



Spatiotemporal evolution of *Reaumuria* (Tamaricaceae) in Central Asia: insights from molecular biogeography

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Abstract

Reaumuria is an arid adapted genus with a distribution center in Central Asia; its evolution and dispersal is investigated in this paper. Eighteen species of *Reaumuria* and nine species of two other genera in the Tamaricaceae, *Tamarix* and *Myricaria*, were sampled, and four markers ITS, *rps16*, *psbB-psbH*, and *trnL-trnF* were sequenced. The reconstructed phylogenetic tree is fundamentally consistent with previous morphological classification, except that *R. soongorica*, sometimes considered to be a separate genus or subgenus, is completely nested within *Reaumuria*. The ancestral area of the genus is suggested to be western Central Asia, and distributions in the Iran-Mediterranean area and the Tianshan and Pamir-Alai mountains are inferred as dispersals. Westward dispersals to the Iran-Mediterranean were ancient Oligocene to Miocene, whereas dispersals eastward were recent. The spatiotemporal evolution of *Reaumuria* is used as a link to abiotic paleoclimatic and geological events, in particular, increased aridity beginning at the Eocene-Oligocene Transition (EOT), and as a result of uplift of the Himalayas and Qinghai-Tibet Plateau (QTP). The diversification of the two sections (22.51–19.78 Ma) suggests a response to increasing aridification in response to QTP uplift and expansion.

Key words: *Reaumuria*, Central Asia, molecular clock, evolution, EOT, QTP

Introduction

Central Asia occupies a vast area of the Eurasian continent, from across the Caspian Sea to the Daxinan Mountains in northeastern China, and northward to Siberia and the Altai mountains and southward to the Kunlun and Hindukush mountains and the Iranian Plateau. In brief it includes in the western part, five countries of Middle Asia, and in the east, an arid area consisting of northwestern China and southern Mongolia, see Fig. 1. According to the floristic divisions of Grubov (1999), the Central Asian flora can be divided into three parts, Mongolian, Junggar–Turanian, and Tibetan, although most of the area of Tibet is considered to belong to East Asia (Wu and Wu, 1996). The vegetation of Central Asia is fundamentally grassland and desert; however, it also includes montane forests of the Tianshan and Pamir-Alai ranges. Central Asia is characterized by an arid climate with low annual precipitation, and is arid-hot in summer and cold in winter (Grubov, 1999). The ancestor of the sister arid-adapted families Tamaricaceae and Frankeniaceae may have been an inhabitant of the northern latitudinal arid zone that existed in southern Asia before QTP uplift (Guo et al., 2008), and adapted to saline environments associated with borders of the Paratethys Sea. The subsequent spatiotemporal evolution of organisms in this region is related in large part to the process of increasing aridification and continentality, controlled by factors such as westward

retreat of the inland Paratethys Sea, and uplift of the Himalayas and Qinghai-Tibetan Plateau (QTP) (Guo et al., 2008; Clift et al., 2010; Miao et al., 2012). Concerning paleoclimates, the most remarkable events were global aridification and cooling, with the start of glaciation in Antarctica, at the Eocene-Oligocene Transition (EOT) (Liu et al., 2009b; Xiao et al., 2010; Buerki et al., 2013); the deposition of hundreds of meters of dust sediments in the Chinese Loess Plateau under influence of the dry winter Asian monsoon, beginning at the Oligocene-Miocene boundary and strengthening thereafter (Guo et al., 2008); and further global climatic cooling and deterioration, such as at 8~7 Ma (Quade et al., 1989, Willis and McElwain, 1992; Coleman and Hodges, 1995; Li and Fang, 1998; Shi et al., 1998, 1999), and strengthening of the monsoons in East and South Asia (An et al., 2001; Miao et al., 2012). Based on climate simulations, Miao et al. (2012) recognized QTP uplift or global climate cooling during 17~5 Ma, as controlling factors likely to have influenced aridification in Central Asia.

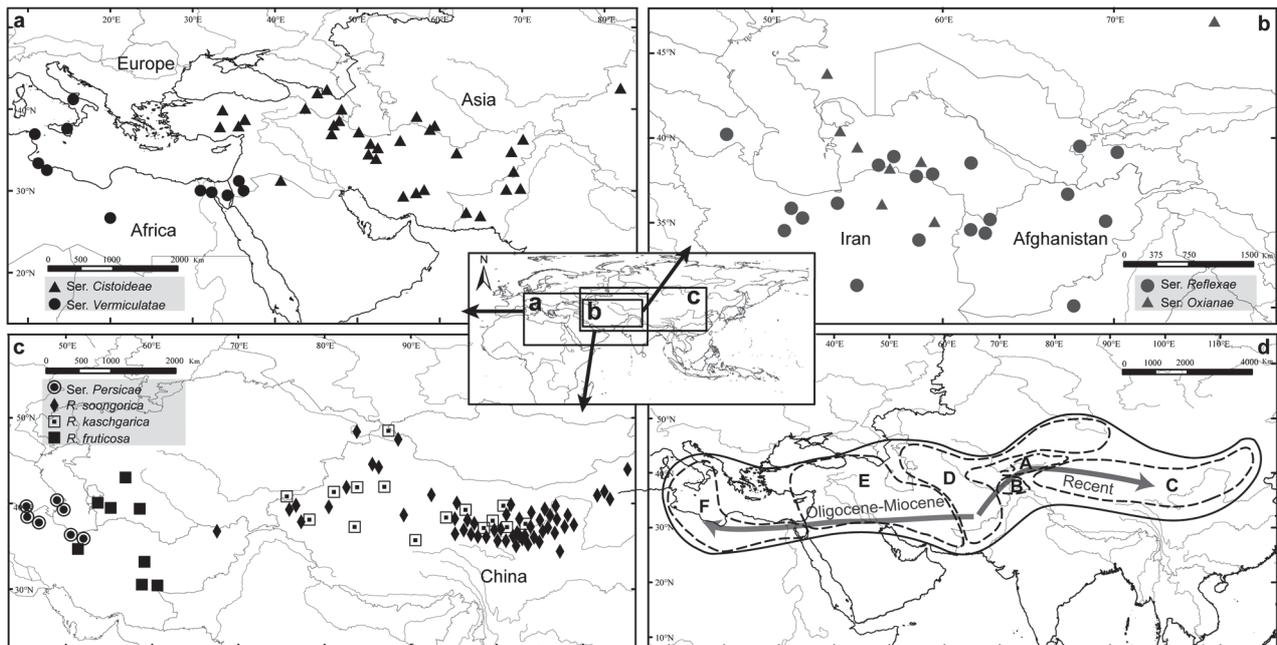


FIGURE 1. Distribution of *Reaumuria*, modified from Hao et al. (2013). Three species of series *Kaschgaricae* are detailed in Fig. 1c, six distribution areas, and westward and eastward dispersals conducted from biogeographical history reconstruction are illustrated in Fig. 1d.

Reaumuria, belonging to Tamaricaceae, comprises 12–21 species (Gorshkova, 1949; Gaskin, et al., 2004; Yang and Gaskin, 2006; Hao et al. 2013). The generic distribution center is Central Asia, with taxa extending into western Asia, southern Europe, and northern Africa. A taxonomic question exists regarding the genus; using *R. soongarica* and its character of solitary flowers as the type species for the new name *Hololachna soongarica*, Ehrenberg in 1827 separated this genus from *Reaumuria*. Maximowicz (1889) later returned it as a subgenus, but most authors have agreed that it should be only a species (e.g. Schiman-Czeika, 1964; Mabberly, 1993; Gaskin, 2003; Yang and Gaskin, 2006). Nevertheless, DNA sequence evidence in one case did seem to support its status as a genus (Gaskin et al., 2004). Also, there had been no world classification monograph regarding these taxa up until very recently (Hao et al., 2013), only regional ones, such as of the Former Union Soviet, China, Mongolia, Iran, and Pakistan. Twenty-one species are currently recognized.

Therefore, we sampled about 18 species from *Reaumuria*, in attempt to: (1) reconstruct a phylogenetic tree to confirm morphological classification; (2) date the diversification epoch and reconstruct biogeographic history of the genus; and (3) reveal this genus as a useful model for elucidation of floristic origins in Central Asia.

Materials and Methods

Taxon sampled

Eighteen species (43 samples) of *Reaumuria*, with outgroups consisting of 4 species of *Tamarix* and 5 of

Myricaria, or in total, 27 species and 52 samples served as sources of DNA material, see Table 1. The herbaria utilized in China are as follows: PE (Institute of Botany, Chinese Academy of Sciences (CAS), Beijing), and XJBI (Xinjiang Institute of Ecology and Geography, CAS, Urumqi, Xinjiang); Russia: LE (Komarov Botanical Institute, Russian Academy of Sciences, St. Petersburg, Russia), MW (Moscow University, Moscow, Russia), and MAH (Main Botanical Garden, Russian Academy of Sciences, Russia).

DNA sequencing

Total genomic DNA was extracted using the CTAB method (Doyle and Doyle, 1987). The polymerase chain reaction (PCR) was used for amplification of double stranded DNA. The 25 µl reaction system contained 0.25 µl of Ex Taq, 2.5 µl of 10× Ex Taq buffer (Mg²⁺ concentration of 25 mM), 2.0 µl of dNTP mix (2.5 mM concentration for each dNTP), 1 µl of the forward and reverse primers at 5 µmol/µl, and 0.5 µl of template DNA. The following primers were used: for ITS: ITS1-*f* (5'-AGA AGT CGT AAC AAG GTT TCC GTA GC-3') and ITS4-*r* (5'-TCC TCC GCT TAT TGA TAT GC-3'), for *trnL-trnF*: *trnL-f* (5'-CGA AAT CGG TAG ACG CTA CG-3') and *trnF-r* (5'-ATT TGA ACT GGT GAC ACG AG -3'), for the intron of *rps16*: *rps16-f* (5'-GTG GTA GAA AGC AAC GTG CGA CTT-3'), and for *rps16-r* (5'-TCG GGA TCG AAC ATC AAT TGC AAC-3') (Oxelman et al., 1997); and the intergenic spacer *psbB-psbH*: *psbB-psbH-r* (5'-TTCAACAGTTTGTGTAGCCA-3') and *psbB-psbH-f* (5'-AGATGTTTTTGCTGGTATTGA-3') (Xu et al. 2000).

The protocol for amplification consisted of an initial hotstart at 95 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 10 min. PCR products were purified using the PEG precipitation procedure (Johnson and Soltis, 1995). They were sequenced using an ABI Prism 3770 Genetic Analyzer (Shanghai Sangon Biological Engineering Technology & Service, Shanghai, China). Sequences were aligned using CLUSTAL X software (Thompson et al., 1997), and then adjusted by hand. All gaps were treated as missing characters. Finally, a combined dataset consisting of ITS and the three cpDNA sequences was prepared for phylogenetic analysis.

Phylogenetic analysis and divergence time estimates

The sequence dataset comprised a total of 3269 aligned nucleotide characters from the four genes, ITS, *trnL-trnF*, *rps16*, and *psbB-psbH*. The incongruence length difference (ILD) test of the four gene datasets for the same 60 taxa was carried out in PAUP* (Farris et al., 1994), to assess potential conflicts between different DNA fragments. This test was implemented with 100 partition-homogeneity test replicates, using a heuristic search option with simple addition of taxa, TBR branch swapping and MaxTrees set to 1000. A value of 0.222 from the incongruence length difference (ILD) tests (Farris et al., 1994) showed that the four gene datasets were not incongruent.

Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian inference. ML analysis was performed with PAUP; clade support was estimated with 1,000 heuristic bootstrap replicates (100 random addition cycles per replicate, with tree bisection-reconnection and branch-swapping) (Felsenstein, 1985; Hillis and Bull 1993). For ML analysis, Modeltest 3.06 (Posada and Crandall, 1998) was used to estimate the appropriate model of DNA substitution for the sequence data. The model selected by the Akaike information criterion (AIC) was GTR+I+G. The related parameters of Modeltest were used for the ML analysis.

Bayesian inference and divergence time estimates were conducted using a Bayesian method implemented in BEAST v1.7.5 (<http://beast.bio.ed.ac.uk/>), employing a relaxed clock model (Drummond et al., 2006; Drummond and Rambaut, 2007). We used the uncorrelated lognormal clock with a Yule process for the speciation model, GTR+I+G for the substitution model (estimated for the dataset), and a normal distribution with SD of 1 as priors on the calibration nodes to accommodate calibration uncertainty. The normal priors are constrained to the root of all taxa, namely the family Tamaricaceae 70 Ma, Stdev=1, and genus *Tamarix* constraint 25 Ma for two priors are: uniform lower=25, upper=70; lognormal mean=0, Stdev=1, offset=25. A Markov chain Monte Carlo was run for 50 million generations and sampled every 1,000 generations. Two independent runs for two priors of *Tamarix* constraint were performed to confirm the convergence of the analysis. The two runs were combined using TreeCombiner v1.7.5 (<http://beast.bio.ed.ac.uk/>). The last 40 million generations were used to construct the maximum clade credibility tree and the associated 95% highest posterior density distributions around the estimated node ages under TreeAnnotator v1.7.5 (<http://beast.bio.ed.ac.uk/>).

TABLE 1. Voucher information for *Reaumuria* species and two genera *Myricaria* and *Tamarix* as outgroups

Species	Voucher	Source	GenBank			
			ITS	<i>trnL-trnF</i>	<i>rps16</i>	<i>psbB-psbH</i>
<i>Reaumuria</i> L.						
<i>R. alternifolia</i> (Labill.) Britt. 1	I. Rusanovich, 19880616 (MHA)	Vostochnaya, Russia	KJ729611	KJ729765	KJ729714	KJ729663
<i>R. alternifolia</i> (Labill.) Britt. 2	A. P. Khokhriakov, 19960808 (MHA)	OHu-Narman, Erzurum, Turkey	KJ729612	KJ729766	KJ729715	KJ729664
<i>R. alternifolia</i> (Labill.) Britt. 3	B. Wakaksh, 19630716 (LE)	Armenia, Caucasus	KJ729613	KJ729767	KJ729716	KJ729665
<i>R. alternifolia</i> (Labill.) Britt. 4	L.A. Kramarenko, 19850528 (MW)	Navoi, Uzbekistan	KJ729614	KJ729768	KJ729717	KJ729666
<i>R. babataghi</i> Botsch.	Li & P.I. Tzukervanik 19790519-275 (LE)	Ridge Babatag, Uzbekistan	KJ729615	KJ729769	KJ729718	KJ729667
<i>R. badghysi</i> E. Kor. 1	T. Gorelova s.n. 19780607 (LE)	Badhыз, Turkmenistan	KJ729616	KJ729770	KJ729719	KJ729668
<i>R. badghysi</i> E. Kor. 2	T. Gorelova s.n. 19770916 (LE)	lake Er-Ojlan-duz, Badkhyz, Turkmenistan	KJ729617	-----	KJ729720	KJ729669
<i>R. botschantzevii</i> Zuckerw. & Kurbanov 1	D. Kurbanov s.n. 19810516 (LE)	Kyurendag, Turkmenistan	KJ729618	KJ729771	KJ729721	KJ729670
<i>R. botschantzevii</i> Zuckerw. & Kurbanov 2	D. Kurbanov s.n. 19810516 (LE)	Akooba, Kjurendag, Turkmenistan	KJ729619	KJ729772	KJ729722	KJ729671
<i>R. cistoide</i> Adam.	B. Alezesh, 19610628 (LE)	Nakhichevan, Caucasus	KJ729620	KJ729773	KJ729723	KJ729672
<i>R. fruticosa</i> Bge. 1	V.P. Bochantsev, 19700710 104 (LE)	Kyzyl Kum desert, Uzbekistan	KJ729621	KJ729774	KJ729724	KJ729673
<i>R. fruticosa</i> Bge. 2	Proskuryakova, 19700520 (MW)	Mella-Kara, Turkmenistan	KJ729622	KJ729775	KJ729725	KJ729674
<i>R. hirtella</i> Jaub. & Spach 1	A. Danin, 19790514 (MHA)	Judean desert, Israel	KJ729623	KJ729776	KJ729726	KJ729675
<i>R. hirtella</i> Jaub. & Spach 2	V.P. Bochantsev, 19620507 (LE)	Abu Zenima, Egypt	KJ729624	KJ729777	-----	KJ729676
<i>R. hirtella</i> Jaub. & Spach 3	W. Frey, 19800708-763 (LE)	Amman, Jordan	KJ729625	KJ729778	KJ729727	KJ729677
<i>R. hypericoides</i> Willd. 1	Ju. Trouba, 19780721-702 (LE)	Azerbaijan, Caucasus	KJ729626	KJ729779	KJ729728	KJ729678
<i>R. hypericoides</i> Willd. 2	Gogina, 19660718-1508 (MW)	Azerbaijan, Caucasus	KJ729627	KJ729780	KJ729729	KJ729679
<i>R. kaschgarica</i> Rupr.	V.P. Bochantsev, T.A. Aidarova, I.T. Sudnitsina, 19790814 (LE)	Narynskaya, Kirgizstan	KJ729628	KJ729781	KJ729730	KJ729680

TABLE 1. (Continued)

Species	Voucher	Source	GenBank			
			ITS	<i>trnL-trnF</i>	<i>rps16</i>	<i>psbB-psbH</i>
<i>R. korovinii</i> Botsch. & Lincz.	T. Gorelova, 19790926 (LE)	Badkhyz, Turkmenistan	KJ729629	KJ729782	KJ729731	KJ729681
<i>R. mucronata</i> Jaub. & Spach	V.P. Bochantsev, 19620406 (LE)	W. desert of Mediterranean coast, Egypt	KJ729630	KJ729783	KJ729732	KJ729682
<i>R. oxiata</i> (Ldb.) Boiss.	B. Shelbaev s.n. 19680626 (LE)	Karakalpakia, Uzbekistan	KJ729631	-----	KJ729733	KJ729683
<i>R. persica</i> Boiss. 1	L. Smoljcaninora, An. Fedorov, 19520831 (MHA)	Diza, Nahichevan, Caucasus	KJ729632	KJ729784	KJ729734	KJ729684
<i>R. persica</i> Boiss. 2	Ya. Tschkprzhs, 19590729 (LE)	Armenia, Caucasus	KJ729633	KJ729785	KJ729735	KJ729685
<i>R. persica</i> Boiss. 3	L. Smoljcaninora, 19520831 (MW)	Nakhichevan, Azerbaijan, Caucasus	KJ729634	KJ729786	KJ729736	KJ729686
<i>R. reflexa</i> Lipsky 1	F. Hasanov, 19830625 (LE)	Kugitang Mountains, Uzbekistan	KJ729635	KJ729787	KJ729737	KJ729687
<i>R. reflexa</i> Lipsky 2	D. Kurbanov, 19830525 (LE)	Kyurendag, Turkmenistan	KJ729636	KJ729788	KJ729738	KJ729688
<i>R. sogdiana</i> Kom. 1	V.P. Bochantsev 19790819-275 (MW)	Ala-Areha, Kirgizstan	KJ729637	KJ729789	KJ729739	-----
<i>R. sogdiana</i> Kom. 2	T. Egorova, 19600711-1778 (LE)	Zarafshan, Uzbekistan	KJ729638	KJ729790	KJ729740	KJ729689
<i>R. soongarica</i> (Pall.) Maxim. 1	M.G. Pimenov, E.G. Kluikov 79 19890731-79 (MW)	S. Kaviuksu River, S. slope of the Susamyr range, Kirgizstan	KJ729639	KJ729791	KJ729741	KJ729690
<i>R. soongarica</i> (Pall.) Maxim. 2	C.A. Tubtsnov, 19890726-8 (MW)	Dundgovi, Mongolia	KJ729640	KJ729792	KJ729742	KJ729691
<i>R. soongarica</i> (Pall.) Maxim. 3	L.I. Ivanina, 19780713-600 (LE)	Borohudzir, Tadzhikistan	KJ729641	KJ729793	KJ729743	KJ729692
<i>R. soongarica</i> (Pall.) Maxim. 4	V.P. Bochantsev, R.A. Aidarova 19790821 (LE)	Issyk-Kul, Kirgizstan	KJ729642	KJ729794	KJ729744	KJ729693
<i>R. soongarica</i> (Pall.) Maxim. 5	I.O. Baitulin, N.K. Aralbaev, S.T. Nesterova, 19970629 (LE)	Northern Ciszaissania, Kazakhstan	KJ729643	KJ729795	KJ729745	KJ729694
<i>R. soongarica</i> (Pall.) Maxim. 6	C.A. Tchbanov, 19890726-6 (MW)	Dundgovi, Mongolia	KJ729644	KJ729796	KJ729746	KJ729695
<i>R. turkestanica</i> Gorschk. 1	T. Chtskern, 197007 (MW)	Namtso, China	KJ729645	KJ729797	KJ729747	KJ729696
<i>R. turkestanica</i> Gorschk. 2	A. Sukhorukov, 20010615 (MW)	Respublica, Uzbekistan	KJ729646	KJ729798	KJ729748	KJ729697

TABLE 1. (Continued)

Species	Voucher	Source	GenBank			
			ITS	<i>trnL-trnF</i>	<i>rps16</i>	<i>psbB-psbH</i>
<i>R. turkestanica</i> Gorschk. 3	Wendelbo, Foroughi, 19740909, 14632 (LE)	Kavir Protected Area, Tehran, Iran	KJ729647	KJ729799	KJ729749	KJ729698
<i>R. turkestanica</i> Gorschk. 4	V.P. Bochantsev, 19850722 (LE)	Shurob, Uzbekistan	KJ729648	KJ729800	KJ729750	KJ729699
<i>R. turkestanica</i> Gorschk. 5	P.I. Tzukervanik, 19790519-275 (LE)	Babatag, Uzbekistan	KJ729649	KJ729801	KJ729751	KJ729700
<i>R. turkestanica</i> Gorschk. 6	A. Schkhorukov, 20010615 (MW)	Gulistan, Syrdaria, Uzbekistan	KJ729650	KJ729802	KJ729752	KJ729701
<i>R. turkestanica</i> Gorschk. 7	R. Kamelin s.n. 19700725 (LE)	Talasskaya valley, Kirgizstan	KJ729651	KJ729803	KJ729753	KJ729702
<i>R. vermiculata</i> Linn. 1	D. Podlech, 19840601-38447 (LE)	Sfax, Tunisia	KJ729652	KJ729804	KJ729754	KJ729703
<i>R. vermiculata</i> Linn. 2	U. Pratov, 19780606-293 (LE)	Gardetal Haiba, Libya	KJ729653	KJ729805	KJ729755	KJ729704
<i>Myricaria</i> Desv.						
<i>M. alopecuroides</i> Schrenk	P. Yan 3650 (SHI)	Tashikurgan, Xinjiang, China	KJ729654	KJ729806	KJ729756	KJ729705
<i>M. bracteata</i> Royle	Y.H. Wu 36461 (HNWP)	Nuomuhong, Dulan, Qinghai, China	KJ729655	KJ729807	KJ729757	KJ729706
<i>M. dahurica</i> DC.	V. Kchvaev, 19880817-1239-1(MW)	Altai, Siberia	KJ729656	KJ729808	KJ729758	KJ729707
<i>M. longifolia</i> Ehrenb.	V.V. Stupin, 20070803-S-189 (MW)	Hovsgol, Mongolia	KJ729657	KJ729809	KJ729759	KJ729708
<i>M. squamosa</i> Desv.	P. Yan 4002 (SHI)	Bandir, Tashkurgan, Xinjiang, China	KJ729658	KJ729810	KJ729760	KJ729709
<i>Tamarix</i> L.						
<i>T. Karakalensis</i> Freyn	K.B. Blinkovsky 12 VIII 1953 (LE)	C. Kopetdag, Ashkhabad, Turcominia	KJ729659	KJ729811	KJ729761	KJ729710
<i>T. Laxa</i> Willd.	O.N. Demina 18 V 2001 (LE)	Orlovsky, Bostov, Russia	KJ729660	-----	KJ729762	KJ729711
<i>T. Meyeri</i> Boiss.	M.R. Tanybaeva 12 V 2007 (LE)	Turkestan Ridge, Kirgiztan	KJ729661	KJ729812	KJ729763	KJ729712
<i>T. Ramosissima</i> Kar. ex Boiss.	N.A. Brykova s.n. 10 VII 1998 (LE)	Orlovsky, Bostov, Russia	KJ729662	KJ729813	KJ729764	KJ729713

Meanwhile, since 70 Ma root age of family is not directly come from exact fossil, it probably should be expanded the age from the root. This was implemented by employing so-named “soft maximum bound” strategy (Yang and Rannala 2006; Ho 2007; Sanders and Lee 2007; Ho and Phillips 2009; Inoue 2010) to defense the uncertainty resulting from root 70 Ma and used normal distribution. We treated root age as exponent distribution (offset=70, mean=71.75, 70x2.5%) and Gamma distribution (offset=70) respectively. *Tamarix* was constrained 25 Ma as prior lognormal distribution, with a Birth-Death process for the speciation model, strict clock, and HKY + Gamma for the substitution model in terms of rule of soft maximum bound.

Divergence time estimate constraint

Tamaricaceae is included in the Caryophyllales (AGP 2009; Reveal 2011). The earliest reliable fossil record is of *Tamarix*, Miocene at 25 Ma, from the Yunnan province of China (IB-NIGP, 1978). Even though Tamaricaceae currently has no reliable earlier macrofossil record, an estimate of its age can be approximated by considering its nature and the history of related families. Tiffney (1985) has made the generalization that extant woody families originated during the Cretaceous to early Eocene, while herbaceous families appeared during the late Oligocene to Miocene. According to pollen records and Angiosperm molecular dating, families related to the Tamaricaceae, such as Polygonaceae and Amaranthaceae / Chenopodiaceae, have an approximate root age of ca. 65 Ma (Muller 1984), and Caryophyllaceae of ca. 60-80 Ma (Eggens 2006; Foir et al. 2005). In the light of these and the general age of woody families, Tamaricaceae might have a crown age of about 70 Ma, and this was chosen as the basis for our molecular dating.

Ancestral area reconstruction

Operational areas

As seen from the distributions of *Reaumuria* at sectional and series levels (Fig. 1a,b,c), most species occur in Central Asia. We have followed the floristic divisions of Grubov (1999) in dividing Central Asia into eastern and western parts, and separate the Tianshan and Pamir-Alai Mountains from Central Asia due to the distinctiveness of their montane vegetation as compared to grasslands and desert. In addition, the distribution of *Reaumuria* was considered, especially its endemism. In fine, six areas were distinguished for the present biogeographical study, namely, A: Tianshan; B: Pamir-Alai; C: eastern Central Asia, including arid northwestern China and Mongolia; D: western Central Asia, namely the Turan-Junggar sensu Grubov (1999), including five countries of Central Asia of the former Soviet Russia; E: Iran-Turkey; and F: the Mediterranean, including southern Europe and northern Africa. See Fig. 1d.

Ancestral area reconstruction

To infer vicariance and dispersal events, three methods were used: a parsimony-based method (Diva v.1.1; Ronquist, 1997) and S-Diva (or Bayes-DIVA, Nylander et al., 2008; Yu et al. 2010), and a maximum likelihood-based DEC model (Lagrange v.2.0.1; Ree et al. 2005; Ree and Smith, 2008). S-Diva adds a statistical attribute, and is an improvement over Diva. The two popular approaches S-Diva and Lagrange were employed to assess the relevant explanatory processes of vicariance, dispersal, and extinction in biogeography.

S-Diva S-Diva determines statistical support for ancestral range reconstructions using multiple trees from Bayesian analysis (Nylander et al., 2008). This has the advantage that uncertainties in phylogenetic inference can be taken into account. In general, 100 Bayesian MCMC trees with stable topologies from BEAST were input into the S-Diva program RASP (Yu et al. 2010). Here for *Reaumuria*, the 781 last stable Bayesian inference trees by BEAST were input to the program RASP. Biogeographical events such as dispersal, vicariance and extinction were calculated under Tree View Form in RASP.

Lagrange Parametric likelihood analysis, with a dispersal–extinction–cladogenesis model (Ree et al. 2005), was implemented in Lagrange v. 2.0.1 (Ree and Smith 2008). This methodology calculates the likelihood of biogeographical routes and areas occupied by the most recent common ancestor (MRCA) for a given phylogenetic tree topology and the present distribution of taxa. Therefore, dispersal and vicariance of lineages, represented by connection areas, can be estimated by the probabilities. This is a form of MRCA area reconstruction differing from the parsimony approach of Diva and S-Diva.

Corresponding to the time dimension of the S-DIVA results, the calculations of biogeographical events including dispersal, vicariance and extinction, were produced in RASP.

Results

Phylogenetic analysis and divergence time estimates

The phylogenetic trees obtained from ML and Bayesian inference in BEAST had an approximately identical topology over *Reaumuria* and the other two genera. Thus, the Bayesian inference tree from BEAST is presented in Fig. 2. This tree shows that *Reaumuria* is monophyletic with a high posterior probability of 1.00. Results of divergence time estimates used for three priors of root ages, normal, Gamma and exponent distribution, are shown in Table 2. Due to the roughly similarity of these estimated values, a consistent dating is offered. The result of normal distribution is chosen in following discussion. The estimated crown age of *Reaumuria* is 32.95 (95% HPD: 14.16–55.43) Ma (node 1), and the estimated crown ages of the two sections (nodes 2 and 3) are respectively 22.91 (95% HPD: 9.78–40.75) Ma and 19.78 (95% HPD: 7.52–37.52) Ma. The crown ages of the five series (nodes 5~9) ranged from ca. 13.13 to 6.72 (3.21) Ma, during middle-late Miocene, and the clade with six samples of *R. soongorica* was dated to only 1.62 (95% HPD: 0.44–3.68) Ma.

Hololachna as a genus or subgenus, being representative of *R. soongorica*, is entirely included within *Reaumuria*. In the phylogeny, the clades at nodes 2 and 3 correspond to the two sections in the genus, and the five clades at nodes 5~9 correspond to the five series. In spite of a few needed changes listed below, the phylogenetic relationships of the taxa are fundamentally consistent with previous morphological systematics. A detailed classification is offered in the Appendix.

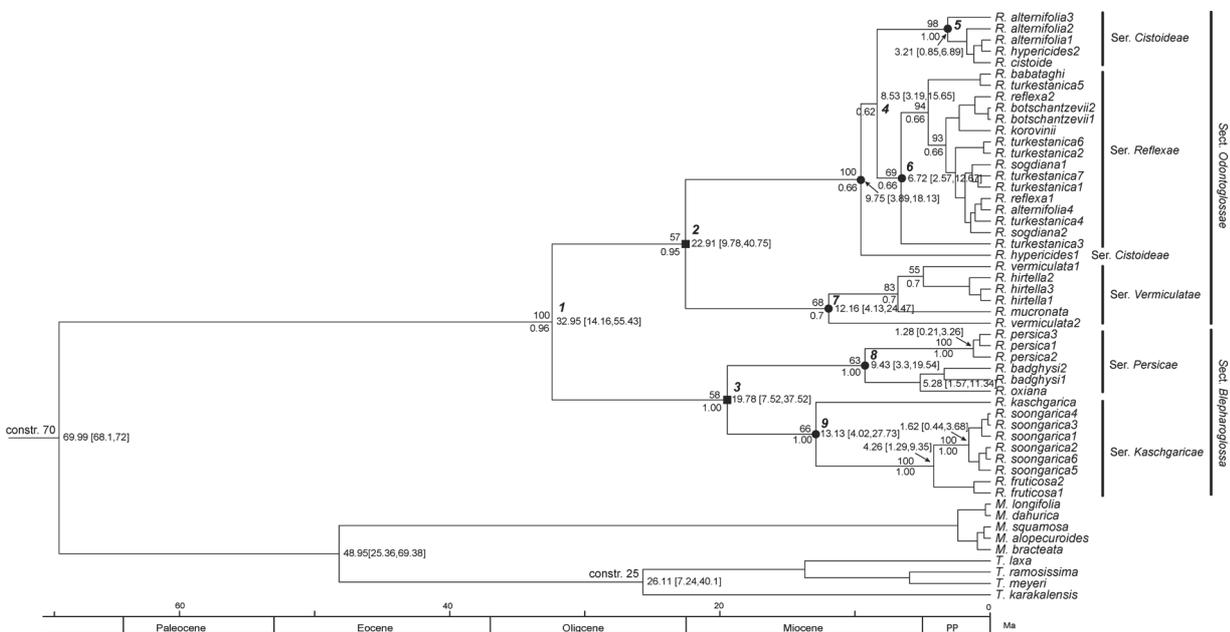


FIGURE 2. Phylogenetic tree and chronogram using BEAST Bayesian inference. Values at left of nodes on the tree are bootstrap support above, and posterior probability below. At the right of nodes are the estimated dating values and their 95% HPD from BEAST. Node numbers (1~9) are in italic at the right of nodes. The classification system derived from tree construction, including two sections and five series, is shown on the right of figure, and detailed in the Appendix.

TABLE 2. Results of divergence time estimates used for three priors of root ages, normal, Gamma and exponent distribution, are shown by node values and their 95% HPD intervals. Node numbers refer to Fig. 2.

root prior	1	2	3	4	5	6	7	8	9
normal	32.95 14.16–55.43	22.91 9.78–40.75	19.78 7.52–37.52	8.53 3.19–15.65	3.21 0.85–6.89	6.72 2.57–12.67	12.16 4.13–24.47	9.43 3.3–19.54	13.13 4.02–27.73
Gamma	34.5 28.64–39.03	26.73 22.22–30.65	19.68 16.37–23.14	7.92 6.4–9.62	6.88 5.3–8.68	6.33 5.01–7.75	15.61 12.83–18.37	14.66 11.5–17.87	13.8 10.87–16.87
exponent	36.54 29.83–43.27	28.32 23.03–33.72	20.85 16.87–25.28	8.41 6.61–10.36	7.28 5.32–9.23	6.73 5.22–8.41	16.56 13.38–20.11	14.6 11.3218.39	15.5 12.15–19.51

Optimization of ancestral distributions

The results of Lagrange were ignored due to frequent uncertainties at many nodes for the estimated ages of *Reaumuria*. Thus, only the S-Diva result is shown in Fig. 3. Western Central Asia (D) appeared at many nodes of the genus, sections, and series, only being absent for distributions in the Mediterranean (F), corresponding to series *Vermiculatae*. Distributions in Iran-Turkey (E) are marked as dispersals. Therefore, we confirm western Central Asia as the place of origin of *Reaumuria*.

Four dispersals were indicated to have originated from western Central Asia (D), as marked in Fig. 3. On the other hand, many dispersals appeared in the Tianshan Mountains (A), the Pamir-Alai (B), and/or eastern Central Asia (C), shown within species, such as *R. turkestanica*, *R. soongorica*, and *C. kaschgarica*.

Biogeographical events corresponding to time dimensions are shown in Fig. 4. Eight or more dispersal events occurred since about 7-6 Ma, with four at 10 Ma. This indicates that the main events were recent, since late Miocene-Pliocene.

Discussion

Phylogenetic clades and morphological classification

In the phylogenetic tree of Fig. 2, two major groups and five phylogenetic clades are identified. Approximately, the two groups correspond to the sections sensu Gorshkova (1949) and Hao et al. (2013), with the exception that representatives of the lone species *R. soongorica* in section or subgenus *Hololachna* are entirely nested within section *Blepharoglossa* Niedenzu of subgenus *Odontoglossa* Niedenzu. Since the six samples of the species are all nested in *Reaumuria*, the creation of *Hololachna* is obviously unjustified. We propose two sections in the genus, and the five phylogenetic clades roughly correspond to the existing series (Gorshkova, 1949; Hao et al. 2013). However, series *Oxianae* and *Persicae* should be combined into a single unit, *Persicae* Gorschk, and *R. soongorica*, *R. kaschgarica*, and *R. fruticosa* should be placed in series *Kaschgaricae* Gorschk (see Appendix). A classification system outline in terms of the current phylogenetic analysis is presented in the Appendix.

Linkage between Tertiary evolutionary timescale and abiotic EOT climate change and QTP uplift

As mentioned, we dated the crown age of *Reaumuria* as 32.95 (95% HPD: 14.16-55.43) Ma, and that of the two sections *Odontoglossa* and *Blepharoglossa* at 22.51 (95% HPD: 9.78-40.75) Ma and 19.78 (95% HPD: 7.52-37.52) Ma respectively, near the beginning of the Miocene. The first of these ages may roughly coincide with the EOT, an abrupt cooling that occurred at about 33.7 Ma. At the time of this shift, the earth's climate changed from warm, with temperatures of 20°, to about 5° C cooler within present temperate latitudes, and a decrease of perhaps half that in the tropics (Liu et al., 2009b; Hren et al., 2013). It was accompanied by the expansion of ice sheets on the Antarctic continent, a global sea-level drop (e.g. Miller et al., 1991; Katz et al., 2008), declining atmospheric CO₂ concentration, deepening of the calcite compensation depth, and biotic reorganizations (Xiao et al., 2010). The EOT has been demonstrated to have had a great impact relating to aridification and cooling (Dupont-Nivet et al., 2007), and to biodiversity (Katz et al., 2008; Liu et al., 2009b), with examples from the Neotropical floras (Jaramillo et al., 2006) and southern hemisphere landmasses (Coetsee and Muller, 1984). Recently, a botanical example has been contributed of sapindaceous lineages (Buerki et al., 2013) mainly from southeastern Asia, including many dispersal events and the emergence of new genera around the EOT. The distribution of *Reaumuria*, located in the broad Central Asian range of 20-50° N and 10-120° E (Fig. 1), surely also has the possibility to have been affected by cooling at the EOT, which may have aided its diversification. In addition, Paratethys retreat westward at the beginning of the Oligocene 34-32 Ma (Popov, 2004; Hou et al., 2011), would have been a source of increasing aridity and thus a possible contributing factor for diversification.

Also, during the early Miocene 23-19 Ma, land appeared behind a rapid regression of the Paratethys Sea in the eastern region (Rögl, 1998; Hou et al., 2011), and *Reaumuria* species had an environmental setting allowing their colonization into western Central Asia and dispersal westward along the southern coast.

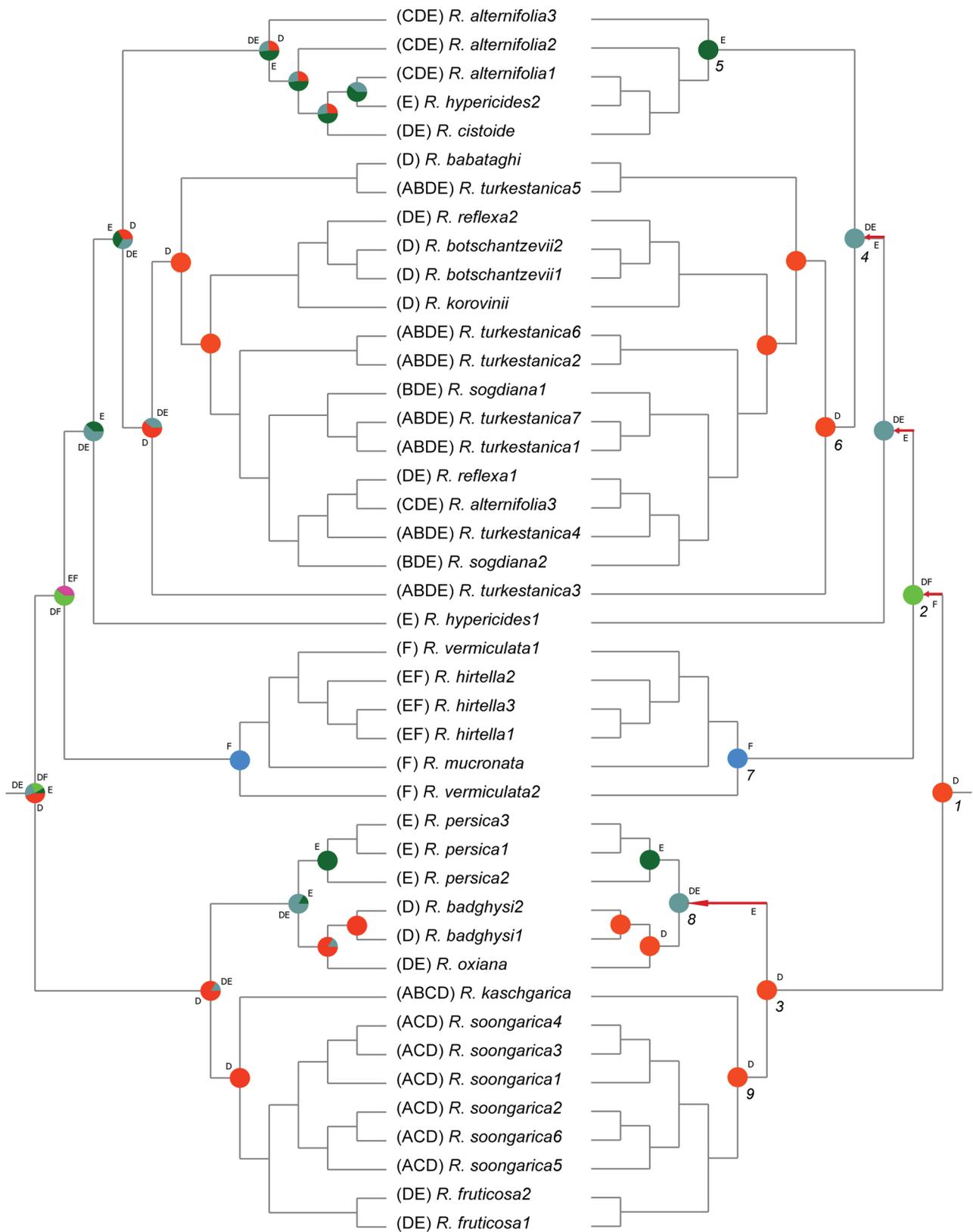


FIGURE 3. Reconstructions of ancestral areas performed with S-DIVA on the left, Lagrange on the right, for several nodes, and the most similar states (areas) with largest frequencies calculated by RASP, are illustrated on the right. Pie charts at the internal nodes represent the calculated probabilities (relative frequencies) of alternative ancestral area reconstructions. In the right figure, several dispersals are indicated by arrows on the branches. Node numbers (1~9) are in italics at the right of the node, the same as in Fig. 2. Area letters as stated in the text: A: Tianshan Mountains; B: Pamir-Alai mountains; C: eastern Central Asia; D: western Central Asia; E: Iran-Turkey; and F: Mediterranean. A detailed description is in the text.

Secondly, a great geological event, uplift of the Himalayas and QTP, is inferred to have progressed during the Oligocene and Miocene, resulting in origin of the modern Asian monsoons at about the Oligocene-Miocene boundary (Sun and Wang, 2005; Guo et al., 2008). Uplift of the QTP and Himalayas blocked warm and humid airflow from the Indian Ocean and progressively changed the climatic pattern of the Asian interior, converting it from humid and coastal to continental. *Reaumuria* diversification may have responded to this Himalayan and QTP motion, with early Miocene ages of diversification for the two sections *Odontoglossa* and *Blepharoglossa*, respectively 22.51 (95% HPD: 9.78–40.75) Ma and 19.78 (95% HPD: 7.52–37.52) Ma (see Fig. 2, node 2 and 3, two solid square nodes) by our dating. As morphological diversifications, section *Odontoglossa* developed flattened leaves and sparse bracts, whereas section *Blepharoglossa* gained slender, fleshy, apetiolate leaves, with sessile bracts, and orbicular sepals, all of which can probably be considered drought adaptations and may therefore have been accentuated by Himalayan and QTP uplift, forming an arid adapted morphological imprint on sectional diversification of the genus.

Thirdly, diversification at the series level, identified by the five phylogenetic clades (nodes 5–9 with solid circle in Fig. 2), was dated to middle–late Miocene 13.13–6.72 (3.21) Ma; during middle–late Miocene 17–5 Ma, the climate in Central Asia was drying to an increasing extent (Guo, et al., 2008; Miao, et al., 2012). In addition, even though it was once treated as a lone member of the subgenus *Hololachne* Ehrenb., *R. soongorica*, with all our six samples as a clade (Fig. 2), has an estimated crown age of only ca. 1.62 Ma. Its morphological characters of fewer stamens, about 7–10 (15 in other species in the genus), and small, short-cylindric leaves, as compared with the characters of many stamens and large, flattened leaves of most species, could probably be explained as derived attributes, in concordance with its estimated youthful age.

Evolutionary spatiotemporal pattern

Ancestral area reconstruction (Figs. 3–4) offers two useful kinds of information, ancestral place, and subsequent migration events, or dispersals. In general, taxa with Central Asia as their area of distribution concentration are regarded to have originated there, but the exact place is usually not clear. Since we subdivided Central Asia into western and eastern portions, and designated the Tianshan and Pamir-Alai mountains as defined operating areas, we are enabled to reconstruct the biogeographic history of *Reaumuria* in Central Asia in greater detail. Western Central Asia is defined as the ancestral area for the genus, see Fig. 3, comprising the Junggar and Turanian lowlands.

From Figs. 2-3, western Central Asia (D), namely the Turan-Junggar, is not only the generic ancestral area, but also the location of the MRCA of many groups within *Reaumuria*. Early dispersals appeared at the time range of 32.95–22.91 Ma (D→F, node 1→2), and resulted in the dispersal and emergence of series *Vermiculatae*; one dispersal occurred at 22.91–(9.75)8.53 Ma (D→E, node 2→4) and induced series *Cistoideae*, and one at 19.78–9.43 Ma (D→E, node 3→8) and yielded series *Persiae*. However, most dispersals occurred subsequent to 14 Ma, particularly after ca. 6 Ma. Distributions in A, B and C are indicated as dispersals from D (Fig. 3), and are shown within species, especially *R. turkestanica*, *R. soongorica*, and *R. kaschgarica*.

It is easily seen that westward dispersals to Iran-Turkey (E) and the southern Mediterranean (F) from western Central Asia (D) are ancient, from Oligocene to early-middle Miocene at series and section level, whereas dispersals eastward were very recent and within species in Fig. 3. From a paleogeographic perspective, we speculate that the early dispersals were probably due to resemblances between western Central Asia and Iran-Turkey and the southern Mediterranean at the time, all of which were located on the southern coast of the Paratethys in the Oligocene to early Miocene (Rögl, 1998; Hou et al., 2011), whereas during the middle-late Miocene, in Central Asia as a union, arid climates of the western and eastern parts had considerable similarity (Guo, et al., 2008; Miao, et al., 2012), resulting in frequent and easy dispersals eastward to eastern Central Asia (China and Mongolia), as well as westward from western Central Asia (D).

In contrast with the floristic origins of Central Asian taxa such as *Atraphaxis* (Polygonaceae) originating from the Tianshan Mountains (Wulff, 1945) and then dispersing to grasslands and desert, *Reaumuria* is here found to have originated from the lowland Turanian grasslands, and then dispersed to the highlands of the Tianshan and Pamir-Alai mountains. This was probably determined by the arid attributes of *Reaumuria*, as compared with the montane moist origin of *Atraphaxis*. Essentially, *Reaumuria* is an arid taxon, an ancient member of the Central Asian flora.

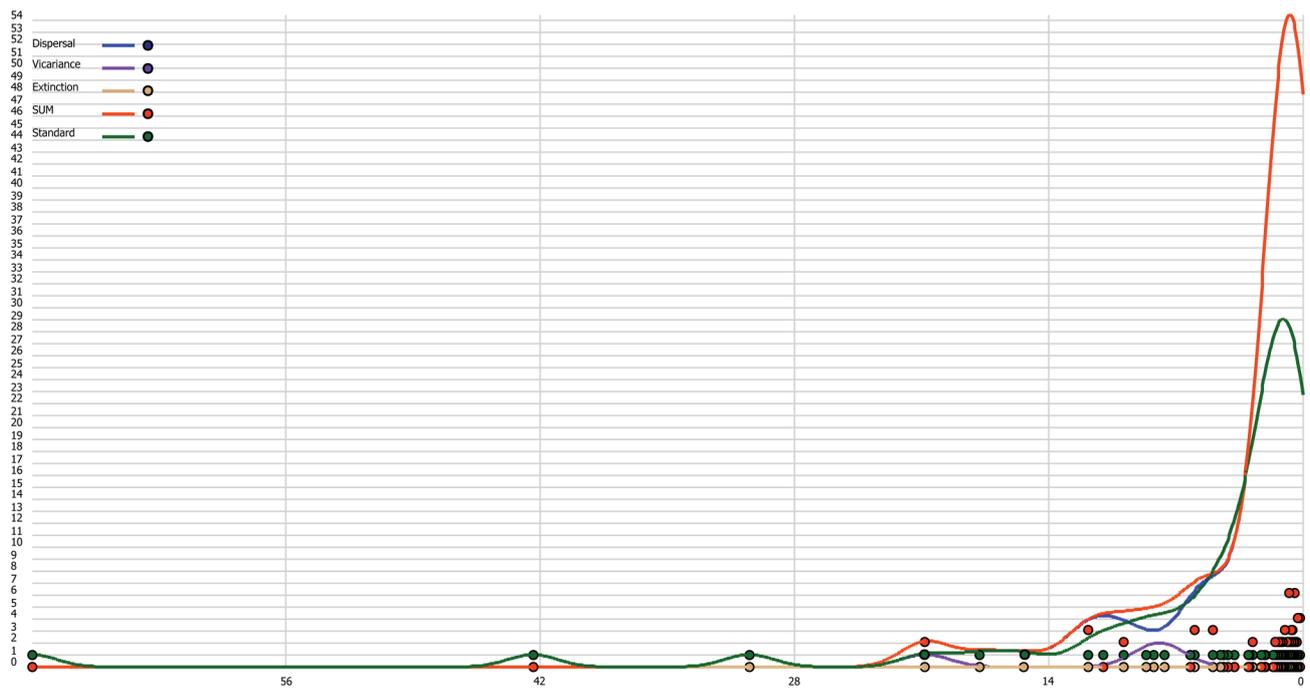


FIGURE 4. For S-DIVA results, the calculations of its biogeographical events including dispersal, vicariance and extinction, produced in RASP, are shown.

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Appendix An outlook of the classification system for *Reaumuria*.

Section 1. *Odontoglossa* Niedenzu

Leaves flat, obsolete petiolate; bracts sparse.

Series 1. *Reflexae* Gorschk.

- (1) *R. babataghi* Botsch.
- (2) *R. turkestanica* Gorschk.
- (3) *R. reflexa* Lipsky
- (4) *R. botschantzevii* Zuckerw. & Kurbanov
- (5) *R. korovinii* Botsch. & Lincz.
- (6) *R. sogdiana* Kom.

Series 2. *Cistoideae* Gorschk.

- (7) *R. alternifolia* (Labill.) Britt.
- (8) *R. cistoides* Adam.
- (9) *R. hypericoides* Willd.

Series 3. *Vermiculatae* Gorschk.

- (10) *R. vermiculata* Linn.
- (11) *R. mucronata* Jaub. & Spach
- (12) *R. hirtella* Jaub. & Spach

Section 2. *Blepharoglossa* Niedenzu

Leaves terete or subterete, fleshy, sessile in axils; bracts mostly dense, appressed to calyx.

Series 4. *Kaschgaricae* (Gorschk.) M.L. Zhang

Leaves short, subterete, thick, bracts sparse, short.

- (13) *R. kaschgarica* Rupr.
- (14) *R. soongarica* (Pall.) Maxim.
- (15) *R. fruticosa* Bge.

Series 5. *Persicae* (Gorschk.) M.L. Zhang

Bracts numerous, imbricate, or forming involucre.

- (16) *R. persica* Boiss.
- (17) *R. oxiana* (Ldb.) Boiss.
- (18) *R. badhysi* E. Kor.