

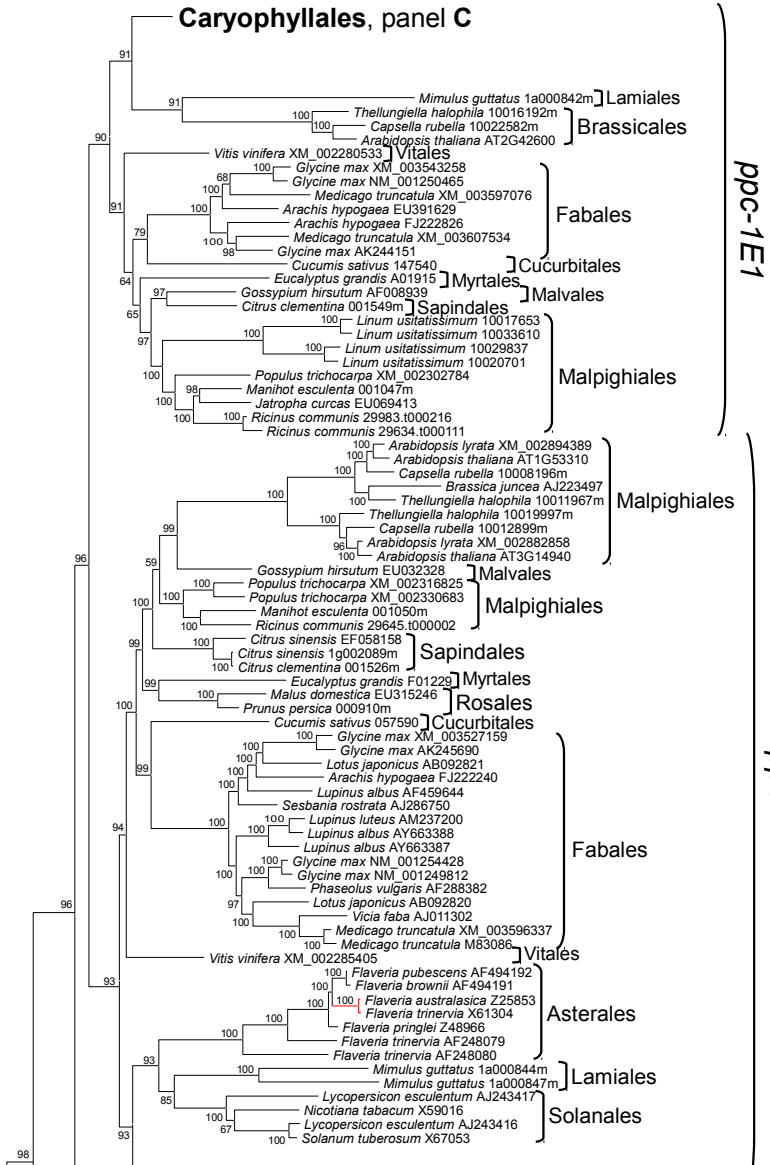
Supplementary Material for:

## Shared origins of a key enzyme during the evolution of C<sub>4</sub> and CAM metabolism

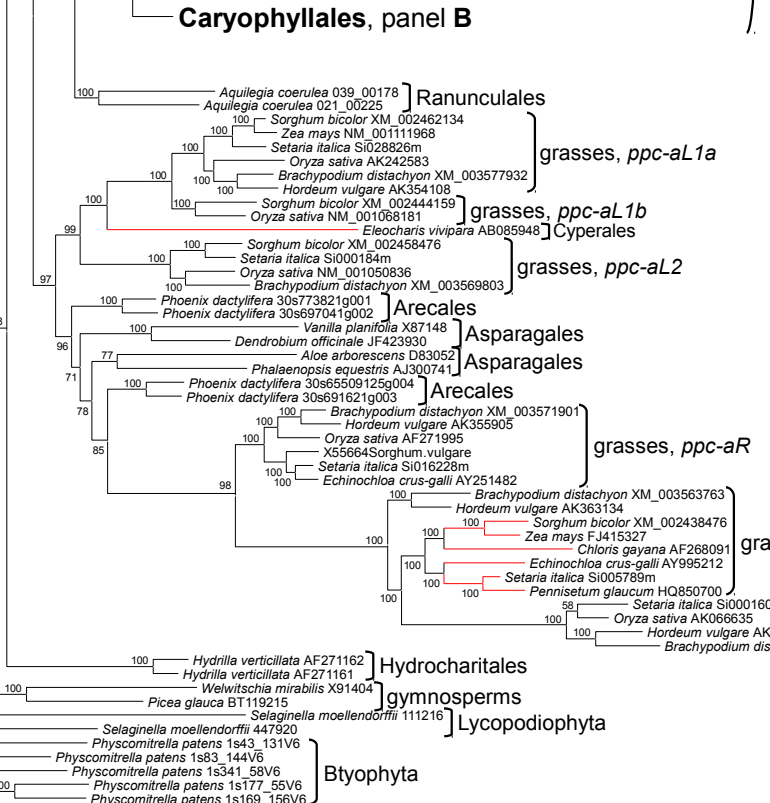
Pascal-Antoine Christin, Monica Arakaki, Colin P. Osborne, Andrea Bräutigam, Rowan F. Sage, Julian M. Hibberd, Steven Kelly, Sarah Covshoff, Gane Ka-Shu Wong, Lillian Hancock, Erika J. Edwards

A

Caryophyllales, panel C

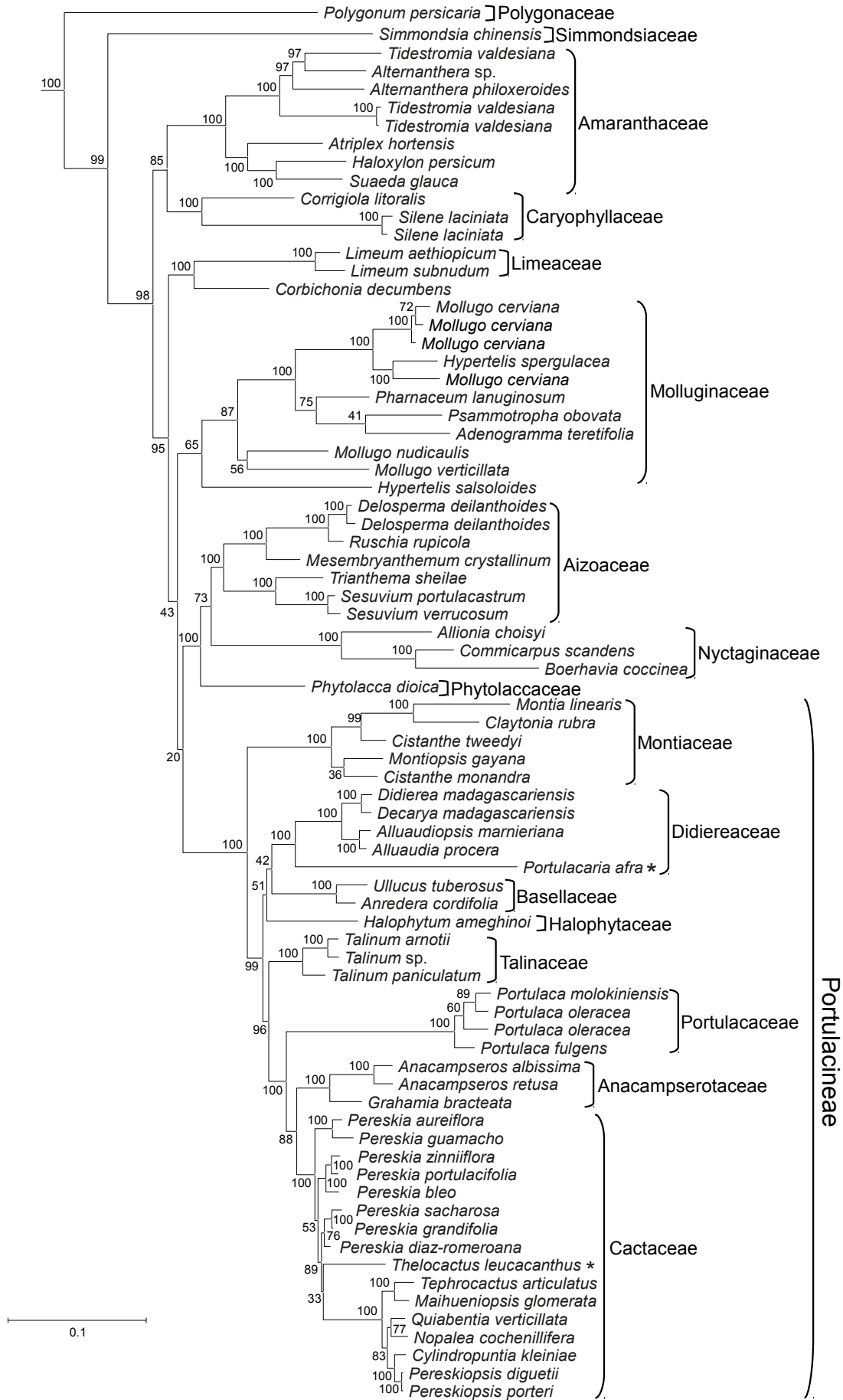


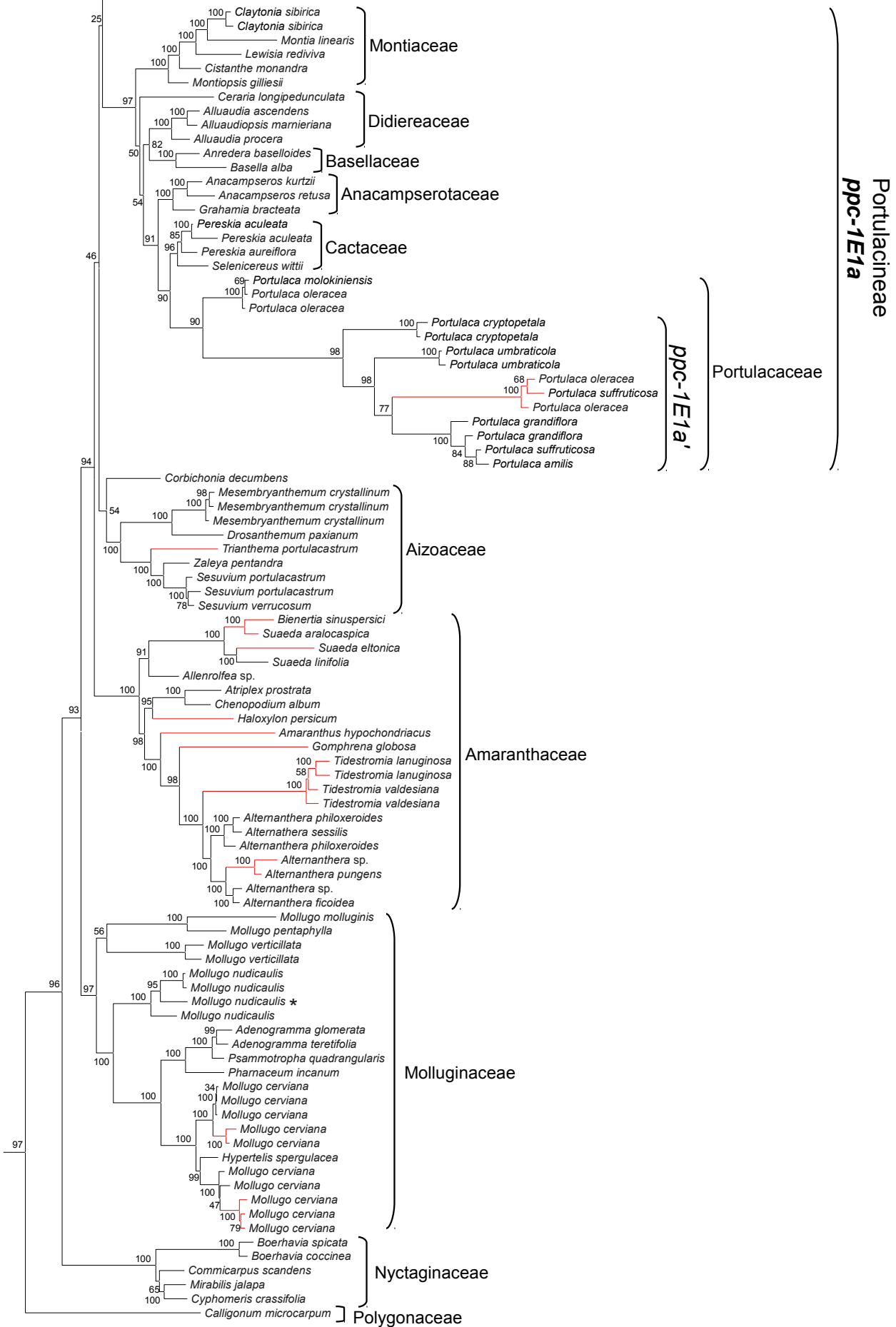
Caryophyllales, panel B

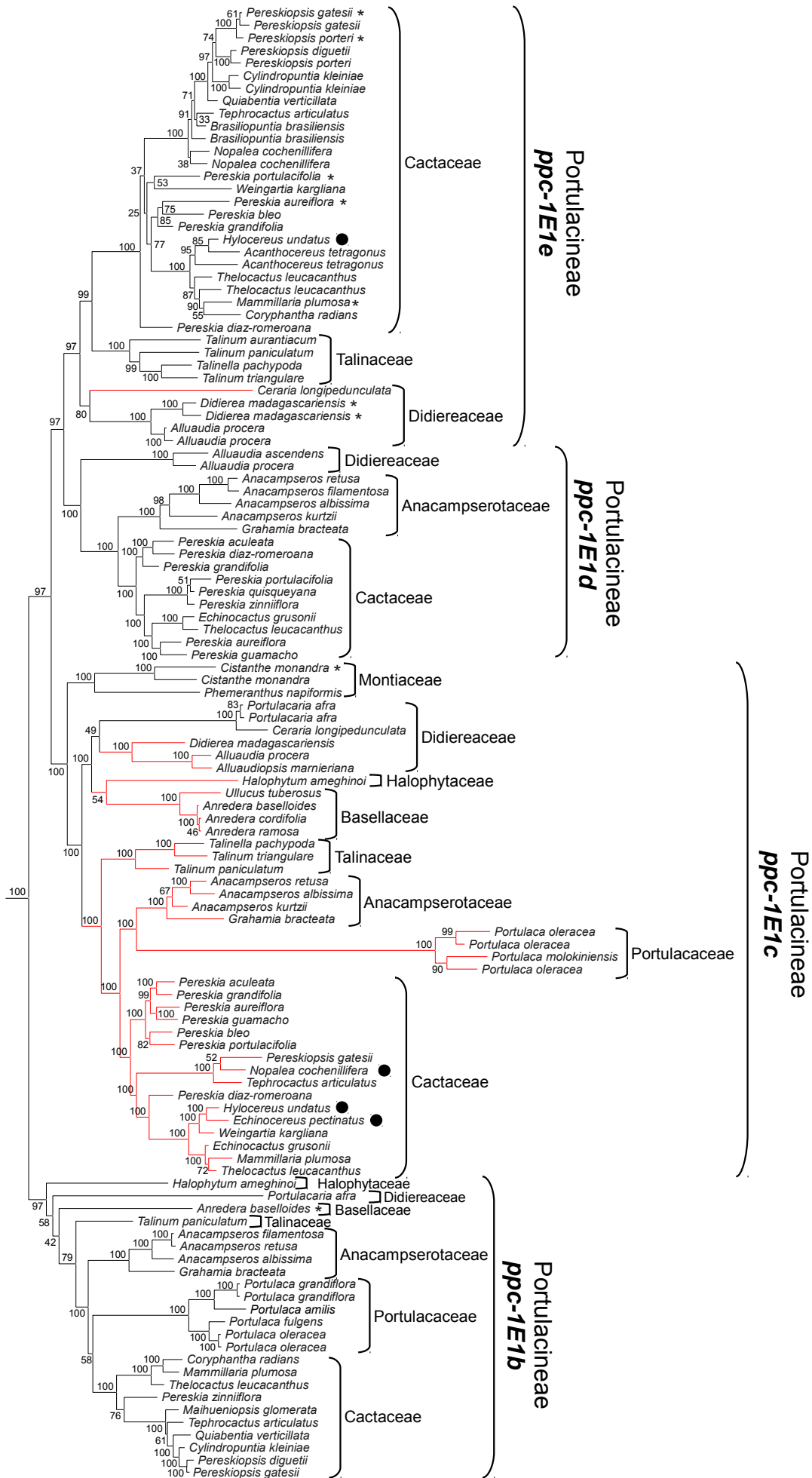


0.1

monocots

**B**

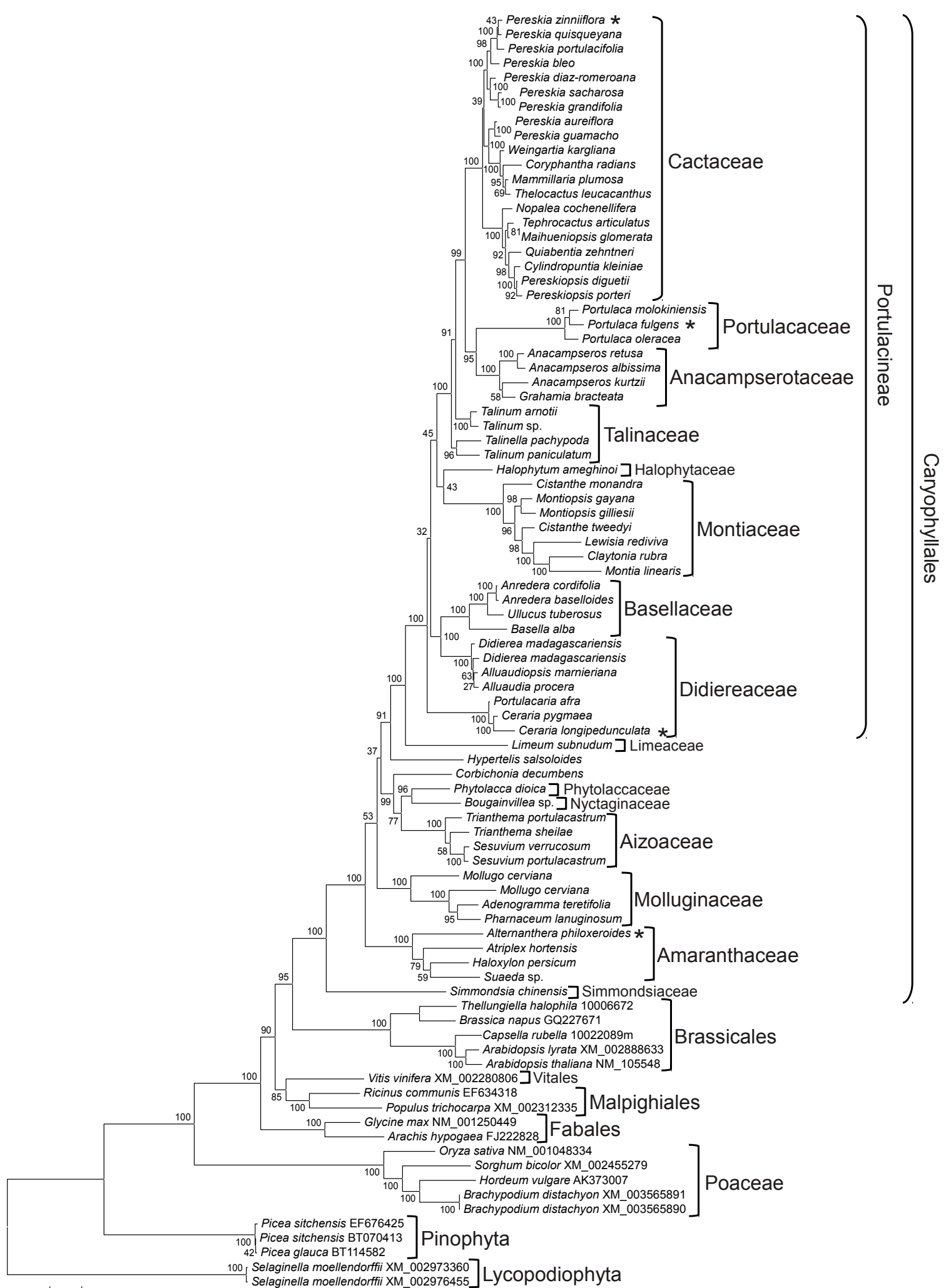
**C****panel D**

**D**

0.1

**Supplementary Figure 1: Phylogenetic relationships among *ppc-1* genes.**

This phylogenetic tree was obtained through Bayesian inference on nucleotide sequences. Names of taxonomic groups and gene lineages are indicated on the right. Branches in lineages presenting a Ser780 are highlighted in red. Bayesian support values are indicated near branches. Asterisks indicate putative pseudogenes with one or several stop codons in the coding sequence. Black circles indicate sequences that were isolated from cacti cDNA. **A** Complete phylogenetic tree, **B** *ppc-1E2* of Caryophyllales, **C** and **D** *ppc-1E1* of Caryophyllales.

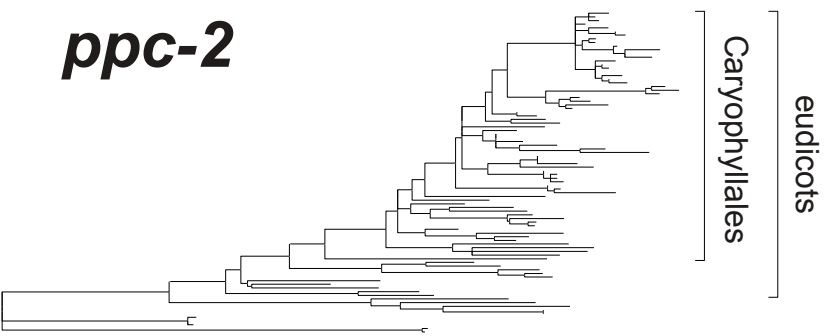
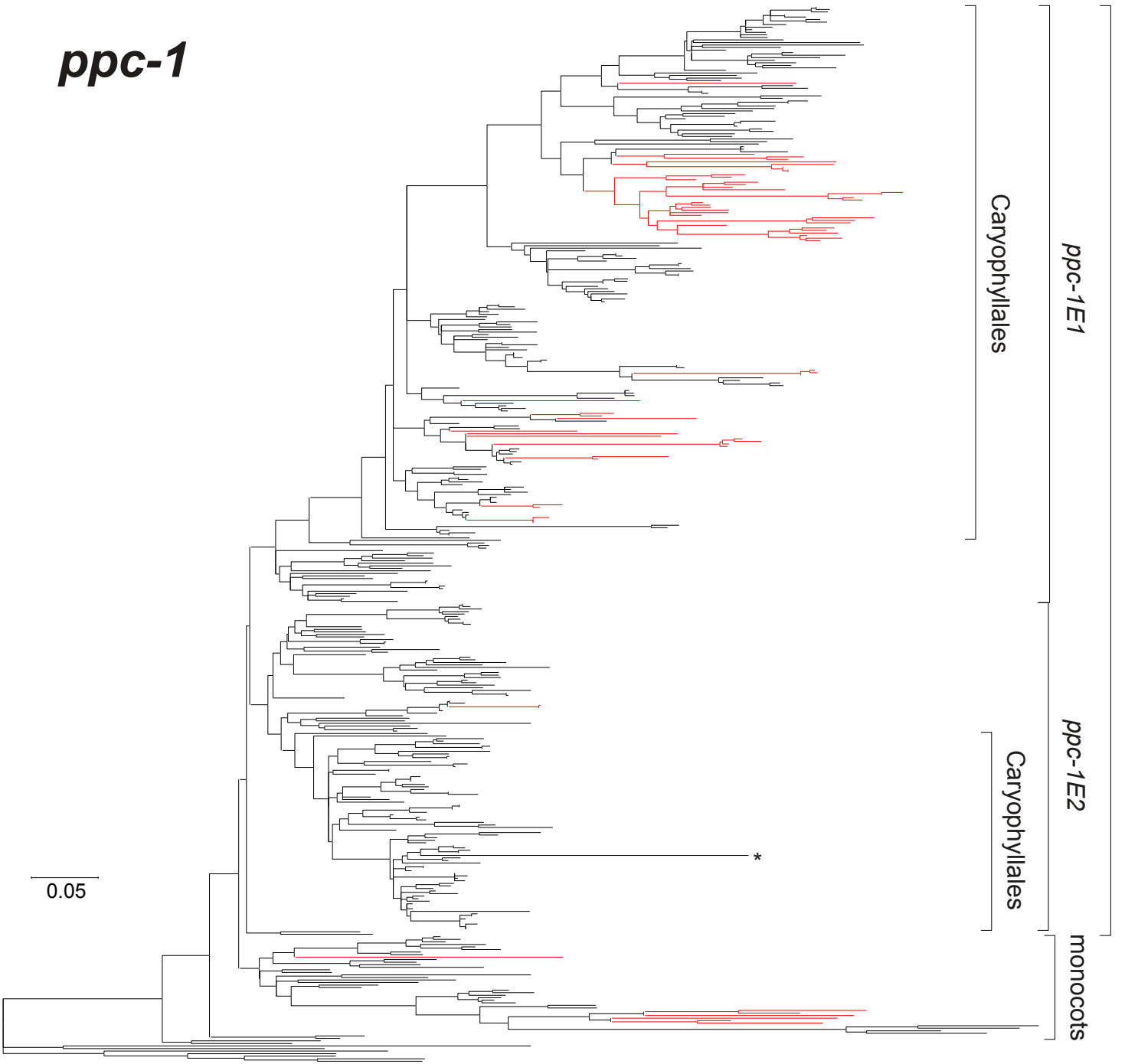


0.05

**Supplementary Figure 2: Phylogenetic relationships among *ppc-2* genes.**

This phylogenetic tree was obtained through Bayesian inference on nucleotide sequences. Names of taxonomic groups are indicated on the right. Bayesian support values are indicated near branches.





**Supplementary Figure 3: Amino acid changes on genes encoding PEPC.**

The topology was inferred on nucleotide sequences, but branch lengths were estimated based on amino acid sequences. The branch lengths inferred on nucleotide sequences, together with all species names and support values, are available in Figures S1 and S2. The names of the main groups are indicated on the right. Groups of genes containing a Ser780 are highlighted by red branches. The asterisk highlights a pseudogene with multiple stop codons.

**Supplementary Table 1: Sample of Caryophyllales (excluding Portulacineae) used for analyses of PEPC encoding genes.**

Species	Voucher	<i>ppc-1E1</i>	<i>ppc-1E2</i>	<i>ppc-2</i>
<b>Simmondsiaceae</b>				
<i>Simmondsia chinensis</i> (Link) C.K. Schneid.	Clement 235 (YU)	-	KJ161696	KJ161762
<b>Polygonaceae</b>				
<i>Calligonum microcarpum</i> I.G. Borshch.	Sage 0509-17	KJ161675	-	-
<i>Polygonum persicaria</i> L.	-	-	KJ161697	-
<b>Caryophyllaceae</b>				
<i>Corrigiola litoralis</i> L.	FR-0082024 (FR)	-	FN868276	-
<i>Silene laciniata</i> Cav.	Clement 236 (Y)	-	KJ161698, KJ161699	-
<b>Amaranthaceae</b>				
<i>Allenrolfea</i> sp.	Christin s.n. (BRU)	KJ161676	-	-
<i>Alternanthera ficoidea</i> (L.) P. Beauv.	-	AY950666	-	-
<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	Sage 0509-13	KJ161677, KJ161678	KJ161700	KJ161763
<i>Alternanthera pungens</i> Kunth	-	AY950665	-	-
<i>Alternanthera sessilis</i> (L.) R. Br.	-	AY950667	-	-
<i>Alternanthera</i> sp.	Sage 0509-16	KJ161679	KJ161701	-
<i>Amaranthus hypochondriacus</i> L.	-	Z68125	-	-
<i>Atriplex hortensis</i> L.	-	-	KJ161702	KJ161764
<i>Atriplex prostrata</i> Boucher ex DC.	-	KJ161680	-	-
<i>Bieneria sinuspersici</i> Akhani	-	DQ538352	-	-
<i>Chenopodium album</i> L.	-	KJ161681	-	-
<i>Gomphrena globosa</i> L.	-	KJ161682	-	-
<i>Haloxylon persicum</i> Bunge ex Boiss. & Buhse	Sage 0509-H8	KJ161683	KJ161703	KJ161765
<i>Suaeda aralocaspica</i> Freitag & Schutze	-	DQ538353	-	-
<i>Suaeda eltonica</i> Iljin	-	DQ538354	-	-
<i>Suaeda glauca</i> (Bunge) Bunge	-	-	EU629170	-
<i>Suaeda linifolia</i> Pall.	-	DQ538355	-	-
<i>Suaeda</i> sp.	Christin s.n. (BRU)	-	-	KJ161766
<i>Tidestromia lanuginosa</i> (Nutt.) Standl.	Higgins et al. 21768 (NY)	KJ161684, KJ161685	-	-
<i>Tidestromia valdesiana</i> Sánchez-del Pino & Flores Oliv.	Sanchez del Pino (NY)	KJ161686, KJ161687	KJ161704, KJ161705, KJ161706	-
<b>Aizoaceae</b>				
<i>Delosperma deilanthoides</i> S.A. Hammer	998369/0 (ZSS)	-	KJ161707, KJ161708	-
<i>Drosanthemum paxianum</i> Schwantes	-	Y17844	-	-
<i>Mesembryanthemum crystallinum</i> L.	-	X13660, AJ312642, AJ312643	-	-
<i>Ruschia rupicola</i> (Engl.) Schwantes	Brack 1508 (ZSS)	-	KJ161709	-
<i>Sesuvium portulacastrum</i> (L.) L.	-	KJ161688	KJ161710	KJ161767
<i>Sesuvium verrucosum</i> Raf.	Sage 0509-06	-	KJ161711	KJ161768
<i>Trianthema portulacastrum</i> L.	Christin 163 (BRU)	KJ161689	-	KJ161769
<i>Trianthema sheilae</i> A.G.Mill. & J.A.Nyberg	-	-	KJ161712	KJ161770
<i>Zaleya pentandra</i> (L.) C. Jeffrey	-	KJ161690	-	-

**Incertae sedis**

<i>Corbichonia decumbens</i> (Forssk.) Exell	-	FN868270	FN868271	KJ161771
<i>Hypertelis salsoloides</i> (Burch.) Adamson	Ogburn 143 (BRU)	-	KJ161713	KJ161772

**Nyctaginaceae**

<i>Allionia choisyi</i> Standl.	Douglas 2187 (DUKE)	-	KJ161714	-
<i>Boerhavia coccinea</i> Mill.	Douglas 2056 (NY)	KJ161691	KJ161715	-
<i>Boerhavia spicata</i> Choisy	Douglas 2171 (NY)	KJ161692	-	-
<i>Bougainvillea</i> sp.	Cultivated, Botanical Garden Zürich	-	-	KJ161773
<i>Commicarpus scandens</i> (L.) Standl.	-	KJ161693	KJ161716	-
<i>Cyphomeris crassifolia</i> (Standl.) Standl.	Douglas 2203	KJ161694	-	-
<i>Mirabilis jalapa</i> L.	-	KJ161695	-	-

**Phytolaccaceae**

<i>Phytolacca dioica</i> L.	871040/0 (ZSS)	-	KJ161717	KJ161774
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**Limeaceae**

<i>Limeum aethiopicum</i> Burm.f.		-	FN868277	-
<i>Limeum subnudum</i> Friedrich	Ogburn 154 (BRU)	-	KJ161718	KJ161775

**Molluginaceae**

<i>Adenogramma glomerata</i> Druce	Ogburn 142 (BRU)	FN868266	-	-
<i>Adenogramma teretifolia</i> Adamson	Ogburn 156 (BRU)	FN868267	KJ161719	KJ161776
<i>Hypertelis spergulacea</i> E. Mey. ex Fenzl	Giess et al. 5366 (K)	FN868290	FN868289	-
<i>Mollugo cerviana</i> (L.) Ser.	Atwood & Welsh 10684 (NY)	FN868297, FN868296	KJ161720	KJ161777
<i>Mollugo cerviana</i> (L.) Ser.	Shekhawat 2008	FN868294, FN868295	-	-
<i>Mollugo cerviana</i> (L.) Ser.	Thulin et al. 11211 (UPS)	FN868298, FN868299	-	-
<i>Mollugo cerviana</i> (L.) Ser.	Potgieter 225 (K)	FN868301	FN868287	-
<i>Mollugo cerviana</i> (L.) Ser.	J. Sánchez Sánchez s.n. (G)	FN868300	FN868288	KJ161778
<i>Mollugo cerviana</i> (L.) Ser.	Lazarides & Palmer 243 (CANB)	FN868291	FN868285	-
<i>Mollugo cerviana</i> (L.) Ser.	G. Leach 2008 (CANB)	FN868292	-	-
<i>Mollugo cerviana</i> (L.) Ser.	Smyth 213 (CANB)	FN868293	-	-
<i>Mollugo molluginis</i> (F.Muell.) Druce	Telford 11746 (CANB)	FN868275	-	-
<i>Mollugo nudicaulis</i> Lam.	Liogier 16585 (NY)	FN868284	-	-
<i>Mollugo nudicaulis</i> Lam.	Devi Sept 1961 (CANB)	FN868281, FN868280	FN868282	-
<i>Mollugo nudicaulis</i> Lam.	Thulin & Bashir-Mohamed 6759 (UPS)	FN868283	-	-
<i>Mollugo pentaphylla</i> L.	Nee 42741 (NY)	FN868272	-	-
<i>Mollugo verticillata</i> L.	Sage & Sage 8-2007 (TRT)	FN868273	KJ161721	-
<i>Mollugo verticillata</i> L.	M. Nee 37372 (G)	FN868274	-	-
<i>Pharnaceum incanum</i> L.	Ogburn 148 (BRU)	FN868268	-	-
<i>Pharnaceum lanuginosum</i> J.C. Manning & Goldblatt	Ogburn 161 (BRU)	-	KJ161722	KJ161779
<i>Psammotropha obovata</i> Adamson	Hilliard & Burtt 7095 (K)	-	FN868278	-
<i>Psammotropha quadrangularis</i> (L. f.) Fenzl	Ogburn 160 (BRU)	FN868269	-	-

**Supplementary Table 2: List of Portulacineae genes encoding PEPC analysed**

Species	Voucher	<i>ppc-1E1a</i>	<i>ppc-1E1b</i>	<i>ppc-1E1c</i>	<i>ppc-1E1d</i>	<i>ppc-1E1e</i>	<i>ppc-1E2</i>	<i>ppc-2</i>
<b>Montiaceae</b>								
<i>Cistanthe monandra</i> (Nutt.) Hershkovitz	Ogburn 137 (BRU)	KJ161552	-	KJ161591, KJ161592	-	-	KJ161723	KJ161780
<i>Cistanthe tweedyi</i> (A. Gray) Hersk.	Ogburn 182 (BRU)	-	-	-	-	-	KJ161724	KJ161781
<i>Claytonia rubra</i> (Howell) Tidestr.	Ogburn 183 (BRU)	-	-	-	-	-	KJ161725	KJ161782
<i>Lewisia rediviva</i> Pursh	Ogburn 185 (BRU)	KJ161553	-	-	-	-	-	KJ161783
<i>Montia linearis</i> (Douglas ex Hook.) Greene	Ogburn 287 (BRU)	KJ161554	-	-	-	-	KJ161726	KJ161784
<i>Claytonia sibirica</i> (L.) Howell	Ogburn n.a. (BRU) - Brown greenhouse	KJ161555, KJ161556	-	-	-	-	-	-
<i>Montiopsis gayana</i> (Barnéoud) D.I. Ford	Ogburn 268 (BRU)	-	-	-	-	-	KJ161727	KJ161785
<i>Montiopsis gilliesii</i> (Hook. & Arn.) D.I. Ford	Ogburn 166 (BRU)	KJ161557	-	-	-	-	-	KJ161786
<i>Phemeranthus napiformis</i> (DC.) Raf.	Ogburn 226 (BRU)	-	-	KJ161593	-	-	-	-
<b>Halophytaceae</b>								
<i>Halophytum ameghinoi</i> (Speg.) Speg.	Edwards 255 (BRU)	-	KJ161569	KJ161594	-	-	KJ161728	KJ161787
<b>Basellaceae</b>								
<i>Anredera baselloides</i> (Kunth) Baill.	Ogburn 256 (BRU)	KJ161558	KJ161570	KJ161595	-	-	-	KJ161788
<i>Anredera cordifolia</i> (Ten.) Steenis	913635/b (ZSS) - Hofstätter s/n, Madagascar	-	-	KJ161596	-	-	KJ161729	KJ161789
<i>Anredera ramosa</i> (Moq.) Eliasson	981287/0 (ZSS) - Mostul 1254, Mexico, Veracruz	-	-	KJ161597	-	-	-	-
<i>Basella alba</i> L.	Endara 1638 (FLAS) - Kanapaha Bot. Garden, Gainesville, FL	KJ161559	-	-	-	-	-	KJ161790
<i>Ullucus tuberosus</i> Caldas	101123/0 (ZSS) - Bot. Garden Bonn	-	-	KJ161598	-	-	KJ161730	KJ161791
<b>Didiereaceae</b>								
<i>Alluaudia ascendens</i> (Drake) Drake	823558/0 (ZSS) - received from Hauenstein, cultivated	KJ161560	-	-	KJ161625	-	-	-
<i>Alluaudia procera</i> (Drake) Drake	995323/0 (ZSS) - cultivated	KJ161561	-	KJ161599	KJ161626	KJ161642, KJ161643	KJ161731	KJ161792
<i>Alluaudiopsis marnieriana</i> Rauh	812213/0 (ZSS) - Bot. Garden Heidelberg	KJ161562	-	KJ161600	-	-	KJ161732	KJ161793
<i>Ceraria longipedunculata</i> Merxm. & Podlech	901396/0 (ZSS) - Lavranos & Bleck s/n, Namibia	KJ161563	-	KJ161601	-	KJ161644	-	KJ161794
<i>Ceraria pygmaea</i> (Pillans) G.D.Rowley	Lavranos 22309 (ZSS)	-	-	-	-	-	-	KJ161795
<i>Decarya madagascariensis</i> Choux	Rauh 7592 (ZSS)	-	-	-	-	-	KJ161733	KJ161796
<i>Didierea madagascariensis</i> Baill.	931246/0 (ZSS) - Rauh M1803, Madagascar	-	-	KJ161602	-	KJ161645, KJ161646	KJ161734	KJ161797
<i>Portulacaria afra</i> Jacq.	995326/0 (ZSS) - cultivated	-	KJ161571	KJ161603, KJ161604	-	-	KJ161735	KJ161798
<b>Talinaceae</b>								
<i>Talinum arnotii</i> Hook. f.	932096 (ZSS)	-	-	-	-	-	KJ161736	KJ161799
<i>Talinum aurantiacum</i> Engelm.	Ogburn 267 (BRU)	-	-	-	-	KJ161647	-	-
<i>Talinum paniculatum</i> (Jacq.) Gaertn.	941354/0 (ZSS) - Leuenberger & Egli 4364, Argentina, Catamarca	-	KJ161572	KJ161605	-	KJ161648	KJ161737	KJ161800
<i>Talinum</i> sp.	961663/a (ZSS)	-	-	-	-	-	KJ161738	KJ161802
<i>Talinum triangulare</i> (Jacq.) Willd.	Ogburn n.a. (BRU) - Brown greenhouse	-	-	KJ161606	-	-	-	-
<i>Talinella pachypoda</i> Egli	952546/0 (ZSS) - Hoffman 347, Madagascar	-	-	KJ161607	-	KJ161649	-	KJ161801
<b>Anacampserotaceae</b>								
<i>Anacampseros albissima</i> Marloth	Ogburn 255 (BRU)	-	KJ161573	KJ161608	KJ161627	-	KJ161739	KJ161803
<i>Anacampseros filamentosa</i> Sims	Ogburn 36 (MO)	-	KJ161574	-	KJ161628	-	-	-
<i>Anacampseros kurtzii</i> Bacigalupo	Spriggs n.a. (BRU) - Brown greenhouse	KJ161564	-	KJ161609	KJ161629	-	-	KJ161804

<i>Anacampseros retusa</i> Poelln.	803518/a (ZSS) - Cape Province	KJ161565	KJ161575	KJ161610	KJ161630	-	KJ161740	KJ161805
<i>Grahamia bracteata</i> Gillies ex Hook.	Ogburn n.a. (BRU) - Brown greenhouse	KJ161566	KJ161576	KJ161611	KJ161631	-	KJ161741	KJ161806
<b>Portulacaceae</b>								
<i>Portulaca fulgens</i> Griseb.	995813/0 (ZSS) - Leuenberger & Eggli 4896, Argentina, Tucuman	-	KJ161577	-	-	-	KJ161742	KJ161807
<i>Portulaca grandiflora</i> Hook.	998914/0 (ZSS) - Hunkeler 591, Argentina, Catamarca	-	KJ161578, KJ161579	-	-	-	-	-
<i>Portulaca molokiniensis</i> Hobdy	921793/0 (ZSS) - Wong n.a., Hawaii, Molokini Island	-	-	-	-	-	KJ161743	KJ161808
<i>Portulaca oleracea</i> L.	Sage 0509-01	-	KJ161580	-	-	-	KJ161744, KJ161809	KJ161745
<b>Cactaceae - Pereskia</b>								
<i>Pereskia aculeata</i> Mill.	Ogburn n.a. (BRU) - Brown greenhouse	X95860, KJ161567	-	KJ161612	KJ161632	-	-	-
<i>Pereskia aureiflora</i> F. Ritter	166-54-83-20 (B) - R. Nyffeler	KJ161568	-	KJ161613	KJ161633	KJ161650	KJ161746	KJ161810
<i>Pereskia bleo</i> (Kunth) DC.	Ogburn n.a. (BRU) - Brown greenhouse	-	-	KJ161614	-	KJ161651	KJ161747	KJ161811
<i>Pereskia diaz-romeroana</i> Cárdenas	Arakaki n.a. (BRU) - Brown greenhouse	-	-	KJ161615	KJ161634	KJ161652	KJ161748	KJ161812
<i>Pereskia grandifolia</i> Haw.	Ogburn n.a. (BRU) - Brown greenhouse	-	-	KJ161616	KJ161635	KJ161653	KJ161749	KJ161813
<i>Pereskia guamacho</i> F.A.C. Weber	Arakaki n.a. (BRU) - Brown greenhouse	-	-	KJ161617	KJ161636	-	KJ161750	KJ161814
<i>Pereskia portulacifolia</i> (L.) DC.	Ogburn n.a. (BRU) - Brown greenhouse	-	-	KJ161618	KJ161637	KJ161654	KJ161751	KJ161815
<i>Pereskia quisqueyana</i> Alain	Ogburn n.a. (BRU) - Brown greenhouse	-	-	-	KJ161638	-	-	KJ161816
<i>Pereskia sacharosa</i> Griseb.	Arakaki n.a. (BRU) - Brown greenhouse	-	-	-	-	-	KJ161752	KJ161817
<i>Pereskia zinniflora</i> DC.	842526/a (ZSS) - Cuba, Sancti Spiritus	-	KJ161581	-	KJ161639	-	KJ161753	KJ161818
<b>Cactaceae - Opuntioideae</b>								
<i>Brasiliopuntia brasiliensis</i> (Willd.) A. Berger	Ogburn n.a. (BRU) - Brown greenhouse	-	-	-	-	KJ161655, KJ161656	-	-
<i>Cylindropuntia kleiniae</i> (DC.) F.M. Knuth aff	Ogburn n.a. (BRU) - Brown greenhouse	-	KJ161582	-	-	KJ161657, KJ161658	KJ161754	KJ161819
<i>Maihueiopsis glomerata</i> (Haw.) R. Kiesling	Arakaki 1905 (BRU) - 55 from Mesa Garden Nursery 1.932 (D. Ferguson 280), Argentina, Salta	-	KJ161583	-	-	-	KJ161755	KJ161820
<i>Nopalea cochenellifera</i> (DC.) F.M. Knuth aff	Ogburn n.a. (BRU) - Brown greenhouse	-	-	KJ161830	-	KJ161659, KJ161660	KJ161756	KJ161821
<i>Pereskiaopsis diguetii</i> (F.A.C. Weber) Britton & Rose	942160/b (ZSS) - Lomelí Sención & Díaz L. s/n, Mexico, Jalisco	-	KJ161584	-	-	KJ161661	KJ161757	KJ161822
<i>Pereskiaopsis gatesii</i> Baxter	Ogburn n.a. (BRU) - Brown greenhouse	-	KJ161585	KJ161619	-	KJ161662, KJ161663	-	-
<i>Pereskiaopsis porteri</i> (Brandege ex F.A.C. Weber) Britton & Rose	Ogburn n.a. (BRU) - Brown greenhouse	-	-	-	-	KJ161664, KJ161665	KJ161758	KJ161823
<i>Quiabentia verticillata</i> (Vaupel) Vaupel ex Berger	Ogburn n.a. (BRU) - Brown greenhouse	-	KJ161586	-	-	KJ161666	-	-
<i>Quiabentia zehntneri</i> (Britton & Rose) Britton & Rose	864369 (ZSS)	-	-	-	-	-	KJ161759	KJ161824
<i>Tephrocactus articulatus</i> (Pfeiff.) Backeb.	Spriggs n.a. (BRU) - Brown greenhouse	-	KJ161587	KJ161620	-	KJ161667	KJ161760	KJ161825
<b>Cactaceae - Cactoideae</b>								
<i>Acanthocereus tetragonus</i> (L.) Hummelinck	892219 (ZSS) - brought by R. Nyffeler	-	-	-	-	KJ161668, KJ161669	-	-
<i>Coryphantha radians</i> (DC.) Britton & Rose	Arakaki 1898 (BRU) - from Mesa Garden Nursery 125 (S.Brack 679), Mexico, Hidalgo	-	KJ161588	-	-	KJ161670	-	KJ161826

<i>Echinocactus grusonii</i> Hildm.	Spriggs n.a. (BRU) - Brown greenhouse	-	-	KJ161621	KJ161640	-	-	-
<i>Echinocereus pectinatus</i> (Scheidw.) Engelm.	-	-	-	KJ161831	-	-	-	-
<i>Hylocereus undatus</i> (Haw.) Britton & Rose	-	-	-	JF966382	-	JF966381	-	-
<i>Mammillaria plumosa</i> F.A.C. Weber	Arakaki 1907 - from Mesa Garden Nursery 845.5 (S. Brack 327), Mexico, Coahuila	-	-	KJ161589	KJ161622	-	KJ161671	-
<i>Selenicereus wittii</i> (K. Schum.) G.D. Rowley	-	-	-	-	-	-	-	-
<i>Thelocactus leucacanthus</i> (Zucc. Ex Pfeiff.) Britton & Rose	Arakaki 1912 (BRU) - from Mesa Garden Nursery 1273 (S. Brack 514), Mexico, Hidalgo	-	-	KJ161590	KJ161623	KJ161641	KJ161672, KJ161761	KJ161828
<i>Weingartia kargliana</i> Rausch	782218/b (ZSS) - Rausch 677, Bolivia, N of Tupiza	-	-	KJ161624	-	KJ161674	-	KJ161829

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**Supplementary Table 3: Additional primers used for PCR amplification of Caryophyllales genes encoding PEPC.**

<b>Name</b>	<b>Primer</b>	<b>Target</b>
<i>ppc-1588-For</i>	GGKGCATACATYATCTCCATG	all <i>ppc-1E1</i>
<i>ppc-1621-For</i>	TCRGATGTGCTTGCTGTTGAG	all <i>ppc-1E1</i>
<i>ppc-1792-For</i>	ATGATTGGRTACTCTGATTC	all <i>ppc-1E1</i>
<i>ppc-2261-For</i>	TGAACATTGGAAGCCGTCC	all <i>ppc-1E1</i>
<i>ppc-2761-Rev</i>	CTTGGATAAGTGAGGCCTCTC	<i>ppc-1E1a</i>
<i>ppc-2831-Rev</i>	CAGGAGCATACTCACTGGAGG	<i>ppc-1E1b</i>
<i>ppc-2846-Rev</i>	GGTGTCTCAAGGCCAGGAG	all <i>ppc-1E1</i>
<i>ppc-2874-Rev</i>	GCAGCAATACCTTTCATGGTG	<i>ppc-1E1c</i>
<i>ppc-2748-Rev</i>	ATGCCATYAGTTCTTTGGATAAG	<i>ppc-1E1c</i>
<i>ppc-1363-For</i>	ATTCGYCAAGAATCTGACCG	<i>ppc-1E2</i>
<i>ppc-2452-Rev</i>	GTRACCCTAAAGAAAGGCCATTC	<i>ppc-1E2</i>
<i>ppc-1884_For</i>	GCARGGAGARATGGTRCAGGC	<i>ppc-2</i>
<i>ppc-2633-Rev</i>	ATRGTGATSAGCAATGCRTCTC	<i>ppc-2</i>
<i>ppc-2651-Rev</i>	AGCAGCAATYCCATTAATAG	<i>ppc-2</i>



**Supplementary Table 4: Water treatment of *Portulaca oleracea* plants**

Date	Group A	Group B
05.03.2012	watered <sup>1</sup>	watered <sup>1</sup>
08.03.2012	watered	
11.03.2012	watered	
13.03.2012	watered	
15.03.2012	watered	watered
17.03.2012	watered	
19.03.2012	watered	
22.03.2012	watered	watered
26.03.2012	watered	
31.03.2012	watered <sup>1</sup>	watered <sup>1</sup>
04.04.2012	watered	
06.04.2012	sampled	sampled

<sup>1</sup>Nutrients added

**Supplementary Table 5: Sequencing and mapping statistics.**

Time Condition	Day		Night		Day		Night	
	Watered				Drought			
Individual	1	2	1	2	3	4	3	4
Pairs of reads sequenced	11,980,617	7,440,608	7,519,679	11,832,946	7,311,777	8,168,471	8,659,554	7,785,604
Pairs of reads used in assemblies	10,017,935	5,886,163	5,708,027	6,352,153	6,286,010	6,352,153	7,403,534	6,957,525
Contigs	47,047	44,957	43,749	66,755	40,334	56,987	48,471	55,248
Percent reads aligned	89.11	86.84	86.98	80.58	88.3	86.96	87.45	86.34

**Supplementary Table 6: Expression levels in rpm of C<sub>4</sub>-related genes in day and night samples of *Portulaca oleracea* plants grown in different conditions<sup>1</sup>.**

Time Condition Individual	Day		Night		Day		Night	
	Watered				Drought			
	1	2	1	2	3	4	3	4
ALA-AT-1	2,382	1,095	3,723	2,722	775	306	651	539
ASP-AT-1	83	105	175	134	78	202	83	96
ASP-AT-2	743	500	444	222	438	162	120	90
ASP-AT-3	0	0	0	0	0	0	0	0
ASP-AT-4	2,111	1,430	1,141	798	680	650	329	375
β-CA-1	0	0	0	0	0	0	0	0
β-CA-2	714	649	273	298	332	423	255	253
β-CA-3	658	325	291	139	208	66	53	28
β-CA-4	20,880	10,296	9,775	6,476	10,818	2,021	1,631	1,505
NAD-MDH-1	22	12	15	19	16	74	40	35
NAD-MDH-2	675	532	363	197	630	628	207	179
NAD-MDH-3	1171	1168	292	239	837	371	146	132
NAD-ME-1	643	666	534	501	648	499	535	560
NAD-ME-2	1131	990	947	804	838	617	636	591
NADP-MDH-1	1,956	2,300	20	2,359	1,357	1,793	1,812	2,263
NADP-MDH-2	13	231	40	11	4	21	10	20
NADP-MDH-3	648	520	534	523	939	764	1,141	1,246
NADP-ME-1	1,184	896	215	313	1,021	651	385	281
NADP-ME-2	0	0	0	17	0	0	0	0
NADP-ME-3	42	19	102	0	75	86	211	257
PCK-1	21	13	28	77	10	11	41	48
PPDK-1	26,696	24,814	21,010	21,105	29,855	18,778	11,463	10,164

<sup>1</sup> ALA-AT = alanine aminotransferase, ASP-AT = aspartate aminotransferase, β-CA = β-carbonic anhydrase, NAD-MDH = NAD-malate dehydrogenase, NAD-ME = NAD-malic enzyme, NADP-MDH = NADP-malate dehydrogenase, NADP-ME = NADP-malic enzyme, PCK = phosphoenolpyruvate carboxykinase, PPDK = pyruvate, phosphate dikinase.