SUPPLEMENTARY MATERIALS

Comparative Analysis of *Actaea* **Chloroplast Genomes and Molecular Marker Development for the Identification of Authentic Cimicifugae Rhizoma**

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Figure S1: Validation of the complete chloroplast (cp) genomes of three *Actaea* species, *A. simplex, A. dahurica,* and *A. biternata* using raw read mapping. LSC, large single copy region; SSC, small single copy region; IRa, inverted repeat a; IRb, inverted repeat b.



Figure S2: Codon frequencies and RSCU (relative synonymous codon usage) values of three *Actaea* species, *A. simplex*, *A. dahurica*, and *A. biternata*. (A) Amino acid frequencies in protein-coding genes. (B) RSCU values of 20 amino acids and stop codons in 78 protein-coding genes.



Figure S3: Distribution of simple sequence repeats (SSRs) in cp genomes of three *Actaea* species. (a) Number of SSRs per unit length. (b) Distribution of SSRs in exons, introns and intergenic spacer (IGS) regions. (c) Distribution of different types of SSRs.



Figure S4. Comparison of complete cp genomes of six *Actaea* species using the MAUVE algorithm. Local collinear blocks colored in red indicate syntenic regions, while histograms within each block represent the degree of sequence similarity. Arrows indicate the large single copy (LSC), inverted repeat (IR), and small single copy (SSC) regions. Green color blocks means tRNAs with intron. And red color block indicates rRNAs.



Figure S5: Comparison of the position of junctions between the large single copy (LSC) region, inverted repeat (IR) regions (IRa and IRb), and small single copy (SSC) region in the cp genomes of six *Actaea* species.



Figure S6: Comparison of non-synonymous substitution rate (Ka), synonymous substitution rate (Ks) and Ka/Ks ratio of 67 conserved protein-coding genes among the cp genomes of *A. simplex, A. dahurica,* and *A. biternata*. Blue and orange bars indicate Ka and Ks values, respectively, while gray bars indicate Ka/Ks ratios. Genes with Ka or Ks values of 0 are not shown.



Figure S7: Phylogenetic analysis of nine species within the tribe Cimicifugeae. The phylogenetic tree was constructed with the maximum likelihood (ML) method and Bayesian Inference (BI) using whole cp genome sequences of six *Actaea* species and one *Eranthis* species. *Beesia calthifolia* and *Anemonopsis macrophylla* were used as outgroups. ML topology is shown with bootstrap (BS) values (%) and BI posterior probability (PP) values at each node. Black triangles indicate the cp genomes of three *Actaea* species investigated in this study.

three Actaea species.								
	A. simplex	A. dahurica	A. biternata					
Number of input reads	7,455,916	7,348,266	8,077,362					
Number of trimmed reads	6,415,201	6,240,189	7,211,294					
Total raw bases (bp)	2,196,684,451	2,184,464,508	2,347,534,148					
Trimmed bases (bp)	1,500,015,928	1,462,941,174	1,706,161,420					
Number of aligned reads	22,304	52,247	194,903					
Coverage (×)	31.51	74.67	280.52					
cp genome length (bp)	159,624	159,523	159,789					

Table S1: Raw read and assembly information of the chloroplast (cp) genomes of

Primer name	Primer sequence (5'→3')	Junction ⁺
LI_F	CCCGGGACCAAGTTACGATT	
LI_R	AGTTTGATTCTTCGTCGCCGT	LSC/IKa
IS_F	ACTTCCGAAACGAAGGGGAC	$\mathbf{ID}_{\mathbf{a}} / \mathbf{SS} C$
IS_R	AAATATGTAGGCGGAGGGCG	IKa/55C
SI_F	CGTTGCTCAGGTTGTTTCGT	CC/IDh
SI_R	GGATCCACCGAAGAAGACCC	55C/1KD
IL_F	AACCCTGTAGACCATCCCCA	IDb/I CC
IL_R	GTTCAATTCCCGTCGTTCGC	

Table S2: Primers used for PCR-based sequence validation of cp junctions in three Actaea species.

⁺LSC, large single copy region; SSC, small single copy region; IRa, inverted repeat a; IRb, inverted repeat b.

Species Junction ⁺		PCR product size Start position (bp) (bp)		End position (bp)	Identity (%)
	LSC/IRa	202	88,697	88,898	100
A	IRa/SSC	240	114,964	115,203	100
A. simplex	SSC/IRb	558	132,791	133,348	100
	IRb/LSC	364	159,324	63	100
	LSC/IRa	207	88,625	88,831	100
A delución	IRa/SSC	467	114,922	115,388	100
A. aunurica	SSC/IRb	556	132,663	133,218	100
	IRb/LSC	379	159,219	74	100
	LSC/IRa	213	88,921	89,133	100
A 1.1.	IRa/SSC	517	115,201	115,717	100
A. biternata	SSC/IRb	562	132,950	133,511	100
	IRb/LSC	374	159,489	73	100

Table S3: Information of PCR-based sequencing.

⁺LSC, large single copy region; SSC, small single copy region; IRa, inverted repeat a; IRb, inverted repeat b.

Gene group	Gene name
Photosystem I	psaA, B, C, I, J, ycf3+, ycf4
Photosystem II	psbA, B, C, D, E, F, H, I, J, K, L, M, N, T, Z
Cytochrome b6/f	petA, B^{\S} , D^{\S} , G, L, N
ATP synthase	atpA, B, E, F§, H, I
Rubisco	rbcL
NADH oxidoreductase	ndhA§, B§.I, C, D, E, F, G, HI, I, J, K
Large ribosomal subunit proteins	rpl2, 14, 16 [§] , 20, 22, 33, 36
Small ribosomal subunit proteins	rps2, 3, 4, 7 [¶] , 8, 11, 12 ^{+, ¶, ¥} , 14, 15, 18, 19
RNA polymerase	<i>rpoA, B, C</i> 1§, C2
Protein-coding genes with unknown function	ycf1, ycf2
Other genes	accD, ccsA, cemA, clpP ⁺ , matK
Ribosomal RNA (rRNA) genes	rrn16¶, 23¶, 4.5¶, 5¶
Transfer RNA (tRNA) genes	<pre>trnA-UGC^{®, ¶}, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnfM-CAU, trnG-GCC,trnG-UCC, trnH-GUG, trnI-CAU, trnI-GAU^{®, ¶},trnK-UUU, trnL-CAA, trnL-UAA, trnL-UAG, trnL-CAA[¶], trnM-CAU, trnN-GUU[¶], trnP-UGG, trnQ- UUG, trnR-ACG[¶], trnR-UCU, trnS-GCU, trnS-GGA, trnS- UGA, trnT-GGU, trnT-UGU, trnV-GAC[¶], trnW-CCA, trnY-GUA</pre>

Table S4: Genes present in the cp genomes of three *Actaea* species.

[§]Genes containing a single intron.
 [†]Genes containing two introns.
 [¶]Genes with two copies in inverted repeats (IRs).
 [¥]Trans-splicing gene.

	Gene	Location ⁺	Exon I	Intron I (bp)§	Exon II	Intron II (bp)	Exon III
	name		(00)s				(66)
1	trnk-UUU	LSC	37	2544 (2551, 2543)	35		
2	rps16	LSC	40	892 (892, 907)	221		
3	trnG-UCC	LSC	23	694 (688, 688)	48		
4	atpF	LSC	144	728 (723, 727)	411		
5	rpoC1	LSC	435	754 (759, 754)	1620		
6	ycf3	LSC	126	713 (715, 715)	226	757 (744, 745)	155
7	trnL-UAA	LSC	35	508 (505, 502)	50		
8	trnV-UAC	LSC	38	581 (582, 581)	37		
9	rps12	LSC	232		26		114
10	clpP	LSC	69	812 (813, 807)	291	646 (636, 633)	246
11	petB	LSC	6	803 (800, 799)	642		
12	petD	LSC	8	723	496		
13	rpl16	LSC	9	999 (998, 1004)	402		
14	ndhB	IR	777	700	756		
15	trnI-GAU	IR	42	941	35		
16	trnA-UGC	IR	38	803 (803, 800)	35		
17	ndhA	IR	551	905 (920, 917)	541		

Table S5: Gene intron in cp genomes of three Actaea speceis.

[†]LSC, large single copy region; SSC, small single copy region; IR, inverted repeat.

[§]Numbers in parentheses represent the length of corresponding sequences in *A. dahurica* and *A. biternata*.

A. simplex	Position ⁺	Repeat unit length (bp)	Repeat unit sequence	Repeat number	Location [§]
1	IGS (trnK-UUU, rps16)	23	TTTTTTCGTTATTTATTTTA TAT	2	LSC
2	IGS (rps16, trnQ-UUG)	12	ΑΤΑΤΑΤΑΤΑΤΑΤΑ	2	LSC
3	IGS (rps16, trnQ-UUG)	16	AGATTGTTCAAGACAA	2	LSC
4	IGS (rps16, trnQ-UUG)	8	GTAATAAA	3	LSC
5	IGS (trnS-GCU, trnG- UCC)	13	AATTGAATAAAAA	2	LSC
6	IGS (atpH, atpI)	19	TTCATTAGTCTGAATCCTT	2	LSC
7	IGS (petN, psbM)	13	TACGAGAATCCAG	2	LSC
8	IGS (petN, psbM)	15	TTTTACAAATATGAA	2	LSC
9	IGS (trnS-GGA, rps4)	17	ATACTGCAATAATATGA	2	LSC
10	IGS (trnL-UAA, trnF- GAA)	25	TCTTTCACAAAAGGATCG TGGGAGA	2	LSC
11	Exon (accD)	15	AATGACTATTCATCT	2	LSC
12	IGS (psal, ycf4)	17	TATGTAGATATCCATAG	2	LSC
13	Intron (<i>rpl16</i>)	15	TTAATGCTTTATTAC	2	LSC
14	Exon (<i>ycf</i> 2)	21	TCTTTTTGTCCAAGTCGCT TC	3	IR
15	Exon (<i>ycf</i> 2)	18	TATTGATGATAGTGACGA	3	IR
16	IGS (rps12, trnV-GAC)	16	ATTTTATTGATTTATT	2	IR
17	IGS (rrn5, trnR-ACG)	15	AATATGAAAAAAAAA	2	IR
18	IGS (ndhF, trnL-UAG)	21	ACAAATAGAACAAGTAA AAAA	2	SSC
19	IGS (ndhF, trnL-UAG)	20	TTTATTCGTATTAATATTA A	2	SSC
20	IGS (ndhE, ndhG)	22	AATTAACTAAAATATAAT ATAA	2	SSC
21	Exon (<i>ndhG</i>)	12	GTATTTAGTATT	2	SSC

Table S6: Tandem repeats detected in the cp genome of *A. simplex*.

[†]IGS, intergenic spacer region.

^sLSC, large single copy region; SSC, small single copy region; IR, inverted repeat.

A. dahurica	Position ⁺	Repeat unit length (bp)	Repeat unit sequence	Repeat number	Location [§]
1	IGS (trnH-GUG, psbA)	12	AATATATACAAA	2	LSC
2	IGS (atpH, atpI)	21	TAATACAAGCATGAATAC AAA	2	LSC
3	IGS (atpH, atpI)	19	TTCATTAGTCTGAATCCTT	2	LSC
4	IGS (petN, psbM)	13	TACGAGAATCCAG	2	LSC
5	IGS (petN, psbM)	15	TTTTACAAATATGAA	2	LSC
6	IGS (psbM, trnD-GUC)	8	AATCATAT	3	LSC
7	IGS (trnE-UUC, trnT- GGU)	8	ATTTCTAT	3	LSC
8	IGS (trnE-UUC, trnT- GGU)	15	TTCTTCAATTCAACT	2	LSC
9	IGS (psbZ, trnG-GCC)	18	ATATATT	2	LSC
10	IGS (ycf3, trnS-GGA)	17	ATACTGCAATAATATGA	2	LSC
11	IGS (trnL-UAA, trnL- UAA)	25	TCTTTCACAAAAGGATCG TGGGAGA	2	LSC
12	IGS (ndhC, trnV-UAC)	13	TATATTTTATTTGA	2	LSC
13	IGS (ndhC, trnV-UAC)	10	TATTATTATC	2	LSC
14	IGS (ndhC, trnV-UAC)	19	GTACAGTACAAATATAGT A	2	LSC
15	IGS (atpB, rbcL)	16	TTATTATTCATTATTA	2	LSC
16	IGS (rbcL, accD)	15	AATGACTATTCATCT	2	LSC
17	IGS (rbcL, accD)	17	TTTAGTTTATAGTAATC	2	LSC
18	IGS (psal, ycf4)	17	TATGTAGATATCCATAG	2	LSC
19	Intron (<i>rpl16</i>)	15	TTAATGCTTTATTAC	2	LSC
20	Exon (<i>ycf</i> 2)	21	TCTTTTTGTCCAAGTCGCT TC	3	IR
21	Exon (ycf2)	18	TATTGATGATAGTGACGA	4	IR
22	IGS (rps12, trnV-GAC)	16	ATTTTATTGATTTATT	2	IR
23	IGS (rrn5, trnR-ACG)	13	AATATGAAAAAAA	2	IR

Table S7: Tandem repeats detected in the cp genome of *A. dahurica*.

[†]IGS, intergenic spacer region.

^sLSC, large single copy region; SSC, small single copy region; IR, inverted repeat.

A. biternata	Position ⁺	Repeat unit length (bp)	Repeat unit sequence	Repeat number	Location [§]
1	Intron (<i>rps16</i>)	9	TATAGTATA	2	LSC
2	IGS (rps16, trnQ-UUG)	21	ATAATAATACATATTTAT	2	LSC
3	IGS (rps16, trnQ-UUG)	28	TTTAGAGATAAGGAATAT ATAATATAAA	2	LSC
4	IGS (rps16, trnQ-UUG)	17	AATAGAAAGAAAACAAA	2	LSC
5	IGS (atpH, atpI)	19	TTCATTAGTCTGAATCCTT	2	LSC
6	IGS (petN, psbM)	15	TTTTACAAATATGAA	2	LSC
7	IGS (trnT-GGU, psbD)	25	TATTATCTATATAACGATA TGGATA	2	LSC
8	IGS (psbZ, trnG-GCC)	24	TATAAATATATAATGTTAT AGTTA	2	LSC
9	IGS (ycf3, trnS-GGA)	17	ATACTGCAATAATATGA	2	LSC
10	IGS (ndhC,trnV-UAC)	13	ATTTTTTTTTATTAT	2	LSC
11	IGS (rbcL, accD)	19	AGTTCCTATATTTATTTG	2	LSC
12	IGS (rbcL, accD)	46	TATAATATATATAGACTTG ATCTAGATATAAAAAA GATCTTATCTT	2	LSC
13	IGS (rbcL, accD)	15	AATGACTATTCATCT	2	LSC
14	IGS (psal, ycf4)	17	TATGTAGATATCCATAG	2	LSC
15	Exon (<i>ycf</i> 2)	18	TATTGATGATAGTGACGA	4	IR
16	IGS (rps12, trnV-GAC)	16	ATTTTATTGATTTATT	2	IR
17	IGS (rrn5, trnR-ACG)	12	AATATGAAAAAA	2	IR
18	IGS (ccsA, ndhD)	15	TAGGAATAGCATATA	2	SSC
19	IGS (ndhE, ndhG)	22	ААТТААСТААААТАТААТ СТАА	2	SSC
20	Exon (ycf1)	15	TTTTTATTTTCACTA	2	SSC

Table S8: Tandem re	peats detected in the c	p genome of A. hiternata.
	peaks detected in the e	generate er in enternann

⁺IGS, intergenic spacer region. [§]LSC, large single copy region; SSC, small single copy region; IR, inverted repeat.

A. heracleifolia	Collection site	Voucher number
1	Mungoksodo-dong, Taebaek-si, Gangwon-do	KIOM201501013910
2	Daeseong, Hwagae, Hadong, Gyeongsangnam-do,	KIOM201401009497
3	Ungyo, Bangnim, Pyeongchang, Gangwon-do	KIOM201401010685
4	Singi-ri, Jinbu, Pyeongchang, Gangwon-do	KIOM201401011037
A. simplex		
5	Gohan, Gohan, Jeongseon, Gangwon-do	KIOM201401010623
6	Ungyo, Bangnim, Pyeongchang, Gangwon-do	KIOM201401010683
7	Gohan, Gohan, Jeongseon, Gangwon-do	KIOM201401010894
8	Taegi, Dunnae, Hoengseong, Gangwon-do	KIOM201401011006
A. dahurica		
9	Mungoksodo, Taebaek, Gangwon-do	KIOM201501013910
10	Jeoksang, Muju, Jeollabuk-do	KIOM200601000057
11	Gohan, Gohan, Jeongseon, Gangwon-do	KIOM201401010625
12	Mungoksodo, Taebaek, Gangwon-do	KIOM201501013910
A. biternata		
13	Aewol, Jeju, Jeju-do	KIOM201001003333
14	Aewol, Jeju, Jeju-do	KIOM201001003331
15	Aewol, Jeju, Jeju-do	KIOM201101003949
16	Aewol, Jeju, Jeju-do	KIOM201101003950
A. asiatica		
17	Icheon, Sangbuk, Ulju, Ulsan	KIOM201401009748
18	Sannae, Namwon, Jeollabuk-do	KIOM200801001156
19	Hyeol, Taebaek, Gangwon-do	KIOM201401009950
20	Danjibong, Gimcheon, Gyeongsangbuk-do	KIOM201501011463

Table S9: List of *Actaea* accession numbers used in this study.

Family	Taxon	GenBank accession number					
	Actaea heracleifolia	NC_042253					
	Actaea simplex	MN623225					
	Actaea dahurica	MN623226					
	Actaea biternata	MN623227					
Cimicifugeae	Actaea asiatica	NC_041525					
	Actaea vaginata	NC_041543					
	Eranthis stellata	NC_041536					
	Beesia calthifolia	NC_041531					
	Anemonopsis macrophylla	NC_041527					

Table S10: Accession numbers of cp genomes used for phylogenetic analysis.

	Model	f(a)	f(c)	f(g)	f(t)	kappa	titv	Ra	Rb	Rc	Rd	Re	Rf	pInv	Gamma		
AIC	GTR+I+G	0.3	0.19	0.18	0.31	2.4	1.13	0.77	1.28	0.24	0.2	1.34	1	0.488	0.216		
	Model			-lnL*		К		AI	С	delta		weig	ght	cum	Weight		
	GTR+I+G		11	.0141.7		26		22033	35.4	0		0.545852		0.5	45852		
-	ГVM+I+G		11	0142.9		25		22033	35.7	0.376	98	0.452	208	0.9	97932		
1	ГIM1+I+G		11	0149.8		24		22034	47.6	12.218	892	0.001	213	0.9	99145		
TI	PM1uf+I+G		11	0151.1		23		22034	48.3	12.91	71	0.000	855		1		
	GTR+I		11	0163.6		25		22032	77.2	41.852	256	4.46E	-10		1		
	TVM+I		11	0164.8		24		22032	77.6	42.253	366	3.65E	-10		1		
	GTR+G		11	0167.2		25		22038	34.3	48.94	602	1.28E	-11		1		
	TVM+G		11	0168.4		24		22038	34.8	49.44	26	1.00E	-11		1		
	TIM1+I		11	0171.7		23		22038	39.4	54.07156		9.90E	-13		1		
]	ГРМ1uf+I		11	0173.1		22		22039	90.1	54.77704		6.96E-13		96E-13			
	TIM1+G		11	0175.3		23		22039	96.6	61.28	992	2.68E	-14		1		
Т	PM1uf+G		11	0176.7		22		22039	97.4	62.014	496	1.87E	-14		1		
1	ГIM2+I+G		1	10344		24		2207	736	400.6717		400.6717 5		5.40E	-88		1
TI	PM2uf+I+G		11	0345.2		23		22073	36.4	401.04	438 4.48E-88		-88		1		
	TIM2+I		11	0365.9		23		22072	77.7	442.34	458	4.82E	-97		1		
1	ГРМ2uf+I		11	0367.1		22		22072	78.1	442.75	549	3.93E	-97		1		
	TIM2+G		11	0368.2		23		22078	32.4	447.0	647	4.55E	-98		1		
Т	PM2uf+G		11	0369.5		22		22078	32.9	447.5	511	3.55E	-98		1		
	TrN+I+G		11	0368.5		23		2207	783	447.6	685	3.37E	-98		1		
]	HKY+I+G		11	0369.8		22		22078	33.7	448.3	448.3158 2.44E-98		-98		1		
1	ГIM3+I+G		11	0368.4		24		220784.7		220784.7 449.373		49.3737 1.43E-98		-98		1	
TI	PM3uf+I+G		11	0369.7		23		22078	35.4	450.09	450.0925 1.00E-98		-98		1		
	TrN+I		11	.0390.3		22		22082	24.5	489.1	569	3.28E-	-107		1		
	HKY+I		11	.0391.6		21		22082	220825.2 489.8519		519	2.33E	-107		1		

Table S11: Selection of the best-fitting substitution model for 65 conserved cp genes using jModelTest.

*-lnL, negative log likelihood; K, number of estimated parameters; AIC, Akaike Information Criterion; delta, AIC difference; weight, AIC weight; cumWeight, cumulative AIC weight.

	Model	f(a)	f(c)	f(g)	f(t)	kappa	titv	Ra	Rb	Rc	Rd	Re	Rf	pInv	Gamma
AIC	TVM+I+G	0.3	0.19	0.18	0.31	1.64	0.76	0.90	1.18	0.63	0.18	1.18	1	0.851	0.851
	Model			-lnL*			AIC		delta		weight		cumWeight		
	TVM+I+G			289185.5398			578421.0796		0		0.684392		0.684392		
	GTR+I+G		289185.3138			26		578422.6277		1.54806		0.315608		1	
	TVM+I		289222.7514			24		578493.5027		72.42312		1.28E-16		1	
	GTR+I		289222.5696			25		578495.1392		74.05954		5.67E-17		1	
	TPM1uf+I+G		289347.6132			23		578741.2265		320.14686		2.07E-70		1	
	TIM1+I+G		289347.7098			24		578743.4196		322.33994		6.92E-71		1	
	TPM1uf+I		289387.4426			22	578818.8852			397.80558		2.84E-87		1	
	TIM1+I		289387.2983			23	578820.5966		399.51696		1.21E-87		1		
	TVM+G		289	399.979	97	24		578847	.9595	426.87	7984	1.38E	-93		1
	GTR+G		289	399.772	73	25		578849	.5546	428.47	7502	6.21E	-94		1
	TPM3uf+I+G		289552.8185			23	579151.6371		730.55744		1.57E-159		1		
	TIM3+I+G		289552.9781			24	579153.9561		732.87652		4.93E-160		1		
	TPM1uf+G		289555.0684			22	579154.1367		733.0571		4.51E-160		1		
	TIM1+G		289554.8922			23		579155.7845		734.70484		1.98E-160		1	
	TPM2uf+I+G		289571.4373			23		579188.8746		767.795		1.29E-167		1	
	HKY+I+G		289572.964			22		579189.9281		768.84844		7.62E-168			1
	TIM2+I+G		289571.3345			24	579190.669		769.58934		5.26E-168			1	
	TrN+I+G		289575.0821			23	579196.1642		775.08454		3.37E-169			1	
	TPM3uf+I		289589.1743			22	579222.3486		801.26896		6.95E-175		1		
	TIM3+I		289588.9459			23	579223.8918		802.81222		3.21E-175			1	
	TPM2uf+I		289608.0302			22		579260.0603		838.98072		4.50E-183			1
	TIM2+I		289607.8925			23		579261.785		840.70542		1.90E-183			1
	HKY+I		289612.2148			21		579266.4296		845.35002		1.86E-184			1
	TrN+I		289612.0516			22		579268.1031		847.0235		8.06E-185			1

 Table S12: Selection of the best-fitting substitution model for whole cp genomes using jModelTest.

*-InL, negative log likelihood; K, number of estimated parameters; AIC, Akaike Information Criterion; delta, AIC difference; weight, AIC weight; cumWeight, cumulative AIC weight.