JIPB Journal of Integrative Plant Biology

Tertiary montane origin of the Central Asian flora, evidence inferred from cpDNA sequences of *Atraphaxis* (Polygonaceae)

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Research Article

Abstract *Atraphaxis* has approximately 25 species and a distribution center in Central Asia. It has been previously used to hypothesize an origin from montane forest. We sampled 18 species covering three sections within the genus and sequenced five cpDNA spacers, *atp*B-*rbc*L, *psb*K-*psb*I, *psb*A-*trn*H, *rbc*L, and *trn*L-*trn*F. BEAST was used to reconstruct phylogenetic relationship and time divergences, and S-DIVA and Lagrange were used, based on distribution area and ecotype data, for reconstruction of ancestral areas and events. Our results appear compatible with designation of three taxonomic sections within the genus. The generic stem and crown ages were Eocene, approximately 47 Ma, and Oligocene 27 Ma, respectively. The origin of *Atraphaxis* is confirmed as montane, with an ancestral area consisting of the Junggar Basin and uplands of the Pamir-Tianshan-Alatau-Altai mountain chains, and ancestral ecotype of montane forest. Two remarkable paleogeographic events, shrinkage of the inland Paratethys Sea at the boundary of the late Oligocene and early Miocene, and the time intervals of cooling and drying of global climate from 24 (22) Ma onward likely facilitated early diversification of *Atraphaxis*, while rapid uplift of the Tianshan Mountains during the late Miocene may have promoted later diversification.

Keywords: Allopatric diversification; *Atraphaxis*; biogeography; Central Asia flora; molecular clock; montane origin; phylogeny; Polygonaceae
Citation: Zhang ML, Sanderson SC, Sun YX, Byalt VV, Hao XL (2014) Tertiary montane origin of the Central Asian flora, evidence inferred from cpDNA sequences of *Atraphaxis* (Polygonaceae). **J Integr Plant Biol** 56: 1125–1135. doi: 10.1111/jipb.12226
Edited by: Hongya Gu, Peking University, China
Received Mar. 31, 2014; **Accepted** Jun. 10, 2014
Available online on Jun. 12, 2014 at www.wileyonlinelibrary.com/journal/jipb

INTRODUCTION

Atraphaxis, a shrub genus of the family Polygonaceae, includes approximately 25 species and occurs mainly in Central Asia, with a few taxa expanding to northern China in East Asia, Iran and Turkey in western Asia, and northern Africa and southeastern Europe (Pavlov 1936; Lovelius 1978; Bao and Li 1993; Bao and Grabovskaya-Borodina 2003; Figure 1). As shrubs, *Atraphaxis* species can act as the dominant elements in the vegetation of desert areas. For instance, in mountain front and lower montane zones of the northern slope of the Tianshan Range in China, there are communities titled Form. *Atraphaxis frutescens*, Form. *Atraphaxis pungens*, and Form. *Atraphaxis virgata*, comprised predominantly by these *Atraphaxis* species (Vegetation Exp Team 1978; Wu and Wang 1980; Hu 2004).

Systematically, *Atraphaxis*, *Calligonum*, and *Pteropyrum* formerly constituted Tribe Atraphaxideae (Dammer 1893), or subtribe Atraphaxidinae (Jaretzky 1925; Hong 1995), and a Tibetan genus, *Parapteropyrum* (Li 1981; Bao and Li 1993), was a subsequent addition to the group. However, this tribe, including these four genera, was shown to be non-monophyletic in terms of a recent molecular phylogeny (Lamb-Frye and Kron 2003; Sanchez and Kron 2008, 2009, 2011; Tavakkoli et al. 2010; Sanchez et al. 2009, 2011; Sun and Zhang 2012). As a result, *Atraphaxis* is now placed in tribe Polygoneae, while *Calligonum* and *Pteropyrum* are located in tribe Calligoneae, distant from *Atraphaxis* (Sanchez et al. 2009, 2011b; Tavakkoli et al. 2010; Sun and Zhang 2012), and *Parapteropyrum* is included in Fagopyreae (Sanchez et al. 2011a, 2011b). These investigations were at or above the generic level, and no more than 5–6 species were sampled from *Atraphaxis*, and thus no detailed phylogeny within *Atraphaxis* has been carried out.

Central Asian phytogeography is always an attractive subject, and many hypotheses, theories, and conclusions concerning origin, evolution, and dispersal have been contributed by Russian botanists (e.g. Wulff 1944; Tahktajan 1969; Grubov 1999). Popov (1938, see Wulff 1944) proposed three evolutionary stages for the flora of this region, from Cretaceous to Early Tertiary, Later Tertiary, and from the Later Tertiary onward. Wulff (1944) and Iljin (1937) suggested that the Central Asian flora originated from the Mediterranean rather than Africa; Grubov (1999) thought it to be ancient and of native origin. However, most of these inferences were based only on morphological characters and, consequently, few definitive works have approached the core of the origin, evolution, and dispersal of the flora. However, recent investigations relating to it, for example, studies on *Hippophae rhamnoides* (Jia et al. 2012) and *Artemisia* (Miao et al. 2011), have provided evidence supporting some of the hypotheses on the flora's origin and evolution.

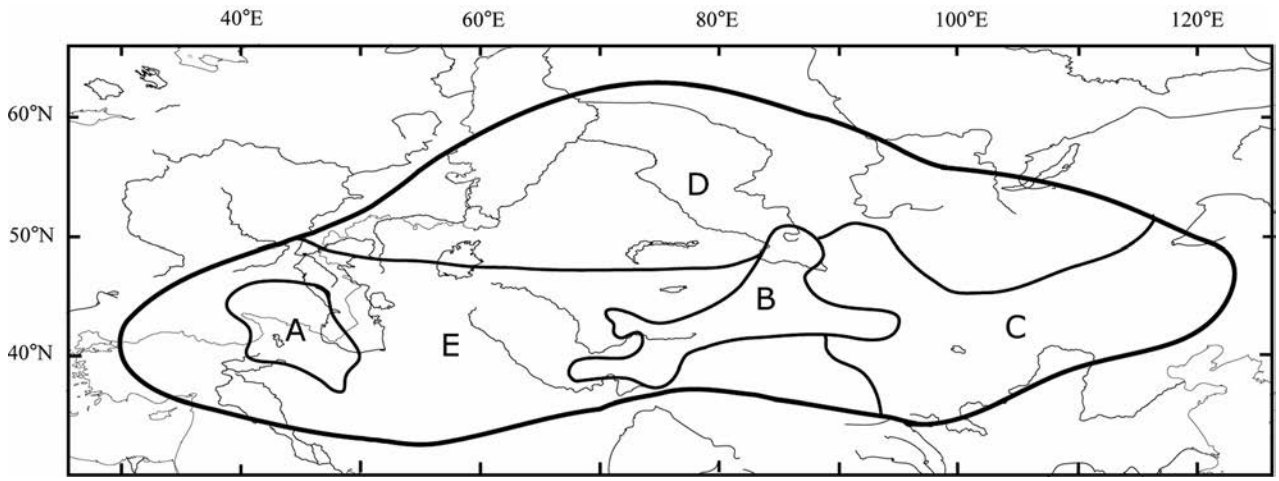

Figure 1. Distribution of *Atraphaxis*, divided into five areas
A, Caucasus; B, Junggar Basin and uplands of the Pamir-Tianshan-Alatau-Altai mountains; C, Mongolia; D, Siberia; E, Turan.

Atraphaxis was regarded as a key group by which to explore these origins by Kransnov (1868, see Wulff 1944). He found that a lower floristic zone in the Tianshan Mountains has a considerable similarity to the wet lowlands of the Caspian–Aral Sea, and believed that the Tertiary mesic montane flora of the Tianshan and other mountains was the primary source of the extant flora of these lowlands. Kransnov regarded *Atraphaxis muschketovii*, a tall shrub occurring in the forest margins of the Tianshan Mountains, as the most primitive species in *Atraphaxis*. Clearly, this genus is a promising example for verifying the hypothesis of a montane origin of the Central Asian flora. However, after Kransnov, there was no further progress in dealing with the origin and evolution of the genus.

After sampling most of the *Atraphaxis* species, this investigation using molecular approaches, attempts to: (i) determine a molecular phylogeny for confirmation of the previous classification, and use it as a basis of biogeography; (ii) explore the spatiotemporal evolution of the genus, especially a more exact time and place of origin; and (iii) examine the hypothesis of a montane origin of this genus and the Central Asian flora.

RESULTS

Phylogenetic analysis and divergence time estimates

Our phylogenetic tree primarily indicates that *Atraphaxis* is monophyletic with high maximum parsimony (mp = 100) and posterior probability (pp = 1.00). Three sections, namely, *Tragopyrum*, *Atraphaxis*, and *Physopyrum*, labeled in Figure 2, and previously classified within the genus (Pavlov 1936; Lovelius 1978; Bao and Li 1993), were identified, although *Atraphaxis karatavensis* was placed outside of section *Atraphaxis*. However, this section was found to be nested within section *Tragopyrum*. Nodes representing six phylogenetic clades are labeled in the phylogenetic tree (Figure 2), and section *Tragopyrum* included three (clades 3, 5, 6). However, the three have weak support (mp < 70 and pp < 0.70), and *Atraphaxis kopetdaghensis* and *Atraphaxis jrtyschensis* are not included in them.

In addition, species widely distributed in the locations of the Turanian or Mongolian floras formed clades in terms of these floras, see Table 1 and Figure 2. For example, in section *Tragopyrum*, with the clade 3 samples all being from the Mongolian flora (China), and clades 1, 5, and 6 from the Turanian flora, which would indicate allopatric diversification.

BEAST dating results for the stem and crown ages of *Atraphaxis* were, respectively, 47.45 Ma (95% highest posterior density (HPD): 35–64.87) and 26.67 Ma (95% HPD: 11.88–43.49). Thus, the time of origin of *Atraphaxis* can be dated to the Eocene, with initial divergence of clades in late Oligocene. These estimates seem earlier than the stem age of 35.2–40.8 Ma and crown age of 19.6–22.6 Ma reported by Schuster et al. (2013). The nodal times of all six of the identified clades fall into the time interval of 19–7 Ma, indicating that most diversification of infrageneric groups occurred in the Miocene. As seen in Figure 2, section *Physopyrum* and clades 3, 5, and 6 of section *Tragopyrum* emerged relatively early, whereas clade 4 of section *Atraphaxis* is relatively young, with dated ages of approximately 7 Ma.

Ancestral area and ecotype reconstructions

For ancestral area reconstruction, the results estimated from S-DIVA and Lagrange are somewhat different, especially at some nodes (Figure 3, left). For example, at the root node of *Atraphaxis*, S-DIVA results indicate B (most likely), and Lagrange suggests ABCDE (most likely) or BDE, therefore, B should probably be selected. Similarly, BE would be chosen at node 2. At node 1, B occurs in many unions of areas, and so it would appear to be the ancestral area. On the whole, B, the Junggar and uplands of the Pamir-Tianshan-Alatau-Altai chain, is indicated to be the ancestral area of *Atraphaxis*, and it is the most recent common ancestor (MRCA) location of many of the groups. The S-DIVA result seems better than that of Lagrange. Two dispersals occurred from the ancestor of the genus to

Table 1. Voucher information for the *Atraphaxis* and outgroups

Species	Voucher	Source	GenBank accession numbers (*atp*B-*rbc*L, *psb*K-*psb*I, *psb*A-*trn*H,*rbc*L, *trn*L-F)
Atraphaxis bracteata A. Los.	M.L. Zhang 0811 (XJBI)	TBG, Xinjiang, China	JQ009204, JQ009242, JQ009223, JQ009261, JQ009279
A. compacta Ledeb.	Y.X. Sun 0806 (XJBI)	Urumchi, Xinjiang, China	JQ009206, JQ009244, JQ009225, JQ009263, JQ009281
A. irtyschensis Yang et Han	M.Z. Chen 0821 (XJBI)	MBG, Gansu, China	JQ009208, JQ009246, JQ009227, JQ009265, JQ009283
A. manshurica Kitag.	M.Z. Chen 0822 (XJBI)	Lanzhou, Gansu, China	JQ009211, JQ009249, JQ009230, JQ009268, JQ009286
A. pungens (Bieb.) Jaub. et Spach	M.L. Zhang 0812 (XJBI)	TBG, Xinjiang, China	JQ009210, JQ009248, JQ009229, JQ009267, JQ009285
A. spinosa L.	M.Z. Chen 0823 (XJBI)	MBG, Gansu, China	JQ009207, JQ009245, JQ009226, JQ009264, JQ009282
A. replicata Lam.	B.R. Pan 0871 (XJBI)	Altai, Xinjiang, China	JQ009205, JQ009243, JQ009224, JQ009262, JQ009280
A. virgata (Regel) Krassn.	B.R. Pan 0881 (XJBI)	Tuoli, Xinjiang, China	JQ009209, JQ009247, JQ009228, JQ009266, JQ009284
A. badghysi Kult. 1	A. Meschcheryakov (LE)	Lake Er-Ojlan-duz, Badkhyz, Turcomania	KJ820685, KJ820697, KJ820707, KJ820717, ------
A. badghysi Kult. 2	V. P. Bochantsev 259 (LE)	Nomaksaar, Badkhyz, Turcomania	KJ820686, KJ820698, 00000000, KJ820718, ------
A. caucasica (Hoff.) Parl.	T. Popova 326 (LE)	Akhaltziksky Distr., Georgia	KJ820687, KJ820699, KJ820708, KJ820719, KJ820724
A. decipiens Jaub.	E. I. Rachkovskaya 5576 (LE)	Karaganda, Kazakhstan	KJ820720, KJ820700, KJ820709, 00000000, KJ820725
A. frutescens (L.) C. Koch	I. O. Baitulin, N. K. Aralbaiev s.n. (LE)	Zajsanskaya, E. Kazakhstan	KJ820689, KJ820701, 00000000, KJ820721, ------
A. frutescens (L.) C. Koch 2	M. Lomonosova, N. Medvedeva 547(LE)	Ulug-Khemsky Distr. Tuva, Russia	KJ820690, KJ820702, KJ820710, KJ820722, KJ820726
A. karatavensis Lipsch	V. P. Botchantsev, R. Kamelin 392 (LE)	N slope of Ridge Nurutau, Uzbekistan	KJ820691, KJ820703, KJ820711, ------, KJ820727
A. kopetdaghensis Koval.	Meschcheryakov A. s.n. (LE)	C. Kopetdag, Karaagach, Turcomania	KJ820692, ------, KJ820712, ------, KJ820728
A. laetivirens (Ledeb.) Jaub. Et Spach	V. I. Vassilevich, E. M. Lavrenko 834 (LE)	Mts. Shipu-Tau Semipalatinsk, Kazakhstan	KJ820693, KJ820704, KJ820713, ------, ------
A. muschketovii Krassn 2	V. P. Goloskokov s.n. (LE)	Zailijsky, Kazakhstan	KJ820694, KJ820705, KJ820714, ------, KJ820729
A. pyrifolia Bunge	Mansarova s.n. (LE)	Valley of Kondara, Tadzhikistan	KJ820695, KJ820706, KJ820715, ------, ------
A. teretifolia (M. Pop) Kom.	V. I. Grubov s.n. (LE)	Mouth of River Betpak-dala, Kazakhstan	KJ820696, 00000000, KJ820716, ------, ------
Calligonum aphyllum (Pall.) Gürke	M.L. Zhang 0841	TBG, Xinjiang, China	JQ009215, JQ009253, JQ009234, JQ009272, JQ009290
C. arborescens Litv.	M.L. Zhang 0842	TBG, Xinjiang, China	JQ009219, JQ009257, JQ009238, JQ009276, JQ009294
C. densum Borszcz.	M.L. Zhang 0843	TBG, Xinjiang, China	JQ009216, JQ009254, JQ009235, JQ009273, JQ009291
C. junceum (Fisch. et Mey.) Litv.	M.L. Zhang 0844	TBG, Xinjiang, China	JQ009214, JQ009252, JQ009233, JQ009271, JQ009289
C. leucocladum (Schrenk) Bge.	M.L. Zhang 0845	TBG, Xinjiang, China	JQ009218, JQ009256, JQ009237, JQ009275, JQ009293
C. mongolicum Turcz	M.L. Zhang 0846	TBG, Xinjiang, China	JQ009220, JQ009258, JQ009239, JQ009277, JQ009295
C. roborowskii A. Los.	M.L. Zhang 0847	TBG, Xinjiang, China	JQ009213, JQ009251, JQ009232, JQ009270 JQ009288
C. rubicundum Bge.	M.L. Zhang 0848	TBG, Xinjiang, China	JQ009212, JQ009250, JQ009231, JQ009269, JQ009287
C. zaidamense A. Los.	M.L. Zhang 0849	TBG, Xinjiang, China	JQ009217, JQ009255, JQ009236, JQ009274, JQ009292
Polygonum tibeticum A. J. Li	Z.Z. Zhou 0801	Jiacha, Tibet, China	JQ009221, JQ009259, JQ009240, JQ009278, JQ009296
P. aucherii Jaub. et Spach	A.L. Ashirova, F. Kerimovaet al. s.n. (LE)	Kaakhnishsky, Turcoman (LE)	JQ009222, JQ009260, JQ009241, 00000000, KJ820723
P. aviculare L.		GenBank	HQ843161.1, EU749799.1, FJ503034.1, EF653761.1, HQ843161.1

LE, Herbarium of Vascular Plants, Komarov Botanical Institute of Russian Academy of Sciences; MBG, Minqin Botanical Garden, Gansu Province, China; PE, Herbarium of Institute of Botany, the Chinese Academy of Sciences, Beijing, China; TBG, Turpan Botanical Garden, Xinjiang Province, China.

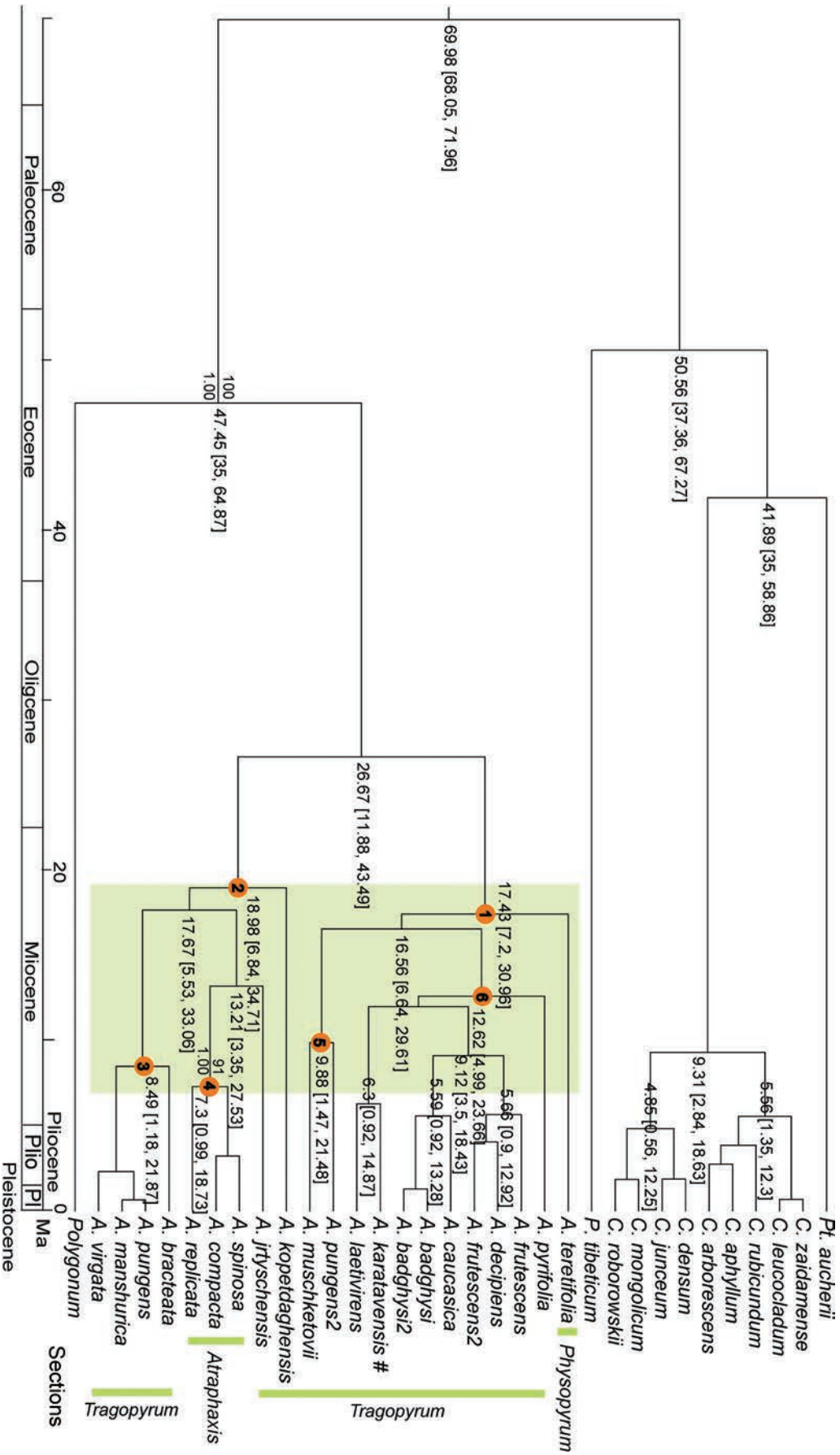

Figure 2. Continued.

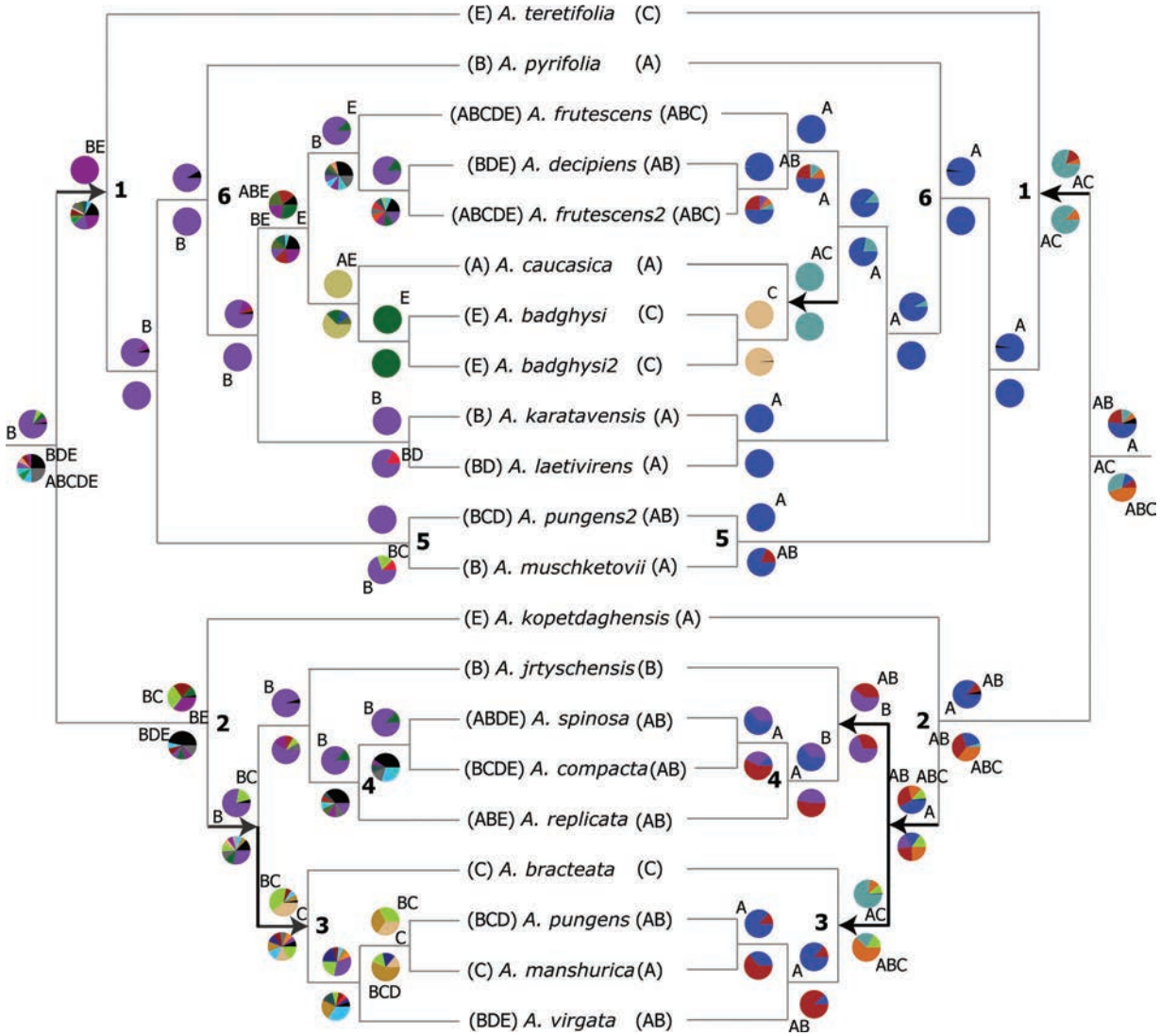

Figure 3. Ancestral area and ecotype reconstructions, left, and right, ecotype, performed with S-DIVA and Lagrange
Pie charts above and below branches resulted from S-DIVA and Lagrange, respectively. Two dispersals for areas (left) are shown with arrowheads, one is from *Atraphaxis* ancestral area B to node 1 BE, another is from node 2 to node 3. Four dispersals for ecotypes (right) are also shown with four arrowheads. Operational areas, as stated in Figure 1: A, Caucasus; B, Junggar and uplands of the Pamir-Tianshan-Alatau-Altai; C, Mongolia; D, Siberia; E, Turan. Ecotype labels: A, montane forest; B, steppe; C, desert.

node 1 (from B to E, Turan), and from node 2 to node 3 (from B to C, Mongolia).

The ancestral ecotype reconstruction by S-DIVA and Lagrange is relatively simple (Figure 3, right). The results show that the ecotypes of the root node of *Atraphaxis* and most of the MRCAs are obviously A, montane forest. Four dispersals are shown in Figure 3 (right), all from A to B (steppe) or C (desert), for instance, from the ancestor of

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Figure 2. Chronogram of *Atraphaxis* and outgroups *Polygonum*, *Calligonum*, *Pteropyrum*, and *Parapteropyrum*, as performed by BEAST
Dates of mean estimated times and interval for nodes (right) with 95% highest posterior density, and the maximum parsimony and posterior probability values are shown on the left above and below nodes, respectively (e.g. node 4). The six phylogenetic clades are labeled with circles near the nodes, and their diversification interval from 19 to 7 Ma is illustrated with the green shadow. The dating timescale and geological stratigraphic period are shown. The three sections within *Atraphaxis* are shown at the right of the figure, according to the classification system of Lovelius (1978) and Bao and Li (1993).

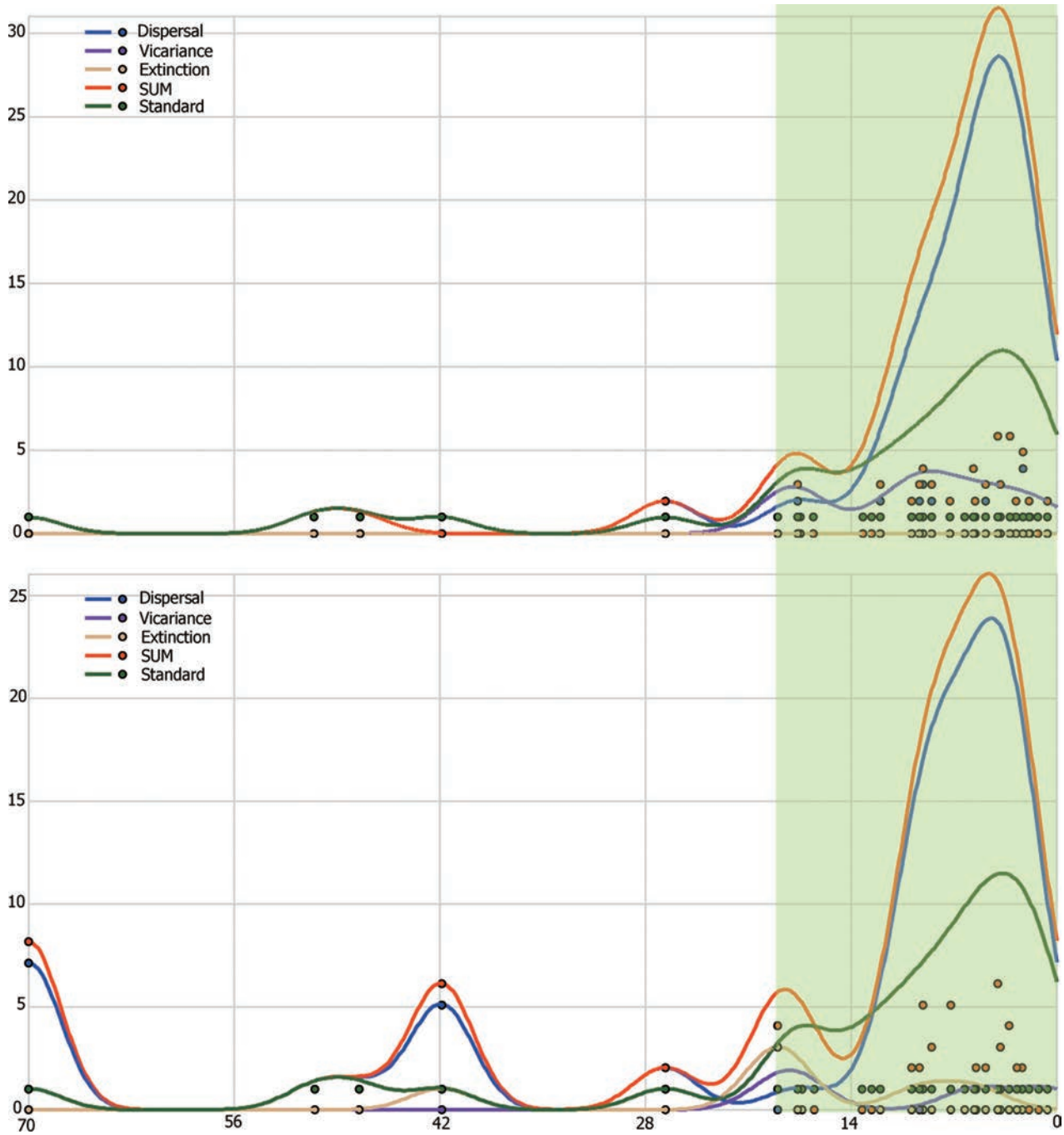

Figure 4. Diagram annotating biogeographical events of vicariance, dispersal, and extinction in Figures 2 and 3, calculated under S-DIVA (RASP)

The upper is for distribution area and the lower is ecotype. For both, starting from 19 and 11 Ma to the present are notable time nodes and intervals of rich and dense occurrences.

the genus to node 1 (A → C), and from node 2 to node 3 (A → C).

Concerning the appearance time range of biogeographical events, vicariance, dispersal, and extinction (Figure 4), the results of distribution area and ecotype by S-DIVA event calculation indicated that an important time node was at 19 Ma, where the species had a rich and notable diversification, and starting from 11 Ma to the present was another dense interval of events. Both the aspects of distribution area and ecotype analyses confirmed that a remarkable speciation and diversification took place during the period of middle and late Miocene.

DISCUSSION

Phylogenetic clades versus classification

In *Atraphaxis*, classification of the two sections (or subgenera) should have been expected to be stable, because it is based on a series of distinctive morphological characters. As mentioned, Section *Tragopyrum* has a perianth of five segments, eight stamens, three styles, and a trigonous achene, while section *Atraphaxis* has a perianth of four segments, six stamens, and two styles, and a compressed and lenticular achene (Pavlov 1936; Lovelius 1978; Bao and Li 1993). A third section, *Physopyrum*, only includes one species, *Atraphaxis teretifolia*, which was used to establish the genus *Physopyrum* by Mikhail G. Popov in 1935. Pavlov (1936) put this species in section *Tragopyrum* of the genus *Atraphaxis*, whereas Lovelius (1978) raised it to sectional level. Our phylogenetic evidence best supports Lovelius (1978). As described by Lovelius (1978), *A. teretifolia* has several morphological characteristics distinguishing it from the other two sections, namely fleshy, terete, verrucous leaves, a raceme inflorescence, flowers with three interior and two exterior of five perianth segments, and with eight stamens and three styles. Moreover, *A. teretifolia* is shown to be an early diverged taxon from the *Atraphaxis* ancestor (Figure 2). Therefore, three sections within *Atraphaxis* would be justifiable. However, variation and irreconcilable taxonomical treatments in section *Tragopyrum* have remained. In particular, Lovelius' (1978) classification within section *Tragopyrum* is not supported by this phylogenetic analysis, which seems due to his overmany divisions of series and subsection rank, as suggested by Bao and Li (1993).

In addition, a balanced classification should probably be performed covering the whole Central Asian region, instead of only the western Central Asia (Middle Asia) Turanian flora (Pavlov 1936; Lovelius 1978), or the eastern Central Asia Mongolian flora (Bao and Li 1993; Bao and Grabovskaya-Borodina 2003).

Tertiary origin and evolution

As mentioned, our dated times of stem age of approximately 47 Ma and crown age of approximately 27 Ma for *Atraphaxis*, are greater than the recent dating of 35.2–40.8 Ma and 19.6–22.6 Ma, respectively, by Schuster et al. (2013). We believe that this discrepancy results in part from the different sequences used, but is probably mainly due to the present analysis adding more species samples within the genus, which in practice can change the tree topology and sequence variability. In any case, both we and Schuster et al. (2013) hypothesize an origin and diversification of *Atraphaxis* in the Older Tertiary Eocene-Oligocene. This hypothesis of an Eocene-Oligocene origin of *Atraphaxis* is similar to that for *Artemisia* (Asteraceae) (Miao et al. 2011), a cosmopolitan grassland genus mainly inhabiting arid and semiarid regions, that originated in the late Eocene, with development mainly in the Miocene, in arid and semiarid Central Asia.

The ancestral diversification of *Atraphaxis* is dated to approximately 27 Ma at the boundary of the Oligocene and Miocene. Central Asia during this period had just experienced a remarkable paleogeographic event, shrinkage of the great inland Paratethys Sea (Ramstein et al. 1997; Hrbbek and Meyer 2003). Such an event would certainly have provided large areas for colonization and likely influenced the divergence of Central Asian *Atraphaxis*.

As mentioned above, there is an allopatric diversification of *Atraphaxis* between the Mongolian and Turanian floras (Figure 2). This would be due to floral and vegetational differences between species of the two regions (Wu and Wang 1983; Wu and Wu 1998; Grubov 1999), and to the divergence of ecological factors such as temperature, precipitation, and soils (Hou 1988; Agakhanjanz and Breckle 1995). Thus, the allopatric diversification of *Atraphaxis* seems best explained by the background of flora and vegetation and by ecological factors. According to Wu and Wu (1998), the Central Asia flora can be divided into eastern and western parts, the eastern belonging to the warm temperate desert region, that is Kashgar-Mongolia, with many shared endemic species (Liu 1982, 1995; Zhao and Zhu 2003), and the western part belonging to the temperate desert region, with vegetation made up of *Artemesia* spp., the shrubs *Haloxylon* and *Salsola* spp., as well as a rich variety of species of Chenepodiaceae (Wu and Wang 1983; Hou 1988). In our six identified phylogenetic clades (Figure 2), clade 4 representing section *Atraphaxis* is revealed to be young, with a dated diversification age of approximately 7 Ma. This is in agreement with morphological differentiation, because section *Tragopyrum* is primitive with a perianth of five segments, and eight stamens, three styles, and a trigonous achene, while section *Atraphaxis* is derived with a perianth of four segments, and with six stamens, two styles, and a compressed achene (Bao and Li 1993). The dated diversification time of approximately 7 Ma is associated with the intensified cooling and drying of global climate at approximately 8–7 Ma (6) (see Sun and Zhang 2008; Sun et al. 2010; Miao et al. 2012) and a major episode of uplift in the Tianshan Mountains (Sun et al. 2004).

From the event analyses (Figure 4), all events of dispersal, vicariance, and extinction had a dense time range after 19 Ma, especially from 11 Ma to the present. Based on Guo et al. (2002, 2008), climatic cooling and drying during 24–5 Ma (22) shaped the arid and semiarid belts of northwestern China. The dispersal, vicariance, and extinction of *Atraphaxis* were likely affected by this climatic cooling and drying. Therefore, our *Atraphaxis* scenario of biogeographical event emergences is consistent with the formation of these climatic belts in the Miocene. Typical dispersal events for areas are obvious in Figure 3, one is from *Atraphaxis* ancestral area B to node 1 BE during Oligocene–Miocene 26.67–17.43 Ma, that is, from Junggar and uplands of the Pamir-Tianshan-Alatau-Altai (B) to Turan (E), and in terms of ecotypes, montane (A) to desert (C); another is from node 2 to node 3 during Miocene 18.98–8.49 Ma, from the uplands (B) to Mongolia (C), which is also from a montane (A) to a desert ecotype (C). These spatiotemporal events depict the speciation process from the Junggar and uplands of the Pamir-Tianshan-Alatau-Altai westward and eastward, to steppe and/or desert, and constitute a case of Tertiary montane origin and diversification.

Even though Popov's (1938) proposal of three evolutionary stages for the Central Asian flora, namely, from Cretaceous to Early Tertiary, the Later Tertiary, and from Later Tertiary onward, is understandable, there is no exact timescale to annotate these stages and process, and few examples have been offered. Our contribution of the generic divergence time

(stem age) of Eocene (ca. 47 Ma), with an ancestral area presence (crown age) at the boundary of the Oligocene and Miocene (ca. 27 Ma), internal diversification of six phylogenetic clades at approximately 11–7 Ma (19), and, for example, dispersal and vicariance events, serves as not only a practical case supporting Popov's (1938) proposal, but also elaborates some time nodes and intervals quantitatively, on the verge of revealing the essential characteristics and history of the Central Asian flora.

Montane origin and adaptive radiation from the Tianshan Mountains and adjacent area in Central Asia

Our ancestral area reconstruction (Figure 3) indicates that *Atraphaxis* has the ancestral area B, namely, the Junggar Basin and uplands of the Pamir-Tianshan-Alatau-Altai chain, and has the ancestral ecotype A, montane forest, both jointly supporting the suggestions of Kransnov (1868, see Wulff 1944), of a montane origin of this genus. Even though *Atraphaxis muschketowi*, a suggested primitive species in the genus by Kransnov (1868), was not located in a primitive position on the phylogenetic tree (Figure 2), our conclusion clearly supports his hypothesis of the montane origin.

In section *Atraphaxis*, *Atraphaxis compacta*, *Atraphaxis spinosa*, and *Atraphaxis replicata* have ecotypes of montane (A) and steppe (B; Figure 3). According to our inferences, the A montane ecotype located at the ancestral location, node 3, is primitive in this section, and steppe species have evolved from montane species. Moreover, a montane ecotype appears at most nodes of the genus, and the uplands of the Pamir-Tianshan-Alatau-Altai chain are located in the central portion of the Central Asian steppe and desert. Most of these mountain ranges are located in the interaction zones between cratonic basins and have experienced episodes of uplift and erosion due to long range effects of the ongoing India–Asia collision (Buslov 2009), but the Tianshan and perhaps others were uplifted in a more dramatic fashion beginning in late Meiocene approximately 7 Ma (Sun et al. 2004). The Pamirs, being further to the south, had an earlier major uplift (Ducea et al. 2003). Rain shadow areas associated with mountain ranges were probably the most ancient xeric sites, and arid adapted plants developing there should have been able to move into the Central Asian plains as the Paratethys Sea withdrew and the landscape there became arid. Thus, the steppe and desert distributions would best be explained as a descent from a montane distribution by adaptive radiation. Once the ecological conditions became approximately matched, species from the low and middle mountains could have easily migrated to neighboring steppe or desert.

An inferred montane origin and diversification of *Atraphaxis*, in fact, may suggest that as they became uplifted, the Tianshan range and adjacent mountains have continued to play an important role in the Central Asian flora. The Tianshan Mountains, located in the central part of Central Asia, and running from eastern Xinjiang to western Kazakstan and Kirghistan across more than 3,000 km, in the south linking with the southern Pamir-Alai ranges and in the north with the Altau-Tarbagatai-Altai, constitute the majority of the Central Asian forest zone (Wulff 1944; Wu and Wang 1983; Agakhanjanz and Breckle 1995; Grubov 1999). The Tianshan Mountains have a rich flora and high species numbers. According to the statistics of Agakhanjanz and Breckle (1995), the central portion of the range has 1,870 species, the western portion has 2,812, and the northern portion 2,230. The Ili Valley-Tianshan Mountains in Xinjiang of China is referred to as a "humidity island" because of its richer rainfall and vegetation (Hu 2004), as compared to other parts of northwestern China (Hu 2004; Chen 2010). Owing to the diverse flora, vegetation, paleogeography, and unique location of these mountains in Central Asia, they have been suggested as a center of origin and diversification, and are recognized as a conservation hotspot of global biodiversity (Wulff 1944; Wu and Wang 1983; Agakhanjanz and Breckle 1995; Grubov 1999; Hu 2004; Chen 2010; Zachos and Habel 2011). Our recent phylogeographical studies, such as those on *Clematis sibirica* and *Clematis songorica* (Ranunculaceae) (Zhang et al. 2013, 2014), the *Delphinium naviculare* species group (Ranunculaceae) (Zhang and Zhang 2012), the *Aconitum nemorum* (Ranunculaceae) species group (Jiang et al. 2014), and *Ribes meyeri* (Saxifragaceae) (Xie and Zhang 2013), have shown that the Tianshan Mountains are unquestionably a haplotype diversity center and Quaternary Pleistocene refugium. Presently, *Atraphaxis* contributes a valid example of an Early Tertiary montane origin, and diversification in the Tianshan Mountains at the generic level. Such a case has been lacking thus far.

With a concentrated distribution in Central Asia, and origination and diversification from montane habitats, *Atraphaxis* can be usefully employed to explore the floristic relationships among Central Asia and adjacent areas. Owing to its native origin, its distributions in other areas would be dispersals, migrations, or adaptive radiation (Figure 3). This is different from species or groups occurring in Central Asia and adjacent regions of Eurasia, such as the Himalayas, East Asia, or the Mediterranean, and having different places of origin. For instance, *H. rhamnoides* (Elaeagnaceae) (Jia et al. 2012) and *Myricaria* (Tamaricaceae) (Zhang et al. 2014) are both speculated to have originated from the Himalayas and migrated to Central Asia. Endemic to the Mediterranean, *Anagyris* (Leguminosae) (Ortega-Olivencia and Catalán 2009), is inferred to have evolved in Central Asia and migrated to that region. Also, *Calophaca* (Leguminosae) (ML Zhang, unpubl. data, 2013) appears to have probably originated from Central Asia in the Pamir–western Tianshan mountains and migrated to East Asia, although the contrasting direction of origination from East Asia and migration to Central Asia could be possible (Wulff 1944; Wu and Wang 1983; Grubov 1999). However, many informative phylogenetic and biogeographical cases have doubtlessly not yet emerged.

MATERIALS AND METHODS

Taxon sampling

We examined a total of 24 samples belonging to 18 species from *Atraphaxis*, with outgroups consisting of one species each of *Polygonum*, *Parapteropyrum*, and *Pteropyrum*, and nine of *Calligonum* (Table 1), as done previously (Sun and Zhang 2012). Leaf materials from the botanical garden or field were dried with silica gel.

DNA sequencing and alignment

Total genomic DNA was extracted using the cetyltrimethylammonium bromide method (Doyle and Doyle 1987). The

polymerase chain reaction (PCR) was used for dsDNA amplification. Each 25 μL reaction contained 0.25 μL of Ex Taq (2.5 U/μL), 2.5 μL of 10 × Ex Taq buffer (Mg^{2+} concentration of 25 mmol/L), 2.0 μL of deoxyribonucleotide triphosphate (dNTP) mix (at 2.5 mmol/L concentration for each dNTP), and 1 μL each of forward and reverse primers at 5 μmol/μL. The following primers were used: *trn*L-*trn*F (Taberlet et al. 1991), *atp*B-*rbc*L (Janssens et al. 2006), *psb*AF (Sang et al. 1997), and *trn*H-*trn*R (Tate and Simpson 2003). Those for *psb*A-*trn*H intergenic spacer (IGS), *psb*K, and *psb*I were provided by Kim Ki-Joong for the IGS between *psb*K and *psb*I, 1FS (Lamb-Frye and Kron 2003), and *rbc*L-1460R for *rbc*L are all described in Sun and Zhang (2012). For PCR amplifications, predenaturation was first conducted at 94 °C for 3 min, followed by 30 cycles of: (i) denaturation at 94 °C for 30 s; (ii) annealing at 48–54 °C for 30 s; and (iii) extension at 72 °C for 1 min. At the end of the cycles, a final extension was used at 72 °C for 10 min. PCR products were purified using the polyethylene glycol precipitation procedure (Johnson and Soltis, 1995). Sequencing reactions were performed by Beijing Sanbo Biological Engineering Technology and Service Corporation (Beijing, China). Sequences were aligned using CLUSTAL X software (Thompson et al. 1997), and then adjusted by hand. All gaps were treated as missing characteristics. Finally, a combined five gene dataset including *atp*B-*rbc*L, *psb*K-*psb*I, *psb*A-*trn*H, *rbc*L, and *trn*L-*trn*F was assembled for phylogenetic analysis.

Phylogenetic analysis and divergence time estimates

Bayesian phylogenetic analysis and divergence time estimates were together implemented in BEAST 1.5.4 (Drummond et al. 2006; Drummond and Rambaut 2007). We used the uncorrelated lognormal relaxed clock model with a Yule process for the speciation model, GTR + I + G for the substitution model (estimated for the dataset). Recently, a comprehensive dating of Polygonaceae (Schuster et al. 2013) has been carried out, in terms of its outline and pollen fossil of Polygonaceae (Gray 1964; Muller 1981), and we utilized four constraints: (i) the root of Polygonaceae at approximately 70 Ma (prior normal, mean = 70 Ma, *SD* = 1); (ii) a pollen fossil of *Calligonum* 5.3–2.6 Ma (prior exponential); (iii) Calligoneae 63–35 Ma (stem-crown of Schuster et al. 2013, prior I exponential offset 35, mean 63; prior II uniform, lower 35, upper 70); and (iv) *Polygonum-Atraphaxis* 39–35 Ma (stem-crown of Schuster et al. 2013, prior I exponential offset 35, mean 39; prior II uniform, lower 35, upper 70). A Markov chain Monte Carlo (MCMC) was run for 50 million generations and sampled every 1,000 generations, and two independent runs for priors I and II were performed to confirm convergence of the analysis. The stationarity of each run was examined using the effective sampling size of each parameter (>200). Two runs were combined used LogCombiner version 1.7.5. The last 80 million generations were used to construct the maximum clade credibility tree and associated 95% HPD distributions around the estimated node ages using the program TreeAnnotator 1.5.4, and visualized using FigTree 1.3.1.

Areas

Our distribution areas are fundamentally defined in terms of the floristic divisions of Grubov (1999). Two large areas are the Mongolian and Turanian provinces. The Mongolian province is composed of the Kashgar (Tarim Basin), and the Mongolian Plateau; the main vegetation of this province is desert. The Turanian province includes the Middle Asian plains as well as Iran and Turkey, and the predominant vegetation is also desert.

In Central Asia, there is a mountain chain which consists of the Pamir, Tianshan, Alatau, and Altai ranges, located at the boundary of the Mongolian and Turanian floral regions, and which is rich in *Atraphaxis* species. We treated it combined with the Junggar as a unit of the distribution areas. Being far away from this mountain chain, and located west of Central Asia, the Caucasus is also regarded as a unit. Siberia, located north of Central Asia, has a vegetation zone of steppe; the west part (north of Kazakhstan and Caucasus) is temperate steppe, and the east part (north of Mongolia) is dry steppe. Thus, although Siberia has several species of the southern Central Asian desert, its steppe vegetation is different from that of the Central Asian desert, so we treated it as a unit as well. Thus, five distribution areas are finally defined, their boundaries decided on the basis of Asian topography, vegetation and flora, see Figure 1: (A) Caucasus, (B) Junggar and uplands of the Pamir–Tianshan–Alatau–Altai, (C) Mongolia, (D) Siberia, (E) Turan.

Ecotype

Three ecotypes, in the light of the vegetation types in Central Asia, are montane forest (A), steppe (B), and desert (C).

Ancestral area reconstructions

We used two methods to infer vicariance and dispersal events, a Bayesian parsimony-based method (S-DIVA), and a maximum likelihood-based DEC model (Lagrange version 2.0.1) (Ree et al. 2005; Ree and Smith 2008).

DIVA is an event-based method that optimizes ancestral distributions by assuming a vicariance explanation while incorporating the potential contributions of dispersal and extinction (Ronquist 1997). Bayes-DIVA, based on DIVA, calculates the posterior distribution of a Bayesian MCMC sample of tree topologies (Nylander et al. 2008). Bayes-DIVA is also referred to as S-DIVA, and can be performed in RASP (Reconstruct Ancestral State in Phylogenies) version 2.0 beta (http://mnh.scu.edu.cn/soft/blog/RASP). The BEAST molecular dating tree (Figure 2) was treated as a fully resolved phylogram for using basis of S-DIVA, with 1,001 post-burnin trees derived from the BEAST analysis to use for ancestral area reconstruction in the program RASP. Multiple samples of the same taxon in a clade are combined into one branch, or many identical terminal areas in a clade are regarded as one; consequently, the tree is reduced to maximum simplicity. RASP was performed with various constraints of maximum areas of two at each node, to infer possible ancestral areas and potential vicariance and dispersal events.

We also employed parametric likelihood analysis with a dispersal-extinction-cladogenesis model (Ree et al. 2005), as implemented in Lagrange version 2.0.1 (Ree and Smith 2008). This analysis calculates the likelihood of biogeographical routes and areas occupied by the MRCA for a given phylogenetic tree topology and the present distributions of taxa. Maximum likelihood parameters are estimated for rates of migratory events between areas (range expansions) and local extinctions within areas (range contraction). Like S-DIVA, Lagrange is used to explore the three most relevant processes of the biogeographical history of a lineage, namely, vicariance,

dispersal, and extinction (e.g. Clayton et al. 2009; Almeida et al. 2012; Mao et al. 2012).

To describe these biogeographical events at a time node or within time intervals in Figures 2 and 3, we used the single area model under "Event model" in Tree View of S-DIVA (RASP) for calculation. This calculation was entirely on the basis of RASP, using 1,001 post-burnin trees, a BEAST final tree and distribution data, with the result shown in a "diagram". For ancestral area reconstruction of the distribution areas and ecotypes, see Figure 3; results are shown in Figure 4.

ACKNOWLEDGEMENTS

We are grateful to the herbaria staffs of Komarov Botanical Institute (St Petersburg), Russian Academy of Sciences, Moscow University (Moscow), Main Botanical Garden (Moscow), Russian Academy of Sciences, Institute of Botany, the Chinese Academy of Sciences (CAS), Xinjiang Institute of Ecology and Geography, CAS, Northwest Institute of Plateau Biology, CAS, Shihezi University, for our accommodation in checking of specimens. This work was supported by the China National Key Basic Research Programs (2012FY111500, 2014CB954201), CAS Important Direction for Knowledge Innovation Project (KZCX2-EW-305), and Xinjiang Institute of Ecology and Geography, CAS.

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