]	Tanzania	-9.04,32.48	3 x different plants collected as live cuttings
		PHI1[1]	Philippines	15.94,121.01	3 x different plants collected as live cuttings

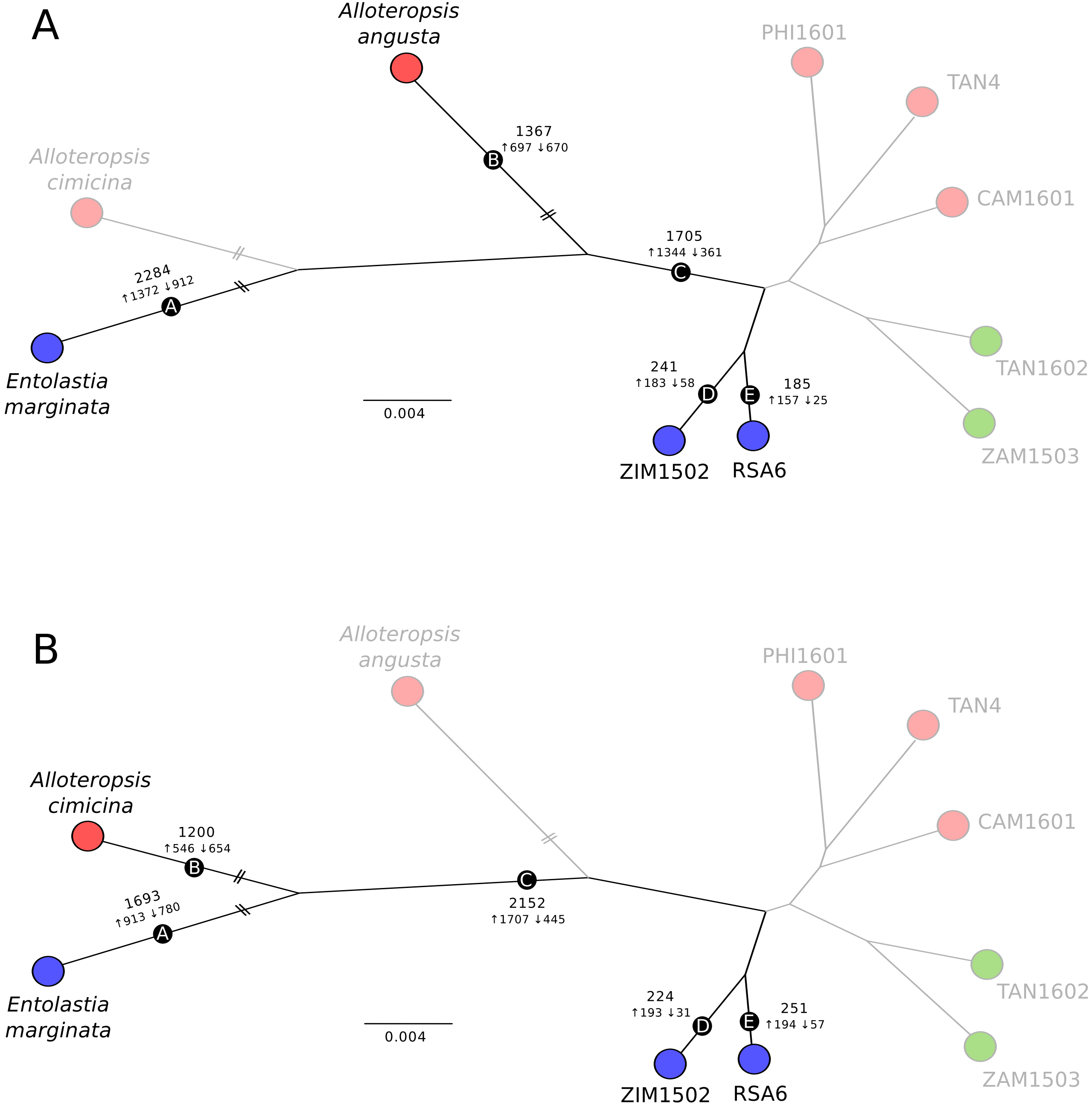
[1] Data retrieved from Dunning *et al.* (2019)

Table S3: RNA-Seq data and mapping statistics for ten populations with triplicates.

Pop.	Sample	raw PE reads	Gbp	Clean PE reads	Percentage of clean reads mapped
ACIM2	ACIM2-1	5967276	1.29	5810460	43.26
	ACIM2-2	3501357	0.76	3419398	41.14
	ACIM2-3	3102005	0.67	3033994	33.47
EMAR1	EMAR1-2	3928822	0.85	3849825	37.56
	EMAR1-4A	3898055	0.84	3810471	34.58
	EMAR1-4B	2500722	0.54	2439927	34.74
AANG1	AANG1-6A	4618386	1.00	4332270	34.08
	AANG1-6B	5997583	1.30	5836626	32.09
	AANG1-6C	6009876	1.30	5866831	46.05
ZIM2	ZIM2-1	2815050	0.61	2622817	57.19
	ZIM2-5	3357287	0.73	3236198	57.55
	ZIM2-10	5308033	1.15	4989635	64.43
RSA6	RSA6-1	5284768	1.14	5193728	60.35
	RSA6-7	4397850	0.95	4299225	68.02
	RSA6-9	8129836	1.76	7964933	69.32
TAN1602	TAN1602-1	10558384	2.28	10304872	59.92
	TAN1602-2	8101223	1.75	7890990	64.87
	TAN1602-3	2623196	0.57	2494198	54.53
ZAM3	ZAM3-2	11178860	2.41	10897999	64.05
	ZAM3-4	5146745	1.11	4992771	62.46
	ZAM3-8	6118516	1.32	5853276	61.77
CMR1	CMR1-2	12525719	2.71	12273903	65.09
	CMR1-7	4385675	0.95	4282371	57.92
	CMR1-10	2724267	0.59	2652363	65.58
TAN4	TAN4-1	6581391	1.42	6393229	60.82
	TAN4-3	7547915	1.63	7335642	62.60
	TAN4-8	6089921	1.32	5932271	60.97
PHI1	PHI1-1	4911514	1.06	4705864	68.31
	PHI1-13	4643128	1.00	4441009	68.07
	PHI1-17	5167456	1.12	5003163	68.31

Captions of supplementary figures

Figure S1: Phylogenetic patterns of changes in gene expression in (A) *Alloteropsis angusta* and (B) *Alloteropsis cimicina*. For each branch of the unrooted phylogeny from Fig. 1, the number of differentially expressed genes is indicated, with numbers next to arrows indicating those that are consistently up- or down-regulated as you move along the tree from the outgroup *Entolasia marginata*. Each population has three biological replicates, and colors indicate the photosynthetic type (blue = C_3; green = C_3+C_4; red = C_4). Scale indicates number of nucleotide substitutions per site, truncated branches are highlighted by two bars. For each tree, taxa excluded from the analyses are greyed.


A
Alloteropsis angusta
PHI1601
TAN4
CAM1601
Alloteropsis cimicina
1367
↑697 ↓670
B
1705
↑1344 ↓361
C
2284
↑1372 ↓912
A
Entolastia marginata
0.004
241
↑183 ↓58
D
E
185
↑157 ↓25
TAN1602
ZAM1503
ZIM1502
RSA6
B
Alloteropsis angusta
PHI1601
TAN4
CAM1601
Alloteropsis cimicina
1200
↑546 ↓654
B
1693
↑913 ↓780
A
C
2152
↑1707 ↓445
Entolastia marginata
0.004
224
↑193 ↓31
D
E
251
↑194 ↓57
TAN1602
ZAM1503
ZIM1502
RSA6