Molecular phylogeny and biogeography of the narrow endemic *Coelonema* and affinitive *Draba* (Brassicaceae) based on two DNA regions

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ABSTRACT

To clarify the relationship between two genera, *Draba* and the narrow genus *Coelonema*, endemic to the QilianMountains of the northeastern Qinghai-Tibet Plateau, phylogenetic analyses were conducted using nuclear ribosomal DNA ITS, and the chloroplast DNA *trnL*, from *Coelonema draboides* and 30 species of *Draba* representing eight sections, including 25 species of Chinese *Draba*, seven of which were endemic to the study region. The results unambiguously support several previously published proposals to unite *Coelonema* with *Draba* and accommodate *C. draboides* in the latter genus on the basis of morphological re-examination. Our molecular data presented here also provide evidence that these two genera should be combined as a monophyletic group with high support. In addition, it is estimated that *Draba* may have originated about 1.36–2.71 Mya, with *C. draboides* diverging from *Draba* about 0.15–0.31 Mya, based on the molecular calibration of ITS datasets. The assumed speciation and rapid expansion of these two genera is likely to have occurred in the eastern edge of the QilianMountains area according to molecular phylogeny and estimated divergence times, which correspond well with the known geological and paleobotanical histories of the Qinghai-Tibet Plateau.

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1. Introduction

The Qinghai-Tibet Plateau (QTP) is the largest, highest and youngest plateau in the world, covering an area of more than 2.5×10^6 km^2 at an average elevation of about 4000 m above sea level (Zheng, 1996). Despite significant impacts from both the ice sheet that covered almost the entire plateau during the Quaternary and the uplifting of the QTP since the Tertiary, which must have resulted in large-scale recession and extinction of the flora (Li et al., 1995), the QTP is characterised by an exceptionally young flora with more than 12,000 species of 1500 genera and 189 families. It is estimated that about 50 genera and more than 25% of the total number of species are endemic to this region (Wu, 1980, 1987; Wang et al., 1993; Wu and Wu, 1996). These endemic genera of the QTP, which have been classified primarily on morphological characteristics, are hypothesized to be closely related, and to have originated from local or adjacently distributed genera (Wu, 1980, 1987; Wu et al., 1995). If this hypothesis is correct, it should be possible to find the sympatrically or immediately specific progenitors

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of all endemics in the region around the QTP. However, it is still difficult to identify the close relatives of some endemics, especially some endemic genera, using purely morphological features.

As a monotypic genus of the Brassicaceae, *Coelonema* Maxim., which is endemic to the QilianMountains of the northeastern QTP, was initially established by Maximovicz (1880). *Coelonema draboides* Maxim., the only representative of the monotypic genus *Coelonema*, is a perennial, stoloniferous herb, which produces many stems covered with persistent petiolar remains from previous years. Stems reach a height of 4–8 cm before flowering, with simple and forked trichomes. Plants flower from June to July forming a rosette, and silicles mature by about August. Typical habitats of *C. draboides* are alpine meadow, rocky crevices, and shrubland within cryospheric regions at altitudes of 3500–4000 m above sea level (Zhou, 1987, 2001). It is distributed narrowly in the Huzhu and Datong Counties of Qinghai Province, and in Tianzhu County of Gansu Province in the QilianMountains. *Draba* L. is the largest genus in the family Brassicaceae and a member of the tribe Arbideae, includes about 370 described species distributed worldwide, especially in the subarctic to arctic regions, and in alpine or mountainous areas of the temperate regions of the Northern Hemisphere (Al-Shehbaz, 1987; Al-Shehbaz, et al., 2006; Warwick and Al-Shehbaz, 2006; Koch et al., 2007; Jordon-Thaden et al., 2010). *Draba* is notorious for its taxonomic complexity, the current sectional classification at the infrageneric level being highly controversial. Schulz (1927, 1936) divided this genus into 17 sections according to the presence or absence of stem leaves and flower colour etc., but some botanists (e.g. Fernald, 1934; Al-Shehbaz, 1987) have criticized this artificial sectional classification. For Chinese *Draba*, comprising 48 species (16 endemic), Zhou (1987) divided the group into the following four sections following Schulz's (1927, 1936) and Walters's (1964) sectional delimitation: *Chrysodraba* DC., *Phyllodraba* O. E. Schulz, *Draba* and *Drabella* DC.

Zhou (1987) connected *Coelonema* with *Draba,* and delimited *Armoracia* Gaertn. within the tribe *Drabeae* of Brassicaceae, and proposed that it was closely related to *Draba* on the basis of morphological characters (Maximovicz, 1880; Ho et al., 1997). However, *Coelonema* was still treated as an independent genus (Zhou, 1987, 2001), because it differed from *Draba,* but only to the extent that the former genus included stoloniferous perennial species with flattened bases to their staminal filaments, while *Draba* consisted of annuals, biennials, or perennials with slender or slightly flattened staminal filaments (Al-Shehbaz, 2004). Despite these differences, *Coelonema* was transferred to *Draba*, and *C. draboides* was renamed *Draba draboides*, since there were no other significant differences in morphological characters (Appel and Al-Shehbaz, 2003; Al-Shehbaz, 2004). As a result, the phylogenetic relationship between *Coelonema* and *Draba* has not yet been explicitly addressed. DNA sequences are particularly informative for resolving the true relationships among problematic genera, especially where morphological characters are lacking or ambiguous (Francisco-Ortega et al., 1997, 2001; Panero et al., 1999; Park et al., 2001). The internal transcribed spacers (ITS) region of nuclear ribosomal DNA and the *trnL* (UAA) intron (*trnL*) region of noncoding chloroplast DNA have been shown to be suitable markers for phylogenetic reconstructions within a genus, or for closely related genera in the QTP (Chen et al., 2005a,b; Liu et al., 2000, 2001, 2002, 2006; Wang and Liu, 2004;Wang et al., 2005, 2007; Zhang et al., 2008). Moreover, these two regions have also been used for the phylogenetic analyses of foreign *Draba* (Widmer and Baltisberger, 1999; Koch and Al-Shehbaz, 2002; Scheen et al., 2002; Beilstein and Windham, 2003; Grundt et al., 2004). In addition, major goals of modern biogeography are to reconstruct the phylogenies of genera and evaluate their origin and evolution in the context of the geological and paleoclimatic histories of their distribution areas (Avise, 2000). However, neither divergence time nor biogeographic analyses of *Coelonema* and Chinese *Draba* have been performed in any previous studies.

Hence, the main aims of the study presented here were to apply molecular phylogenetic approaches based on nuclear ITS and plastid *trnL* sequence data (focusing on the narrow endemic *Coelonema* as well as Chinese *Draba*) in order to: (1) clarify the phylogenetic relationships between *Coelonema* and *Draba*, (2) infer the divergence time of these two genera using a molecular calibration, and (3) assess putative correlations between the origins of the two genera and geological events.

2. Materials and methods

2.1. Plant materials

Twenty-four species (including varieties treated as species), belonging to *Coelonema* and *Draba* were newly sampled (Table 1). The species of *Draba* represent all sections of this genus in China, according to Zhou (1987, 2001). Among the studied species of these two genera, eight species endemic to China were sampled, including *C. draboides*, *Draba handelii*, *Draba ladyginii*, *Draba rockii*, *Draba senilis* etc. Voucher specimens were deposited in the herbarium of the Northwest Plateau Institute of Biology (HNWP), Chinese Academy of Sciences. Sequence data for eight species of *Draba* were retrieved from GenBank (indicated by "[a]" in Table 1); their origins and reasons for their selection are given in Koch and Al-Shehbaz (2002; see Fig. 2) and Koch et al. (2005). *Arabis alpina* L. and *Erophila verna* (L.) Chev. were chosen as outgroups based on the results of previous studies (Schulz, 1927; Koch and Al-Shehbaz, 2002). Table 1 lists the origins of the taxonomic material, voucher information, and GenBank accession numbers of the species studied. The gross morphological characters of *Coelonema* and *Draba* are compared in Table 2.

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from silica-gel-dried leaves or other material from herbarium specimens using the $2\times$ CTAB procedure following Doyle and Doyle (1987) or a CASsuper Plant Genomic DNA Isolation Kit, following the manufacturer's protocol (Casarray, Shanghai, China). The nuclear ITS and plastid *trnL* regions were amplified with universal primers

Table 1

List of taxa, origin of materials, voucher information and GenBank accession numbers. (The infrageneric classification system is according to Schulz (1927) and Zhou (1987, 2001)).

Genus Section	Species	Locality	Voucher	GenBank Accesssion No.	
				ITS region	trnL (UAA) intron
Coelonema Maxim.	*C. draboides* Maxim.	Huzhu, Qinghai 3500	Chen006		
Draba L.					
*Chrysodraba*DC.	*D. alpina* L.	Huzhu, Qinghai 3600	1788		
	D. incompta Steven	Qiemo, Xinjiang 4180	2626		
	D. involucrata (W. W. Smith) W. W. Smith[(a)]	Qumalai, Qinghai 4600	00770		
	D. involucrata (W. W. Smith) W. W. Smith [(b)a]	India	Kli26	DQ518393	DQ180237
	D. oreades Schulz	Maqin, Qinghai 4340	665		
	D. rockii O. E. Schulz	Baqing, Xizang 4730	4059		
	D. senilis O. E. Schulz	Yushu, Qinghai 5085	675		
	D.oligosperma Hook.[a]	U. S. A.	79233	AF146491	AF146939
Phyllodraba O. E. Schulz	*D. elata* Hook. F. et Thoms.	Cuona, Xizang 4100	75–947		
Draba	*D. altaica* (C. A. Mey.) Bunge	Yushu, Qinghai 4850	531		
	D. glomerata Royle	Maduo, Qinghai 4660	243		
	D. handelii O. E. Schulz	Dingri, Xizang 5200	5727		
	D. ladyginii Pohle[(a)]	Yushu, Qinghai 3900	651		
	D. ladyginii Pohle[(b)]	Haibei, Qinghai 3400	L483		
	D. lanceolata Royle	Yuanqu, Shanxi 2500	0310		
	D. lanceolata Royle *var..chingii* O. E. Schulz	Menyuan, Qinghai 3800	L476		
	D. lanceolata Royle *var leiocarpa* O. E. Schulz	Dulan, Qinghai 3500	L558		
	D. lichiangensis W. W. Smith	Maqin, Qinghai 4780	776		
	D. mongolica Turcz.[(a)]	Datong, Qinghai 3650	5680		
	D. mongolica Turcz.[(b)]	Hainan, Qinghai 3300	L499		
	D. parviflora (E. Regel) O. E. Schulz	Dangci, Xizang 4280	079		
	D. subamplexicaulis C. A. Mey	Maqin, Qinghai 3950	840		
	D. incana L.	Dulan, Qinghai 3540	L547		
	D.lasiophylla Royle[a]	India	Kli27	DQ518394	DQ180238
*Drabella*DC.	*D. ellipsoidea* Hook. f. et Thoms.	Jiuzhi, Qinghai 3800	530		
	D. eriopoda Turcz.[(a)]	Hainan, Qinghai 4000	6399		
	D. eriopoda Turcz.[(b)]	Haibei, Qinghai 3420	L481		
	D. eriopoda Turcz.[(c)]	Hainan, Qinghai 3750	L497		
	D. nemorosa L.[(a)]	Guyuan, Ningxia 2450	76–00900		
	D. nemorosa L.[(b)]	Maqin, Qinghai 3700	L513		
	D. stenocarpa Hook. F. et Thoms.	Dari, Qinghai 4150	1712		
Calodraba O. E. Schulz	*D. peruviana* (DC.) O. E. Schulz[a]	Peru	4440	AF146468	AF146915
	D.hitchcockii Rollins[a]	U. S. A.	83263	AF146487	AF146935
Rhabdodraba O. E. Schulz	*D.schusteri* O. E. Schulz[a]	Peru	6033	AF146453	AF146901
*Leucodraba*DC.	*D.dubia* Suter[a]	Switzerland	1999b	AF120722	AF120738
*Aizopsis*DC.	*D.aizoides* L.[a]	Austria	OSBU#5199	AF146512	AF146958
Arabis L.	*A.alpina* L.[a]	Romania	3618	AF137559	AY034180
*Erophila*DC.	E. verna (L.) Chev.[a]	Germany	Lys0048	DQ249862	DQ479871

(a) and (b), both in text and table, indicate the same plant collected from different locations.

c and d, both in text and table, indicate universal primers.

[a] Indicates sequences of these species that were downloaded from GenBank.

ITS1 and ITS4 (White et al., 1990), and c and d (Taberlet et al., 1991), respectively. Polymerase chain reactions (PCRs) were carried out in a Biometra thermal cycler with 25 μl reaction mixtures, containing 10 ng– 20 ng genomic DNA, 50 mM Tris-HCL, 1.5 mM MgCl$_2$, 0.5 mM dNTPs, 1.25 μM of each primer and 0.75 U *Taq*DNA polymerase (CA Sarray, Shanghai, China). PCRs were performed with the following programs: initial denaturation at 94 °C for 4 min; 36 cycles of 94 °C for 45 s, 56 °C (45 °C for *trnL*) for 50 s, 72 °C for 1 min; and a final extension step at 72 °C for 7 min. All successfully amplified DNA fragments were purified using a CASpure PCR Purification Kit following the protocol recommended by the manufacturer (CASarray, Shanghai, China). Sequencing primers were the same as those used for amplifying the corresponding regions, but the reaction volumes were scaled down to 10 μl. Sequencing reactions were carried out in a Biometra thermal cycler (Tpersonal 48) using a DYEnamic ET terminator Cycle Sequencing Kit (Amersham Biosciences Corp.) following the manufacturer's protocol. Sequencing products were cleaned using Autoseq 96 plates and then analysed with a MegaBACE500 DNA Analysis System (Amersham Biosciences Corp.). Both DNA strands were sequenced using forward and reverse primers, giving an overlap of at least 70%. Consequently, and since materials of some species were sampled from more than one locality, 28 sequences of ITS and *trnL* were obtained from 24 species of *Coelonema* and *Draba*.

2.3. Data analysis

All sequences were aligned using ClustalX with additional minor manual adjustments. Sequences' boundaries were determined by comparison with published sequences of the genus *Draba* retrieved from Genbank (Koch and Al-Shehbaz, 2002). Each sequence dataset was subjected to maximum parsimony (MP) and maximum likelihood (ML) analyses using

Table 2
Comparison of gross morphological characters of *Coelonema* and *Draba* according to Zhou (1987, 2001).

Genus Characters	*Coelonema*	*Draba*
Life form	herbs perennial	herbs perennial, rarely annual, biennial
Stem	erect or decumbent	erect or ascending, sometimes prostrate
Leaf	basal leaves subsessile or shortly petiolate, rosulate, simple, entire, ciliate, with persistent petiolar bases; cauline leaves similar to basal ones	basal leaves petiolate, often rosulate, simple, entire or toothed, rarely lobed; cauline leaves petiolate or sessile, cuneate or auriculate at base, entire or dentate, sometimes absent
Anthotaxy	racemes several flowered, ebracteate of basally bracteate	racemes bracteate or ebracteate, elongated or not in fruit
Sepal	broadly ovate or oblong, ascending, pubescent, unequal, base of lateral pair subsaccate, margin membranous	ovate, oblong, or elliptic, base of lateral pair not saccate or subsaccate, margin usually membranous
Petal	yellow, longer than sepals; blade obovate, apex subemarginate; claw short, obscurely differentiated form blade	yellow, white, pink purple, orange (or rarely red); blade obovate, spatulate, oblong, oblanceolate, orbicular, or linear, apex obtuse, rounded, or rarely emarginate; claw obscurely to strongly differentiated from blade
Stamen	6, erect, tetradymamous; hollow filaments filiform, dilated at base, winged, toothless; anthers ovate, obtuse at apex	6, tetradynamous; solid filaments thin, dilated or not at base, wingless; anthers ovate or oblong, obtuse at apex
Gland	median glands absent; lateral-glands lunar	median glands present or absent; lateralglands toothlike, semiannular, or annular
Ovules	8–10 per ovary	4 to numerous per ovary
Fruit	dehiscent silicles, broadly ovate to ovate-oblong, latiseptate, sessile; valves papery, prominently veined, glabrous or puberulent, smooth; replum rounded; septum complete; style to 0.7 mm; stigma capitate, entire	dehiscent, silicles or rarely siliques, ovate, elliptic, oblong, orbicular, ovoid, globose, lanceolate, or linear, latiseptate or terete, sometimes spirally twisted; valves distinctly or obscurely veined, glabrous or pubescent; replum rounded; septum complete, membranous, translucent; style distinct or obsolete, glabrous; stigma capitate, entire or slightly 2-lobed
Seed	blackish, biseriate, wingless, oblong, flattened; seed coat reticulate; cotyledons accumbent	biseriate, wingless (or rarely winged), oblong, ovate, or orbicular, flattened; seed coat minutely reticulate, not mucilaginous when wetted; cotyledons accumbent

PAUP*4.0b10 (Swofford, 2003) and Bayesian analysis using MrBayes 3.0 (Huelsenbeck and Ronquist, 2001). Modeltest, Version 3.06 (Posada and Crandall, 1998), was used to select parameters and assumptions for ML analyses. In MP analysis, characters were equally weighted and unordered (Fitch, 1971), with all gaps treated as missing data. Heuristic searches were conducted with 100 replicates of random addition of sequences, in combination with ACCTRAN character optimization, MULPARS + TBR branch-swapping and STEEPEST DESCENT options to search for multiple islands of most-parsimonious trees (Maddison, 1991). The relative support for individual clades was evaluated by bootstrap analysis (Felsenstein, 1985). Bootstrap values (BS) were calculated from 1000 replicates using a heuristic search with simple addition of sequence, TBR branch-swapping, and MULPARS options. For Bayesian analyses using a common model of GTR + I + G, four simultaneous Monte Carlo Markov chains (MCMCs) were run for two million generations, saving a tree every 100 generation. A majority-rule consensus tree was calculated by PAUP* from the last 16,001 out of the 20,001 trees sampled. The first 4000 trees (burn-in) were excluded to avoid trees that might have been sampled prior to the convergence of the Markov chains. The Bayesian posterior probabilities (BPP) of each topological bipartition were also estimated according to their frequency across all 16,001 sampled trees (Larget and Simon, 1999). Support for each branch was assessed using both BP and BPP. The incongruence length difference (ILD) test (Farris et al., 1995) was used to detect potential conflicts between the phylogenetic indications provided by the nuclear and plastid datasets, and to determine whether it would be valid to combine them. The ILD test was implemented as a partition homogeneity test in the PAUP* program (Sinauer Associates Inc., Sunderland, MA, USA), in which 1000 replicates were each heuristically searched with 100 random sequence additions.

An ML analysis was also performed with PAUP. The best-fit ML substitution model for each dataset was selected using Modeltest, and these optimal models were then used to parameterize likelihood searches in PAUP, with the simple addition of sequences, TBR branch-swapping, MULTREES and COLLAPSE options selected. Based on ITS sequence data, the molecular clock hypothesis was tested using a likelihood-ratio (LR) test (Huelsenbeck and Rannala, 1997) in PAUP to compare the log-likelihood of the ML trees with and without a molecular clock assumption. When the LR test rejected a molecular clock hypothesis, the ML tree based on the ITS sequence was subjected to non-parametric rate smoothing (NPRS; Sanderson, 1997) using the default settings in TreeEdit v1.0a8 (Rambaut and Charleston, 2000; Richardson et al., 2001) to estimate divergence times.

3. Results

3.1. ITS and trnL sequences

The lengths of unaligned ITS and *trnL* sequences varied from 662 to 666 base pairs (bp) and from 356 to 359 bp, and the total alignments were 668 bp and 359 bp long among species of *Coelonema* and *Draba*, respectively. The mean pairwise

distance between *C. draboides* and the *Draba* species ranged from 0.301% (*C. draboides vs. Draba lanceolata var. leiocarpa*) to 4.834% (*C. draboides vs. Draba nemorosa*[a]) for ITS and from 0 to 2.535% (*C. draboides vs. Draba aizoides*) for *trnL*. The highest pairwise distance found within species of *Draba* was 5.438% for ITS (*D. nemorosa*[a] *vs. Draba glomerata* and *D. lanceolata var. chingii*) and 2.817% for *trnL* (*D. aizoides vs. Draba incompta* and *D. nemorosa*[b]). The ingroup (*Coelonema* and *Draba*) species showed a pairwise distance variation of 5.430%–8.540% for ITS and 1.690%–3.380% for *trnL* with two outgroup taxa, *A. alpina* and *E. verna* (pairwise distance matrix not shown).

3.2. Phylogenetic analysis and divergence time

The nuclear ITS matrix used for the analyses contained 668 characters, of which 544 were constant, 79 variable but parsimony-uninformative, and 45 phylogenetically informative when the gaps were treated as missing data. MP analyses yielded over 2208 trees with a length of 176 steps, a consistency index (CI) of 0.784 and a retention index (RI) of 0.779. The 50% majority-rule consensus tree derived from Bayesian analysis is shown in Fig. 1, with BPP values noted above branches. The topologies of the ML tree (not shown; −ln 1976.987, the best-fit model being SYM + G) and the Bayesian 50% majority-rule consensus tree were mostly congruent with the strict consensus tree (not shown). Many of the nodes along the spine of
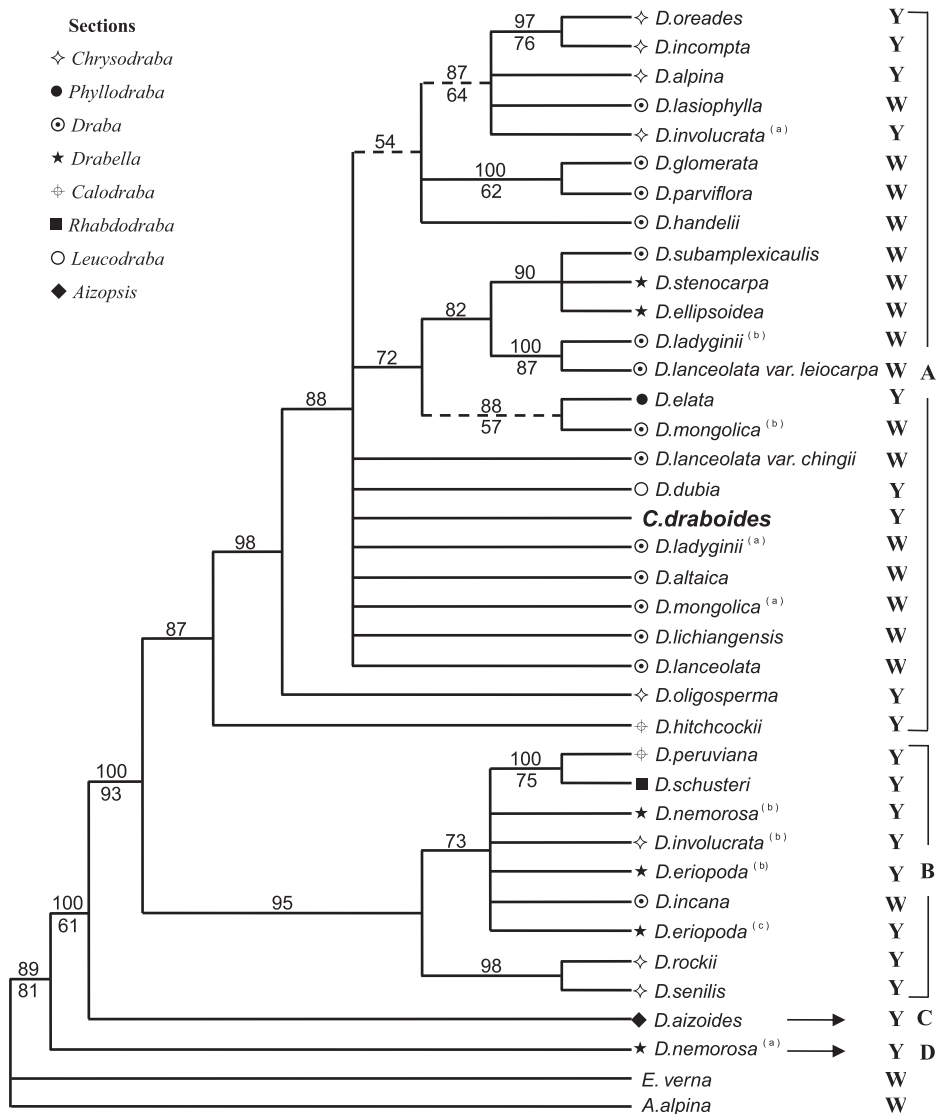

Fig. 1. The 50% majority-rule consensus tree derived from Bayesian analysis of the nuclear internal transcribed spacer (ITS) dataset. Numbers above and below the branches indicate the Bayesian posterior probabilities (BPP) and the maximum parsimony bootstrap (BS) values with >50% support based on 1000 replicates, respectively. Dashed lines denote branches that collapse in the strict consensus tree from the parsimony analysis. The sections following each species are indicated. Petal colour is indicated by Y (yellow) and W (white).

the tree in Fig. 1 have moderate or strong BPP and BS (below branches) values. In common with previous reports based on morphological characters (Appel and Al-Shehbaz, 2003; Al-Shehbaz, 2004), the present analyses strongly support the notion that *C. draboides* should be accommodated within *Draba* (BS = 81%; BPP = 89%).

When gaps were excluded, the aligned plastid *trnL* data included 359 total characters, of which 13 were variable, and only 11 were potentially informative. The Bayesian 50% majority-rule consensus tree is shown in Fig. 2. Parsimony analysis identified only four MP trees with 28 steps (CI = 0.893; RI = 0.943). The ML analysis resulted in a tree with a likelihood score of –ln 670.542 (not shown; the best-fit model being HKY + G). The topological results of three methods were largely congruent, and also strongly support the placement of *Coelonema*, which should be united with *Draba* (BS = 87%; BPP = 66%) in accordance with the ITS results.

Since the ILD test showed potential conflicts between the nuclear ITS and plastid *trnL* datasets ($P = 0.01$), the data for two fragments were not combined. Although Koch and Al-Shehbaz (2002) demonstrated that the genus *Erophila* should be integrated into *Draba*, our results from the ITS tree (Fig. 1; BS = 81%; BPP = 89%) and the *trnL* tree (Fig. 2; BS = 66%; BPP = 87%) don't support this conclusion. The assumption of a molecular clock using the ITS sequences matrix could not be rejected on the basis of an LR test (1976.987 vs. 1995.452, d.f. = 36, $P > 0.05$). However, no reliable fossil record for the tribe *Drabeae* is available and it is not possible to calibrate a related node to estimate the divergence time for *C. draboides*. Based on the ITS clock used by Koch and Al-Shehbaz (2002) for *Draba*, in which a 1% divergence corresponded to 0.5–1.0 million years, *Draba* may have originated about 1.36–2.71 million years ago (Mya) and *C. draboides* is estimated to have diverged from about 0.15 to 0.31 Mya (Figure not shown).
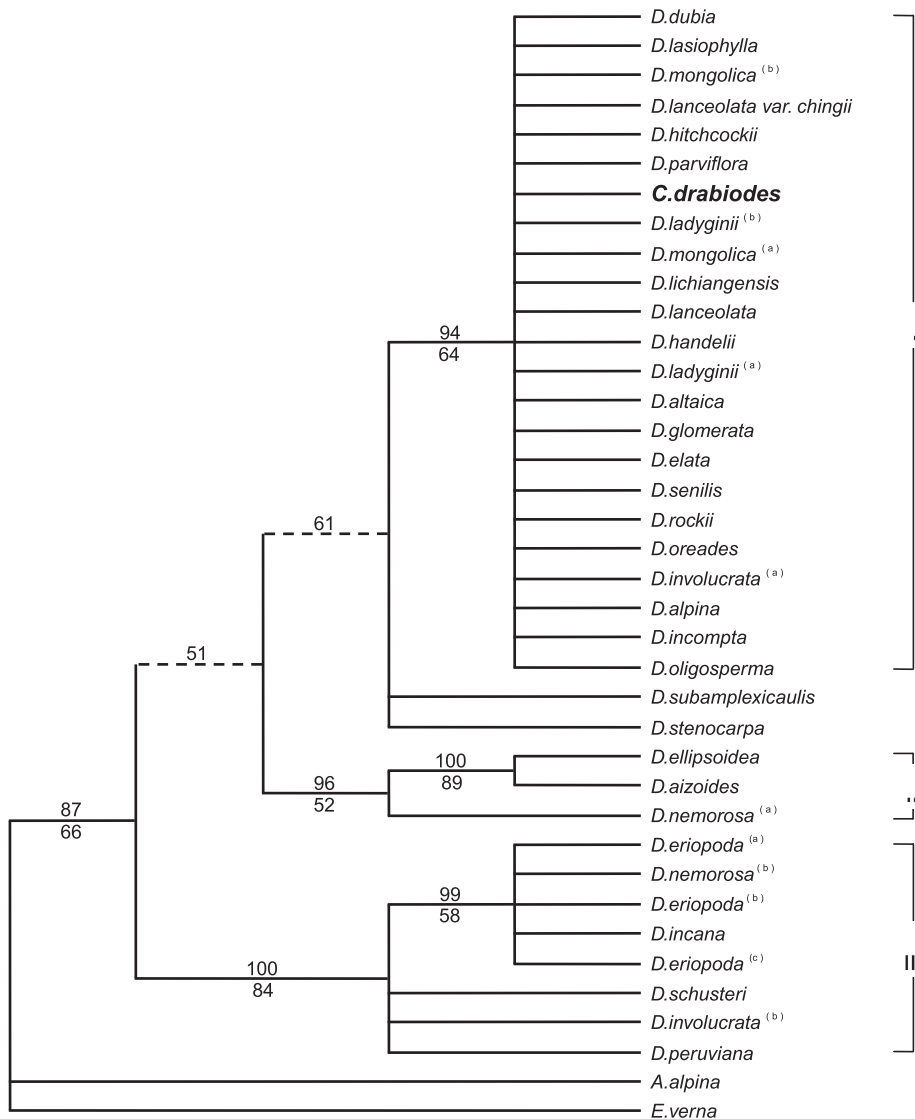

Fig. 2. The 50% majority-rule consensus tree derived from Bayesian analysis of the *trnL* matrix. See Fig. 1 for further details.

4. Discussion

4.1. Phylogenetic relationships of Coelonema and Draba

Based on the analysis of nuclear ITS sequences, western North American and South American *Draba* have previously been reported to be monophyletic taxa by Koch and Al-Shehbaz (2002) and by Beilstein and Windham (2003). And then, Jordon-Thaden et al. (2010) tested that *Draba* represented a monophyletic evolutionary lineage within the tribe *Arbideae*. Similarly, our present results from the analyses of molecular data also indicate *Coelonema* and *Draba* to be monophyletic. In the phylogenetic tree based on ITS sequence data, 31 species of these two genera fall tentatively into four clades (Fig. 1): *C. draboides* is grouped with the 22 species of *Draba* (Group A; BPP = 87%), Group B comprises eight species of *Draba* (BPP = 95%), whereas Groups C and D consist of *D. aizoides* and *D. nemorosa*[a] with high supports (BS = 93%, BPP = 100%; BS = 61%, BPP = 100%), respectively. In common with the results of Beilstein and Windham (2003), a basal clade is also formed by Group D with *D. nemorosa*[a]. On the basis of 36 sets of sequence data for 30 species, three groups (I, II and III) have been provisionally identified within the *trnL* tree (Fig. 2). *C. draboides* forms a clade with 20 species of *Draba* in Group I (BS = 64%, BPP = 94%), Group II comprises three species of *Draba* (BS = 52%; BPP = 96%), and six species of *Draba* form Group III with high BS and BPP values (BS = 84%; BPP = 100%). These results strongly indicate that *Coelonema* should not be treated as a monotypic genus endemic to the QilianMountains, and that *C. draboides* should certainly be nested within *Draba*. These results also support the conclusions drawn by Appel and Al-Shehbaz (2003) and Al-Shehbaz (2004), on the basis of morphological re-examination, that *C. draboides* should be accommodated within *Draba* and renamed *D. draboides*.

It is well-known that the genus *Draba*, which includes about 370 species primarily distributed in the arctic-alpine region of the Northern Hemisphere, with some 80 species in South and Central America and 48 species (16 endemic) in China, is the largest and most taxonomically problematic genus in the Brassicaceae (Al-Shehbaz, 1987; Zhou, 1987, 2001; Koch and Al-Shehbaz, 2002; Al-Shehbaz, et al., 2006; Warwick and Al-Shehbaz, 2006; Koch et al., 2007; Jordon-Thaden et al., 2010). The phylogenetic relationships among species of *Draba* have been investigated in many studies comparing gross morphology, floral anatomy, chromosomes and molecular data (Schulz, 1927; Mulligan, 1976; Zhou, 1987, 2001; Brochmann et al., 1992; Koch and Al-Shehbaz, 2002; Scheen et al., 2002; Beilstein and Windham, 2003; Grundt et al., 2004, 2006; Jordon-Thaden et al., 2010). However, until the present study of 25 out of 48 Chinese *Draba* species, including seven endemic species and seven foreign species (Table 1), the molecular phylogeny of the Chinese species of *Draba* had not been investigated. Koch and Al-Shehbaz (2002) found little support for Schulz's (1927) sections of South American species of *Draba*, using ITS and *trnL* data. Although the species in the present study were subdivided into eight sections of *Draba* according to Schulz (1927) and Zhou (1987), our results don't generally support the infrageneric classification, with the possible exception of some subclades, for example *Draba oreades* and *D. incompta* (BS = 76%, BPP = 97%) and *D. rockii* and *D. senilis* (BPP = 98%) (Fig. 1). Moreover, petal colour among species of *Draba* in the field has been considered as a taxonomically important character when combined with information on chromosome number (Mulligan, 1976; Zhou, 2001; Beilstein and Windham, 2003). Mulligan (1976) proposed that native North American *Draba* could be split into three groups: the first comprising all white-flowered taxa with euploid ($x = 8$) chromosome numbers and partial interfertility; the second comprising all yellow-flowered species with euploid ($x = 8$) chromosome numbers; and the third consisting of yellow-flowered species with aneuploid ($x = 9, 10, 12$, etc.) chromosome numbers. Furthermore, western North American *Draba* were divided into well-supported clades; one of taxa with a chromosome number based on $x = 8$, and one of taxa whose chromosome base numbers appear to be aneuploid, deviating from $x = 8$. Our analyses of nuclear ITS and *trnL* regions clearly do not support these proposed divisions. According to the ITS Bayesian tree (Fig. 1), Group A includes not only of white-petalled, euploid species, e.g. *D. lanceolata* ($2n = 32$; Zhou, 2001), but also yellow-petalled, euploid species, e.g. *Draba alpine* ($x = 8$; Brochmann et al., 1993) and aneuploid species, e.g. *C. draboides* ($2n \approx 42$; Huang et al., 1996) and *D. oreades* ($2n = 40$; Zhou, 2001); while Group B includes both yellow-petalled euploid species, e.g. *Draba eriopoda* ($x = 8$; Huang et al., 1996) and white-petalled euploid species, e.g. *Draba incana* ($x = 8$; Brochmann et al., 1993). Moreover, since the hypothesized base chromosome number for *Draba* is $x = 8$, yellow-flowered, euploid species have been considered ancestral in the genus (Mulligan, 1976; Koch and Al-Shehbaz, 2002). Since *D. nemorosa*[a] is a yellow-flowered, euploid species with base chromosome number $x = 8$ (Beilstein and Windham, 2003) and is situated in a basal clade (Group D) (Fig. 1), it is proposed as an ancestral taxon in *Draba*.

Since the monotypic *Coelonema*, which is endemic to the QilianMountains of the northeastern QTP, has been recognized to be closely related to *Draba* (Maximovicz, 1880; Ho et al., 1997), both *Coelonema* and *Draba* have been positioned within tribe *Drabeae* of the family Brassicaceae, along with the genus *Armoracia* (Zhou, 1987). Various Chinese floras state that *Coelonema* differs constitutionally from *Draba* by the former including species with winged, toothless, hollow and filiform filaments, as well as lunar lateral-glands; whereas species included in *Draba* have wingless, solid and thin filaments, together with toothlike, semiannular or annular lateral-glands (Table 2). Apart from these trivial differences, these two genera are not otherwise separable on morphological grounds (Table 2). After a critical examination of the holotypes of *C. draboides*, and numerous other species of Asian *Draba*, Al-Shehbaz (2004) held these alleged differences to be insignificant. The same conclusion was reached by Appel and Al-Shehbaz (2003), who justified uniting *Coelonema* with *Draba*, and accommodating *C. draboides* within *Draba* and renaming it *D. draboides*. The results of our study on the molecular phylogeny of *Coelonema* and *Draba* are clearly in agreement with these proposals (Figs. 1 and 2). However, the infrageneric position of *D. draboides* remains indistinct and further studies using more species of *Draba* are necessary to resolve this issue.

4.2. Biogeographical implications

Several botanists have documented the close relationships between the floras of Central Asia and western North America (Parks and Wendel, 1993; Koch and Al-Shehbaz, 2002), thereby establishing complex speciation and migration scenarios for plants. The Bering land-bridge, which putatively connected Asia and North America in several periods during the late Tertiary and Pleistocene (Parrish, 1987), probably served as a migration route for several Brassicaceae taxa. Koch and Al-Shehbaz (2002) have suggested that some *Draba* taxa may have reached North America from the high mountains of Central Asia and circumarctic areas, via the Bering Bridge. And then, some analyses of the geographic origin indicated that *Draba* originated somewhere within the regions between the central Asian and Caucasus Mountains (Jordon-Thaden, 2009; Jordon-Thaden et al., 2010). In addition, palynological analyses of samples from the Venezuelan Andes have indicated the occurrence of *Draba* in Holocene deposits formed in the deglaciaion events of the late Pleistocene (Salgado et al., 1988). Our present study, which has focused on species of *Draba* in the QTP and several other taxa from the Americas and Europe, has produced results, based on the ITS tree (Fig. 1; BS = 61%, BPP = 100%), which leads us to propose, firstly, that Group D should include *D. nemorosa*[a] from the eastern edge of the QilianMountains as an ancestral clade, and secondly that based on the ITS clock, *Draba* originated about 1.36–2.71 Mya. We therefore hypothesize the eastern edge of the QilianMountains to be a centre of origin for some *Draba* taxa from which others were derived during the late Pliocene or early Pleistocene; a hypothesis which corresponds well with those of Wu (1965) and Wang (1989).

Since *Draba* has strong affinities with high alpine regions (e.g. the Alps, Himalayas and Rocky Mountains), its evolution appears to have been influenced by periodic glaciation and deglaciation events throughout the Pleistocene 0.01–1.8 Mya (Koch et al., 2003). During this period, *Coelonema* endemic to the QilianMountains, and *Draba* in the QTP, would, in addition to glacial–interglacial cycles, also have been affected by the uplifting of the QTP since the Quaternary (approximately 2 Mya). Geological and paleobotanical studies of the QTP area, therefore provide a good context in which to develop a plausible scenario regarding the divergence and evolution of *Coelonema* and *Draba*. The first large-scale uplifting of the QTP occurred approximately 3.4 Mya, accompanied by the formation of the largest glaciers in the Northern Hemisphere (Li et al., 1995). The plateau was strongly uplifted again about 2.5 Mya during the most widespread ice-age to have occurred in the Northern Hemisphere, when glaciers developed in the major mountain chains of the QTP area (including the QilianMountains), and which continued from the late Pliocene of the Tertiary to the Quaternary (Fang et al., 1995). These events are in agreement with our proposal that the derived centre of some taxa of *Draba*, and putative refugia for some alpine plants proposed in other studies (Zhang et al., 2005; Chen et al., 2008a, b) occurred in the eastern edge of the QilianMountains area. The third phase of plateau uplifting occurred about 1.6 Mya (Lower-Pleistocene), which was a climatic turning point and a period when the vegetation was replaced by apline shrub and meadow (Shi et al., 1998). However, Su et al. (2005) suggest that the Qilian-Mountains' glaciers retreated due to a relatively warm climate, which was followed by the development of an alpine steppe flora (including some species of Brassicaceae) from the middle of the Middle-Pleistocene to the early Upper-Pleistocene (about 0.36–0.15 Mya). Our estimate of the timing of the divergence of *C. draboides* from *Draba*, between 0.15 and 0.31 Mya (Middle-Upper Pleistocene), corresponds well with both this viewpoint and the hypothesis of Chen et al. (2005c). Further support for this proposal for the divergence of *Draba* species has been presented by Grundt et al. (2004, 2006), who speculated that four circumpolar species (*D. Lactea*, *Draba fladnizensis*, *Draba nivalis* and *Draba subcapitata*) of *Draba* probably had origins in the Pleistocene. Furthermore, since the "Gonghe tectonic movement" about 0.15 Mya, the QilianMountains have been subjected to repeated periods of uplift and a continuously dry climate (Li and Fang, 1998). Most ancestral species of *C. draboides* might therefore have suffered extinctions, while currently surviving ancestor-like species, such as *C. draboides*, may have become extremely endangered and narrowly distributed because of changes to their habitats. Contemporarily, Koch and Al-Shehbaz (2002) suggested a radiation in the New World as short branches of South American *Draba* at ITS tree. And then nearly half species of the *Draba* were found in the New World (Al-Shehbaz, 1987). Moreover, low levels of genetic differentiation within and among the species suggests the expansion to have been recent (Grundt et al., 2004). We found only very low levels of genetic variation in the present study, based on ITS sequences (5.4%), and *trnL* sequences (2.8%), as well as short branches in the ITS and *trnL* trees (Figs. 1 and 2). Thus, it is possible that numerous species of *Draba* expanded rapidly from putative refugia during interglacial or postglacial periods, when impacts from climatic oscillations also occurred, during the uplifting of the QTP in the late Pliocene and Quaternary.

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