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Phylogeography of a Tertiary relict plant, *Meconopsis cambrica* (Papaveraceae), implies the existence of northern refugia for a temperate herb

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Abstract

The perennial herb *Meconopsis cambrica*, a western European endemic, is the only European species of the otherwise Himalayan genus *Meconopsis* and has been interpreted as a Tertiary relict species. Using *rbc*L and ITS sequence variation, we date the split between *M. cambrica* and its sister clade *Papaver* s.str. to the Middle to Upper Miocene (12.8 My, 6.4-19.2 My HPD). Within *M. cambrica*, cpDNA sequence variation reveals the existence of two groups of populations with a comparable level of genetic variation: a northern group from Great Britain, the Massif Central, the western Pyrenees and the Iberian System, and a southern group from the central and eastern Pyrenees. Populations from the Cantabrian Mountains were found in both groups. Based on ITS sequence variation, the divergence between these two groups can be dated to 1.5 My (0.4-2.8 My HPD), and the age of the British populations is estimated as 0.37 My (0.0-0.9 My HPD). AFLP results confirm the distinctive nature of the populations from Britain, the Massif Central and the central and eastern Pyrenees. These patterns of latitudinal variation of *M. cambrica* differ from patterns of longitudinal differentiation found in many other temperate species and imply glacial survival of the northern populations in northerly refugia. The primary differentiation into northern and southern cpDNA groups dates to near the onset of the Quaternary and suggests that an ancient phylogeographic pattern has survived through several glacial periods. Our data provide evidence that the species has persisted for a long period with a highly fragmented and probably very localized distribution.

Introduction

The phylogeographic analysis of widespread plant and animal species in the temperate regions of western Eurasia has resulted in the recognition of a pattern in which species generally have more genetic variation in the more southerly temperate regions than in the colder latitudes into which they expanded after the last Ice Age. Populations in the warmer parts of the temperate zone also tend to possess greater phylogeographic subdivision, arguing for the survival and divergence of taxa here through many Ice Ages and, in many species, for the presence of refugial sources for postglacial colonization (Hewitt 2004). Although the recognition of this pattern is based on a multitude of studies, important modifications have been suggested. It has been recognised that genetic structure at both the leading edge and the rear edge of populations is likely to have been shaped not only by Quaternary range shifts but also by other forces such as natural selection or hybridization (e.g. Eckert *et al.* 2008; Nieto Feliner 2011). In addition, the likely existence of ‘cryptic’ or ‘northerly’ refugia, mainly in Central and Eastern Europe, has recently been much discussed (Stewart & Lister 2001; Bhagwat & Willis 2008; Birks & Willis 2008; Provan & Bennett 2008; Stewart *et al.* 2010). Despite the discovery of evidence for northern refugia, patterns observed in Europe are generally of longitudinal rather than latitudinal differentiation, and genetic differentiation in the north has most commonly been explained in the time-frame of Last Glacial Maximum (LGM) and subsequent range expansion from the south. Where substantial genetic differentiation between populations has been detected in regions north of hypothetical refugia, it has been interpreted as resulting from the existence of different phylogroups with a long history of isolation (Abbott *et al.* 2000; Brochmann *et al.* 2003).

Meconopsis cambrica (L.) Vig. is traditionally regarded as the only European species of an otherwise Himalayan genus. Although molecular analyses had shown that *M. cambrica* is sister to *Papaver* s.str. (excl. *Papaver* sects. *Argemonidium*, *Californicum*, *Horrida* and *Meconella*; see Kadereit *et al.* 1997) and not to Asian *Meconopsis* (Kadereit *et al.* 1997; Carolan *et al.* 2006), Kadereit *et al.* (1997) interpreted *Meconopsis* as paraphyletic in relation to a polyphyletic *Papaver*, and accordingly considered the European *M. cambrica* as a relict species of Tertiary times descended from a postulated widespread Eurasian *Meconopsis*. Their study made no attempt to estimate the age of *M. cambrica*. The natural distribution of *M. cambrica* comprises montane areas in Spain and France, and both lowland and upland areas in Great Britain and Ireland (Fig. 1; Tutin *et al.* 1993), but the species has become naturalized in parts of Central and Northern Europe (Jalas & Suominen 1991; Valtueña *et al*. 2011). The presence of *M. cambrica* in western Europe as a putative Tertiary relic is notable; the Tertiary relics of western Eurasia are concentrated in the Black Sea area (Milne & Abbott 2002) although there are other western European examples such as *Borderea pyrenaica* (Picó & Riba 2002), *Ramonda myconi* (Segarra-Moragues *et al.* 2007) and *Prunus lusitanica* (Pulido *et al*. 2008; Calleja *et al.* 2009). Within Europe *M. cambrica* has an unusual distribution. It was classified by Finnie *et al*. (2007) in the *Erysimum duriaei* element of species which are concentrated in northern Spain, the Pyrenees, south-eastern France and the south-western Alps, but of the 140 species allocated to this element in their study only two (*Arabis scabra*, *Meconopsis cambrica*) extent north to Britain although another three are members of the small group of species which occur in Ireland but not in Britain.

We chose *M. cambrica* to investigate whether a species of presumably great and pre-Quaternary age shows patterns of geographical differentiation that predate the LGM and Holocene or even the onset of the Quaternary. In recent years some studies of Mediterranean tree species have suggested the existence of ancient patterns of differentiation (Petit *et al.* 2005; Grivet *et al.* 2006; Hampe & Petit 2007; Magri *et al.* 2007). The persistence of traces of ancient differentiation was explained by three factors: 1) low rates of evolution as a consequence of long generation times in long-lived species, 2) genetic stability without speciation maintaining species as relatively uniform, and 3) habitat stability because these species are distributed in the Mediterranean area where the climatic oscillations of the Quaternary were less severe than in higher latitudes of Europe (Hampe & Petit 2007). There have been very few studies which have examined the possibility that similar differentiation might be present in herbaceous perennials. In this paper we use different molecular markers (ITS, cpDNA, AFLP) and methodologies (maximum parsimony, Bayesian analysis) to (1) date the origin of *M. cambrica* and its diversification to test the hypothesis (Kadereit *et al*. 1997) of its Tertiary origin, and (2) reconstruct and date the phylogeographic history of the species in order to test whether ancient patterns of differentiation can be recognized or whether the species conforms to a pattern of southern survival and more or less recent northward expansion.

Material and methods

Species studied

The winter-green perennial herb *Meconopsis cambrica* is classified by Preston & Hill (1997) as a species with an Oceanic Boreo-temperate range, but it might equally well be interpreted as an Oceanic Temperate species which extends into the boreal-montane zone, and the latter interpretation is followed here. Temperate species are those of the broad-leaved forest zone whereas boreal-montane species are those of the coniferous forest zone. In continental Europe *M. cambrica* is most frequent in shady and humid or wet places, often close to streams and rivers, in beech (*Fagus sylvatica*) forests at altitudes between 700 and 1500 m (for details of the habitats in which the material for this study were obtained see Valtueña *et al*. 2011, Table 1). It may extend above the upper limit of beech woodland into the *Pinus sylvestris* zone and it is recorded at altitudes up to 2000 m in the western Pyrenees, reaching its upper limit on montane cliff ledges (Villar 1982, 1986). In Great Britain its occurrences lie outside the probable native range of beech, but the habitats are otherwise essentially similar. It is found in moist, mildly base-rich woodland on stream and valley sides at low altitudes (<250 m), and at higher altitudes (up to 600 m) on rocky ground by upland streams and in tall-herb communities on base-rich rock ledges. Most of the associates at the montane sites we sampled were species with boreo-temperate and temperate ranges, although the sites are known for their arctic-montane flora. The deciduous woodland habitats are those of a temperate species but the extension above this zone indicates boreo-temperate affinities; clearly these categories are in any case continua and some species do not fit neatly into them. The problem of assessing the phytogeographical affinities of the species are compounded by its relict distribution, the total disafforestation of the upland landscapes in which it grows in Britain and the blurring of vegetation zones in highly oceanic climates (Tuhkanen 1987). In cultivation in Britain the species can be a “persistent and troublesome weed” and naturalized populations are well established, especially by roads and streams, although Halliday (1997) suggested that they are “seldom far from houses”. This suggests that its native range is not limited solely by climatic factors, and this will hamper any attempt to model its climatic requirements.

Sample sites

We sampled (6-) 9-15 individuals from 17 populations (206 individuals) of *M. cambrica* across its entire native range except Ireland (Fig. 1; Table 1). In Great Britain, we included only samples from populations which in an earlier analysis (Valtueña *et al.* 2011) had been identified as comprising native plants without introgression from introduced genotypes. *Papaver atlanticum* and *P. bracteatum* cultivated in the Botanic Garden of Mainz University were used as outgroup species in the analyses based on DNA sequences (see below). These two species were chosen to represent *Papaver* s.str. which is sister group to *M. cambrica* (Kadereit *et al.* 1997; Carolan *et al.* 2006).

DNA extraction and sequencing

Genomic DNA was extracted using the NucleoSpin Plant DNA extraction kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. The nuclear ribosomal Internal Transcriber Spacer region (including ITS1, 5.8S and ITS2) was amplified using primers ITS-A and ITS-4 (White *et al.* 1990), the chloroplast *psb*J-*pet*A region was amplified using primers *psb*J and *pet*A (Shaw *et al.* 2007), and the chloroplast *trn*Q-5'*rps*16 region was amplified using primers *trn*Q$^{(UUG)}$ and *rps*16x1 (Shaw *et al*. 2007). Amplification and sequencing was carried out as described in Zhang *et al*. (2007). The number of samples per population is given in Table 1. Sequences were edited and manually aligned using the program SEQUENCHER vs. 4.8 (GENECODES Corp., Ann Arbor, Michigan, USA). All sequences were submitted to GenBank (see supplementary material, Table S1).

AFLPs

AFLP products were obtained following the methodology described in Valtueña *et al*. (2011). The number of samples per population is given in Table 1. Error rates were calculated using replicated extractions of 14 samples.

Molecular Dating

In order to obtain calibration points in the Old World clade (OWC) of Papaveraceae subf. Papaveroideae (containing *Papaver, Meconopsis*, *Stylomecon* and *Roemeria*; Kadereit *et al.* 1997), the split between this clade and the New World clade of the subfamily (containing *Argemone*, *Romneya*, *Canbya*, *Platystemon*, *Meconella* and *Hesperomecon*) had to be dated in a first step. To do this, *rbc*L sequences of 87 taxa representing all families of Ranunculales, major clades of the Eudicots (selected from Savolainen *et al*. 2000) as sister to Ranunculales, and 19 species belonging to 14 genera of subf. Papaveroideae (supplementary material, Table S2) were obtained from GenBank, except the *M. cambrica* sequence that was obtained following the protocol described in Zhang *et al.* (2007). For the estimation of divergence times, a clock-enforced tree was calculated with the appropriate model of DNA substitution for the inference of phylogenetic relationships under ML estimated using Modeltest 3.06 (Posada & Crandall 1998). The GRT+I+G model selected by the Akaike Information Criterion (AIC; Posada & Buckley 2004) was chosen [settings: Lset Base=(0.2725 0.1846 0.2239) Nst=6 Rmat=(1.6329 3.3564 0.4514 1.2378 4.4616) Rates=gamma Shape=0.7817 Pinvar=0.4847]. Maximum Likelihood heuristic searches and bootstrap analyses were performed in PAUP* 4.0b10 (Swofford 2003) with 100 replicated heuristic searches, 10 random addition sequences, tree-bisection-reconnection (TBR) branch swapping, best only and MULTREES on. The program Modeltest 3.06 (Posada & Crandall 1998) was used to perform a likelihood ratio test (Felsenstein 1988) comparing the log-likelihood scores of trees with and without enforcing a molecular clock. There was a significant difference between the two scores at the 0.01 level indicating that a molecular clock was rejected.

Bayesian Evolutionary Analysis by Sampling Trees BEAST v1.6 by Drummond & Rambaut (2007) was used to estimate the age of the OWC, using a fossil assigned to Papaveroideae and published group ages of eudicots for calibration. Appropriate taxon groups to define calibration nodes and to set prior distributions for these nodes were defined in Bayesian Evolutionary Analysis Utility BEAUti v1.6 (implemented in BEAST, Drummond & Rambaut 2007). Two points of calibration were selected, the root of Eudicots and the root of subf. Papaveroideae. Age estimates obtained from molecular studies indicate that Eudicots are 123-139 million years (My) old (Bell *et al.* 2010), so the root of this clade was set to a normally distributed prior with a mean of 130 My and a standard deviation of 3 My. The oldest known fossil of Papaveroideae, *Palaeoaster*, has been assigned to the Latest Cretaceous (74.5-64.5 My, Smith 2001). Accordingly, subf. Papaveroideae was defined as monophyletic and its root was set with a log normal prior distribution and a zero offset of 74.5 My. The zero offset sets a strict minimum age of a clade. The substitution model parameters were the same as used in the ML analyses, and the gamma distribution was modeled with six categories. A relaxed uncorrelated lognormal clock was used and a birth and death prior was set for branch lengths. Other priors were in default settings and the Markov Chain Monte Carlo (MCMC; Drummond *et al.* 2002) was initiated on a random starting tree. Runs were performed with 20,000,000 iterations and a sample frequency of 1,000. The first runs were used to examine the MCMC performance, and operators were adjusted as suggested by the output analysis. Finally, four BEAST runs of 10,000,000 generations and a sample frequency of 1000 were performed. After assessing convergence in Tracer v1.5 (Rambaut & Drummond 2007) as described in the BEAST manual (Drummond *et al.* 2007) the four tree files were combined with LogCombiner v1.6 (Drummond & Rambaut 2007) with a burnin of 10% to reach satisfactory Effective Sample Sizes (ESS) and the maximum clade credibility tree was summarized in TreeAnnotator v1.6 (Drummond & Rambaut 2007) with a posterior probability (PP) limit of 0.8 and summarizing mean node heights. The split between the two clades of subf. Papaveroideae was estimated to 52 My (73-31 My), and the crown group age of the OWC to 26 My (44-10 My; supplementary material, Fig. S1).

For estimating the age of different nodes in the *M. cambrica* lineage, an ITS data set of 68 samples of subf. Papaveroideae (including the eight different sequences of *M. cambrica*, see Table 1) and three outgroup taxa (supplementary material, Table S3) was analyzed with BEAST (Drummond & Rambaut 2007). The OWC was defined as monophyletic, its stem group age was set to a normally distributed prior with a mean of 52 My and a standard deviation of 10 My, and its crown group age was set to a normally distributed prior with a mean of 26 My and a standard deviation of 8 My. The remaining settings and the calculation procedure were the same as in the *rbc*L data set described above.

Phylogenetic analysis, network construction and phylogeography

Phylogenetic relationships were analyzed using Maximum Parsimony for the ITS data set and a combined matrix of the ITS, *psb*J-*pet*A and *trn*Q-5´*rps*16 data. An inversion of 11bp in position 242-252 of *psb*J-*pet*A was coded as a single character and gaps were considered as a fifth state. Searches for the most parsimonious trees were executed in PAUP* v4.0b10 by a heuristic search with tree-bisection-reconnection (TBR) branch swapping on best only and MULTREES on. *Papaver atlanticum* and *P. bracteatum* were used as outgroup species. Branch support was assessed with 1000 bootstrap (BS) replicates with 10 random taxon additions each and TBR and MULTREES on. Consistency and retention indices were calculated to assess the amount of homoplasy present in the data set. Additionally, a Bayesian analysis of both datasets was carried out using BEAST. The settings and the calculation procedure were the same as described above.

The cpDNA data set (*psb*J-*pet*A, *trn*Q-5´*rps*16) was analyzed under statistical parsimony as implemented in the program TCS 1.21 (Clement *et al.* 2000) to estimate relationships among chloroplast haplotypes. The connection limit was 200 steps to include the outgroup taxa and gaps were coded as a fifth state.

AFLP products were scored manually with GeneMarker 1.5 (GeneMarker, SoftGenetics, LLC) for the presence/absence of fragments between 70 and 450 bp in size. Fragments that could not be scored unambiguously were excluded. The resulting presence/absence (i.e. 1/0) matrix was used for further analysis. A mismatch error rate was calculated as the ‘number of genotype mismatches’ divided by the ‘number of replicate pairs’ and the ‘number of loci’ (Paun *et al.* 2008).

Population genetic structure was analyzed using ‘Bayesian Analysis of Population Structure’ (BAPS, vers. 5.1; Corander *et al.* 2008). The procedure was run 10 times for each value of K = 2-17 with 17 as the assumed maximum number of populations present in the sample. Admixture analyses (Corander & Marttinen 2006) were run with 100 iterations to estimate admixture coefficients for individuals, 200 reference individuals from each population and 20 iterations to estimate admixture coefficients for reference individuals. To confirm groups found in the BAPS analysis, a Principal Coordinates Analysis (PCA), a non-hierarchical grouping technique without prior knowledge of the source location of the sampled individuals, was made with GenAlEx v.6.4 (Peakall & Smouse 2006).

GenAlEx v.6.4 (Peakall & Smouse 2006) was used to calculate different diversity parameters (number of fragments, number of private alleles, percentage of polymorphic loci and H_E) at the population and group levels. Two groups levels were used in the analysis, the first using the six groups defined by the BAPS analysis and the second using the two main groups defined by the haplotypes (see below). POPGENE v.1.32 (Yeh & Boyle 1997) was used to calculate gene diversity (H_S and H_T) and genetic differentiation (F_{ST}, Weir & Cockerham 1984) at species and group level.

Results

Age of the Meconopsis cambrica *lineage*

As mentioned above, the split between the two clades of subf. Papaveroideae was estimated to 52 My (73-31 My), and the crown group age of the Old World clade (*Papaver*, *Meconopsis*, *Stylomecon* and *Roemeria*; Kadereit *et al.* 1997) to 26 My (44-10 My; supplementary material, Fig. S1). The BEAST chronogram of the ITS data set is shown in Fig. 2. The lineage of *M. cambrica* has maximum support (1.00 PP), and the stem group age of *M. cambrica* (node 1 in Fig. 2) was found to be 12.8 million years (My; 6.4-19.2 My 95% Highest Posterior Density confidence interval, HPD). In Fig. 2, the individuals from Great Britain were sister to the remaining individuals of the species (0.93 PP), and the age of this node (node 2) was determined as 1.5 My (0.4-2.8 My HPD). The crown group age of *M. cambrica* in Great Britain (node 3) was dated to 0.37 My (0.0-0.9 My HPD). Considering that in the larger sample of *M. cambrica* for the ITS and cpDNA analyses the sister group relationship between British *M. cambrica* and the remainder of the species sampled was not identified (Fig. 3), we prefer to interpret the results cautiously and to accept the crown group age of *M. cambrica* in Great Britain (i.e. 0.37 My; 0.0-0.9 My HPD) as the estimated age of the British clade of the species.

Phylogeography and statistical parsimony

The ITS region of *M. cambrica* was 649 to 650 bp long. The ITS alignment including the two outgroup species was 660bp long and contained 75 variable and 28 phylogenetically informative positions. The length of the combined ITS, *psb*J-*pet*A and *trn*Q-5´*rps*16 sequences was between 1583 and 1585bp. The alignment including the outgroup species was 1607bp long and contained 124 variable and 44 phylogenetically informative positions.

In the ITS analysis, the two individuals analyzed had the same ITS sequence in all populations except CA3 (with sequences B and C). Seven populations had identical sequences (A), one sequence (G) was shared by three populations, two sequences (B, F) were shared by two populations, and four sequences (C, D, E, H) were found in only one population (see Table 1). The topology of the MP tree and the BEAST tree (Fig. 3A) was identical, and only three clades with high Bayesian support were identified and formed part of a large polytomy. Two of these clades comprised only two populations (IB2 and PY1; CA1 and one plant of CA3) and the third clade contained all four British populations.

The topology of the trees obtained in the MP and the Bayesian analyses of the combined ITS-cpDNA dataset was essentially the same (Fig. 3B). In both analyses, the two clades defined by the two main haplotypes (see below) are weakly supported. In the Bayesian analysis (Fig. 3B, dotted line) the British clade is sister (PP 1.00) to a clade containing the Massif Central (MC1, MC2) and western Pyrenees (PY1, PY2) populations plus one Cantabrian population (CA2). These two clades together are sister to the Iberian System (IB1-3) populations. In the MP analysis, the clade containing the

British, Massif Central, western Pyrenees and Cantabrian populations was not resolved (Fig. 3B).

The statistical parsimony analysis of the cpDNA data set yielded a single network with seven different haplotypes clustering in two main groups, henceforth called haplotype A and haplotype B (Table 1, Fig. 4). In addition to sequence variation, the two groups are defined by an inversion of 11 bp length in position 242-252 of *psb*J-*pet*A. Haplotype A is present in the British Isles, Massif Central, western Pyrenees, Iberian System and one Cantabrian population, whereas haplotype B is found in the Cantabrian and central and eastern Pyrenean populations (Fig. 1). Haplotype A comprises three different haplotypes: A1, A2 and A3 (Fig. 4). A1 is most frequent and was found in British, Massif Central, one western Pyrenean and one Cantabrian population. A2 differs by two mutational steps from A1 and is limited to the Iberian System populations. A3 was only found in one western Pyrenean population. Haplotype B comprises four different haplotypes which differed by one to three mutational steps. The distribution of these haplotypes among populations is shown in Fig. 4.

AFLP analysis

The six AFLP primers generated 319 fragments with a percentage mismatch error rate of 1.17 ± 0.63. The mean number of fragments per individual was 201.4 ± 3.1 (range: 195-213). The individual clustering BAPS analysis resulted in a best partition of $K = 6$ (probability of the number of clusters 0.99, Fig. 5). These six clusters represent (1) two populations from the Cantabrian Mountains (CA1, CA2), (2) the remaining population from this area (CA3), (3) the western Pyrenees plus the Iberian System (PY1-2; IB1-3), (4) the central and eastern Pyrenees (PY3-5), (5) the Massif Central (MC1-2), and (6) Great Britain (GB1-4). In the admixture analysis all individuals were unambiguously assigned to their respective group without any probability of being misplaced. The PCA analysis produced results largely congruent with the BAPS analysis. The first two axes (Fig. 6) provide a separation of populations which most closely resembles the BAPS analysis with $K = 4$; it shows a central and somewhat overlapping group of Cantabrian, Iberian and western Pyrenean populations which clearly differ from the distinctive populations in the central and eastern Pyrenees, Massif Central and Britain. The two Massif Central populations, although distinct from all others, do not appear to be closely allied. CA3 is separated from CA1-2 on the third axis, as in the BAPS analysis (K=6), but unlike that analysis the PCA splits the western Pyrenean plants (PY1-2) from remaining Cantabrian and Iberian populations (CA1-2, IB1-3).

The results from the AFLP analyses at population level and cluster level are shown in Table 2. At population level, the number of fragments varied from 205 (MC2) to 227 (PY3). Five populations did not have any private alleles, and the maximum number of private alleles (four) was found in population GB4. The lowest percentage of polymorphic loci was 3.76 (GB2, IB1), and only three populations showed values >10 (CA3, PY2, PY3). Expected heterozygosity varied from 0.012 (GB2) to 0.047 (PY3). The two Cantabrian clusters did not have any private alleles, and the largest number of private alleles (12) was found in the British cluster. The Iberian plus western Pyrenees and central and eastern Pyrenees clusters had similar values for the different parameters calculated and had the highest values for percentage of polymorphic loci and expected heterozygosity. In contrast to this, the Massif Central cluster had the lowest values for number of alleles, percentage of polymorphic loci and expected heterozygosity. The group including all populations with haplotype A had higher values in the number of alleles, number of private alleles and percentage of polymorphic loci than the group of populations with haplotype B. However, expected heterozygosity was higher in the group of populations with haplotype B.

Gene diversity and differentiation indices are shown in Table 3. The H_T index varied between 0.044 (central and eastern Pyrenees cluster) and 0.027 (British cluster), and the H_S index varied between 0.028 (Cantabrian and central and eastern Pyrenees clusters) and 0.017 (Massif Central cluster). The highest differentiation among populations within clusters was found in the Massif Central cluster (F_{ST} = 0.553) and the lowest in the British cluster (F_{ST} = 0.245). The group of populations with haplotype B had greater gene diversity and lower differentiation among populations than the group of populations with haplotype A (Table 3).

Discussion

Relationships and age of Meconopsis cambrica

The sistergroup relationship between *M. cambrica* and *Papaver* s.str. found here (Fig. 2) confirms earlier results by Kadereit *et al*. (1997) and Carolan *et al*. (2006). The split between *M. cambrica* and *Papaver* s.str. was dated to the Middle to Upper Miocene (12.8 My, 6.4-19.2 My HPD), confirming the Tertiary origin of *M. cambrica* suspected by Kadereit *et al*. (1997). Whereas Kadereit *et al*. (1997) assumed that the genus *Meconopsis* represented a paraphyletic base group which gave rise to a polyphyletic *Papaver*, recent ontogenetic studies (Kadereit & Erbar 2011) have shown that the style of *M. cambrica* is most likely to have originated from a stigmatic disc as found in *Papaver* and thus in parallel to the style found in the two other lineages of *Meconopsis*. We have discussed the taxonomic implications of these results elsewhere (Kadereit *et al*., in press). Within *M. cambrica*, the ITS analysis (Fig. 2) resolved only the British individuals as a clade with moderate support (PP 0.93). The crown group diversification of the species was dated to 1.5 My (0.4-2.8 My HPD), and the diversification of the British populations to 0.37 My (0.0-0.9 My HPD). These dates indicate that the diversification of extant *M. cambrica* may have started near the onset (1.8 - 2.6 mya) of the Quaternary, and that the diversification of the British populations may have taken place in the second half of the Quaternary. However, the wide confidence intervals of our dates suggest that they should be interpreted very cautiously and they preclude us from explaining any cladogenetic event in terms of any particular palaeoclimatic or palaeogeographic scenario.

Phylogeography of Meconopsis cambrica

The analysis of cpDNA sequence variation revealed the existence of two major groups additionally supported by an 11bp inversion in the *psb*J-*pet*A region (Fig. 4). The first group, hereafter referred to as the northern group, includes all material from Great Britain, the Massif Central and the Iberian System together with two populations from the western Pyrenees and one population (CA2) from the Cantabrian Mountains. The second group, hereafter referred to as the southern group, is formed by three populations from the central and eastern Pyrenees and two populations (CA1, CA3) from the Cantabrian Mountains. This grouping is neither supported nor contradicted by the ITS data, which resulted in an essentially unresolved polytomy (Fig. 3A). When the split into the northern and southern groups is accepted as the primary split in the species, the age of this split is equivalent to the age of the crown group diversification of the species calculated on the basis of ITS sequence variation (Fig. 2), i.e. 1.5 My (0.4-2.8 My HPD). This age range, located around the onset of the Quaternary, falls into the same range as that estimated for *Primula* sect. *Auricula* (Zhang *et al*. 2004; Comes & Kadereit 2003). The primary split of *M. cambrica* into the northern and southern groups implies an identical age for these two groups. Considering that the two groups contain broadly comparable levels of genetic variation (Northern group: 6 ITS sequences, 3 haplotypes, 277 fragments and 44.83% PPL in 12 populations; Southern group: 4 ITS sequences, 4 haplotypes, 256 fragments and 34.48% PPL in 5 populations), this seems plausible.

The latitudinal subdivision of *M. cambrica* into a northern and southern group, which probably originated near the onset of the Quaternary, with a northern group which is likely to have persisted the Quaternary glacials in northern latitudes (see below), represents an unusual phylogeographic pattern for a (more or less) temperate plant species. However, a similar pattern has been identified in *Meum athamanticum* (Huck *et al*. 2009), a species which, like *M. cambrica*, is found in the mountains of Europe rather than the Boreal zonobiome and also reaches its northern native limit in the British Isles.

Bhagwat & Willis (2008) reviewed the traits of tree species with northerly refugia and concluded that they are cold-tolerant and have a present-day northerly distribution beyond 60° N, can reproduce vegetatively, are small-seeded and wind-dispersed and are habitat generalists. Some of these characteristics fit some of the species for which evidence for northern refugia was first detected, such as *Pinus sylvestris* (Sinclair *et al.* 1999) and *Picea abies* (Lagercrantz & Ryman 1990). Apart from their possible cold-tolerance, neither *Meconopsis cambrica* nor *Meum athamanticum* have any of the ecological characteristics identified by Bhagwat & Willis (2008) for tree species with 'northerly' refugia.

Northern group

The northern group comprises 12 populations from essentially four different geographical regions. These are four populations from Great Britain, two populations from the Massif Central, two populations from the western Pyrenees, and three populations from the Iberian System. The unexpected placement of one population from the Cantabrian Mountains in the northern group will be discussed further below. In the BAPS analysis of AFLP variation, Great Britain and the Massif Central were identified as separate groups, and the populations from the western Pyrenees and the Iberian System grouped together (Fig. 5).

Apart from the fact that no admixture among these three groups could be detected in the AFLP data, there is evidence that the extant *M. cambrica* populations in the three areas are the result of a long history of independent evolution. Private AFLP fragments were found in all three regions (Great Britain: 12; western Pyrenees and Iberian System: 9; Massif Central: 4), the western Pyrenees and Iberian system populations are variable for cpDNA haplotypes (three) and ITS sequences (four), and the populations from Great Britain are variable for ITS sequences (two). These results are most surprising for the populations from Britain. Considering the presence of 12 private AFLP fragments and two different ITS sequences in this group, and considering that the diversification of the British populations was dated to 0.37 My (0.0-0.9 My HPD), it seems likely that the ancestor(s) of the British populations survived one or several glacial cycles without any gene exchange with other groups of populations identified by us. Genetic variation in the native British populations as a result of hybridization with introduced material can be excluded on the basis of earlier findings by Valtueña *et al.* (2011) who found no evidence of hybridization in the four populations analyzed here.

In situ survival of the ancestors of the extant British populations can be ruled out, as the sites in North Wales were north of the glacial limit at the LGM and *M. cambrica* is not a species which can have been expected to survive on the immediate periphery of a glacial area. We therefore suggest that the British populations must have migrated in response to climate change to areas where no other populations of the species occurred. As Britain was contiguous with mainland Europe in glacial periods of low sea-levels, the refugial areas may have been located in areas which are now outside the current island of Britain. The northerly cryptic tree refugia postulated by recent authors (Stewart & Lister 2001; Bhagwat & Willis 2008, Provan & Bennett 2008), described by Birks & Willis (2008) as providing "adequate soil moisture, temperatures warm enough to permit tree growth, shelter from strong winds and an absence of permafrost and periglacial activity", would also appear to offer potential refugia for the Tertiary relic *M. cambrica*. In the absence of any fossil evidence of *M. cambrica* it is impossible to identify the exact locations of such refugia, although Birks & Willis (2008) map possible sites for LGM tree refugia in Central and Eastern Europe in a broad latitudinal band centered on 50° N. The distinctness of the British populations in combination with their likely glacial history implies that the species has persisted for a long period with a highly fragmented and probably very localized distribution. Refugial areas around 50° N would still be considerably to the north of the extant Massif Central populations of the species which today are the nearest neighbours of the British populations, and would thus fit the hypothesis that the species has had a fragmented and localized distribution in the past. However, it must be admitted that there are potential problems in assuming that the history of *M. cambrica* can be interpreted in terms of its current ecology, as it appears to have a relict native distribution; the expansion of introduced populations in Britain contrasts with the behaviour of the native populations, which may be in long-term decline, and emphasizes our imperfect understanding of the factors limiting the current range.

Southern group

The southern group comprises five populations from two different geographical regions. These are two populations from the Cantabrian Mountains and three populations from the central and eastern Pyrenees. In the BAPS analysis of AFLP variation (Fig. 5), the Pyrenean populations were identified as one cluster, but the material from the Cantabrian Mountains fell into two groups. In that analysis CA1 and CA2 grouped together, but CA3 formed a separate cluster. Interestingly, CA2 grouped with the northern group rather than with its near neighbours CA1 and CA3 in the southern group in the cpDNA analysis (Fig. 4). The lack of genetic homogeneity in the populations from the Cantabrian Mountains may imply episodes of dispersal and probably hybridization with Pyrenean plants. The three populations from the central and western Pyrenees contain seven private AFLP fragments, three different cpDNA haplotypes and one ITS sequence, and the populations from the Cantabrian Mountains contain two different cpDNA haplotypes (not considering CA2), three different ITS sequences but no private alleles. The differences between the Cantabrian and the western Pyrenean populations of *M. cambrica* supports the hypothesis, now generally accepted, that the Iberian peninsula provided multiple glacial refugia (or refugia within refugia) instead of a single large refugium (Gómez & Lunt 2007; Dubreuil *et al*. 2008). There is also growing evidence from phylogenetic studies that the Pyrenees did provide glacial refugia for temperate species, including *Myodes glareolus* (see below), the bushcricket *Ephippiger ephippiger* and humans (Deffontaine *et al.* 2009).

Heterogeneity of the Pyrenean populations

The grouping of the western Pyrenean populations and the populations from the Iberian System with the northern group and of the central and eastern Pyrenean populations with the southern group is a striking feature of the analysis (Figs 3B, 4). Differences between the western as opposed to the central and eastern parts of the Pyrenees are also evident from other sources. First, present-day vegetation in the central and eastern Pyrenees is mainly coniferous forest whereas it is deciduous broad-leaved forest in the western Pyrenees (Lang 1994). Second, several taxa are known to grow either in the western (e.g., *Soldanella villosa*: Zhang *et al*. 2001) or in the central and eastern Pyrenees (e.g., *Gentiana alpina*: Hagen & Kadereit 2000; *Primula hirsuta*: Zhang *et al*. 2004). This pattern is partially supported by the pattern of genetic differentiation in the widespread temperate forest mammal *Myodes glareolus*, in which Deffontaine *et al*. (2009) identified a Basque lineage in the NW Pyrenees (rather than the SW Pyrenees in which we sampled *Meconopsis*) as well as a more widespread Spanish lineage. However, other phylogenetic or phylogeographic analyses of multiple populations/taxa from across the Pyrenees (*Reseda* sect. *Glaucoreseda*: Martin-Bravo *et al*. 2010; *Androsace halleri* complex: Dixon *et al*. 2007) did not reveal the west vs. central/east pattern of relationships which we found, nor is it apparent in an analysis of plant species' distributions at the European scale (Finnie *et al*. 2007). It seems possible that differences between these parts of the Pyrenees are the result of differences in their Quaternary history. Thus, the central and eastern Pyrenees probably were glaciated at least during the Last Glacial Maximum (LGM), but the western Pyrenees (and Iberian System) probably were not (Lang 1994). However, in view of the age estimate for the primary split within *M. cambrica*, the LGM may be irrelevant in explaining patterns of differentiation in *Meconopsis*.

Conclusions

The phylogeographic pattern found in *M. cambrica* – a latitudinal subdivision into a northern and a southern group – deviates strongly from patterns of longitudinal differentiation found in other widespread temperate species from western Eurasia. These latter patterns imply southern survival and northward expansion, although 'southern' has been pushed increasingly north by the detection of cryptic northerly refugia. Our data indicate the glacial or multiple-glacial survival of *M. cambrica* in area(s) north of the southern European refugia. They also indicate that the extant *M. cambrica* populations in these northern areas are the result of a long history of independent evolution probably through several glacial-interglacial oscillations. The differentiation of *M. cambrica* within the Iberian peninsula provides further evidence for multiple refugia in this area. Taken together, our results suggest that this Tertiary relict species has a long history as a localized plant with a fragmented distribution. Studies of other herbaceous perennials with disjunct distributions which span a wide latitudinal range are needed to establish whether this pattern is more widespread than is currently realised.

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Figure Legends

Fig 1 Native distribution (shaded area) and sampled populations of *Meconopsis cambrica* (abbreviations as in Table 1). The dotted line indicates the boundary between the northern and southern groups as defined by cpDNA sequences (squares: haplotype A, circles: haplotype B; see text for explanation). Populations belonging to the same group as defined in the AFLP analysis have the same colour.

Fig 2 BEAST chronogram of subf. Papaveroideae based on ITS sequence variation. Posterior probabilities of clades are indicated above branches (only PP > 0.8). The 95% posterior density distribution of node ages is given in the node bars. Node bars are only given for branches with a PP > 0.8. The scale is in million years. Numbered nodes (1 - 3) are discussed in the text.

Fig 3 MP tree of the ITS data set (A) and strict consensus MP tree of the ITS/cpDNA data set (B). Dotted lines show results obtained with BEAST. Bootstrap branch support >50% is indicated above and BEAST posterior probabilities >80% below branches. Abbreviations of samples as in Table 1.

Fig 4 TCS statistical parsimony network of cpDNA haplotypes found in *Meconopsis cambrica*. Lines connecting haplotypes represent a single mutation with squares representing inferred mutational steps not observed in this study. Names of haplotypes and abbreviations of samples as in Table 1. The black arrow indicates the position of the 11 bp inversion (for explanation see text), circles sizes are equivalent to the number of populations where the haplotypes were found and the broken line indicates the connection to the outgroup species (*Papaver atlanticum* and *P. bracteatum*).

Fig 5 Results of the BAPS analysis of the AFLP data set of *Meconopsis cambrica* from K= 3 to K = 6, admixture analysis for K=6 and best probability for clustering (below). Abbreviation of samples as in Table 1 and colours as in Figure 1.

Fig 6 PCA analysis of the AFLP data set based on genetic distances. Percentage of total variance explained by the first two coordinates is shown on the respective axes. Sample abbreviations as in Table 1. Different colours indicate the different geographical regions studied.

Table 1 Populations of *Meconopsis cambrica* studied. Population abbreviation (Pop), location, number of individuals used (N) in the ITS, *pet*A/*trn*Q and AFLP analyses, ITS sequence type (seq.), cpDNA haplotype (hap.) and BAPS group (group) are indicated.

Pop	Location	ITS		*pet*A / *trn*Q		AFLP	
		N	seq.	N	hap.	N	group
CA1	Spain, Tejedo del Sil, Fontaninas River	2	B	1	B2	13	1
CA2	Spain, Fresnedo, narrow mountain pass between Fresnedo and Paramo	2	A	1	A1	12	1
CA3	Spain, Valle de Lago, beech forest	2	B,C	2	B1	15	2
IB1	Spain, Valdezcaray, bank of mountain stream	2	A	1	A2	13	3
IB2	Spain, Posadas, beech forest close to Oja River	2	F	1	A2	12	3
IB3	Spain, Lugar del Río, Cardenas River	2	E	1	A2	11	3
PY1	Spain, Urbasa, beech forest	2	F	1	A1	12	3
PY2	Spain, Lizárraga, close to Lizárraga Port	2	D	1	A3	9	3
PY3	Spain, Isaba, between Isaba and Zuriza	2	A	1	B4	12	4
PY4	Spain, Bausen, beech forest in path to Serralonga	2	A	1	B1	14	4
PY5	France, Le Rebenty River	2	A	1	B3	11	4
MC1	France, D'Aubrac, beech forest close to Le Merdason River	2	A	1	A1	15	5
MC2	France, Valle de Jordanne, road to Puy Mary	2	A	1	A1	12	5
GB1	Great Britain, Coed Rheidol	2	G	1	A1	6	6
GB2	Great Britain, Devil's Kitchen, Cwm Idwal	2	G	1	A1	14	6
GB3	Great Britain, Cadair Idris, above Llyn Gafr	2	H	1	A1	12	6
GB4	Great Britain, Aberedw woods	2	G	1	A1	13	6

Table 2 Number of alleles (NA), number of private alleles (NPA), percentage of polymorphic loci (PPL) and expected heterozygosity (H_E) for all populations, the six groups obtained in the BAPS analysis and the two groups obtained in the analysis of cpDNA sequence data; population means in parenthesis.

Population	NA	NPA	PPL	H_E
CA1	211	0	6.90	0.023
CA2	212	0	8.15	0.032
CA3	222	0	14.42	0.044
IB1	206	1	3.76	0.013
IB2	214	3	7.84	0.025
IB3	216	1	6.58	0.015
PY1	214	0	5.33	0.018
PY2	225	2	12.85	0.042
PY3	227	2	15.99	0.047
PY4	218	1	8.46	0.021
PY5	216	1	7.21	0.015
MC1	207	0	5.64	0.020
MC2	205	2	4.70	0.015
GB1	221	2	9.09	0.029
GB2	208	2	3.76	0.012
GB3	213	1	6.58	0.019
GB4	216	4	8.15	0.020
BAPS cluster				
1 [Cantabrian 1]	216 (211.5)	0 (0.0)	11.91 (7.52)	0.040 (0.028)
2 [Cantabrian 2]	222 (-)	0 (-)	14.42 (-)	0.044 (-)
3 [Iberian + W Pyrenees]	240 (215.0)	9 (1.4)	23.20 (7.27)	0.049 (0.025)
4 [Pyrenees]	240 (220.3)	7 (1.3)	22.26 (10.55)	0.050 (0.028)
5 [Massif Central]	213 (206.0)	4 (1.0)	10.66 (5.17)	0.037 (0.017)
6 [Great Britain]	242 (214.5)	12 (1.8)	21.63 (6.90)	0.034 (0.020)
cpDNA groups				
Haplotype A	277 (213.1)	28 (1.5)	44.83 (6.87)	0.076 (0.022)
Haplotype B	256 (218.8)	8 (0.8)	34.48 (10.60)	0.077 (0.030)

Table 3 AFLP gene diversity (H_S, H_T) and differentiation (F_{ST}) for the whole data set, the five BAPS groups with more than one population (numbered as in Table 1) and the two groups obtained in the analysis of cpDNA sequence data

Data set	H_T	H_S	F_ST
Meconopsis cambrica	0.065	0.024	0.629
BAPS cluster			
1 [Cantabrian group]	0.037	0.028	0.255
3 [Iberian group]	0.041	0.025	0.390
4 [Pyrenees group]	0.044	0.028	0.375
5 [Massif Central group]	0.039	0.017	0.553
6 [Great Britain group]	0.027	0.020	0.245
cpDNA groups			
Haplotype A	0.054	0.022	0.602
Haplotype B	0.061	0.030	0.512

Fig 1

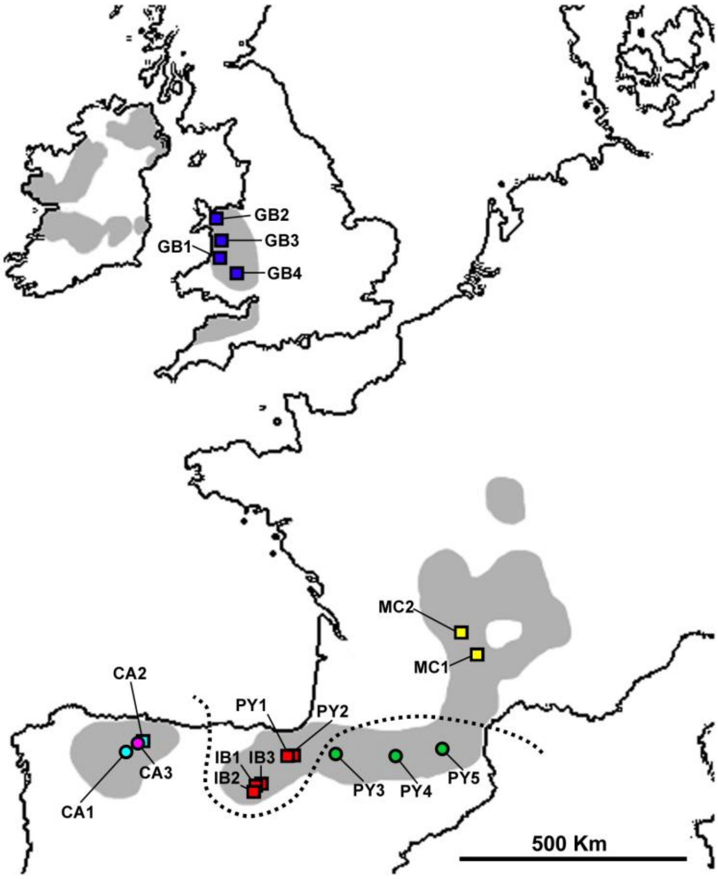

GB2
GB3
GB1
GB4
MC2
MC1
CA2
CA3
CA1
PY1
PY2
IB1
IB3
IB2
PY3
PY4
PY5
500 Km

Fig 2

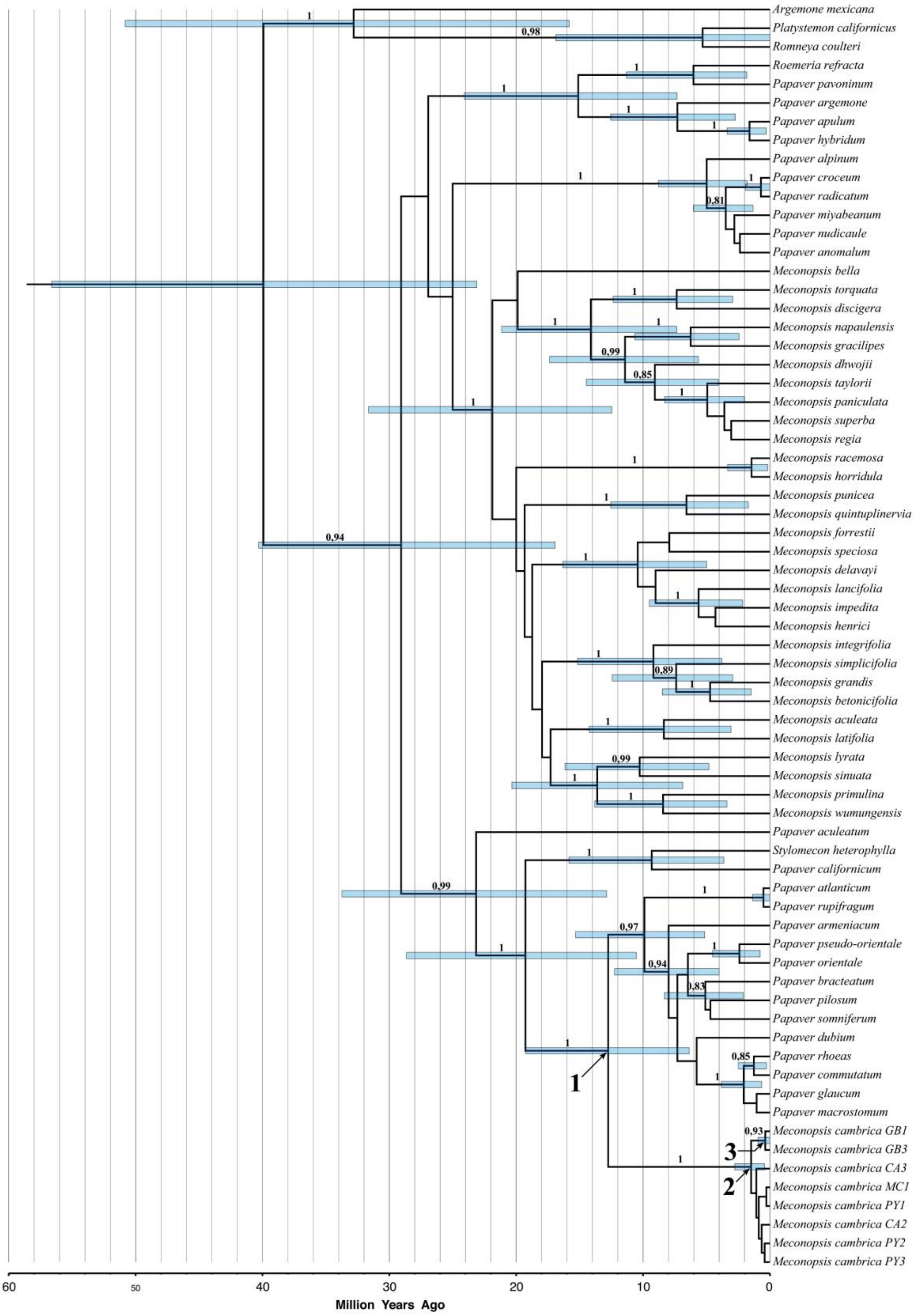

Argemone mexicana
Platystemon californicus
Romneya coulteri
Roemeria refracta
Papaver pavoninum
Papaver argemone
Papaver apulum
Papaver hybridum
Papaver alpinum
Papaver croceum
Papaver radicatum
Papaver miyabeanum
Papaver nudicaule
Papaver anomalum
Meconopsis bella
Meconopsis torquata
Meconopsis discigera
Meconopsis napaulensis
Meconopsis gracilipes
Meconopsis dhwojii
Meconopsis taylorii
Meconopsis paniculata
Meconopsis superba
Meconopsis regia
Meconopsis racemosa
Meconopsis horridula
Meconopsis punicea
Meconopsis quintuplinervia
Meconopsis forrestii
Meconopsis speciosa
Meconopsis delavayi
Meconopsis lancifolia
Meconopsis impedita
Meconopsis henrici
Meconopsis integrifolia
Meconopsis simplicifolia
Meconopsis grandis
Meconopsis betonicifolia
Meconopsis aculeata
Meconopsis latifolia
Meconopsis lyrata
Meconopsis sinuata
Meconopsis primulina
Meconopsis wumungensis
Papaver aculeatum
Stylomecon heterophylla
Papaver californicum
Papaver atlanticum
Papaver rupifragum
Papaver armeniacum
Papaver pseudo-orientale
Papaver orientale
Papaver bracteatum
Papaver pilosum
Papaver somniferum
Papaver dubium
Papaver rhoeas
Papaver commutatum
Papaver glaucum
Papaver macrostomum
Meconopsis cambrica GB1
Meconopsis cambrica GB3
Meconopsis cambrica CA3
Meconopsis cambrica MC1
Meconopsis cambrica PY1
Meconopsis cambrica CA2
Meconopsis cambrica PY2
Meconopsis cambrica PY3
1
0,98
1
1
1
1
1
1
0,81
1
1
1
1
0,99
0,85
1
1
1
1
1
0,94
1
1
1
0,89
1
1
0,99
1
1
0,99
1
1
0,97
1
0,94
0,83
1
0,85
1
1
0,93
1
3
2
60
50
40
30
20
10
0
Million Years Ago

Fig 3

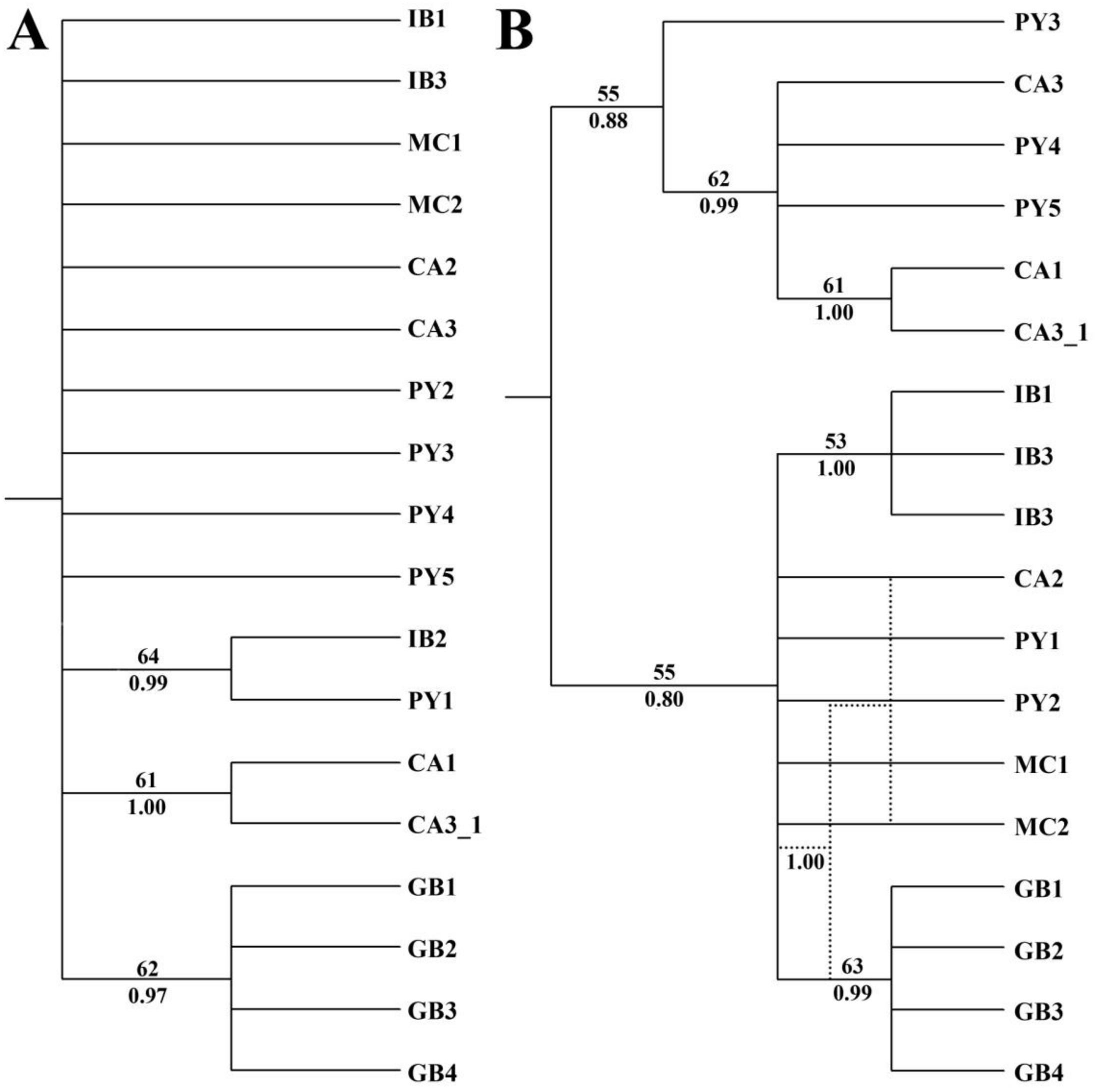

A
IB1
IB3
MC1
MC2
CA2
CA3
PY2
PY3
PY4
PY5
64
0.99
IB2
PY1
61
1.00
CA1
CA3_1
62
0.97
GB1
GB2
GB3
GB4
B
PY3
55
0.88
CA3
PY4
62
0.99
PY5
61
1.00
CA1
CA3_1
IB1
53
1.00
IB3
IB3
CA2
PY1
55
0.80
PY2
MC1
MC2
1.00
GB1
GB2
63
0.99
GB3
GB4

Fig 4

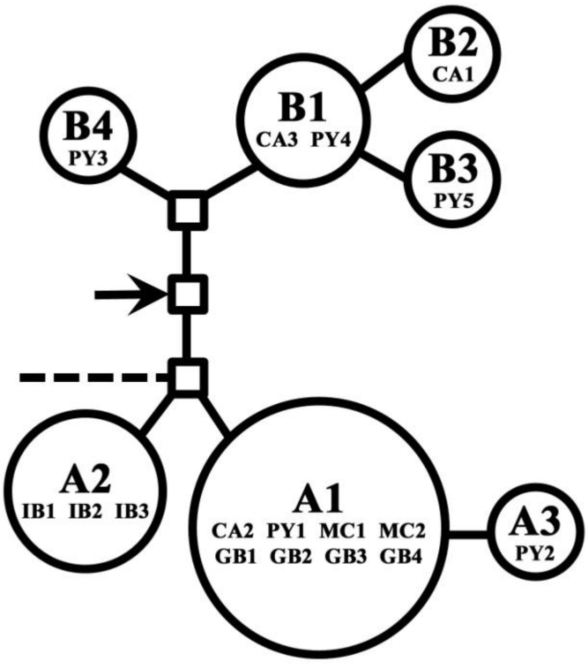
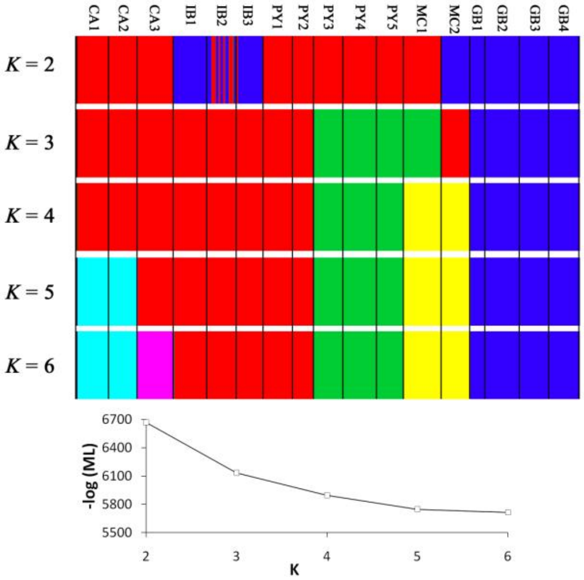
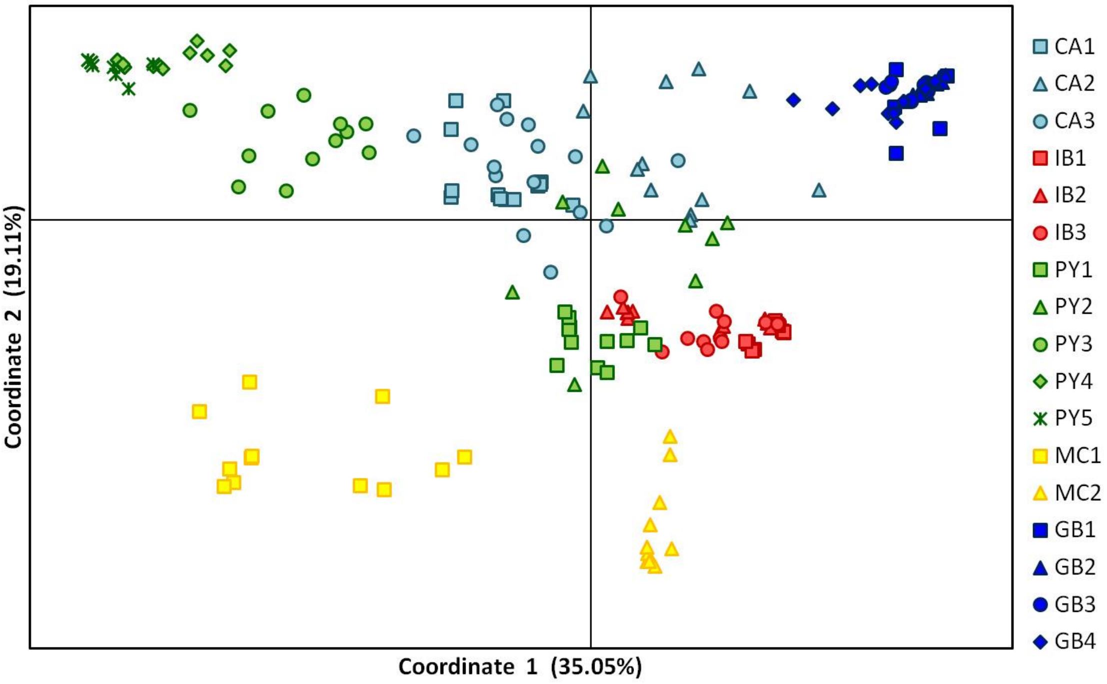

B2
CA1
B1
CA3 PY4
B4
PY3
B3
PY5
A2
IB1 IB2 IB3
A1
CA2 PY1 MC1 MC2
GB1 GB2 GB3 GB4
A3
PY2

Fig 5


CA1
CA2
CA3
IB1
IB2
IB3
PY1
PY2
PY3
PY4
PY5
MC1
MC2
GB1
GB2
GB3
GB4
K = 2
K = 3
K = 4
K = 5
K = 6
6700
6400
6100
5800
5500
-log (ML)
2
3
4
5
6
K

746 **Fig 6**


Coordinate 1 (35.05%)
Coordinate 2 (19.11%)
CA1
CA2
CA3
IB1
IB2
IB3
PY1
PY2
PY3
PY4
PY5
MC1
MC2
GB1
GB2
GB3
GB4

747

Supplementary Material

Table S1 Origin of material of *Meconopsis cambrica* and outgroup taxa studied, including population code (as in Table 1), source of plant material and GenBank accession numbers.

Code	Source	GenBank accession number		
		ITS	psbJ-petA	trnQ-rps16
GB1	AOC, CDP, FJV	JF774135	JF774097	JF774098
GB2	CDP, FJV, TDD	JF774136	JF774087	JF774088
GB3	AOC, CDP, FJV, TDD	JF774137	JF774081	JF774082
GB4	CDP, FJV, RGW	JF774138	JF774083	JF774084
CA1	FJV	JF774155	JF774113	JF774114
CA2	FJV	JF774156	JF774115	JF774116
CA3	FJV	JF774157 JF774158	JF774117 JF774119	JF774118 JF774120
IB1	FJV	JF774150	JF774103	JF774104
IB2	FJV	JF774151	JF774105	JF774106
IB3	FJV	JF774152	JF774107	JF774108
PY1	FJV	JF774159	JF774121	JF774122
PY2	FJV	JF774160	JF774123	JF774124
PY3	FJV	JF774147	JF774125	JF774126
PY4	FJV	JF774148	JF774127	JF774128
PY5	FJV	JF774149	JF774129	JF774130
MC1	FJV	JF774153	JF774109	JF774110
MC2	FJV	JF774154	JF774111	JF774112
Outgroup				
Papaver atlanticum	JWK	JF774161	JF774131	JF774132
Papaver bracteatum	JWK	JF774162	JF774133	JF774134

AOC, Arthur O Chater; CDP, Chris D Preston; FJV, Francisco J Valtueña; JWK, Joachim W Kadereit; RGW, Ray G Woods; TDD, Trevor D Dines.

Table S2 Taxa used in the *rbcL* data set for estimating the stem group age of Old World Papaveroideae, including GenBank accession numbers (GBN)

Order	Family	Species	GBN
Aquifoliales	Aquifoliaceae	*Ilex crenata* THUNB.	L01928
Asterales	Asteraceae	*Cichorium intybus* L.	L13640
Berberidopsidales	Berberidopsidaceae	*Berberidopsis corallina* HOOK.F.	AJ235773
Brassicales	Tropaeolaceae	*Tropaeolum majus* L.	L14706
Buxales	Buxaceae	*Buxus sempervirens* L.	AF093717
Caryophyllales	Caryophyllaceae	*Silene gallica* L.	M83544
Caryophyllales	Frankeniaceae	*Frankenia pulverulenta* L.	Z97638
Celastrales	Parnassiaceae	*Parnassia fimbriata* BANKS	L01939
Chloranthales	Chloranthaceae	*Chloranthus japonicus* SIEBOLD	L12640
Cornales	Cornaceae	*Cornus mas* L.	L11216
Dilleniales	Dilleniaceae	*Dillenia indica* L.	L01903
Dipsacales	Adoxaceae	*Viburnum acerifolia* L.	L01959
Ericales	Ericaceae	*Erica australis* L.	L12617
Ericales	Primulaceae	*Anagallis arvensis* L.	M88343
Fabales	Fabaceae	*Pisum sativum* L.	X03853
Garryales	Eucommiaceae	*Eucommia ulmoides* OLIV.	L01917
Geraniales	Geraniaceae	*Geranium cinereum* CAV.	L14695
Gunnerales	Gunneraceae	*Gunnera manicata* LINDEN	L11186
Lamiales	Lamiaceae	*Lavandula angustifolia* MOENCH	Z37404
Lamiales	Oleaceae	*Jasminum suavissimum* LINDL.	L01929
Malpighiales	Linaceae	*Linum perenne* GUSS.	Z75681
Malvales	Cistaceae	*Cistus revolii* COSTE et SOULIE	Y15140
Myrtales	Melastomataceae	*Clidemia petiolaris* TRIANA	AJ235777
Oxalidales	Oxalidaceae	*Averrhoa carambola* L.	L14692
Proteales	Nelumbonaceae	*Nelumbo lutea* PERS.	M77032
Proteales	Platanaceae	*Platanus occidentalis* L.	L01943
Proteales	Proteaceae	*Roupala macrophylla* POHL	AF093728
Ranunculales	Berberidaceae	*Achlys triphylla* (SM) DC.	L75868
Ranunculales	Berberidaceae	*Berberis thunbergii* DC.	AF139878
Ranunculales	Berberidaceae	*Bongardia chrysogonum* (L.) SPACH	L75870
Ranunculales	Berberidaceae	*Caulophyllum robustum* MAXIM.	AF190441
Ranunculales	Berberidaceae	*Caulophyllum thalictroides* (L.) MICHX.	AF190442
Ranunculales	Berberidaceae	*Diphylleia cymosa* MICHX.	L75866
Ranunculales	Berberidaceae	*Dysosma versipellis* (HANCE) M.CHENG	AF079454
Ranunculales	Berberidaceae	*Epimedium elatum*	DQ851436
Ranunculales	Berberidaceae	*Epimedium koreanum* NAKAI	L75869
Ranunculales	Berberidaceae	*Epimedium platypetalum*	DQ851412
Ranunculales	Berberidaceae	*Jeffersonia diphylla* (L.) PERS.	L75876
Ranunculales	Berberidaceae	*Mahonia bealei* (FORT.) CARR.	L75871
Ranunculales	Berberidaceae	*Nandina domestica* THUNB.	L75843

Table S2 *Continued*

Order	Family	Species	GBN
Ranunculales	Berberidaceae	*Podophyllum peltatum* L.	AF203488
Ranunculales	Berberidaceae	*Ranzania japonica* ITO	L75853
Ranunculales	Berberidaceae	*Sinopodophyllum hexandrum* (ROYLE) YING	AF079455
Ranunculales	Berberidaceae	*Vancouveria chrysantha* GREENE	DQ851445
Ranunculales	Eupteleaceae	*Euptelea polyandra* SIEBOLD et ZUCC.	L12645
Ranunculales	Lardizabalaceae	*Decaisnea fragesii* FRANCH.	L37916
Ranunculales	Menispermaceae	*Menispermum canadense* L.	AF093726
Ranunculales	Papaveraceae	*Argemone mexicana* L.	U86621
Ranunculales	Papaveraceae	*Chelidonium majus* L.	DQ912892
Ranunculales	Papaveraceae	*Corydalis ambigua* CHAM. et SCHLTDL.	DQ912902
Ranunculales	Papaveraceae	*Corydalis flavula* (RAF.) DC.	DQ006097
Ranunculales	Papaveraceae	*Corydalis incise* (THUNB.) PERS.	DQ912903
Ranunculales	Papaveraceae	*Corydalis nobilis* (L.) PERS.	AF093722
Ranunculales	Papaveraceae	*Dendromecon rigida* BENTH.	U86623
Ranunculales	Papaveraceae	*Dicranostigma franchetianum* MAXIM.	U86624
Ranunculales	Papaveraceae	*Eschscholzia californica* CHAM.	DQ912895
Ranunculales	Papaveraceae	*Glaucium flavum* CRANTZ	U86626
Ranunculales	Papaveraceae	*Hunnemannia fumariifolia* SWEET	U86627
Ranunculales	Papaveraceae	*Hypecoum imberbe* SIBTH. et SM.	U86628
Ranunculales	Papaveraceae	*Lamprocapnos spectabilis* (L.) FUKUHARA	L08761
Ranunculales	Papaveraceae	*Macleaya cordata* (WILLD.) R.BR.	U86629
Ranunculales	Papaveraceae	*Meconopsis cambrica* (L.) VIG.	JN634856
Ranunculales	Papaveraceae	*Papaver bracteatum* LINDL.	DQ912897
Ranunculales	Papaveraceae	*Papaver nudicaule* L.	DQ912898
Ranunculales	Papaveraceae	*Papaver orientale* L.	DQ912899
Ranunculales	Papaveraceae	*Papaver radicatum* ROTTB.	DQ912893
Ranunculales	Papaveraceae	*Papaver rhoeas* L.	DQ912900
Ranunculales	Papaveraceae	*Papaver somniferum* L.	DQ912894
Ranunculales	Papaveraceae	*Platystemon californicus* BENTH.	U86630
Ranunculales	Papaveraceae	*Pteridophyllum racemosum* SIEBOLD et ZUCC.	U86631
Ranunculales	Papaveraceae	*Romneya coulteri* HARV.	U86632
Ranunculales	Papaveraceae	*Sanguinaria canadensis* L.	L01951
Ranunculales	Papaveraceae	*Stylophorum diphyllum* NUTT.	U86633
Ranunculales	Ranunculaceae	*Caltha palustris* L.	L02431
Ranunculales	Ranunculaceae	*Hydrastis canadense* L.	L75849
Ranunculales	Ranunculaceae	*Xanthorhiza simplicissima* MARSHALL	L12669
Rosales	Rosaceae	*Dryas drummondi* RICHARDS.	U59818
Sabiales	Sabiaceae	*Sabia* COLEBR. sp.	L12662
Santalales	Olacaceae	*Heisteria parvifolia* SM.	AJ131771
Sapindales	Anacardiaceae	*Pistacia vera* L.	AJ235786

Table S2 *Continued*

Order	Family	Species	GBN
Saxifragales	Altingiaceae	*Liquidambar formosana* HANCE	AJ131772
Saxifragales	Cercidiphyllaceae	*Cercidiphyllum japonicum* SIEBOLD et ZUCC.	L11673
Saxifragales	Crassulaceae	*Kalanchoe daigremontana* HAMET et PERRIER	L11189
Saxifragales	Hamamelidaceae	*Hamamelis mollis* OLIV.	L01922
Saxifragales	Saxifragaceae	*Saxifraga integrifolia* HOOK.	L01953
Solanales	Solanaceae	*Lycopersicon esculentum* MILL.	L14403
Vitales	Vitaceae	*Vitis aestivalis* MICHX.	L01960

Table S3 Taxa used in the ITS data set for dating *Meconopsis cambrica*, including GenBank accession numbers (GBN). Population code in *Meconopsis cambrica* material is indicated.

Species	GBN	Species	GBN
Argemone mexicana	AY328303	*Meconopsis speciosa*	AY328286
Meconopsis aculeata	AY328263	*Meconopsis superba*	AY328274
Meconopsis bella	AY328279	*Meconopsis taylorii*	AY328275
Meconopsis betonicifolia	DQ250323	*Meconopsis torquata*	AY328378
Meconopsis cambrica (GB1)	JF774135	*Meconopsis wumungensis*	AY328265
Meconopsis cambrica (GB3)	JF774137	*Papaver aculeatum*	DQ250316
Meconopsis cambrica (MC1)	JF774153	*Papaver alpinum*	DQ250261
Meconopsis cambrica (CA2)	JF774156	*Papaver anomalum*	DQ250263
Meconopsis cambrica (CA3)	JF774158	*Papaver apulum*	DQ250300
Meconopsis cambrica (PY1)	JF774159	*Papaver argemone*	DQ250298
Meconopsis cambrica (PY2)	JF774160	*Papaver armeniacum*	DQ250302
Meconopsis cambrica (PY3)	JF774147	*Papaver atlanticum*	DQ250315
Meconopsis delavayi	AY328285	*Papaver bracteatum*	DQ250286
Meconopsis dhwojii	AY328276	*Papaver californicum*	DQ250318
Meconopsis discigera	AY328277	*Papaver commutatum*	DQ250313
Meconopsis forrestii	AY328287	*Papaver croceum*	DQ250257
Meconopsis gracilipes	AY328270	*Papaver dubium*	DQ250319
Meconopsis grandis	AY328290	*Papaver glaucum*	DQ250308
Meconopsis henrici	AY328281	*Papaver hybridum*	DQ250301
Meconopsis horridula	AY328261	*Papaver macrostomum*	DQ250275
Meconopsis impedita	AY328280	*Papaver miyabeanum*	DQ250265
Meconopsis integrifolia	AY328288	*Papaver nudicaule*	DQ250260
Meconopsis lancifolia	AY328282	*Papaver orientale*	DQ250291
Meconopsis latifolia	AY328264	*Papaver pavonium*	DQ250283
Meconopsis lyrata	AY328267	*Papaver pilosum*	DQ250320
Meconopsis nepaulensis	AY328269	*Papaver pseudo-orientale*	DQ250288
Meconopsis paniculata	AY328272	*Papaver radicatum*	DQ250262
Meconopsis primulina	AY328266	*Papaver rhoeas*	DQ250273
Meconopsis punicea	AY328293	*Papaver rupifragum*	DQ250314
Meconopsis quintuplinervia	AY328295	*Papaver somniferum*	DQ250306
Meconopsis racemosa	AY328257	*Platystemon californicus*	AF305341
Meconopsis regia	AY328273	*Roemeria refracta*	DQ250299
Meconopsis simplicifolia	AY328289	*Romneya coulteri*	AF098922
Meconopsis sinuata	AY328268	*Stylomecon heterophylla*	DQ250295

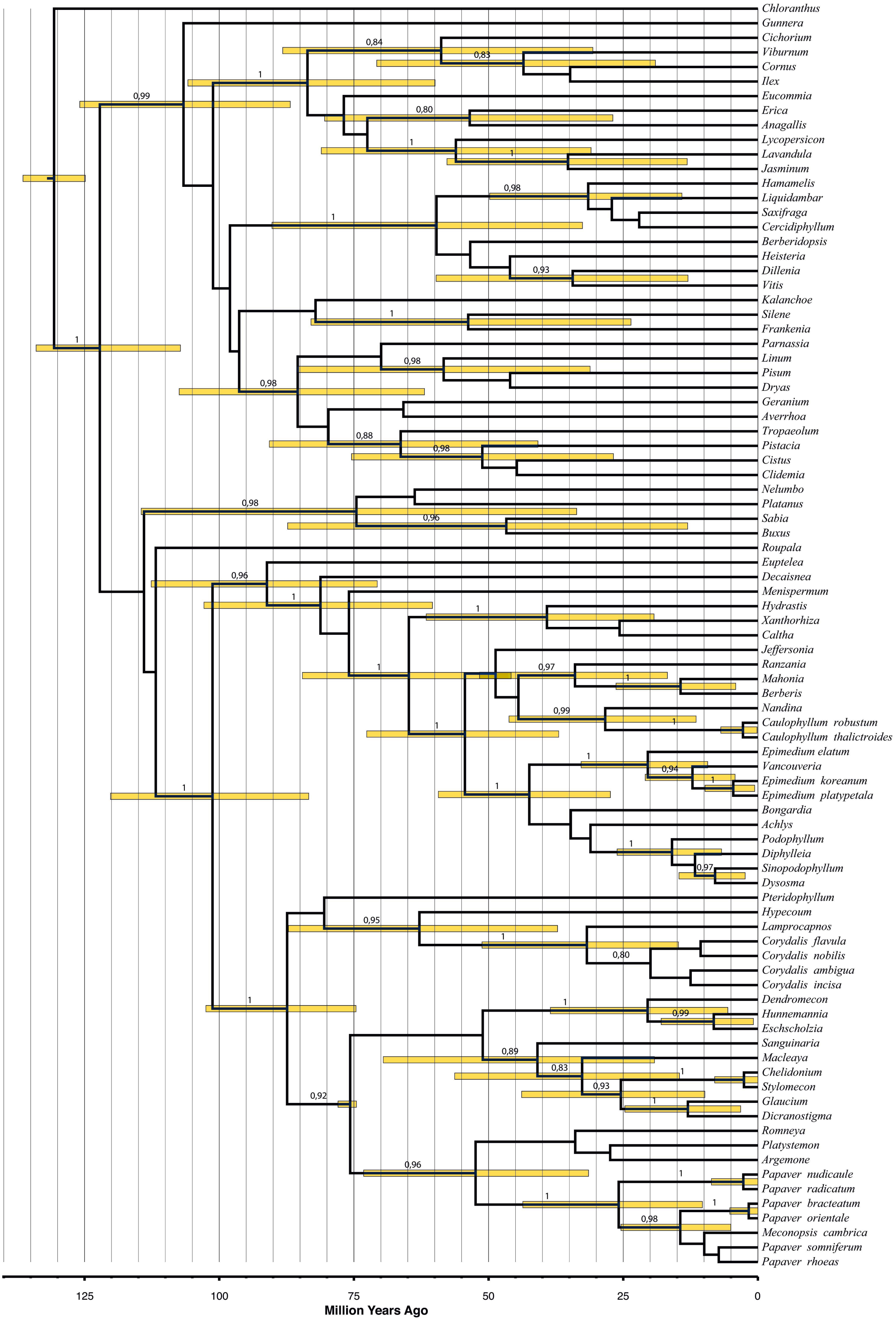


Fig. S1 BEAST chronogram of the Eudicots data set. Posterior probabilities of clades are indicated above branches (only PP. > 0.8). The 95% posterior density distribution of node ages is given in the node bars. Node bars are only given for branches with PP > 0.8. The scale is in million years.