Supplementary Information

Additional file 2:

Table S1. Species, voucher, distribution, and GenBank accession numbers for the samples used in this study.

Species	Voucher	Distribution	GeneBank
Aquilegia alpina L.	F. Prosser 19980716 (ROV)	Europe	PV564728
Aquilegia amurensis Kom.	T.N. Veklich 20200618 (MHA)	Asia	PV564729
Aquilegia apuana (Marchetti) E. Nardi	D. Marchetti & G. Trombetti 20110531 (FI)	Europe	PV564730
Aquilegia aradanica Shaulo & Erst	D. Shaulo & A. Erst 20100703 (NS)	Asia	PV564731
Aquilegia atrata W.D.J. Koch	F. Schuhwerk 19920612 (NS)	Europe	PV564732
Aquilegia atrovinosa Popov ex Gamajun.	H.Y. Xu 20030714 (PE)	Asia	PV564733
Aquilegia aurea Janka	E. Bozilova *s.n.* (LE)	Europe	PV564734
Aquilegia barbaricina Arrigoni & E. Nardi	P.V. Arrigoni & al 050391 (FI).	Europe	PV564735
Aquilegia barnebyi Munz	S. Goodrich 26459 (NY)	North America	PV564736
Aquilegia barykinae Erst, Karakulov & Luferov	A. Karakulov 20100623 (NS)	Asia	PV564737
Aquilegia bertolonii Schott	P. Bamps 19880530 (LE)	Europe	PV564738
Aquilegia borodinii Schischk.	D. Shaulo & al. *s.n.* (NS)	Asia	PV564739
Aquilegia brevistyla Hook.	L. Malyshev 975 (MHA)	North America	PV564740
Aquilegia buergeriana Siebold & Zucc.	F. Konta TNS35846 (NS)	Asia	PV564741
Aquilegia caerulea E.James	Z.D. Chen & H.Z. Kong CL104 (PE)	North America	PV564742
Aquilegia canadensis L.	D. Alta 10508 (NY)	North America	PV564743
Aquilegia caucasica (Ledeb.) Rupr.	M. Patarana 19880726 (LE)	Europe	PV564744
Aquilegia champagnatii Moraldo, Nardi & la Valva	S. Fior, M. Lega 010 (ROV)	Europe	PV564745
Aquilegia chrysantha A. Gray	R.D. Dorn 10298 (NY)	North America	PV564746
Aquilegia coelestis Fed.	G. Lazkov & N. Kentenbaeva 20030612 (LE)	Asia	PV564747
Aquilegia coerulea E. James	A. Erst 20140706 (NS)	North America	PV564748
Aquilegia colchica Kem.-Nath.	L. Vassiljeva 19900705 (LE)	Europe	PV564749
Aquilegia cossoniana (Maire & Sennen) Rivas Mart.	A. Achhal & al. 471607 (FI)	North Africa	PV564750
Aquilegia daingolica Erst & Shaulo	Z. Karamysheva & al. 1321 (LE)	Asia	PV564751
Aquilegia dumeticola Jord.	G. Ferretti 050390 (FI)	Europe	PV564752
Aquilegia ecalcarata Eastw.	H.W. Peng & J. Zhang 210813 (PE)	Asia	PV564753
Aquilegia einseleana F.W. Schultz	F. Prosser & A. Bertolli 20070718 (FI)	Europe	PV564754
Aquilegia elegantula Greene	A. Erst *s.n.* (NS)	North America	PV564755
Aquilegia eximia Van Houtte ex Planch.	A. Eastwood & al. 9692 (PE)	North America	PV564756
Aquilegia flabellata Siebold & Zucc.	T. Konovalova 20010612 (MHA)	Asia	PV564757
Aquilegia flavescens S.Watson	A. Erst *s.n.* (NS)	North America	PV564758
Aquilegia formosa Fisch. ex DC.	I. Koropachinsky & al. 447 (NS)	North America	PV564759
Aquilegia fragrans Benth.	FPH 0471 (PE)	Asia	PV564760
Aquilegia gegica Jabr.-Kolak.	Topotypus *s.n.* (LE)	Europe	PV564761
Aquilegia glandulosa Fisch.	D. Shaulo & A. Erst 20100718 (NS)	Asia	PV564762
Aquilegia grata Maly ex Zimmeter	E. Mayer & al. LJU39336 (LE)	Europe	PV564763
Aquilegia hebeica Erst	H.W. Peng & J. Zhang *s.n.* (PE)	Asia	PV564764

Aquilegia incurvata P.K. Hsiao	Q. Zhang & al. XYB0607-190 (PE)	Asia	PV564765
Aquilegia iulia E. Nardi	S. Fior & M. Lega 014 (ROV)	Europe	PV564766
Aquilegia japonica Nakai & Hara	L.F. Li 201106 (PE)	Asia	PV564767
Aquilegia jonesii Parry	W.A. Weber COLO15467 (NS)	North America	PV564768
Aquilegia jucunda Fisch. & Avé-Lall.	E. Zibzeev & E. Basargin *s.n.* (NS)	Asia	PV564769
Aquilegia karelinii O. et B. Fedscenko	I. Rusanovich 19850616 (MHA)	Asia	PV564771
Aquilegia kansuensis (Brühl) Erst	G.N. Potanin *s.n.* (LE)	Asia	PV564770
Aquilegia kubanica I.M. Vassiljeva	A. Zernov & al. 7627a (MW)	Europe	PV564772
Aquilegia lactiflora Kar. & Kir.	G. Lazkov 20010613 (LE)	Asia	PV564773
Aquilegia laramiensis A. Nelson	A. Nelson *s.n.* (MHA)	North America	PV564774
Aquilegia longissima A. Gray ex S. Wats.	J.L. Emily 170 (PE)	North America	PV564775
Aquilegia lucensis E. Nardi	B. Foggi 19870806 (FI)	Europe	PV564776
Aquilegia magellensis F. Conti & Soldano	C. Ricceri & C. Steinberg 050388 (FI)	Europe	PV564777
Aquilegia moorcroftiana Wall. ex Royle	P. Furse KEW6347 (LE)	Asia	PV564778
Aquilegia nevadensis Boiss. & Reut.	B. Valdes & al. It239288 (FI)	Europe	PV564779
Aquilegia nigricans Baumgarten	H. Riedl 19800713 (LE)	Europe	PV564780
Aquilegia nugorensis Arrigoni & E. Nardi	P.V. Arrigoni & E. Nardi 17 (FI)	Europe	PV564781
Aquilegia nuragica Arrigoni & E. Nardi	P.V. Arrigoni & E. Nardi 10 (FI)	Europe	PV564782
Aquilegia olympica Boissier	M. Kozhin & al. 20110623 (MW)	Europe	PV564783
Aquilegia oxysepala Trautvetter & C.A. Meyer	S. Kazanovsky IRK19296 (NS)	Asia	PV564784
Aquilegia pubescens Coville	I. Koropachinsky & al. 471 (NS)	North America	PV564785
Aquilegia pubiflora Wall. ex Royle	FPH 1835 (PE)	Asia	PV564786
Aquilegia reuteri Boiss.	R. Bernardello 20100728 (FI)	Europe	PV564787
Aquilegia rockii Munz	M.P. Petrov 19570623 (LE)	Asia	PV564788
Aquilegia saximontana Rydberg	M.S. Roh 20040718 (NS)	North America	PV564789
Aquilegia shockleyi Eastw.	J.D. Morefield & al. 3782 (PE)	North America	PV564790
Aquilegia sibirica Lambert	D. Shaulo & al. 20100712 (NS)	Asia	PV564791
Aquilegia sicula (Strobl) E. Nardi	R. Bavazzano & al. 19720522 (FI)	Europe	PV564792
Aquilegia skinneri Hook.	W.L. Wagner & J.C. Solomon 4297 (PE)	North America	PV564793
Aquilegia synakensis Shaulo & Erst	D. Shaulo & A. Erst 20100714 (NS)	Asia	PV564794
Aquilegia thalictrifolia Schott, Kotschy	Lega 016 (ROV)	Europe	PV564795
Aquilegia turczaninowii Kamelin & Gubanov	E. Kondrat'eva 20130307 (NS)	Asia	PV564796
Aquilegia vestinae Pfenninger, Moser	Lega 015 (ROV)	Europe	PV564797
Aquilegia viridiflora Pall.	A. Erst & Yu. Danilov 20100516 (NS)	Asia	PV564798
Aquilegia viscosa Gouan	M. Li & Q. Ge 19970614 (FI)	Europe	PV564799
Aquilegia vitalii Gamojun.	I.T. Rusanovich & L. Kramarenko *s.n.* (MHA)	Asia	PV564800
Aquilegia xinjiangensis Erst sp. Nov.	A.S. Erst 122016 (NS)	Asia	PV564801
Aquilegia yabeana Kitag.	H.W. Peng & J. Zhang SX026 (PE)	Asia	PV564802
Outgroups			
Dichocarpum dalzielii (J.R.Drumm. & Hutch.) W.T. Wang & Hsiao	W. Wang 111 (PE)	Asia	PV546614
Semiaquilegia adoxoides (DC.) Makino	Q. Shao 02 (PE)	Asia	PV546613
Semiaquilegia danxiashanensis L. Wu, J.J. Zhou, Q. Zhang & W.S. Deng		Asia	NC_088573
Thalictrum minus (Siebold & Zucc.) Miq.	Anonymous *s.n.* (NS)	Asia	PV546612
Thalictrum simplex L.	D. Shaulo & A. Erst 20100717 (NS)	Asia & Europe	PV546611
Urophysa rockii Ulbr.	L. Zhang 200910 (PE)	Asia	PV546610

Table S2. Summary of types of structure variations and plastome lengths of Thalictroideae. IR, inverted repeat; LSC, large single-copy; SSC, small single-copy; sIR, short inverted repeat.

Species	Types	Length (bp)						
		Plastome	LSC	IR	SSC	Inversion	sIR1	sIR2
Aquilegia alpina	II	161345	90504	26726	17389	12705	49	NA
Aquilegia amurensis	III	160406	91259	25903	17341	12852	49	754
Aquilegia apuana	II	161573	90705	26736	17396	12853	49	NA
Aquilegia aradanica	I	160973	90505	26607	17254	12812	NA	NA
Aquilegia atrata	II	161344	90505	26726	17387	12705	49	NA
Aquilegia atrovinosa	III	161860	91512	26521	17306	12822	49	752
Aquilegia aurea	II	160253	90679	26097	17380	12848	49	NA
Aquilegia barbaricina	II	161475	90699	26689	17398	12813	49	NA
Aquilegia barnebyi	III	162013	91201	26704	17404	12828	49	755
Aquilegia barykinae	III	159782	91151	25933	16765	12813	49	754
Aquilegia bertolonii	II	161366	90617	26689	17371	12798	49	NA
Aquilegia borodinii	I	161270	90503	26751	17265	12825	NA	NA
Aquilegia brevistyla	III	162202	91159	26834	17375	12766	49	755
Aquilegia buergeriana	II	161451	90677	26703	17368	12662	49	NA
Aquilegia caerulea	III	161856	91248	26577	17454	12791	49	755
Aquilegia canadensis	III	161929	91173	26687	17382	12768	49	755
Aquilegia caucasica	II	161513	90722	26708	17375	12816	49	NA
Aquilegia champagnatii	II	161577	90692	26738	17409	12842	49	NA
Aquilegia chrysantha	III	161900	91067	26690	17453	12662	49	755

Aquilegia coelestis	III	161821	91560	26503	17255	12816	49	752
Aquilegia coerulea	III	161832	91236	26577	17442	12791	49	755
Aquilegia colchica	II	161518	90616	26757	17388	12817	49	NA
Aquilegia cossoniana	II	161327	90558	26699	17371	12813	49	NA
Aquilegia daingolica	I	161275	90522	26751	17251	12812	NA	NA
Aquilegia dumeticola	II	161500	90695	26724	17357	12821	49	NA
Aquilegia ecalcarata	III	162109	91403	26662	17382	12719	49	755
Aquilegia einseleana	II	161549	90672	26739	17399	12835	49	NA
Aquilegia elegantula	III	161854	91242	26577	17458	12791	49	755
Aquilegia eximia	III	161745	91169	26609	17358	12818	49	760
Aquilegia flabellata	III	160083	90996	25863	17361	12592	49	755
Aquilegia flavescens	III	162025	91245	26712	17356	12824	49	755
Aquilegia formosa	III	161842	91199	26613	17417	12817	49	760
Aquilegia fragrans	III	162014	91516	26618	17262	12822	49	755
Aquilegia gegica	II	161537	90645	26745	17402	12826	49	NA
Aquilegia glandulosa	I	161021	90542	26630	17219	12819	NA	NA
Aquilegia grata	II	161578	90747	26724	17383	12847	49	NA
Aquilegia hebeica	II	160521	90437	26691	16702	12841	49	NA
Aquilegia incurvata	III	162480	91642	26723	17392	12846	49	755
Aquilegia iulia	II	161525	90706	26723	17373	12815	49	NA
Aquilegia japonica	II	161474	90662	26728	17356	12851	49	NA
Aquilegia jonesii	III	162276	91259	26803	17411	12826	49	755
Aquilegia jucunda	I	160997	90497	26625	17250	12807	NA	NA
Aquilegia kansuensis	III	161991	91424	26595	17377	12693	49	755
Aquilegia karelinii	III	161765	91411	26500	17354	12810	49	753

Aquilegia kubanica	II	161104	90425	26647	17385	12822	49	NA
Aquilegia lactiflora	III	162284	91498	26724	17338	12781	49	755
Aquilegia laramiensis	III	161985	91225	26694	17372	12803	49	756
Aquilegia longissima	III	162146	91251	26721	17453	12767	49	751
Aquilegia lucensis	II	161574	90705	26736	17397	12853	49	NA
Aquilegia magellensis	II	161328	90586	26710	17322	12733	49	NA
Aquilegia moorcroftiana	III	161995	91456	26628	17283	12686	49	755
Aquilegia nevadensis	II	161368	90615	26690	17373	12790	49	NA
Aquilegia nigricans	II	161361	90679	26657	17368	12790	49	NA
Aquilegia nugorensis	III	161745	91169	26609	17358	12818	49	760
Aquilegia nuragica	II	161473	90639	26718	17398	12816	49	NA
Aquilegia olympica	II	161544	90657	26742	17403	12828	49	NA
Aquilegia oxysepala	II	161442	90616	26724	17378	12829	49	NA
Aquilegia pubescens	III	161887	91200	26614	17459	12820	49	761
Aquilegia pubiflora	III	161941	91391	26633	17284	12687	49	751
Aquilegia reuteri	II	161353	90644	26689	17331	12798	49	NA
Aquilegia rockii	III	162167	91406	26695	17371	12703	49	756
Aquilegia saximontana	III	162074	91249	26700	17425	12785	49	756
Aquilegia shockleyi	III	161894	91137	26687	17383	12772	49	754
Aquilegia sibirica	II	161372	90398	26802	17370	12835	49	NA
Aquilegia sicula	II	161378	90648	26696	17338	12803	49	NA
Aquilegia skinneri	III	162188	91246	26751	17440	12789	49	755
Aquilegia synakensis	II	161422	90610	26722	17368	12843	49	NA
Aquilegia thalictrifolia	II	161549	90691	26736	17386	12853	49	NA
Aquilegia turczaninowii	III	160595	91262	25994	17345	12817	49	754

Aquilegia vestinae	II	161572	90705	26736	17395	12853	49	NA
Aquilegia viridiflora	I	160995	90501	26625	17244	12813	NA	NA
Aquilegia viscosa	III	160503	91486	25853	17311	12811	49	752
Aquilegia vitalii	III	161923	91572	26488	17375	12811	49	777
Aquilegia xinjiangensis	I	161245	90462	26766	17251	12814	NA	NA
Aquilegia yabeana	III	162457	91611	26743	17360	12837	49	755
Outgroups								
Dichocarpum dalzielii	IV	153727	83381	26448	17450	12148	NA	NA
Semiaquilegia adoxoides	III	162227	91225	26829	17344	13121	49	696
Semiaquilegia danxiashanensis	III	160548	89882	26640	17386	12855	49	14
Thalictrum minus	IV	156258	85700	26480	17598	12741	NA	NA
Thalictrum simplex	IV	156211	85662	26481	17587	12743	NA	NA
Urophysa rockii	III	164138	91683	27544	17367	12711	49	876

Table S3. Summary of characteristics of genes in plastomes of Thalictroideae. The "/" symbol is used to distinguish different copies of the gene.

Gene group	Gene	*Aquilegia*			*Urophysa*			*Semiaquilegia*			*Dichocarpum*			*Thalictrum*		
		No. copy	No. introns	No. exon	No. copy	No. introns	No. exon	No. copy	No. introns	No. exon	No. copy	No. introns	No. exon	No. copy	No. introns	No. exon
Subunit Acetyl- CoA-Carboxylate	*accD*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
ATP synthase	*atpA*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	atpB	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	atpE	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	atpF	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
	atpH	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	atpI	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
c-type cytochrome synthesis gene	*ccsA*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
Envelop membrane protein	*cemA*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
ATP-dependent protease subunit gene	*clpP*	2	0	1	2	0	1	2	0	1	1	2	3	1	2	3
Translational initiation factor	*infA*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
Maturase	*matK*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
NADH oxidoreductase	*ndhA*	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
	ndhB	2	1	2	2	1	2	2	1	2	2	1	2	2	1	2
	ndhC	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	ndhD	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	ndhE	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	ndhF	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	ndhG	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	ndhH	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	ndhI	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	ndhJ	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	ndhK	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1

Cytochrome b6/f complex	*petA*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	petB	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
	petD	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
	petG	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	petL	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	petN	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
Photosystem I	*psaA*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psaB	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psaC	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psaI	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psaJ	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
Photosystem II	*psbA*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbB	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbC	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbD	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbE	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbF	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbH	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbI	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbJ	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbK	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbL	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbM	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbN	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbT	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	psbZ	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
Rubisco large subunit	*rbcL*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1

Ribosomal proteins (LSU)	*rpl2*	1	1	2	1	1	2	1	1	2	2	1	2	2	1	2
	rpl14	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rpl16	2	0/1	1/0	2	0	1	2	0/1	1/0	1	1	2	1	1	2
	rpl20	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rpl22	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rpl23	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1
	rpl32	1	1	0	1	1	0	1	1	0	NA	NA	NA	1	1	0
	rpl33	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rpl36	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
RNA polymerase	*rpoA*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rpoB	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rpoC1	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
	rpoC2	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
Ribosomal proteins (SSU)	*rps2*	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rps3	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rps4	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rps7	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1
	rps8	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rps11	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rps12	2	1	2/3	2	1	2/3	2	1	2/3	2	1	2/3	2	1	2/3
	rps14	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rps15	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rps16	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
	rps18	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	rps19	1	0	1	1	0	1	1	0	1	2	0/1	1/0	2	0/1	1/0
Conserved Open reading frames	*ycf1*	2	0/1	1/0	2	0/1	1/0	2	0/1	1/0	2	0/1	1/0	2	0/1	1/0
	ycf2	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1

	ycf3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	ycf4	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	ycf15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2	1	2
rRNA genes	*rrn4.5*	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1
	rrn5	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1
	rrn16	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1
	rrn23	2	0	1	2	0	1	2	0/1	1/2	2	0	1	2	0	1
tRNA genes	*trnA*^{UGC}	2	1	2	2	1	2	2	1	2	2	1	2	2	1	2
	trnC^{GCA}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	trnD^{GUC}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	trnE^{UUC}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	trnF^{GAA}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	trnfM^{CAU}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	trnG^{GCC}	4	0	1	4	0	1	4	0	1	1	0	1	1	0	1
	trnG^{UCC}	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
	trnH^{GUG}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	trnI^{CAU}	6	0	1	2	0	1	2	0	1	2	0	1	2	0	1
	trnI^{GAU}	2	1	2	2	1	2	2	1	2	2	1	2	2	1	2
	trnK^{UUU}	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
	trnL^{CAA}	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1
	trnL^{UAA}	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
	trnL^{UAG}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	trnM^{CAU}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
	trnN^{GUU}	3	0	1	2	0	1	3	0	1	2	0	1	2	0	1

trnP^{UGG}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
trnQ^{UUG}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
trnR^{ACG}	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1
trnR^{UCU}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
trnS^{GCU}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
trnS^{GGA}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
trnS^{UGA}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
trnT^{GGU}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
trnT^{UGU}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
trnV^{GAC}	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1
trnV^{UAC}	1	1	2	1	1	2	1	1	2	1	1	2	1	1	2
trnW^{CCA}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
trnY^{GUA}	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1

Table S4. Comparison of three transition models in ancestral structural variation reconstruction. ARD, all-rates-different likelihood model; ER, equal-rates likelihood model; SYM, symmetrical likelihood model. The optimal clock model is in bold.

Model	lnL	AIC	AICc
ER	**-16.21**	**34.42**	**34.47**
SYM	-14.89	41.78	42.91
ARD	-13.95	51.9	56.49

Table S5. Summary of characteristics of 121 genes in plastome sequences in *Aquilegia*. We also list the references in which makers were used in previously molecular phylogenetic analyses. Pi, nucleotide diversity.

Gene	Size of gene	GC content of gene	Pi of gene	No. introns	Size of exon	GC content of exon	Pi of exon	Reference
trnH^{GUG}	75	58.70%	0	0	75	58.70%	0	
ψrpl16	156	46.00%	0.00698	1	0	0.00%	0	
psbA	1062	43.80%	0.0005	0	1062	43.80%	0.0005	
trnK^{UUU}(included *matK*)	2630	34.80%	0.00135	1	72	54.20%	0	Fior et al. [1], Bastida et al. [2]
rps16	1161	36.30%	0.00049	1	274	38.30%	0.00023	
trnQ^{UUG}	72	63.90%	0	0	72	63.90%	0	
psbK	186	34.40%	0.00014	0	186	34.40%	0.00014	
psbI	111	39.60%	0.00024	0	111	39.60%	0.00024	
trnS^{GCU}	88	51.10%	0	0	88	51.10%	0	
trnG^{UCC}	824	35.70%	0.00122	1	71	53.50%	0	
trnR^{UCU}	72	43.10%	0	0	72	43.10%	0	
atpA	1524	42.30%	0.00107	0	1524	42.30%	0.00107	
atpF	1274	37.60%	0.00017	1	555	38.20%	0.00019	
atpH	246	46.90%	0.00199	0	246	46.90%	0.00199	
atpI	744	38.80%	0.0007	0	744	38.80%	0.0007	
rps2	711	39.40%	0.00043	0	711	39.40%	0.00043	
rpoC2	4176	39.10%	0.00047	0	4176	39.10%	0.00047	
rpoC1	2825	39.10%	0.0002	1	2085	39.90%	0.00017	
rpoB	3213	41.40%	0.00044	0	3213	41.40%	0.00044	
trnC^{GCA}	71	62.00%	0	0	71	62.00%	0	

petN	90	41.10%	0.00114	0	90	41.10%	0.00114	
psbM	105	32.40%	0	0	105	32.40%	0	
trnD^{GUC}	74	62.20%	0	0	74	62.20%	0	
trnY^{GUA}	84	53.60%	0	0	84	53.60%	0	Fior et al. [1]
trnE^{UUC}	73	57.50%	0	0	73	57.50%	0	Fior et al. [1]
trnT^{GGU}	72	51.40%	0	0	72	51.40%	0	
psbD	1062	44.10%	0.00007	0	1062	44.10%	0.00007	
psbC	1422	45.80%	0.00039	0	1422	45.80%	0.00039	
trnS^{UGA}	93	49.50%	0	0	93	49.50%	0	
psbZ	189	33.40%	0.00054	0	189	33.40%	0.00054	Fior et al. [1]
trnG^{GCC}1	71	52.10%	0	0	71	52.10%	0	
trnfM^{CAU}	74	55.40%	0	0	74	55.40%	0	
rps14	303	41.90%	0	0	303	41.90%	0	
psaB	2205	42.40%	0.00054	0	2205	42.40%	0.00054	
psaA	2253	43.70%	0.00005	0	2253	43.70%	0.00005	
ycf3	2012	38.40%	0.00021	2	513	39.60%	0	
trnS^{GGA}	87	52.10%	0.00483	0	87	52.10%	0.00483	
rps4	600	40.00%	0.00004	0	600	40.00%	0.00004	
trnT^{UGU}	73	54.80%	0	0	73	54.80%	0	Fior et al. [1]
trnL^{UAA}	616	36.30%	0.00075	1	85	48.20%	0	Fior et al. [1]
trnF^{GAA}	73	50.70%	0	0	73	50.70%	0	
ndhJ	477	42.80%	0.00022	0	477	42.80%	0.00022	
ndhK	684	40.80%	0.00021	0	684	40.80%	0.00021	
ndhC	363	26.60%	0	0	363	26.60%	0	
trnV^{UAC}	677	40.30%	0.00027	1	81	52.00%	0	

trnM^{CAU}	73	41.10%	0	0	73	41.10%	0
atpE	402	40.30%	0.00031	0	402	40.30%	0.00031
atpB	1497	43.60%	0.00035	0	1497	43.60%	0.00035
rbcL	1428	45.10%	0.00025	0	1428	45.10%	0.00025
*clpP*1	855	42.10%	0.00144	0	855	42.10%	0.00144
rps12	913	40.20%	0.00003	1	372	41.70%	0.00007
rpl20	354	39.30%	0.00063	0	354	39.30%	0.00063
rps18	345	33.50%	0	0	345	33.50%	0
rpl33	207	36.20%	0.0017	0	207	36.20%	0.0017
psaJ	135	43.00%	0.0002	0	135	43.00%	0.0002
trnP^{UGG}	74	48.60%	0	0	74	48.60%	0
trnW^{CCA}	74	52.70%	0	0	74	52.70%	0
petG	114	39.50%	0	0	114	39.50%	0
petL	96	35.40%	0	0	96	35.40%	0
psbE	252	43.70%	0	0	252	43.70%	0
psbF	120	41.70%	0	0	120	41.70%	0
psbL	117	32.50%	0	0	117	32.50%	0
psbJ	123	41.50%	0	0	123	41.50%	0
petA	969	39.60%	0.00062	0	969	39.60%	0.00062
cemA	681	34.50%	0.00076	0	681	34.50%	0.00076
ycf4	555	40.00%	0.00038	0	555	40.00%	0.00038
psaI	111	37.80%	0.00024	0	111	37.80%	0.00024
accD	1647	37.00%	0.00057	0	1647	37.00%	0.00057
trnG^{GCC}2	71	52.10%	0	0	71	52.10%	0
*clpP*2	630	42.30%	0.00153	0	630	42.30%	0.00153
psbB	1527	44.20%	0.00041	0	1527	44.20%	0.00041

psbT	102	37.20%	0.00026	0	102	37.20%	0.00026	Fior et al. [1]
psbN	132	46.20%	0	0	132	46.20%	0	Fior et al. [1]
psbH	222	40.90%	0.00179	0	222	40.90%	0.00179	
petB	1503	37.50%	0.00004	1	648	42.90%	0	
petD	1258	39.60%	0.00021	1	528	40.50%	0.00034	
rpoA	1050	37.30%	0.00037	0	1050	37.30%	0.00037	
rps11	417	47.50%	0.00041	0	417	47.50%	0.00041	
rpl36	114	42.10%	0	0	114	42.10%	0	
trnN^{GUU}1	72	54.20%	0	0	72	54.20%	0	
infA	123	38.90%	0.07967	0	123	38.90%	0.07967	
rps8	405	39.10%	0.00104	0	405	39.10%	0.00104	
rpl14	369	39.60%	0.00007	0	369	39.60%	0.00007	
rpl16	459	45.50%	0.00017	0	459	45.50%	0.00017	
rps3	669	36.90%	0.00078	0	669	36.90%	0.00078	
rpl22	546	36.80%	0.0001	0	546	36.80%	0.0001	
rps19	558	39.10%	0	0	558	39.10%	0	
rpl2	2984	43.30%	0.0011	1	837	45.80%	0.00074	
rpl23	309	38.20%	0.00028	0	309	38.20%	0.00028	
trnI^{CAU}1	73	54.80%	0	0	73	54.80%	0	
trnI^{CAU}2	74	51.40%	0	0	74	51.40%	0	
trnI^{CAU}3	66	51.50%	0.00734	0	66	51.50%	0.00734	
ycf2	6396	39.00%	0.00014	0	6396	39.00%	0.00014	
trnL^{CAA}	81	50.50%	0.00184	0	81	50.50%	0.00184	
trnG^{GCC}3	72	51.40%	0	0	72	51.40%	0	
ndhB	2236	38.20%	0.00004	1	1533	37.70%	0.00003	
rps7	468	41.00%	0.00006	0	468	41.00%	0.00006	

rps12	913	40.20%	0.00003	1	372	41.70%	0.00007
trnV^{GAC}	72	48.60%	0	0	72	48.60%	0
rrn16	1492	56.80%	0	0	1492	56.80%	0
trnI^{GAU}	995	49.60%	0.00003	1	72	59.70%	0
trnA^{UGC}	864	51.60%	0	1	73	56.20%	0
rrn23	2811	55.30%	0	0	2811	55.30%	0
rrn4.5	103	48.50%	0	0	103	48.50%	0
rrn5	121	51.20%	0	0	121	51.20%	0
trnR^{ACG}	74	63.50%	0	0	74	63.50%	0
trnN^{GUU}2	72	54.20%	0	0	72	54.20%	0
ψycf1	1061	38.30%	0.00105	1	0	0.00%	0
ndhF	2225	35.00%	0.00123	0	2225	35.00%	0.00123
ψrpl32	179	22.30%	0	1	0	0.00%	0
trnL^{UAG}	80	57.50%	0	0	80	57.50%	0
ccsA	984	33.10%	0.00206	0	984	33.10%	0.00206
ndhD	3060	36.20%	0.00029	0	3060	36.20%	0.00029
psaC	246	43.10%	0.00011	0	246	43.10%	0.00011
ndhE	306	33.60%	0.00172	0	306	33.60%	0.00172
ndhG	534	37.30%	0.00163	0	534	37.30%	0.00163
ndhI	540	37.00%	0.00015	0	540	37.00%	0.00015
ndhA	2179	35.60%	0.00079	1	1092	36.40%	0.0001
ndhH	1182	40.20%	0.00065	0	1182	40.20%	0.00065
rps15	264	33.70%	0.0001	0	264	33.70%	0.0001
ycf1	6003	33.80%	0.00196	0	6003	33.80%	0.00196

Table S6. Summary of characteristics of 120 intergenic spacer (IGS) regions in plastome sequences in *Aquilegia*. We also list the references in which makers were used in previously molecular phylogenetic analyses. Pi, nucleotide diversity.

IGS	Size of IGS	GC content of IGS	Pi of IGS	Reference
trnH^{GUG}-*ψrpl16*	276	26.70%	0.01189	
ψrpl16-psbA	116	43.20%	0.00371	
psbA-trnK^{UUU}	300	28.70%	0.00011	
trnK^{UUU}-*rps16*	954	29.50%	0.00532	Fior et al. [1]
rps16-trnQ^{UUG}	1555	30.50%	0.00244	Fior et al. [1]
trnQ^{UUG}-*psbK*	348	30.70%	0.00168	
psbK-psbI	477	31.70%	0.00086	
psbI-trnS^{GCU}	152	26.10%	0.00486	
trnS^{GCU}-*trnG*^{UCC}	1432	28.70%	0.00192	
trnG^{UCC}-*trnR*^{UCU}	278	26.00%	0.00053	
trnR^{UCU}-*atpA*	185	21.80%	0.0008	
atpA-atpF	69	31.80%	0.00113	
atpF-atpH	532	33.70%	0.00071	
atpH-atpI	1188	33.30%	0.00065	Fior et al. [1]
atpI-rps2	242	32.50%	0.00201	
rps2-rpoC2	243	34.60%	0.00027	
rpoC2-rpoC1	184	44.20%	0.00046	
rpoC1-rpoB	26	30.80%	0	
rpoB-trnC^{GCA}	1502	33.20%	0.00137	Fior et al. [1]
trnC^{GCA}-*petN*	969	36.80%	0.00107	Fior et al. [1]
petN-psbM	1498	31.00%	0.00119	Fior et al. [1]
psbM-trnD^{GUC}	1295	33.70%	0.00303	Fior et al. [1]
trnD^{GUC}-*trnY*^{GUA}	497	32.90%	0.0008	1st part of *trnD*^{GUC}-*trnT*^{GGU}, Fior et al. [1]
trnY^{GUA}-*trnE*^{UUC}	70	46.70%	0	2nd part of *trnD*^{GUC}-*trnT*^{GGU}, Fior et al. [1]
trnE^{UUC}-*trnT*^{GGU}	849	29.20%	0.00332	3rd part of *trnD*^{GUC}-*trnT*^{GGU}, Fior et al. [1]
trnT^{GGU}-*psbD*	1814	35.30%	0.01197	Fior et al. [1]
psbD-psbC	0	0.00%	0	
psbC-trnS^{UGA}	205	37.60%	0	
trnS^{UGA}-*psbZ*	405	35.80%	0.00059	1st part of *trnS*^{UGA}-*trnG*^{GCC}, Fior et al. [1]
psbZ-trnG^{GCC}1	520	33.00%	0.00121	2nd part of *trnS*^{UGA}-*trnG*^{GCC}, Fior et al. [1]
trnG^{GCC}1-*trnfM*^{CAU}	199	30.80%	0.00448	
trnfM^{CAU}-*rps14*	162	39.80%	0	
rps14-psaB	162	34.20%	0	
psaB-psaA	25	44.00%	0	

psaA-ycf3	715	39.00%	0.00214	
ycf3-trnS^{GGA}	948	31.80%	0.00133	
trnS^{GGA}*-rps4*	386	34.90%	0.00122	
rps4-trnT^{UGU}	428	27.90%	0.00258	1st part of *rps4-trnF*^{GAA}, Fior et al. [1]
trnT^{UGU}*-trnL*^{UAA}	1400	30.20%	0.00139	2nd part of *rps4-trnF*^{GAA}, Fior et al. [1]
trnL^{UAA}*-trnF*^{GAA}	465	37.30%	0.00066	3rd part of *rps4-trnF*^{GAA}, Fior et al. [1]
trnF^{GAA}*-ndhJ*	792	33.10%	0.00345	Fior et al. [1]
ndhJ-ndhK	180	30.40%	0.00023	
ndhK-ndhC	56	22.90%	0	
ndhC-trnV^{UAC}	1592	32.70%	0.00083	Fior et al. [1]
trnV^{UAC}*-trnM*^{CAU}	196	36.90%	0.00092	
trnM^{CAU}*-atpE*	295	32.00%	0.00034	
atpE-atpB	0	0.00%	0	
atpB-rbcL	803	32.70%	0.00032	Hodges and Arnold [3]
*rbcL-clpP*1	1347	33.90%	0.00298	
*clpP*1*-rps12*	2307	36.20%	0.00672	
rps12-rpl20	841	34.70%	0.0005	Fior et al. [1]
rpl20-rps18	348	38.10%	0.00142	
rps18-rpl33	229	26.10%	0.00114	
rpl33-psaJ	456	31.70%	0.00272	
psaJ-trnP^{UGG}	519	28.70%	0.00223	
trnP^{UGG}*-trnW*^{CCA}	205	27.50%	0	
trnW^{CCA}*-petG*	161	34.60%	0	
petG-petL	181	32.20%	0.00263	
petL-psbE	1450	31.90%	0.00102	Fior et al. [1]
psbE-psbF	9	66.70%	0	
psbF-psbL	22	36.40%	0	
psbL-psbJ	137	41.20%	0	
psbJ-petA	526	33.60%	0.00184	
petA-cemA	411	39.40%	0.00264	
cemA-ycf4	957	32.70%	0.00086	
ycf4-psaI	472	35.50%	0.00126	
psaI-accD	760	26.20%	0.0017	Fior et al. [1]
accD-trnG^{GCC}2	1407	37.10%	0.00185	
trnG^{GCC}2*-clpP*2	590	36.00%	0.00215	
*clpP*2*-psbB*	425	36.80%	0.00013	
psbB-psbT	206	38.30%	0.00059	1st part of *psbB-psbH*, Fior et al. [1]
psbT-psbN	105	31.50%	0.00872	2nd part of *psbB-psbH*, Fior et al. [1]
psbN-psbH	102	32.40%	0	3rd part of *psbB-psbH*, Fior et al. [1]
psbH-petB	198	38.10%	0.0026	
petB-petD	223	31.10%	0.00037	
petD-rpoA	348	32.80%	0.00183	
rpoA-rps11	73	25.00%	0.00077	

rps11-rpl36	115	32.20%	0.00046	
rpl36-trnN^{GUU}1	712	35.10%	0.00437	
trnN^{GUU}1-*infA*	698	21.30%	0.01203	
infA-rps8	218	38.30%	0.00212	
rps8-rpl14	254	27.80%	0.00085	
rpl14-rpl16	154	28.70%	0.00861	
rpl16-rps3	184	26.60%	0.01155	
rps3-rpl22	0	0.00%	0	
rpl22-rps19	80	34.80%	0	
rps19-rpl2	73	24.70%	0.00706	
rpl2-rpl23	148	15.40%	0.03055	
rpl23-trnI^{CAU}1	632	39.50%	0.00033	
trnI^{CAU}1-*trnI*^{CAU}2	58	43.10%	0	
trnI^{CAU}2-*trnI*^{CAU}3	68	47.10%	0	
trnI^{CAU}3-*ycf2*	356	33.80%	0.00493	
ycf2-trnL^{CAA}	1048	47.40%	0	
trnL^{CAA}-*trnG*^{GCC}3	742	22.70%	0.0135	
trnG^{GCC}3-*ndhB*	3038	39.10%	0.00003	
ndhB-rps7	326	38.60%	0.00008	
rps7-rps12	58	35.80%	0	
rps12-trnV^{GAC}	1959	41.30%	0.00014	
trnV^{GAC}-*rrn16*	227	43.20%	0.00012	
rrn16-trnI^{GAU}	294	52.00%	0	
trnI^{GAU}-*trnA*^{UGC}	64	54.70%	0	
trnA^{UGC}-*rrn23*	157	42.80%	0.00067	
rrn23-rrn4.5	100	60.20%	0	
rrn4.5-rrn5	231	47.70%	0	
rrn5-trnR^{ACG}	260	43.90%	0	
trnR^{ACG}-*trnN*^{GUU}2	684	42.20%	0.00368	
trnN^{GUU}2-*ψycf1*	973	33.40%	0.00163	
ψycf1-ndhF	998	28.80%	0	
ndhF-ψrpl32	1527	22.30%	0.00496	
ψrpl32-trnL^{UAG}	1171	28.90%	0.00292	Fior et al. [1]
trnL^{UAG}-*ccsA*	133	28.70%	0	
ccsA-ndhD	292	24.90%	0.00767	
ndhD-psaC	490	32.40%	0.00362	
psaC-ndhE	279	29.50%	0.00202	
ndhE-ndhG	306	25.20%	0.00283	
ndhG-ndhI	512	24.70%	0.00139	
ndhI-ndhA	83	33.70%	0.0046	
ndhA-ndhH	1	0.00%	0	
ndhH-rps15	154	23.00%	0	

rps15-ycf1	460	32.10%	0.00232

Literature Cited

1. Fior S, Li M, Oxelman B, Viola R, Hodges SA, Ometto L, et al. Spatiotemporal reconstruction of the *Aquilegia* rapid radiation through next-generation sequencing of rapidly evolving cpDNA regions. New Phytol. 2013;198:579–92.
2. Bastida JM, Alcántara JM, Rey PJ, Vargas P, Herrera CM. Extended phylogeny of *Aquilegia*: The biogeographical and ecological patterns of two simultaneous but contrasting radiations. Plant Syst Evol. 2010;284:171–85.
3. Hodges SA, Arnold ML. Columbines: A geographically widespread species flock. Proc Natl Acad Sci. 1994;91:5129–32.