

Structure-based GC investigation shed new light on ITS2 evolution in *Corydalis* species

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Tables S1. Samples and their ITS2/5.8S rDNA high-throughput sequencing information^a

Sample	Locality ^b	Species	Section	ITS2 copy # in HGS			5.8S copy # in HGS		
				Total #	Singleton #	Depth	Total #	Singleton #	Depth
WJL01	Songlinkou, Balang MT., Xiaojin, Sichuan	<i>Corydalis flexuosa</i>	Sect. <i>Elatae</i>	16	16	16976.90	1625	311	19430.40
WJL03	Xueshanliang Viewing Platform, Chuanzhusi Town, Songpan, Sichuan	<i>Corydalis rheinbabeniana</i>	Sect. <i>Trachycarpae</i>	135	115	21035.90	1063	281	18657.80
WJL04	Menggu Village, Lixian, Sichuan	<i>Corydalis pingwuensis</i>	Sect. <i>Elatae</i>	6	6	10334.40	1122	261	10838.80
WJL06	Baima Snow MT., Deqin, Yunnan	<i>Corydalis lathyrophylla</i>	Sect. <i>Trachycarpae</i>	135	109	19646.60	1704	460	19532.90
WJL07	Baima Snow MT., Deqin, Yunnan	<i>Corydalis kokiana</i>	Sect. <i>Kokianae</i>	5	5	11852.10	1459	378	14296.00
WJL08	Baima Snow MT., Deqin, Yunnan	<i>Corydalis delphinoides</i>	Sect. <i>Davidianae</i>	8	8	14993.40	1189	367	15165.60
WJL11	Laoludu highway, Geza Township, Shangri-la, Yunnan	<i>Corydalis melanochlora</i>	Sect. <i>Clavatae</i>	165	146	34346.90	3161	503	32452.40
WJL12	Longyu Bay, Luanchuan, Henan	<i>Corydalis ophiocarpa</i>	Sect. <i>Cheilanthifoliae</i>	30	26	13650.80	1308	317	12319.10
WJL13	Top of Shika Snow MT., Shangri-La, Yunnan	<i>Corydalis pachypoda</i>	Sect. <i>Chrysocapnos</i>	124	90	22557.70	1581	336	20248.70
WJL14	Diaohaizi, Yajia ridge, Kangding, Sichuan	<i>Corydalis elata</i>	Sect. <i>Elatae</i>	23	23	12846.20	1219	212	12120.90
WJL15	Wanfoshan, Tongdao Dong Autonomous, Huaihua, Hunan	<i>Corydalis balansae</i>	Sect. <i>Sophorocapnos</i>	88	64	34895.00	2842	578	30159.10
WJL16	Daigu Temple, Diabe, Gansu	<i>Corydalis adunca</i>	Sect. <i>Strictae</i>	4	4	39498.20	2721	571	33986.10
WJL17	Longyu Bay, Luanchuan, Henan	<i>Corydalis caudata</i>	Sect. <i>Corydalis</i>	89	74	31992.80	2479	480	32291.70
WJL18	Ximatan, Cangshan, Dali, Yunnan	<i>Corydalis oxypetala</i>	Sect. <i>Fusifformes</i>	4	4	4375.60	480	132	4511.60
WJL19	Laoludu highway, Geza Township, Shangri-la, Yunnan	<i>Corydalis trachycarpa</i>	Sect. <i>Trachycarpae</i>	148	116	15531.20	1186	263	14020.30

WJL20	Laoludu highway, Geza Township, Shangri-la, Yunnan	<i>Corydalis eugeniae</i>	Sect. <i>Trachycarpae</i>	180	136	23911.90	1715	338	22060.30
WJL22	Xueshanliang Viewing Platform, Chuanzhusi Town, Songpan, Sichuan	<i>Corydalis curviflora</i>	Sect. <i>Curviflorae</i>	12	12	16813.20	1824	345	19118.90
cyn17	Lusi, Nanyang City, Henan	<i>Corydalis edulis</i>	Sect. <i>Aulacostigma</i>	115	38	30007.20	3360	249	31175.40
cyn18	Tongtianhe National Forest Park, Fengxian, Shaanxi	<i>Corydalis shensiiana</i>	Sect. <i>Curviflorae</i>	383	190	28945.70	2697	226	28669.30
CJL02	Longbugou Zhonggu, Kangding, Sichuan	<i>Corydalis laucheana</i>	Sect. <i>Fumarioides</i>	230	129	27493.70	2689	263	27573.90
CJL03	Laoludu highway, Geza Township, Shangri-la, Yunnan	<i>Corydalis benecincta</i>	Sect. <i>Benecinctae</i>	39	21	3473.40	290	34	3325.40
CJL04	Jinlan MT., National Forest Park, Xinxian, Xinyang, Henan	<i>Corydalis incisa</i>	Sect. <i>Incisae</i>	187	96	25201.50	3976	236	26280.90
CJL05	Alpine Botanical Garden, Lijiang, Yunnan	<i>Corydalis zhongdianensis</i>	Sect. <i>Fumarioides</i>	58	31	6658.40	719	75	66290
CJL06	Zhongshan Botanical Garden, Nanjing, Jiangsu	<i>Corydalis decumbens</i>	Sect. <i>Duplotuber</i>	15	13	6819.70	598	78	7705.90
CJL07	Bamei Town, Daofu, Sichuan	<i>Corydalis pseudosibirica</i>	Sect. <i>Fumarioides</i>	114	34	19210.20	1316	118	178890
CJL08	Beijing Botanical Garden, Beijing	<i>Corydalis bungeana</i>	Sect. <i>Chinenses</i>	114	68	20865.90	1947	189	21049.80
CJL09	Dongsong Town, Shangri-la, Ganzi, Sichuan	<i>Corydalis yui</i>	Sect. <i>Fumarioides</i>	33	27	17242.50	678	87	17671.10
NJ01	Zhongshan Botanical Garden, Nanjing, Jiangsu	<i>Corydalis sheareri</i>	Sect. <i>Asterostigmata</i>	312	69	169633.00	9879	375	168492.10
Cfa	NCBI	<i>Corydalis fangshanensis</i>	Sect. <i>Thalictrifoliae</i>	38	14	6248.60	1140	106	6953.60

^a The ITS2 sequence data and the sequence-structure alignment of each sample are posted at:

https://figshare.com/articles/dataset/Structure-based_GC_investigation_shed_new_light_on_ITS2_evolution_in_Corydalis_species/21980624

^b Fieldwork was all over China, the sampling localities included small site, county and province

Table S2. Comparison of GC content between ITS2 and 5.8S fragments

Species	ITS2		5.8S	
	GC content (%)	Standard Deviation	GC content (%)	Standard Deviation
<i>Corydalis yui</i>	74.02	0.49	56.01	0.65
<i>Corydalis flexuosa</i>	73.54	1.02	56.14	0.86
<i>Corydalis elata</i>	75.15	0.77	56.81	0.82
<i>Corydalis benecincta</i>	73.06	0.42	55.86	0.53
<i>Corydalis pseudosibirica</i>	71.86	0.63	55.85	0.63
<i>Corydalis melanochlora</i>	73.86	0.67	56.84	0.87
<i>Corydalis ophiocarpa</i>	73.12	0.46	56.16	0.77
<i>Corydalis trachycarpa</i>	72.64	0.63	56.20	0.75
<i>Corydalis pingwuensis</i>	73.46	1.36	56.69	0.78
<i>Corydalis adunca</i>	75.31	0.35	56.30	0.84
<i>Corydalis oxypetala</i>	71.26	0.21	56.75	0.80
<i>Corydalis sheareri</i>	74.78	0.74	56.77	1.00
<i>Corydalis curviflora</i>	74.44	0.91	56.86	0.79
<i>Corydalis laucheana</i>	75.90	0.72	57.08	0.76
<i>Corydalis incisa</i>	73.47	0.58	56.81	0.65
<i>Corydalis zhongdianensis</i>	73.92	0.46	56.20	0.64
<i>Corydalis bungeana</i>	75.83	0.62	57.01	0.67
<i>Corydalis shensiana</i>	72.39	0.78	56.43	0.85
<i>Corydalis rheinbabeniana</i>	72.56	1.42	56.14	0.86
<i>Corydalis pachypoda</i>	72.37	0.64	56.22	0.77
<i>Corydalis balansae</i>	72.89	0.53	56.33	0.84
<i>Corydalis lathyrophylla</i>	72.30	0.73	56.24	0.80
<i>Corydalis eugeniae</i>	73.24	1.14	56.23	0.82
<i>Corydalis caudata</i>	71.50	0.79	56.28	0.79
<i>Corydalis decumbens</i>	76.50	0.92	55.70	0.69
<i>Corydalis edulis</i>	74.06	0.54	55.87	0.88
<i>Corydalis fangshanensis</i>	74.32	0.44	55.58	0.82
<i>Corydalis delphinioides</i>	74.60	0.62	56.31	0.78
<i>Corydalis kokiana</i>	72.17	1.02	56.81	0.82
Average	73.60	0.71	56.36	0.78

Table S3. Comparison of GC content between ITS2 paired and unpaired regions

Species	ITS2 paired regions		ITS2 unpaired regions		K value
	GC content (%)	Standard Deviation	GC content (%)	Standard Deviation	
<i>Corydalis yui</i>	85.83	0.49	62.73	0.96	3.66
<i>Corydalis flexuosa</i>	80.10	0.66	68.69	1.71	9.18
<i>Corydalis elata</i>	83.87	0.62	67.86	0.91	6.86
<i>Corydalis benecincta</i>	82.02	0.71	63.89	0.56	5.14
<i>Corydalis pseudosibirica</i>	86.64	0.47	58.51	1.02	3.73
<i>Corydalis melanochlora</i>	82.68	0.80	65.95	1.01	6.09
<i>Corydalis ophiocarpa</i>	82.84	0.42	64.41	0.86	5.51
<i>Corydalis trachycarpa</i>	82.44	0.74	63.86	0.92	5.01
<i>Corydalis pingwuensis</i>	83.11	0.45	67.43	1.99	12.53
<i>Corydalis adunca</i>	86.00	0.77	67.45	0.10	5.00
<i>Corydalis oxypetala</i>	82.97	0.37	62.36	0.21	5.67
<i>Corydalis sheareri</i>	86.30	0.54	64.49	1.16	3.81
<i>Corydalis curviflora</i>	83.40	0.97	66.74	1.75	10.33
<i>Corydalis laucheana</i>	85.03	0.56	68.48	1.17	5.23
<i>Corydalis incisa</i>	83.73	0.60	61.81	0.85	5.49
<i>Corydalis zhongdianensis</i>	86.76	0.51	62.22	0.68	2.67
<i>Corydalis bungeana</i>	85.69	0.57	66.91	1.01	4.48
<i>Corydalis shensiana</i>	80.87	0.74	65.65	1.21	4.87
<i>Corydalis rheinbabeniana</i>	83.22	0.99	62.27	1.98	7.40
<i>Corydalis pachypoda</i>	81.46	0.79	63.22	0.94	5.50
<i>Corydalis balansae</i>	81.61	0.49	64.96	0.84	6.08
<i>Corydalis lathyrophylla</i>	83.76	0.75	60.77	1.16	5.74
<i>Corydalis eugeniae</i>	84.17	1.00	61.26	1.72	6.60
<i>Corydalis caudata</i>	83.47	0.97	58.64	1.13	6.77
<i>Corydalis decumbens</i>	86.00	0.78	68.64	1.24	6.12
<i>Corydalis edulis</i>	84.35	0.66	64.00	0.70	4.20
<i>Corydalis fangshanensis</i>	84.33	0.49	64.23	0.62	2.24
<i>Corydalis delphinioides</i>	86.29	0.61	65.40	1.11	9.00
<i>Corydalis kokiana</i>	80.99	0.47	65.68	1.37	12.80
Average	83.79	0.65	64.43	1.06	6.13

Table S4. Comparison of current GC and equilibrium GC (GC*) content between the ITS2 paired and unpaired regions

Species	ITS2 paired regions		ITS2 unpaired regions	
	GC content (%)	GC* content (%)	GC content (%)	GC* content (%)
<i>Corydalis yui</i>	85.83	73.76	62.73	62.91
<i>Corydalis flexuosa</i>	80.10	71.22	68.69	71.07
<i>Corydalis elata</i>	83.87	80.72	67.86	66.54
<i>Corydalis benecincta</i>	82.02	76.11	63.89	65.10
<i>Corydalis pseudosibirica</i>	86.64	74.61	58.51	60.76
<i>Corydalis melanochlora</i>	82.68	88.08	65.95	76.92
<i>Corydalis ophiocarpa</i>	82.84	72.82	64.41	64.57
<i>Corydalis trachycarpa</i>	82.44	76.43	63.86	67.96
<i>Corydalis laucheana</i>	85.03	73.23	68.48	69.88
<i>Corydalis incisa</i>	83.73	76.40	61.81	59.85
<i>Corydalis zhongdianensis</i>	86.76	75.69	62.22	65.87
<i>Corydalis bungeana</i>	85.69	73.06	66.91	63.36
<i>Corydalis shensiana</i>	80.87	72.83	65.65	78.79
<i>Corydalis rheinbabeniana</i>	83.22	76.39	62.27	71.36
<i>Corydalis pachypoda</i>	81.46	84.44	63.22	72.89
<i>Corydalis balansae</i>	81.61	78.48	64.96	70.84
<i>Corydalis lathyrophylla</i>	83.76	78.03	60.77	59.22
<i>Corydalis eugeniae</i>	84.17	84.42	61.26	69.20
<i>Corydalis caudata</i>	83.47	79.24	58.64	60.90
Average	83.48	77.16	63.79	67.26

Table S5. Comparison of substitution elements from intermediates to GC or AU base-pairs in an initial states of ITS2 rate matrix

ITS2 matrix	Elements of the ITS2 rate matrix															
	GU→GC	CC→GC	GG→GC	GA→GC	UC→GC	AC→GC	NN→GC ^a	F _(GC) ^a	GU→AU	AG→AU	AA→AU	CU→AU	UU→AU	AC→AU	NN→AU ^a	F _(AU) ^a
<i>Corydalis yui</i>	2.0814	13.8118	12.9177	12.9177	13.8118	12.8725	68.4129	0.45	0.282	2.6635	2.6635	1.7504	1.7504	2.4824	11.5922	0.0118
<i>Corydalis flexuosa</i>	1.9298	8.6349	7.532	7.532	8.6349	7.4393	41.7029	0.436	0.3848	2.5107	2.5107	1.5018	1.5018	2.1631	10.5729	0.0253
<i>Corydalis elata</i>	1.3008	9.2235	8.3515	8.3515	9.2235	5.9733	42.4241	0.4394	0.1972	2.1215	2.1215	1.2662	1.2662	1.3739	8.3465	0.0153
<i>Corydalis benecincta</i>	2.3459	11.7207	10.2196	10.2196	11.7207	17.6533	63.8798	0.4306	0.3989	3.0742	3.0742	1.7376	1.7376	4.6302	14.6527	0.0192
<i>Corydalis pseudosibirica</i>	2.6175	14.4411	13.4395	13.4395	14.4411	18.6593	77.038	0.4511	0.3134	2.6896	2.6896	1.6091	1.6091	3.4752	12.386	0.0101
<i>Corydalis melanochlora</i>	1.1091	8.107	7.1591	7.1591	8.107	4.592	36.2333	0.434	0.1651	2.1355	2.1355	1.0656	1.0656	1.2096	7.7769	0.017
<i>Corydalis ophiocarpa</i>	0.8815	14.7588	13.2883	13.2883	14.7588	4.7765	61.7522	0.441	0.1463	3.5486	3.5486	2.2059	2.2059	1.1485	12.8038	0.0176
<i>Corydalis trachycarpa</i>	1.1079	8.4045	7.8967	7.8967	8.4045	4.7597	38.47	0.4331	0.1972	2.0681	2.0681	1.4057	1.4057	1.1712	8.316	0.019
<i>Corydalis sheareri</i>	3.6867	0.721	0.721	0.721	0.721	0.721	7.2917	0.3692	0.3267	0.0639	0.0639	0.0639	0.0639	0.0639	0.6462	0.0327
<i>Corydalis curviflora</i>	0.3342	0.9581	0.9581	0.9581	0.9581	0.9581	5.1247	0.3531	0.045	0.129	0.129	0.129	0.129	0.129	0.69	0.0475
<i>Corydalis laucheana</i>	1.9557	17.7633	16.702	16.702	17.7633	11.5451	82.4314	0.4548	0.2975	3.5238	3.5238	2.5405	2.5405	2.2903	14.7164	0.0137
<i>Corydalis incisa</i>	1.3846	11.3371	10.3331	10.3331	11.3371	6.4377	51.1627	0.445	0.2065	2.6699	2.6699	1.5412	1.5412	1.5161	10.1448	0.0156
<i>Corydalis zhongdianensis</i>	2.6438	16.2952	15.1062	15.1062	16.2952	20.0838	85.5304	0.4521	0.3173	2.9824	2.9824	1.8129	1.8129	3.6758	13.5837	0.0099
<i>Corydalis bungeana</i>	2.1983	19.4145	18.3343	18.3343	19.4145	13.7283	91.4242	0.4579	0.3091	3.6581	3.6581	2.578	2.578	2.5867	15.368	0.0121
<i>Corydalis shensiana</i>	2.0713	15.4979	13.9021	13.9021	15.4979	13.9391	74.8104	0.4342	0.395	4.3312	4.3312	2.6509	2.6509	3.8956	18.2548	0.0231
<i>Corydalis rheinbabeniana</i>	1.9509	8.218	7.6522	7.6522	8.218	7.736	41.4273	0.4439	0.3326	1.8959	1.8959	1.3048	1.3048	1.7847	8.5187	0.0175
<i>Corydalis pachypoda</i>	2.2414	8.3518	7.4962	7.4962	8.3518	12.7751	46.7125	0.4297	0.4029	2.2523	2.2523	1.3475	1.3475	3.4451	11.0476	0.0208
<i>Corydalis balansae</i>	1.1039	11.4027	10.34	10.34	11.4027	5.2871	49.8764	0.4356	0.2076	2.9592	2.9592	1.9444	1.9444	1.3721	11.3869	0.0213
<i>Corydalis lathyrophylla</i>	2.0937	3.3294	3.1	3.1	3.3294	2.2486	17.2011	0.4382	0.3406	0.7431	0.7431	0.5043	0.5043	0.5019	3.3373	0.0159
<i>Corydalis eugeniae</i>	3.7566	2.9367	2.764	2.764	2.9367	3.9912	19.1492	0.4392	0.5941	0.6327	0.6327	0.4371	0.4371	0.8599	3.5936	0.015
<i>Corydalis caudata</i>	0.6925	6.6386	6.0624	6.0624	6.6386	5.1833	31.2778	0.3746	0.1043	1.6035	1.6035	0.9134	0.9134	1.252	6.3901	0.0136
Average	1.8804	10.0936	9.2512	9.2512	10.0936	8.6362	49.2063	0.4306	0.2840	2.2979	2.2979	1.4433	1.4433	1.9537	9.7202	0.0188

^a NN→GC/AU represent the sum substitution rates from the six intermediates to GC or AU; F_(GC)/ F_(AU) represent the GC or AU frequency in ITS2 rate matrix

Table S6. Comparison of substitution elements from intermediates to GC or AU base-pairs at equilibrium states of ITS2 rate matrix

ITS2 matrix	Elements of the ITS2 rate matrix															
	GU→GC	GG→GC	GA→GC	CC→GC	UC→GC	AC→GC	NN→GC ^a	F _(GC) ^a	GU→AU	AG→AU	AA→AU	CU→AU	UU→AU	AC→AU	NN→AU ^a	F _(AU) ^a
<i>Corydalis yui</i>	0.5438	9.7281	9.7281	6.6459	6.6459	2.2024	35.4942	0.4061	0.2468	1.4103	1.4103	4.415	4.415	0.4674	12.3648	0.0391
<i>Corydalis flexuosa</i>	0.2377	1.3756	1.3756	14.6282	14.6282	1.3756	33.6209	0.3827	0.1087	5.1577	5.1577	6.4597	6.4597	0.485	23.8285	0.0617
<i>Corydalis elata</i>	0.2496	7.9659	7.9659	6.0216	6.0216	0.7533	28.9779	0.4175	0.0501	1.7416	1.7416	1.599	1.599	0.2179	6.9492	0.0242
<i>Corydalis benecincta</i>	0.5937	6.5113	6.5113	19.3535	19.3535	8.806	61.1293	0.3925	0.4555	3.1203	3.1203	4.9965	4.9965	1.4198	18.1089	0.0486
<i>Corydalis pseudosibirica</i>	1.0353	6.0897	6.0897	4.4034	4.4034	2.1526	24.1741	0.4022	0.1784	2.5215	2.5215	1.0492	1.0492	1.2327	8.5525	0.0397
<i>Corydalis melanochlora</i>	0.3247	1.8528	1.8528	1.1736	1.1736	0.4234	6.8009	0.3771	0.04	0.1817	0.1817	0.228	0.228	0.0656	0.925	0.0072
<i>Corydalis ophiocarpa</i>	0.1642	6.9348	6.9348	6.4989	6.4989	0.8324	27.864	0.3787	0.0843	1.4543	1.4543	3.5599	3.5599	0.1863	10.299	0.0435
<i>Corydalis trachycarpa</i>	0.2525	4.7227	4.7227	3.2525	3.2525	0.7011	16.904	0.3876	0.0912	0.7526	0.7526	1.7062	1.7062	0.1622	5.171	0.0324
<i>Corydalis sheareri</i>	0.2131	5.628	5.628	5.628	5.628	5.628	28.3531	0.3498	0.0349	0.9206	0.9206	0.9206	0.9206	0.9206	4.6379	0.0572
<i>Corydalis curviflora</i>	0.1637	5.5714	5.5714	5.5714	5.5714	5.5714	28.0207	0.2991	0.0198	0.6721	0.6721	0.6721	0.6721	0.6721	3.3803	0.0361
<i>Corydalis laucheana</i>	1.3345	2.133	2.133	2.2166	2.2166	1.3468	11.3805	0.385	0.3383	1.0493	1.0493	0.5407	0.5407	0.6375	4.1558	0.0462
<i>Corydalis incisa</i>	0.4907	3.0076	3.0076	0.7464	0.7464	0.5776	8.5763	0.3629	0.1218	0.646	0.646	0.7464	0.7464	0.2488	3.1554	0.0388
<i>Corydalis zhongdianensis</i>	1.0547	6.7744	6.7744	3.2756	3.2756	2.1971	23.3518	0.4014	0.3444	1.0164	1.0164	2.2118	2.2118	0.6817	7.4825	0.0407
<i>Corydalis bungeana</i>	0.752	7.2438	7.2438	7.2332	7.2332	1.9695	31.6755	0.4076	0.3122	2.332	2.332	3.0071	3.0071	0.635	11.6254	0.0546
<i>Corydalis shensiana</i>	0.3448	3.5918	3.5918	1.865	1.865	0.5342	11.7926	0.3441	0.0961	1.0341	1.0341	1.0012	1.0012	0.2962	4.4629	0.0532
<i>Corydalis rheinbabeniana</i>	0.4214	2.8772	2.8772	1.6714	1.6714	0.7024	10.221	0.3681	0.1376	0.467	0.467	0.9391	0.9391	0.1963	3.1461	0.0336
<i>Corydalis pachypoda</i>	0.4799	2.9927	2.9927	1.943	1.943	0.9082	11.2595	0.4049	0.0998	0.2872	0.2872	0.6222	0.6222	0.1342	2.0528	0.0124
<i>Corydalis balansae</i>	0.3119	4.0553	4.0553	4.89	4.89	1.3235	19.526	0.3954	0.11	1.023	1.023	1.4298	1.4298	0.2769	5.2925	0.0292
<i>Corydalis lathyrophylla</i>	1.0384	1.6412	1.6412	1.6004	1.6004	1.0126	8.5342	0.3813	0.2673	0.4903	0.4903	0.4225	0.4225	0.3102	2.4031	0.0301
<i>Corydalis eugeniae</i>	1.458	1.8869	1.8869	1.2379	1.2379	0.9565	8.6641	0.3925	0.2141	0.2995	0.2995	0.2771	0.2771	0.2314	1.5987	0.0139
<i>Corydalis caudata</i>	0.329	2.9839	2.9839	1.3437	1.3437	0.5189	9.5031	0.3504	0.0765	0.4398	0.4398	0.6939	0.6939	0.1698	2.5137	0.0267
Average	0.5616	4.5509	4.5509	4.8191	4.8191	1.9283	21.2297	0.3803	0.1632	1.2865	1.2865	1.7856	1.7856	0.4594	6.7670	0.0366

^a NN→GC/AU represent the sum substitution rates from the six intermediates to GC or AU; F_(GC)/ F_(AU) represent the GC or AU frequency in ITS2 rate matrix

Table S7. Mismatches transformation inferring from the best-fit double-substitution rate matrix at initial states of ITS2 rate matrix

ITS2 matrix	Mismatches transformation							
	AG		AC		GU		CU	
	AG→CG	AG→AU	AC→GC	AC→AU	GU→GC	GU→AU	CU→CG	CU→AU
<i>Corydalis yui</i>	12.9177	2.6635	12.8725	2.4824	2.0814	0.282	13.8118	1.7504
<i>Corydalis flexuosa</i>	7.532	2.5107	7.4393	2.1631	1.9298	0.3848	8.6349	1.5018
<i>Corydalis elata</i>	8.3515	2.1215	5.9733	1.3739	1.3008	0.1972	9.2235	1.2662
<i>Corydalis benecincta</i>	10.2196	3.0742	17.6533	4.6302	2.3459	0.3989	11.7207	1.7376
<i>Corydalis pseudosibirica</i>	13.4395	2.6896	18.6593	3.4752	2.6175	0.3134	14.4411	1.6091
<i>Corydalis melanochlora</i>	7.1591	2.1355	4.592	1.2096	1.1091	0.1651	8.107	1.0656
<i>Corydalis ophiocarpa</i>	13.2883	3.5486	4.7765	1.1485	0.8815	0.1463	14.7588	2.2059
<i>Corydalis trachycarpa</i>	7.8967	2.0681	4.7597	1.1712	1.1079	0.1972	8.4045	1.4057
<i>Corydalis sheareri</i>	0.8578	0.0639	0.721	0.0639	3.6867	0.3267	0.8578	0.0639
<i>Corydalis curviflora</i>	1.1792	0.129	0.9581	0.129	0.3342	0.045	1.1792	0.129
<i>Corydalis laucheana</i>	16.702	3.5238	11.5451	2.2903	1.9557	0.2975	17.7633	2.5405
<i>Corydalis incisa</i>	10.3331	2.6699	6.4377	1.5161	1.3846	0.2065	11.3371	1.5412
<i>Corydalis zhongdianensis</i>	15.1062	2.9824	20.0838	3.6758	2.6438	0.3173	16.2952	1.8129
<i>Corydalis bungeana</i>	18.3343	3.6581	13.7283	2.5867	2.1983	0.3091	19.4145	2.578
<i>Corydalis shensiana</i>	13.9021	4.3312	13.9391	3.8956	2.0713	0.395	15.4979	2.6509
<i>Corydalis rheinbabeniana</i>	7.6522	1.8959	7.736	1.7847	1.9509	0.3326	8.218	1.3048
<i>Corydalis pachypoda</i>	7.4962	2.2523	12.7751	3.4451	2.2414	0.4029	8.3518	1.3475
<i>Corydalis balansae</i>	10.34	2.9592	5.2871	1.3721	1.1039	0.2076	11.4027	1.9444
<i>Corydalis lathyrophylla</i>	3.1	0.7431	2.2486	0.5019	2.0937	0.3406	3.3294	0.5043
<i>Corydalis eugeniae</i>	2.764	0.6327	3.9912	0.8599	3.7566	0.5941	2.9367	0.4371
<i>Corydalis caudata</i>	6.0624	1.6035	5.1833	1.252	0.6925	0.1043	6.6386	0.9134
Average	9.2683	2.2979	8.6362	1.9537	1.8804	0.2840	10.1107	1.4433

Table S8. Mismatches transformation inferring from the best-fit double-substitution rate matrix at equilibrium states ITS2 rate matrix

ITS2 matrix	Mismatches transformation							
	AG		AC		GU		CU	
	AG→CG	AG→AU	AC→GC	AC→AU	GU→GC	GU→AU	CU→CG	CU→AU
<i>Corydalis yui</i>	9.7281	1.4103	2.2024	0.4674	0.5438	0.2468	6.6459	4.415
<i>Corydalis flexuosa</i>	14.1245	5.1577	1.3756	0.485	0.2377	0.1087	14.6282	6.4597
<i>Corydalis elata</i>	7.9659	1.7416	0.7533	0.2179	0.2496	0.0501	6.0216	1.599
<i>Corydalis benecincta</i>	6.5113	3.1203	8.806	1.4198	0.5937	0.4555	19.3535	4.9965
<i>Corydalis pseudosibirica</i>	6.0897	2.5215	2.1526	1.2327	1.0353	0.1784	4.4034	1.0492
<i>Corydalis melanochlora</i>	1.8528	0.1817	0.4234	0.0656	0.3247	0.04	1.1736	0.228
<i>Corydalis ophiocarpa</i>	6.9348	1.4543	0.8324	0.1863	0.1642	0.0843	6.4989	3.5599
<i>Corydalis trachycarpa</i>	4.7227	0.7526	0.7011	0.1622	0.2525	0.0912	3.2525	1.7062
<i>Corydalis sheareri</i>	5.7499	0.9206	5.628	0.9206	0.2131	0.0349	5.7499	0.9206
<i>Corydalis curviflora</i>	8.0881	0.6721	5.5714	0.6721	0.1637	0.0198	8.0881	0.6721
<i>Corydalis laucheana</i>	2.133	1.0493	1.3468	0.6375	1.3345	0.3383	2.2166	0.5407
<i>Corydalis incisa</i>	3.0076	0.646	0.5776	0.2488	0.4907	0.1218	1.4999	0.7464
<i>Corydalis zhongdianensis</i>	6.7744	1.0164	2.1971	0.6817	1.0547	0.3444	3.2756	2.2118
<i>Corydalis bungeana</i>	7.2438	2.332	1.9695	0.635	0.752	0.3122	7.2332	3.0071
<i>Corydalis shensiana</i>	3.5918	1.0341	0.5342	0.2962	0.3448	0.0961	1.865	1.0012
<i>Corydalis rheinbabeniana</i>	2.8772	0.467	0.7024	0.1963	0.4214	0.1376	1.6714	0.9391
<i>Corydalis pachypoda</i>	2.9927	0.2872	0.9082	0.1342	0.4799	0.0998	1.943	0.6222
<i>Corydalis balansae</i>	4.0553	1.023	1.3235	0.2769	0.3119	0.11	4.89	1.4298
<i>Corydalis lathyrophylla</i>	1.6412	0.4903	1.0126	0.3102	1.0384	0.2673	1.6004	0.4225
<i>Corydalis eugeniae</i>	1.8869	0.2995	0.9565	0.2314	1.458	0.2141	1.2379	0.2771
<i>Corydalis caudata</i>	2.9839	0.4398	0.5189	0.1698	0.329	0.0765	1.3437	0.6939
Average	5.2836	1.2865	1.9283	0.4594	0.5616	0.1632	4.9806	1.7856

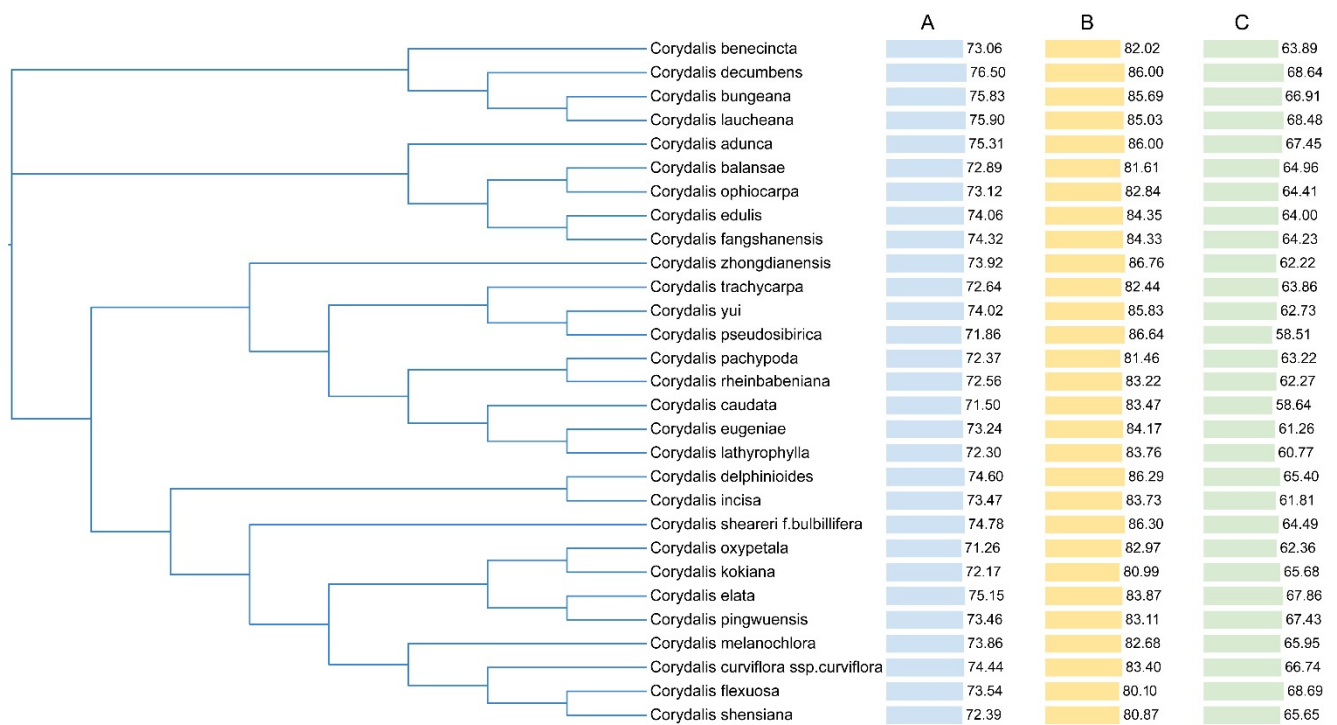


Figure S1. Variation of ITS2 GC content across the 29 *Corydalis* phylogeny. A, B and C value followed species name represents GC content of the entire ITS2 region, ITS2 paired region and ITS2 unpaired region, respectively. The phylogeny was constructed from based on the recent phylogeny of *Corydalis* (Xu et al., 2022).