



# Taxonomy, phylogenetics and biogeography of *Chesneya* (Fabaceae), evidenced from data of three sequences, ITS, *trnS-trnG*, and *rbcl*

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## ARTICLE INFO

### Article history:

Received 21 April 2015

Received in revised form 7 September 2015

Accepted 11 September 2015

Available online xxx

### Keywords:

*Chesneya*  
Biogeography  
Classification  
Evolution  
Molecular clock  
Phylogeny

## ABSTRACT

Plants of Central Asia have played a significant role in the origin of floras of Eurasia and the Northern Hemisphere. *Chesneya*, a small leguminous genus occurring in Central Asia, western Asia, and Tibet, is used to establish phylogenetic relationships and discuss the evolutionary and biogeographical history based on sequence data of ITS and *trnS-trnG* and *rbcl*. We employed BEAST Bayesian inference for dating, and S-DIVA, Lagrange and BBM for ancestral area reconstruction. Our results indicate that *Chesniella* should be a separate genus, while *Spongiocarpella* should be included in *Chesneya*. A classification system within *Chesneya* comprising five sections is presented. The diversification of *Chesneya* (crown age ca. 16.56 Ma) is speculated to have been associated with Qinghai-Tibetan Plateau (QTP) uplift. The following aridification process resulted in the Pliocene diversification of four sections of *Chesneya* during 4.8–2.06 Ma. Ancestral area reconstruction indicates the Himalayas is the ancestral area of *Chesneya* and *Chesniella*, but within Central Asia, the western lowlands, can be inferred as the cradle of most dispersals.

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## 1. Introduction

*Chesneya* Lindl. ex. Endl., a small leguminous genus in tribe Galegeae, comprises 21–30 species (Yakovlev et al., 1996; Lock and Schrire, 2005a,b; Zhu and Laren, 2010) and occurs in Central Asia, western Asia, and Tibet (Xizang), extending to the Pamirian, Iranian, and Mongolian plateaus, with a diversity center in Central Asia (Middle Asia sensu Yakovlev et al., 1996). *Chesneya* species are generally herbaceous or shrubby, inhabiting grasslands, deserts, and montane valleys and meadows.

*Chesneya* was first established in “Genera Plantarum” by John Lindley in 1840. He dedicated *Chesneya* to Francis Rawdon Chesney, a general British soldier (1789–1872), for his plant collections from the Euphrates valley between 1836 and 1858.

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The genus *Kostyczewa* Korshinsky, using *Kostyczewa ternata* Korsh. as type, and described by the Russian Sergei Ivanovitsch Korshinsky in 1896, is the basionym of *Chesneya*.

Borissova (1945) described nine species for *Chesneya* in the *Flora of the USSR*, and established a classification system which included two sections, *Macrocarpon* Borris. and *Microcarpon* Borris. Fruit size, cauline leaf size, and leaf shape, were treated as the key characters for classification. Borissova (1961) later erected section *Microcarpon* as the genus *Chesniella* Borris., Yakovlev et al. (1996) included it within *Chesneya*. Li (1981) treated *Chesneya* classification in China, including ten species, four of them were newly described, and belonging to section *Macrocarpon* mainly distributed in the Qinghai-Tibetan Plateau (QTP), such as *Chesneya nubigena* (D. Don) Ali and *Chesneya spinosa* P. C. Li. Based on these prostrate species in the QTP, Yakovlev and Svazeva (1987) established the genus *Spongiocarpella* Yakovlev & N. Ulziykhutag, and cited the type species as *Spongiocarpella nubigena* (D. Don) Yakovl., based on *Chesneya nubigena*.

Chromosome numbers of *Chesneya parviflora*, *C. nubigena*, *Chesneya rytidosperma* Jaub. & Spach, and *Chesneya elegans* Fomin have been reported to be diploid, generally  $2n = 2x = 16$ , (Jahan et al., 1994; Gu et al., 1993; Sepet et al., 2011), like most species in *Caragana* and *Calophaca* (Moore, 1968; Zhou et al., 2002; Li et al., 1998). Ranjbar and Karamian (2003) used micromorphological evidence of four Iranian species from *Chesneya* section *Macrocarpon*, to compare *Chesneya* and *Gueldenstaedtia*. They suggested that both genera are resemble to *Caragana*, *Calophaca*, and *Halimodendron*, and inferred *Chesneya* to have derived from *Caragana* or *Calophaca* in Eurasia and the former Soviet Union. These five genera constituted tribe Caraganae in tribe Galegeae. However, molecular phylogenetic data has now revealed that *Chesneya*, *Gueldenstaedtia*, *Spongiocarpella*, and *Tibetia* form a clade in Galegeae, whereas *Caragana*, *Calophaca* and *Halimodendron* are members of the tribe Hedysareae (Wojciechowski et al., 1999, 2000; Lock, 2005; Lock and Schrire, 2005a,b). These competing relationships are at the tribal rankings, less concern infrageneric phylogeny and biogeography for *Chesneya* l.s., which, just stated by Lock and Schrire (2005a,b) is lacking.

*Chesneya* species mainly occurs at 37–43° N and 60–80° E, which encompasses the Pamir-Alai, Tianshan, Himalayan, and Hindukush mountains. In these climatically harsh regions, biological spatiotemporal evolution should be related most strongly to abiotic factors, most remarkably to the QTP uplift, and climatic cooling and drying during the Neogene (Willis and McElwain, 2002; Guo et al., 2008; Royden et al., 2008). Even though numerous organisms are distributed along these regions, however, only a few have been investigated linking abiotic factors (e.g. Jia et al., 2012).

Thus, focusing on a series of taxonomic issues of *Chesneya* and relatives, this paper attempts by employing molecular evidence, and phylogenetic and biogeographical approaches. Our aims are: (1) to reassess the previous taxonomy; (2) to infer a biogeographic scenario for the group using molecular dating and ancestral area reconstruction, and (3) to deal with the evolution of the genus in the context of its geological and climatic settings.

## 2. Materials and methods

### 2.1. Taxon sampling

As discussed above, *Chesneya* putatively includes two additional genera, *Chesniella* and *Spongiocarpella*, and since there are different taxonomical treatments for both, we temporarily place them within *Chesneya*. In accordance with current phylogenies (Wojciechowski et al., 1999, 2000; Lock and Schrire, 2005a,b), outgroups sampled included *Gueldenstaedtia*, *Tibetia*, *Caragana*, *Calophaca*, and *Halimodendron*, as well as *Hedysarum* and *Astragalus*, see Table 1. Species samples and vouchers are deposited in herbaria PE (Institute of Botany, Chinese Academy of Sciences, Beijing, China), XIJI (Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China), 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, Moscow, Russia).

### 2.2. DNA sequencing

Total genomic DNA was extracted using the CTAB method (Doyle and Doyle, 1987). Primers for the ITS region, the *trnS-trnG* spacer, and *rbcL* were the same as described in Zhang et al. (2009a,b). The polymerase chain reaction (PCR) was used for amplification of double stranded DNA. The 25  $\mu$ l reaction system contained 0.25  $\mu$ l of Ex Taq, 2.5  $\mu$ l of  $10 \times$  Ex Taq buffer ( $Mg^{2+}$  concentration of 25 mM), 2.0  $\mu$ l of dNTP mix (2.5 mM concentration for each dNTP), 1  $\mu$ l of the forward and reverse primers at 5  $\mu$ mol/ $\mu$ l, and 0.5  $\mu$ l of template DNA. The protocol for amplification consisted of an initial hot start 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) and sequenced using an ABI PRISM 3770 Genetic Analyzer (Shanghai Shengong Biological Engineering Technology & Service, Shanghai, China).

Sequences were aligned with CLUSTAL X software (Thompson et al., 1997) and then adjusted manually. All gaps were treated as missing data. Finally, the combined 3-gene region data set comprised 3953 aligned nucleotide characters.

**Table 1**Voucher information for *Chesneya* s.l. including *Chesniella* Borris. and *Spongiocarpella* Yakovlev & N. Ulziykhutag, and outgroups.

Species	Voucher	Source	Genbank		
			ITS	<i>rbcl</i>	<i>trnS-trnG</i>
<b><i>Chesneya</i> Lindl.</b>					
<i>C. astragalina</i> Jaub. & Spach	Belyanina N., Proskuryakova G., Sofeikova T., 19790521 (MW)	SW Kopet-Dag, Turcoman	KT834921	KT834852	KT834887
<i>C. badachschanica</i> Boriss.	Kamelin R.V., 19860605-874 (LE)	West Pamir	KT834922	KT834853	KT834888
<i>C. borissovae</i> Pavlov	Pavlov V., 19540801-490 (MHA)	Bostandyk, Kazakhstan	KT834923	KT834854	KT834889
<i>C. botschantzevii</i> R. M. Vinogr.	Bochantsev V., 19760517-944 (LE)	Badhyz, Turcoman	KT834924	KT834855	KT834890
<i>C. crassipes</i> Boriss. 1	Kamelin R.V., Sauarov N., Khalimov A., 19860605-888 (LE)	Obi-Matraun (Odudi) canyon, Tajikistan	KT834925	KT834856	KT834891
<i>C. crassipes</i> Boriss. 2	Qinghai-Tibet Vegetation Team, 19760611-12630 (PE)	Zhada, Xizang, China		KT834857	
<i>C. cuneata</i> (Benth.) Ali	Anders O., 19730526-9940 (LE)	Panjshir Tal, Parwan, Afghanistan	KT834926	KT834858	KT834892
<i>C. dawasica</i> Boriss.	Kamelin R.V., Sauarov N., Khalimov A., 19860106-740 (LE)	Darvaz, Turcoman	KT834927	KT834859	KT834893
<i>C. dshungarica</i> Golosk.	Goloskokov V.P., 19560607 (MW)	SW spurs of Dzungar Alatau, Kazakhstan	KT834928	KT834860	KT834894
<i>C. ferganensis</i> Korsh. 1	Kamelin R.V., 19850601-287 (LE)	Sokh's canyon, Pamir-Alai	KT834929	KT834861	KT834895
<i>C. ferganensis</i> Korsh. 2	Gorbunova N.V., 19780307-8 (LE)	Karakalpak, Uzbekistan	KT834930	KT834862	KT834896
<i>C. ferganensis</i> Korsh. 3	Pimenov M.G., Klyuykov E.V., Lazkov G.A., 20000622-36 (MW)	Naryn, Kirghistan	KT834931	KT834863	KT834897
<i>C. gracilis</i> (Boriss.) R. Korn.	Kamelin R.V., Averoyanov L., 19860423-11 (LE)	Koi-Pioz-Tau Mountains, Tajikistan	KT834932	KT834864	KT834898
<i>C. hissarica</i> Boriss.	Koroleva A.S., Nikitin V.A., 19340608-133 (LE)	Varzob, Dushanbe, Tajikistan	KT834933	KT834865	KT834899
<i>C. kopetdaghensis</i> Boriss. 1	Chetscheryakov, 19770609 (LE)	Kopetdag, Turcoman	KT834934	KT834866	KT834900
<i>C. kopetdaghensis</i> Boriss. 2	Sytin A., 19870502 (MHA)	Kara-Kala, Turcoman	KT834935	KT834867	KT834901
<i>C. kschtutica</i> Rassulova & B. A. Sharipova	Ganybaeva M.R., Lazkov G.A. 20070919-1807 (LE)	Kyrkbulak, Kirghistan	KT834936	KT834868	KT834902
<i>C. linczevskiyi</i> Boriss.	Bochantsev V., 19600528-523 (LE)	Kara-Tau, Tajikistan	KT834937	KT834869	KT834903
<i>C. macrosperma</i> R. Kam. in LE	Chukavina A.G., Pogrebnova L.E., 19590908-1265 (LE)	Ridge of Oburdon, Tajikistan	KT834938	KT834870	KT834904
<i>C. mongolica</i> Maxim.	Gubanov I.A., 19890801 (LE)	The junction of Mongolia and Inner Mongolia	KT834939	KT834871	KT834905
<i>C. neplii</i> Boriss.	Varivtseva E.A., Nepli G.N., 19480602-782 (LE)	Gardani-ushty, Tajikistan	KT834940	KT834872	KT834906
<i>C. nubigena</i> (D. Don) Ali	Li B.S., 1990-19856 (PE)	Jilong, Xizang, China	KT834941	KT834873	KT834907
<i>C. parviflora</i> Jaub. & Spach	? (LE)	Caucasus	KT834942	KT834874	KT834908
<i>C. quinata</i> Al. Fed. 1	Bochantsev V., 19740811-262 (LE)	Kokomeren, Kirghistan	KT834943	KT834875	KT834909
<i>C. quinata</i> Federov. 2	Pimenov M.G., Klyuykov E.V. Lazkov G.A., 20000621-6 (MW)	Naryn, Kirghistan	KT834944	KT834876	KT834910
<i>C. spinosa</i> P. C. Li	Li B.S., Li F., Yang Y., 19900916-13809 (PE)	Jilong, Xizang, China	KT834945	KT834877	KT834911
<i>C. tadjikistana</i> Boriss.	Bochantsev V., 19600601-769 (LE)	Panj, Tajikistan	KT834946	KT834878	KT834912
<i>C. ternate</i> (Korsh.) M. Pop.	Pimenov M.G., Klyuykov E.V., 19890806-149 (MW)	Kyzyl-Bulak, Kirghistan	KT834947	KT834879	KT834913
<i>C. tribuloides</i> Nevski.	Bochantsev V., 19850718 (LE)	Gaurdak, Turcoman	KT834948	KT834880	KT834914
<i>C. trijuga</i> Boriss. 1	Lazkov G.A., 20060614 (LE)	Shahimardan, Kirghistan	KT834949	KT834881	KT834915
<i>C. trijuga</i> Boriss. 2	Shafeev Y.G., 19500604 (MW)	Alai mountains, Kirghistan	KT834950	KT834882	KT834916
<i>C. turkestanica</i> Franchet 1	Kamelin R., 19710731 (LE)	Kokh, Tajikistan	KT834951	KT834883	KT834917
<i>C. turkestanica</i> Franchet 2	Pimenov M., Baranov Yu. Sdobnina L., 19710601-613 (MW)	Takob, Varzob, Tajikistan	KT834952	KT834884	KT834918
<i>C. turkestanica</i> Franchet 3	Konovalova T., Shevyreva N., 19920712 (MHA)	Gissar range, Kondar river, Tajikistan	KT834953	KT834885	KT834919
<i>C. villosa</i> (Boriss.) Kamelin & R. M. Vinogr.	Gorbunova N.V., 19800722 (LE)	Terskei Ala-too, Kirghistan		KT834886	KT834920
<b><i>Gueldenstaedtia</i> Fisch.</b>					
<i>Gu. stenophylla</i> Bunge			GQ246109.1		
<i>Gu. verna</i> (Georgi) Boriss.			GQ246108.1	GQ436359.1	
<b><i>Tibetia</i> (Ali) H. P. Tsui</b>					
<i>Ti. himalaica</i> (Baker) H. P. Tsui				JQ933348.1	
<b><i>Calophaca</i> Fisch.</b>					
<i>Cal. soongorica</i> Kar. et Kir.	E.E. Pyoahobeq, L.A. Kpamapehko 05141984 (PE)	Semiipalatinskaya, Tajikistan	FJ537288	FJ537237	FJ537189
<b><i>Caragana</i> Fabr.</b>					
<i>Ca. acanthophylla</i> Kom.	M.L. Zhang 00-154(PE)	Cultivated, Turfan Botanical Garden, Xinjiang, China	FJ537238	FJ537191	FJ537139
<i>Ca. microphylla</i> Lam.	M.L. Zhang et al. 99-214 (PE)	Lhongcheng, Mongolia	FJ537264	FJ537213	FJ537166
<i>Ca. opulens</i> Kom.	M.L. Zhang, Y. Kang 99-123(PE)	Daofu, Sichuan, China	FJ537282	FJ537231	FJ537183

Table 1 (continued)

Species	Voucher	Source	Genbank		
			ITS	<i>rbcl</i>	<i>trnS-trnG</i>
<b>Halimodendron Fisch. ex DC.</b>					
<i>Halimodendron halodendron</i> (Pall.) Voss	M.L. Zhang 00-279(PE)	Cultivated, Urumqi Botanical Garden, Xinjiang, China	FJ537289	FJ537237	FJ537190
<b>Hedysarum Linn.</b>					
<i>Hedysarum alpinum</i> L.	M. Riewe 182(CAS)	Northwest Territories, Canada	FJ537287	FJ537235	FJ537188
<b>Astragalus Linn.</b>					
<i>A. coluteocarpus</i> Boiss.	Qinghai–Xizang Exped. Team 76-8083(PE)	Zada, Ali, Xizang, China	FJ537286		FJ537187

### 2.3. Phylogenetic analysis and divergence time estimation

Congruence between ITS data and the combined cpDNA (*trnS-trnG* and *rbcl*) data was assessed by comparing topologies and support values from MrBayes analyses (Ronquist and Huelsenbeck, 2003), employing Markov chain Monte Carlo runs of 20 million generations sampled every 1000 generations. Majority rule (>50%) consensus trees were constructed after removing the burn-in samples (the first 10% of sampled trees). The Bayesian ITS and cpDNA trees showed no major incongruence supported by posterior probabilities of 0.95 or greater. Therefore, we combined the ITS and cpDNA datasets for further phylogenetic examination.

Phylogenetic analyses were performed using Maximum Likelihood (ML) and Bayesian inference. ML analysis was performed with PAUP v4.0 (Swofford, 2003); clade support was estimated with 1000 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 sequence data. The model selected using the Akaike information criterion (AIC) was GTR + I + G. The related parameters of Modeltest were used for ML analysis.

Bayesian phylogenetic analysis and divergence time estimates were done with BEAST 1.5.4 (Drummond et al., 2006; Drummond and Rambaut, 2007). The uncorrelated lognormal relaxed clock model with a Yule process for the speciation model, and GTR + I + G for the substitution model (estimated from the data set), were used. The minimum age of tree prior (normal distribution Mean = 33.3, Stdev = 2.7) for Hedysareae and Astragaleae, i.e., the ancestral node of *Astragalus* and *Caragana*, was defined as the root and fixed at 33.3 Ma following Lavin et al. (2005, *matK* dating node 76), and a prior of Hedysareae constraint (Lavin et al., 2005; *matK* node 77) using Uniform, lower = 21.1, upper = 29.3. A Markov chain Monte Carlo was run for 30 million generations and sampled every 1000 generations. Two independent runs were performed to confirm convergence of the analysis. The stationarity of each run was examined using the effective sampling size of each parameter (>200). The last 25 million generations were used to construct the maximum clade credibility tree and associated 95% highest posterior density distributions around the estimated node ages using the program TreeAnnotator 1.5.4, and the tree was visualized using FigTree 1.3.1.

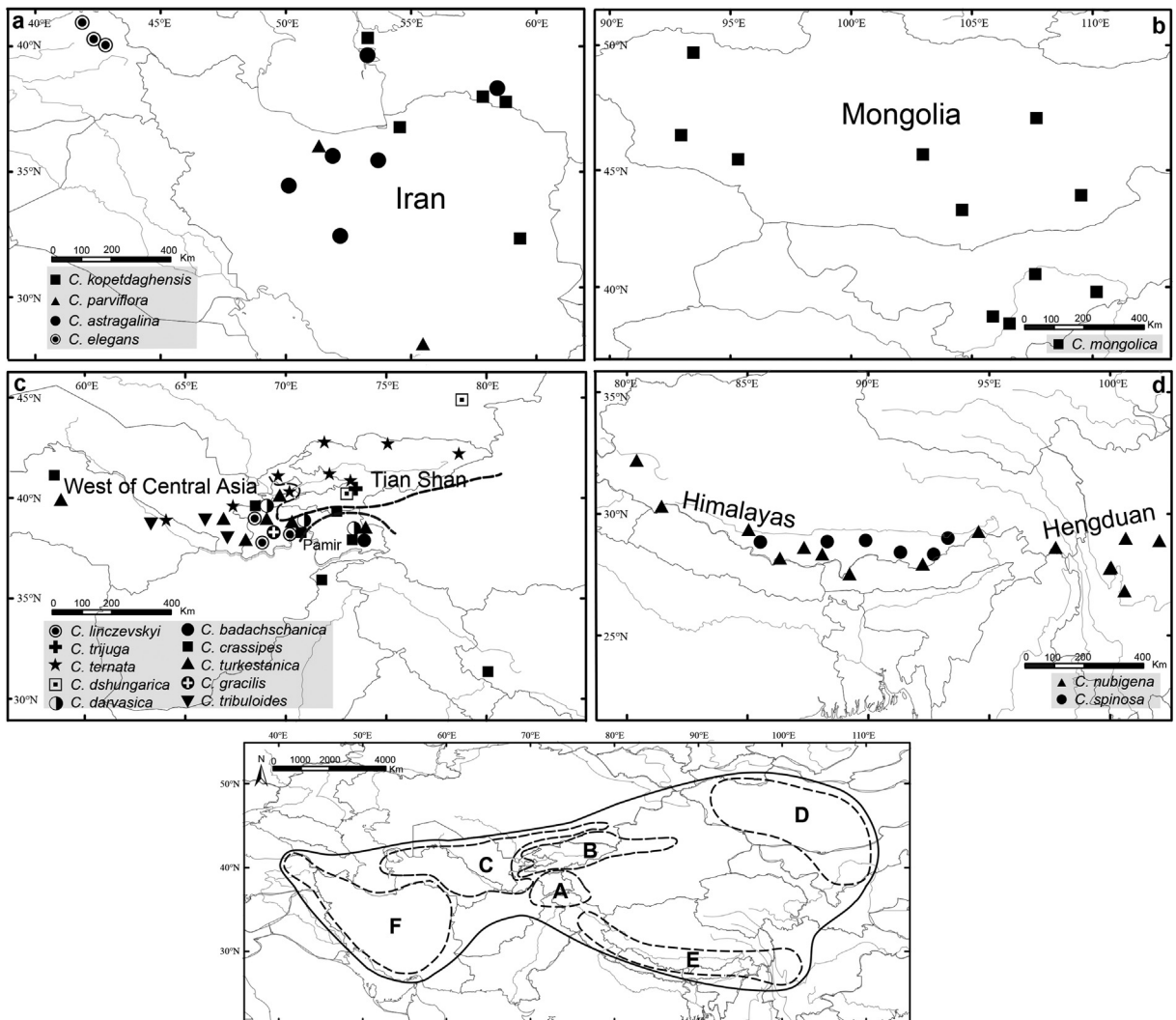
### 2.4. Biogeographical areas

Six biogeographical areas were identified based on the species distribution of *Chesneya*, see Fig. 1. Most of the species are montane, thus the Pamir-Alai, Tianshan, and Himalayan mountains, and Iranian Plateau are regarded as biogeographical areas. These are natural and independent biodiversity regions (Zachos and Habel, 2011) with distinctive vegetation and floras. Thus, the six areas are: A: Pamir-Alai; B: Tianshan Mountains, mainly their western part, and including portions of Kyrgistan and Kazakhstan, since the taxa are absent in the eastern part (see Fig. 1); C: western Central Asia; D: Mongolia, mainly southwestern Mongolia; E: the Himalayas, extending to the eastern Hengduan Mountains in China; F: the Iranian Plateau montane area near the Caspian, and the Caucasus and Turkmenistan.

### 2.5. Ancestral area reconstructions

To infer vicariance and dispersal events, two methods were used: the Bayesian statistic parsimony-based method (S-DIVA) (Nylander et al., 2008), implemented in RASP (Reconstruct Ancestral State in Phylogenies) 2.0 beta (Yu et al., 2010); and a maximum likelihood-based DEC (dispersal extinction cladogenesis) model implemented in Lagrange v.2.0.1 (Ree et al., 2005; Ree and Smith, 2008).

The BEAST molecular dating tree (Fig. 2) was treated as a fully resolved phylogram for use as the basis for S-DIVA, with 701 post-burn-in trees derived from the BEAST analysis used for ancestral area reconstruction in RASP. RASP was performed with various constraints of maximum areas 2 at each node, to infer possible ancestral areas and potential vicariance and dispersal events (e.g. Clayton et al., 2009; Almeida et al., 2012; Mao et al., 2012). Lagrange v. 2.0.1 (Ree and Smith, 2008) was used to calculate the likelihood of biogeographical routes and areas occupied by the most recent common ancestor (MRCA) for the BEAST molecular dating tree (Fig. 2) and the present distributions of taxa. Maximum likelihood parameters were estimated for rates of migratory events between areas (range expansions) and local extinctions within areas (range contractions).



**Fig. 1.** Distribution of *Chesneya* species (a,b,c,d); distribution range and six areas of species occurrence, A: the Pamir-Alai mountains; B: Tianshan Mountains; C: western Central Asia; D: Mongolia; E: Himalayas; F: Iranian Plateau.

In addition, as a comparison with S-DIVA, Bayesian binary MCMC (BBM) was employed. The calculations of biogeographical events, including dispersal, vicariance, and extinction were produced in RASP.

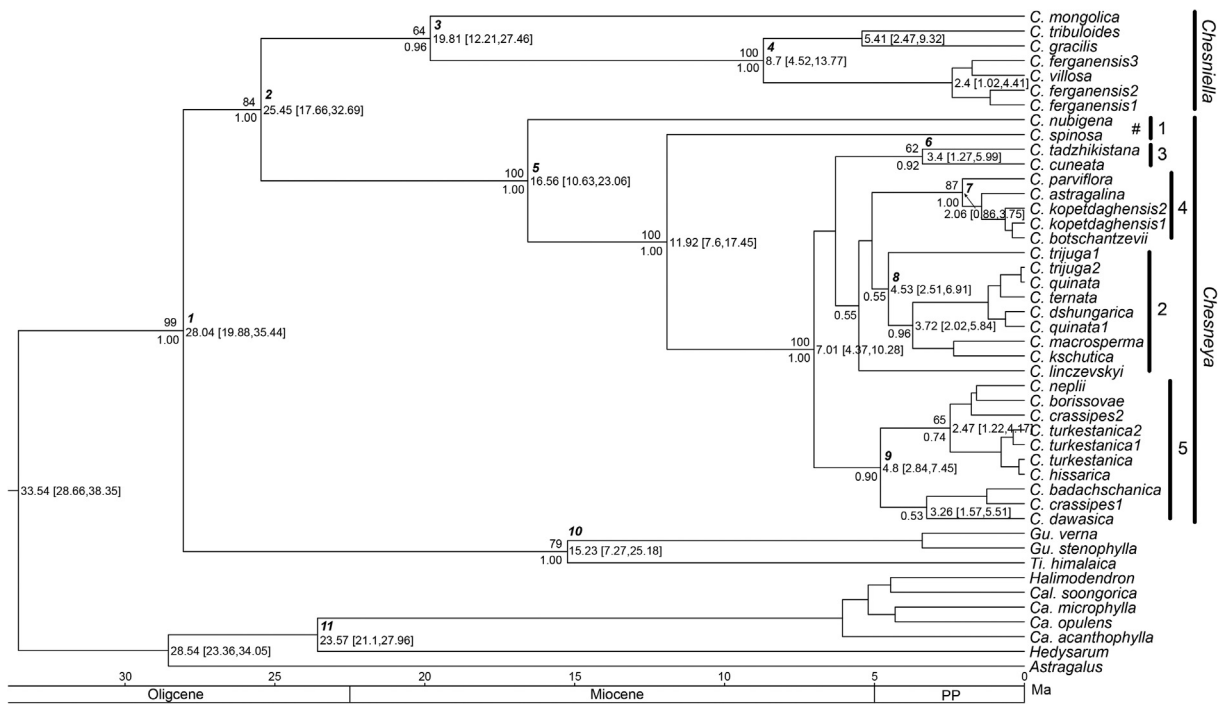
### 3. Results

#### 3.1. Phylogenetic analysis and divergence time estimates

ML analysis yielded one optimal tree, the topology of which was on the whole equivalent to that of the Bayesian tree in BEAST with high support at most nodes in Fig. 2. Since the BEAST tree also has a dating result, it is used to illustrate phylogenetic relationships and dating (see Fig. 2).

The phylogenetic tree (see Fig. 2) shows that *Chesneya* (node 5) is monophyletic and consists of four clades (nodes 6–9) plus *C. nubigena* and *C. spinosa*, as well as *Chesneya lincevskyi*. *Chesneya* + *Chesniella* (node 2) were sister to each other. However, *Spongiocarpella*, represented by *C. nubigena* and *C. spinosa*, was nested in *Chesneya*, and did not form a monophyletic clade. The three species of *Gueldenstaedtia* and *Tibetia* formed a clade (node 10). All of *Chesneya*, *Gueldenstaedtia*, *Spongiocarpella*, and *Tibetia* constituted a clade (node 1) in tribe Galegeae, while the combination (node 11) of *Caragana*, *Calophaca*, *Halimodendron*, and *Hedysarum* was sister to *Astragalus*.

The estimated divergence age of *Chesneya* at node 5 was ca. 16.56 (95% HPD: 10.63–23.06) Ma, and that of *Chesniella* at node 3 was ca. 19.81 (95% HPD: 12.21–27.46) Ma, and both *Gueldenstaedtia* and *Tibetia* at node 10 were ca. 15.23 (95% HPD:



**Fig. 2.** Phylogenetic tree and chronogram from BEAST Bayesian inference. Values at left of nodes are bootstrap support above and posterior probability below, and at the right of nodes are node numbers 1–11 in italics above, and estimated values and their 95% HPD of BEAST below. Two species of the previous *Spongiocarpella* are labeled with “#”, and should be included in *Chesneya*. Classification of five sections within *Chesneya* are: 1: Section *Pulvinatae*, 2: Section *Paucifolinatae*, 3: Section *Caulescentes*, 4: Section *Parviflorae*, 5: Section *Polyphyllae*, detailed in the Appendix.

7.27–25.18) Ma, all around early Miocene. Diversification of the four clades within *Chesneya* (nodes 6–9) with ages of 4.8–2.06 Ma shows that these were recent events in the Pliocene.

### 3.2. Ancestral area reconstructions

The results calculated by S-DIVA, BBM, and Lagrange are roughly consistent, especially at nodes 5–9 within *Chesneya*, see Fig. 3, but in the Lagrange result, there are uncertainties at nodes 1, 2, 3, and 5. Thus, the results of S-DIVA and BBM, as shown in Fig. 3 are used as our main source of information.

At node 2 (Fig. 3), three areas occurred with roughly equal probabilities, CE (Pamir-Alai Mountains and Himalayas), DE (Mongolia and the Himalayas), and BE (Tianshan Mountains and Himalayas), three share E (Himalayas union). This agrees with the BBM result, which indicated E as the ancestral area at this node. At node 3, the ancestor of *Chesniella* was estimated as CD, a dispersal from E (Himalayas). *Chesneya* had its ancestor in E, but except for the two Himalayan species *C. nubigena* and *C. spinosa*, the ancestor of all of the other species had as ancestral area C (western Central Asia). Similarly, within *Chesniella*, except for the Mongolian species *C. mongolica*, all had the ancestral area C.

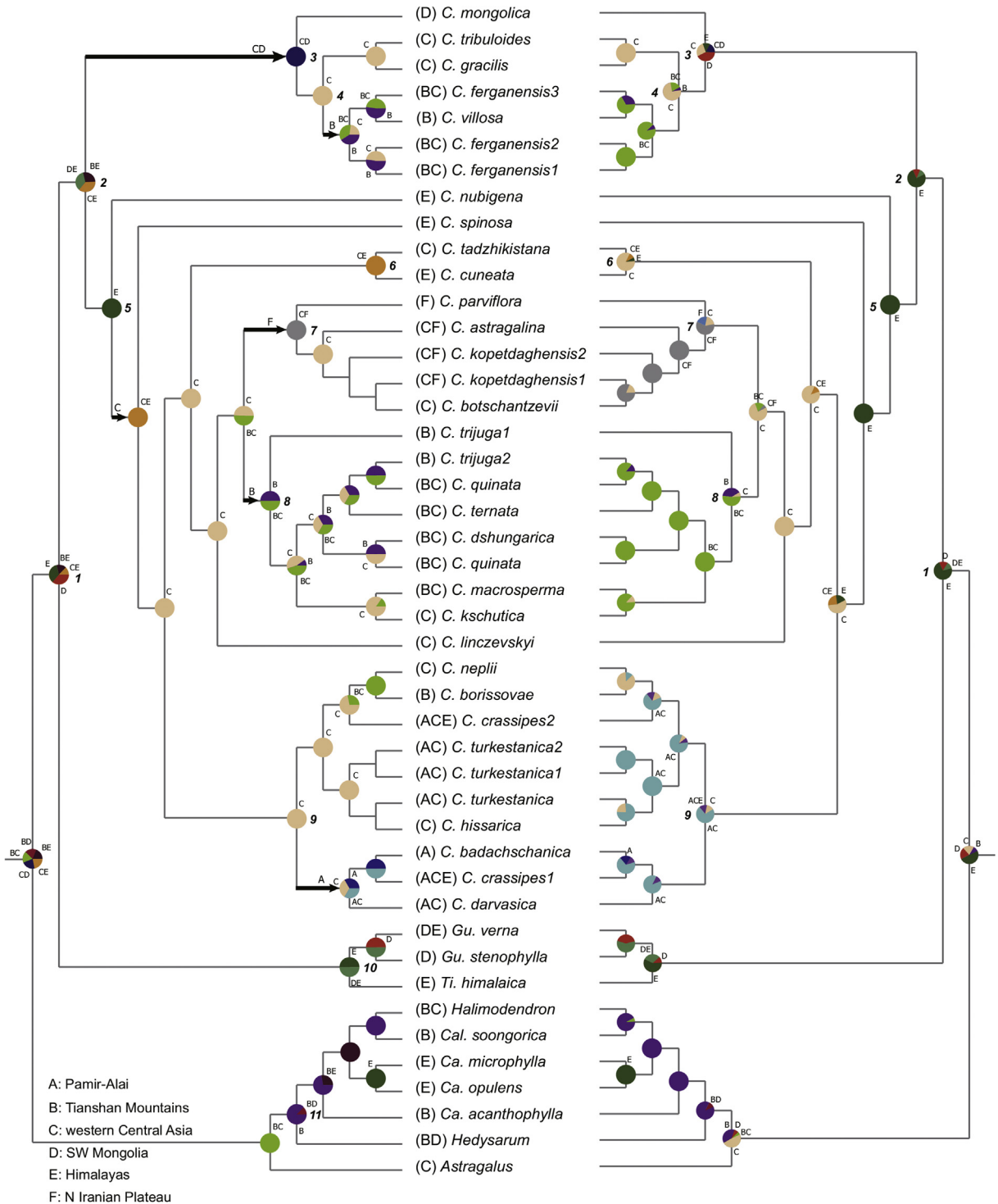
Several dispersals are indicated in Fig. 3. Except for C (western Central Asia), which dispersed from E (Himalayas), the remainder of the dispersals in A, B, D, or F were from C (western Central Asia). This demonstrates that C (western Central Asia) was the ancestral area of the Central Asian taxa.

The numbers of events was six or more since about 6 Ma, whereas the number of dispersals was four at 28 Ma (node 1) and two at about 8.7 Ma (node 4), with an additional six events since the Pliocene ca. 5 Ma. Thus the majority of biogeographical events occurred recently, after the late Miocene in Figs. 2 and 3.

## 4. Discussion

### 4.1. Phylogenetic clades and taxonomy of *Chesneya* and allied groups

Using our phylogenetic tree (Fig. 2), several previous classifications are illuminated. First, *Chesniella* is justified to be a genus instead of a subgenus within *Chesneya*, since it forms a phylogenetic clade (node 3) and is well-supported (bt = 64%, pp = 0.98). Morphologically, *Chesniella* has the distinctive characters of prostrate stems, small leaves and pods, and dense white pubescence on leaves and branches as compared to *Chesneya*. It occurs in western Central Asia, and southwestern Mongolia. Therefore, the separation of *Chesniella* and *Chesneya* by Borissova (1961) and Zhu and Larsen (2010) is preferred,



**Fig. 3.** Reconstructions of ancestral areas performed with S-DIVA (left) and BBM (right). Pie charts at the internal nodes represent the calculated probabilities (relative frequencies) of alternative ancestral area reconstructions. Node numbers are the same as in Fig. 2. In the S-DIVA tree, several dispersals are indicated with arrowheads on the lines. The six biogeographical areas are the same as in Fig. 1b; a detailed description is in the text.

rather than the combination by Yakovlev et al. (1996). *Chesneya nubigena* and *C. spinosa*, included in *Spongiocarpella* by Yakovlev and Svazeva (1987), should be included in *Chesneya*. This is in agreement with Zhu (1998), Qian (1998), and Zhu and Larsen (2010).

Within *Chesneya* (see Fig. 2), the recovery of four clades (clades 6–9) offers a framework for classification of sections. First, *C. nubigena* and *C. spinosa*, endemic to the QTP and Himalayas, have distinctive morphological characters, such as a cushionlike habit, persistent stems with spreading pubescence, and a shorter pod. Even though both are non-monophyletic, they are located at the apparent basal location of phylogenetic tree, forming a taxonomical meaningful group. Next, *Borissova* (1961) established a series *Ternatae* in *Chesneya*, based on species *Chesneya ternata* and *C. linczevskyi*, and so on, with the character of three leaflets. In the present phylogenetic tree, this group (node 8 and *C. linczevskyi*, out of node 8) was recognized with the characteristic of fewer leaves and is given the name section “Paucifolinateae” in [Support Information S1](#). Within *Chesneya*, most species are acaulescent, but a few are caulescent, such as *C. elegans* and *Chesneya tadhikistana*, these should also form a group (node 6 in Fig. 2). The clade at node 7 contains species mainly occurring in Iran, Turkmenistan, Afghanistan, and western Pakistan. A group (node 9) with many species is characterized by acaulescence and has many leaflets, is named “Polyphyllae” in our classification, to equal the previous series *Subacaules* (*Borissova*, 1945) or series *Polyphyllae* (*Borissova*, 1961). The above groups have phylogenetic identity and distinct morphological characters, and should therefore be established at sectional rank. Finally, we offer several representatives of types and important specimens ([Support Information S2](#)), and produce a classification system of five sections for *Chesneya*, see [Support Information S1](#).

Our phylogenetic tree (Fig. 2) shown that *Chesneya*, *Chesniella*, *Gueldenstaedtia*, and *Tibetia* forms a monophyletic group, which is placed in tribe Galegeae, while *Caragana*, *Calophaca*, and *Halimodendron* are included in tribe Hedysareae. This supports previous molecular systematic results (*Wojciechowski et al.*, 1999, 2000; *Lock*, 2005; *Lock and Schrire*, 2005a,b). Establishment of the tribe *Caraganae* (*Ranjbar and Karamian*, 2003) for *Chesneya* and other genera, cannot be supported.

#### 4.2. Diversification of *Chesneya* and allied groups

*Chesneya*, *Chesniella*, *Gueldenstaedtia*, and *Tibetia* constitute a clade within tribe Galegeae, and its ancestral area age (Fig. 2, node 1) is dated to 28.04 (95% HPD: 19.88–35.44) Ma, roughly approaches the estimated age of 29.3 (21.1–35.4) Ma of tribe Hedysareae (*Lavin et al.*, 2005). Diversification for *Chesneya* at node 5 was dated to 16.56 (95% HPD: 10.63–23.06) Ma, *Chesniella* at node 3 to 19.81 (95% HPD: 12.21–27.46) Ma, and both *Gueldenstaedtia* and *Tibetia* at node 14 to 15.23 (95% HPD: 7.27–25.18) Ma. These estimated generic ages are similar to that for *Caragana*, about 16 Ma (*Zhang and Fritsch*, 2010) or around early-middle Miocene, which was inferred to be related to QTP uplift (*Zhang and Fritsch*, 2010). This implies the uplift of the Himalayas and QTP appear to have triggered the origin and diversification of *Chesneya* and other genera. In addition, *Chesneya* and *Chesniella* to have originated at ca. 25.45 Ma (node 2 in Figs. 2 and 3), as well as having the bulk of their species in Central Asia, accordingly, the origin of both genera was most likely coupled with westward retreat of the Paratethys during late Oligocene (*Rögl*, 1998), a period when the climate became increasingly arid.

Ancestral area reconstruction within *Chesneya* (Fig. 3) indicates that the Himalayas (E) (node 5) is the most likely ancestral area. From its Himalayan ancestor (E) (node 2), first developing taxa during 16.56–11.29 Ma were in situ, such as *C. nubigena* and *C. spinosa*, and then dispersed to Central Asia and continuously produced its numerous descent lineages. *Chesneya* lineages from western ancestral Asia (C), dispersed to the Tianshan Mountains (B) (node 8 at ca. 4.53 Ma), and to the Iran-Turcoman area (F) (node 7 at ca. 2.06 Ma). Within *Chesniella*, dispersal from western Central Asia (C) (node 4) to the Tianshan Mountains (B) was at 8.7–2.4 Ma. At least six dispersal events occurred from Pliocene ca. 5 Ma to the present (Fig. 3). According to *Miao et al.* (2012), following Himalayan and QTP uplift, the Central Asian climate underwent cooling and drying, and a long term aridification process was maintained during Mid-late Miocene 17–5 Ma. This climatic setting may have directly induced diversification of four of the sections within *Chesneya* in Central Asia (Figs. 2–3). Meanwhile, the arid climate, and Tianshan Mountains in Central Asia and adjacent regions, allowed dispersal of *Chesneya* and *Chesniella* species along the mountains.

#### 4.3. *Chesneya* and its floristic implication for the Central Asian flora

Central Asian phytogeography is an attractive subject, and Russian botanists have had numerous contributions (e.g. *Wulff*, 1944; *Grubov*, 1999; *Tahktajan*, 1969). *Popov* (1938, see *Wulff*, 1944) proposed three evolutionary stages for the Central Asian flora, from the Cretaceous to early Tertiary, late Tertiary, and from late Tertiary onward. *Chesneya*, with the distribution concentration in Central Asia, offers one case at the second stage (*Popov* 1938). During that epoch, as mentioned above, *Chesneya* diversification was associated with QTP uplift and the Central Asian increasing aridification process, and *Chesniella* and *Tibetia* likewise. Later, diversification of the four sections within *Chesneya* during the Pliocene (Figs. 2,3) fits the scenario of *Popov's* third stage.

The origins of the Central Asian flora, in accordance with its geographic location, can be hypothesized as being from the adjacent areas of East Asia, the Mediterranean, the northern Altai, or Siberia, or could be native to the area. *Wulff* (1944) and *Iljin* (1937) suggested that the Central Asian flora was primarily native or originated from the Mediterranean, instead of from Africa. *Grubov* (1999) thought that the Central Asian flora was ancient instead of young, and its origin was native. In the cases of particular plants, *Caragana* was hypothesized to have been formed in East Asia by *Komarov* (1908), *Nitraria* was inferred to have originated from Africa by *Komarov* (1947), or was native according to *Grubov* (1999), or from the Tethys, i.e., the modern Mediterranean (*Pan et al.*, 2003). A Himalayan origin has been demonstrated with *Hippophae rhamnoides* (*Jia et al.*, 2012), *Myricaria* (*Zhang et al.*, 2014a), and to these can now be added *Chesneya*.



Even *Chesneya* originated from the Himalayas and diversified in the early Miocene, however, most of *Chesneya* extant species in Central Asia and adjacent regions had their ancestry in the lowlands of western Central Asia (C) (Fig. 3), and montane distributions in the highlands of the Pamir-Alai, Tianshan, Iranian Plateau, and Caucasus were later dispersals. This speciation mode is just in contrast to that in *Atraphaxis*, which is inferred to have originated from moist montane habitats of the western Tianshan Mountains and then dispersed to grasslands and deserts (Wulff, 1944; Zhang et al., 2014b).

## Acknowledgments

We are grateful to the herbaria staffs of the Komarov Botanical Institute, Russian Academy of Sciences (St. Petersburg), Moscow University (Moscow), Main Botanical Garden, Russian Academy of Sciences (Moscow), and Institute of Botany, Chinese Academy of Sciences (Beijing) for our convenience in checking of specimens. Many thanks to Dr. James I. Cohen working at Texas A&M International University for his English improvements to the manuscript, and to Prof. Matt Lavin, Prof. Richard M. K. Saunders, and Dr. Michael D. Pirie for their valuable and constructive comments and suggestions to the manuscript. Funding was provided by the China National Key Basic Research Program (2014CB954201), and the Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.bse.2015.09.017>.

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