



Evolutionary history of fumitories (subfamily Fumarioideae, Papaveraceae): An old story shaped by the main geological and climatic events in the Northern Hemisphere [☆]



Miguel A. Pérez-Gutiérrez ^a, Ana T. Romero-García ^a, M. Carmen Fernández ^b, G. Blanca ^a,
María J. Salinas-Bonillo ^c, Víctor N. Suárez-Santiago ^{a,*}

^a Department of Botany, Faculty of Sciences, University of Granada, c/ Severo Ochoa s/n, 18071 Granada, Spain

^b Department of Cell Biology, Faculty of Sciences, University of Granada, c/ Severo Ochoa s/n, 18071 Granada, Spain

^c Department of Biology and Geology, University of Almería, c/ Carretera de Sacramento s/n, 04120 Almería, Spain

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ABSTRACT

Fumitories (subfamily Fumarioideae, Papaveraceae) represent, by their wide mainly northern temperate distribution (also present in South Africa) a suitable plant group to use as a model system for studying biogeographical links between floristic regions of the Northern Hemisphere and also the Southern Hemisphere Cape region. However, the phylogeny of the entire Fumarioideae subfamily is not totally known. In this work, we infer a molecular phylogeny of Fumarioideae, which we use to interpret the biogeographical patterns in the subfamily and to establish biogeographical links between floristic regions, such as those suggested by its different inter- and intra-continental disjunctions. The tribe Hypecoeae is the sister group of tribe Fumarieae, this latter holding a basal grade of monotypic or few-species genera with bisymmetric flowers, and a core group, Core Fumarieae, of more speciose rich genera with zygomorphic flowers. The biogeographical analysis shows a subfamily that originated in East Asia at the end of the Early Cretaceous. From here, ancestral range expansions followed three different directions, one at the beginning of the Late Cretaceous by the ancestor of tribe Hypecoeae towards central Asia, and two during the Cretaceous–Palaeogene transition towards western North America and Indochina by the ancestor of the tribe Fumarieae. The ancestor of Core Fumarieae expanded its range from East Asia into the Himalayas before to the middle Eocene. The uplifts of the Qinghai-Tibetan Plateau together with the zonal climate pattern of the Palaeogene are suggested to be responsible both for the accelerated diversification rate resulting in the origin of the basal lineages of Core Fumarieae as well as for the westward migration of the ancestor of Fumarieae s.str. into the Irano-Turanian region. From here, this latter group reached South Africa during late Eocene and Mediterranean basin during Oligocene. There were two colonization waves of the Mediterranean following two different routes: a northern route during the early Oligocene by the subtribe Sarcocapninae, probably facilitated by the land bridge resulting of the Mediterranean microplate accretion; and a southern route into North Africa, through the Gomphotherium land bridge, taken by the subtribe Fumariinae between late Oligocene and middle Miocene.

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1. Introduction

A key aspect of the biogeographical studies is the elucidation of historical connections between different floristic regions to understand the genesis of plant diversity in these floristic regions (Linder, 2005). The establishment of biogeographical links between

floristic regions requires the evaluation of the current patterns of plant distribution (MacLaughlin, 1994), and therefore key taxa need to be studied. In this sense, the subfamily Fumarioideae Eaton (Papaveraceae Juss.) represents a suitable plant group to use as a model system for studying biogeographical links between Northern Hemisphere floristic regions, involving also the Southern Hemisphere Cape region. On the one hand, Fumarioideae shows a wide, mainly northern temperate distribution (also present in South Africa; Fig. 1) and, on the other hand, its species occupy both forest floor and open-dry habitats, being present in floristic regions

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* Corresponding author.

E-mail address: vsuarez@ugr.es (V.N. Suárez-Santiago).

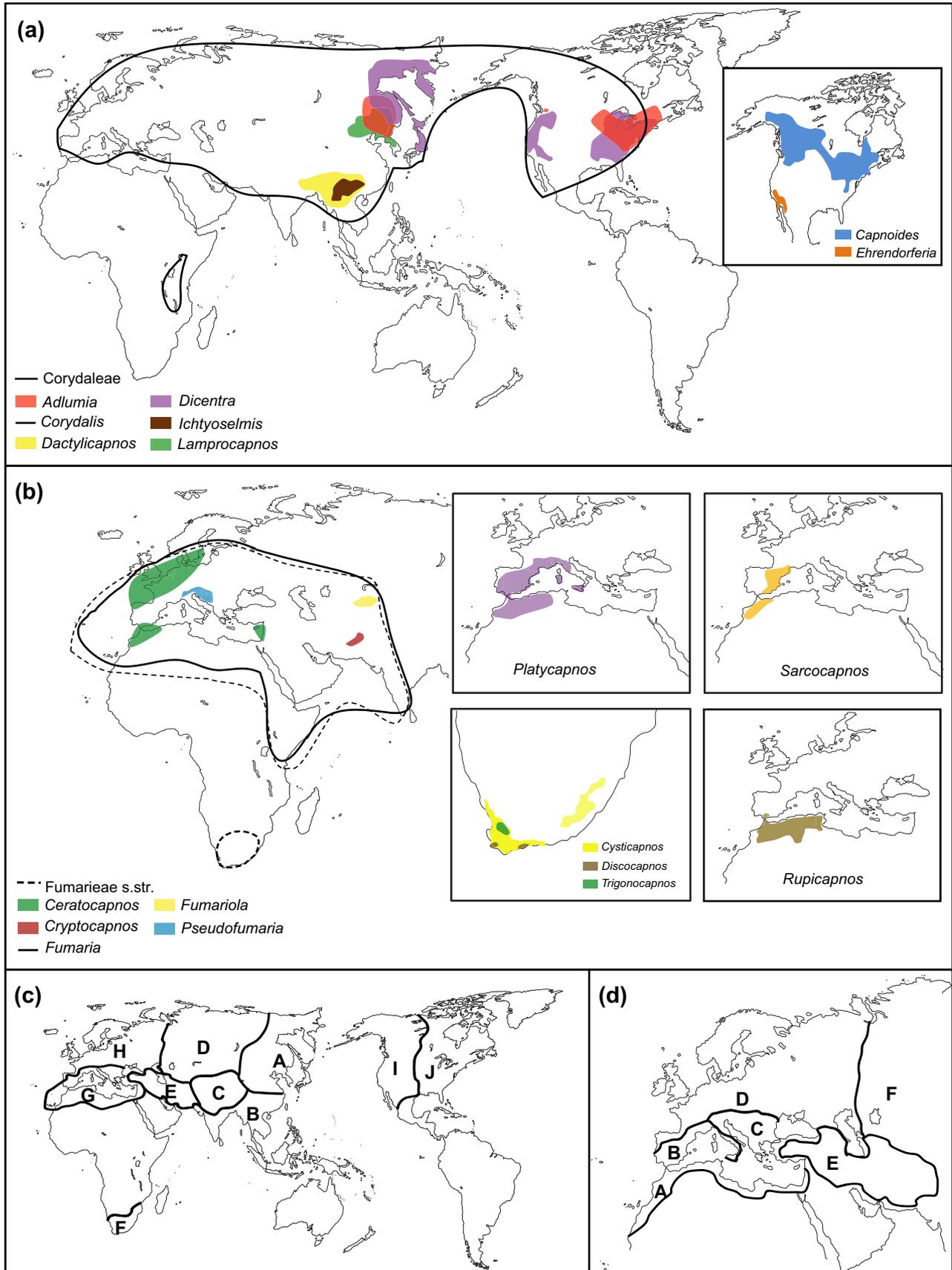


Fig. 1. Geographic distribution of tribe Fumarioideae and selected areas. (a) Distribution of genera from tribe Corydaleae *sensu* Lidén. (b) Distribution of genera from Fumarioideae s.str. (c) Areas defined for the ancestral range reconstruction analysis of the subfamily Fumarioideae: A, East Asia; B, Indochina; C, Himalayas; D, Central Asia; E, Irano-Turanian; F, South Africa; G, Mediterranean; H, Europe; I, western North America; J, eastern North America. (d) Areas defined in the ancestral range-reconstruction analysis focusing on the Mediterranean taxa of Fumarioideae s.str.: A, southern Mediterranean basin; B, Western Mediterranean; C, Eastern Mediterranean; D, Central and Northern Europe; E, Irano-Turanian; F, Central Asia.

with different macroclimatic conditions, enabling floristic connections to be tested between contrasting regions.

Molecular dating and ancestral-area reconstruction methods based on phylogenies provide better approaches to understand the plant biogeographical models and enable them to be interpreted in connection to the climatic geological events over the history of the Earth. This requires prior knowledge of the phylogenetic relationships. In this context, the phylogeny of Fumarioideae is not fully understood, since no complete molecular study has been made, and only some partial phylogenies are available (e.g. Lidén et al., 1995, 1997; Salinas et al., 2003; Pérez-Gutiérrez et al., 2012).

Fumarioideae is one of the two subfamilies (together Papaveroideae Eaton) currently recognized in Papaveraceae (Wang et al., 2009), and includes two genera with controversial phylogenetic affinities in Papaveraceae, *Hypecoum* L. and *Pteridophyllum* Siebold & Zucc. (see Lidén, 1986, 1993a,b; Kadereit et al., 1994; Hoot et al., 1997; Zhang et al., 2008). It includes c. 590 species and 21 genera (Lidén, 1986; Lidén et al., 1997; Zhang et al., 2008) grouped into two tribes (Stevens, 2001 onwards): Fumarieae Dumort. (=Fumariaceae s.str.) and Hypecoae Dumort. (*Hypecoum* + *Pteridophyllum*). Previously *Hypecoum* was placed either in Fumariaceae (as a subfamily or in Papaveraceae subfamily Fumarioideae without suprageneric status, Lidén, 1993a; Zhang et al., 2008; respectively) or close to Fumariaceae s.str. (Takhtajan, 1997), and a sister-clade relationship between both groups was found on the basis of morphology and molecular data (Kadereit et al., 1994; Hoot et al., 1997); *Pteridophyllum* was considered the earliest-diverging lineage of Papaveraceae (Lidén, 1986; Kadereit et al., 1994; Hoot et al., 1997). Wang et al. (2009) seems to solve all these controversies, since they obtained a relationship between *Hypecoum*–*Pteridophyllum* and the rest of Fumarioideae (Fumariaceae s.str.); however, the classification proposed by them should be taken with caution as noted in Pérez-Gutiérrez et al. (2012).

Classification within the tribe Fumarieae includes two main groups previously recognized at the tribal level by Lidén (1986, 1993a) within its subfamily Fumarioideae: tribe Corydaleae Reichenb. (8 genera) and tribe Fumarieae s.str. (11 genera). However, morphological and molecular analyses show Corydaleae to be a non-monophyletic group (Loconte et al., 1995; Lidén et al., 1997). Fumarieae s.str. is monophyletic as it has been asserted both with cladistics analysis of morphological characters (Lidén, 1986) and molecular phylogenetic analysis (Pérez-Gutiérrez et al., 2012). According to Lidén (1986) Fumarieae s.str. can be divided into three subtribes on a morphological basis: Discocapninae Lidén, Fumariinae, and Sarcocapninae Lidén. These three groups of genera were confirmed as three monophyletic lineages by Pérez-Gutiérrez et al. (2012) using chloroplast and nuclear DNA phylogenies.

Few molecular phylogenetic studies are available on the relationships within Fumarioideae. Some of these works have addressed phylogenies on a particular genus (Lidén et al., 1995; Salinas et al., 2003), and no complete molecular phylogeny has been published. The first molecular approach to the phylogeny of Fumarioideae was that of Lidén et al. (1997) using the intron of the chloroplast *rps16* gene. These authors focused mainly on tribe Corydaleae *sensu* Lidén (1986, 1993a), and they not only found Corydaleae to be a non-monophyletic group, but also clarified many relationships among its genera and identified the basal lineages for the subfamily. Taxonomically, this study resulted in the split of *Dicentra* Bernh. in four genera (*Dicentra* s.str., *Ehrendorferia* T. Fukuhara & Lidén, *Ichtyoselmis* Lidén & T. Fukuhara and *Lamprocapnos* Endl.), and the recognition of three subgenera for a monophyletic *Corydalis* DC. [*Cremonocapnos* Wendelbo, *Corydalis* and *Sophorocapnos* (Turcz.) Fukuhara & Lidén]. Within Fumarieae s.str. Lidén et al. (1997) found

Cysticapnos Mill. (hitherto *incertae sedis*) to belong to this group, but as they included only three Fumariaceae s.str. species no further conclusions could be drawn. Pérez-Gutiérrez et al. (2012) concentrated their phylogenetic analysis on the Fumariaceae s.str. (as tribe Fumariaceae *sensu* Lidén, 1986, 1993a) using chloroplast and nuclear DNA markers. These authors asserted the monophyly of the group, confirmed the existence of the three lineages inside, and established the generic relationships. Consequently, to date, no molecular study including all Fumarioideae genera has been published.

In the present work, we infer a molecular phylogeny of Fumarioideae including all its genera in order to analyse the tribal and generic relationship in the subfamily as a whole. This allows us to interpret the biogeographical patterns in the subfamily and to establish biogeographical links between floristic regions, as those suggested by the various existing disjunctions at different taxonomic levels (Lidén, 1986, 1993a; Kadereit et al., 1994, 1995; Fig. 1).

Roughly, Corydaleae *sensu* Lidén (Fig. 1A; Table 1), distributed throughout Asia, North America, and Europe. Three genera show disjunct areas between Asia and North America (*Adlumia* DC., *Corydalis*, and *Dicentra*). Different disjunction patterns can be seen: (i) East Asia/North America, within *Corydalis* subgenus *Sophorocapnos* section *Sophorocapnos* (Turcz.) Popov in Schischkin (widespread in North America), and within *Dicentra* with *D. peregrina* (Rudolph) Makino from East Siberia–Japan and the remainder from North America (within *Dicentra* also a western North America/eastern North America disjunct pattern exists); (ii) East Asia/eastern North America, in *Adlumia* with *A. fungosa* (Ait.) Britton, Sterns & Poggenb. from eastern North America and *A. asiatica* Ohwi restricted to Korea and Manchuria; (iii) East Asia/western North America, in *Corydalis* subgenus *Corydalis* section *Archaeocapnos* Popov ex Michajlova; (iv) Central to Arctic Asia/NW North America, in *Corydalis* subgenus *Corydalis* section *Dactylotuber* (Ruprecht) Popov in Schischkin. While most *Corydalis* are concentrated in the Sino-Himalayan area and the three above-mentioned sections (*Archaeocapnos*, *Dactylotuber*, *Sophorocapnos*) reach North America, many species of different sections reach Europe and south-western Asia, and one species appears in the mountains of East Africa (*C. mildbraedii* Fedde). With regard to rest of the Corydaleae genera, *Lamprocapnos* is exclusive from Asia (NE China, N Korea, and SE Russia), *Ehrendorferia* from western North America, *Dactylicapnos* Wall. is centred in the Himalayan region to W China, and the monotypic *Capnoides* Mill. is present in northern North America. Fumarieae s.str. shows a strong Mediterranean–South Africa–Central Asia disjunction (Fig. 1B), since it is centred in the Mediterranean basin but the lineage *Cysticapnos*–*Discocapnos* Cham. & Schltdl.–*Trigonocapnos* Schltr. (subtribe Discocapninae *sensu* Lidén, 1986, 1993a) is endemic to South Africa, and *Fumariola* Korsh. and *Cryptocapnos* Rech.f. are exclusive from Central Asia (Turkestan and Afghanistan, respectively).

Despite the noteworthy biogeographical pattern of Fumarioideae, very few studies have evaluated it in a phylogenetic framework, most of them being based on cladistics analyses of morphological data (focused either on small groups, e.g. *Rupicapnos* Pomel and *Sarcocapnos* DC., Lidén, 1986; or in a wider context on the whole Papaveraceae family, Zhuang, 1993; Kadereit et al., 1994, 1995). Only one study has centred on Fumariaceae s.str. used a molecular phylogeny to test ancestral ranges (Pérez-Gutiérrez et al., 2012). Therefore, no biogeographical analysis including ancestral-area reconstruction and/or dating methods based on phylogenies has been made for the whole subfamily. The poorly understood phylogeny of Fumarioideae and the lack of fossil records have discouraged such studies.

In the present work, we use seven DNA regions [five from chloroplast DNA (*matK* gene, *trnL* intron, *trnL-F* intergenic spacer,

Table 1
Comparative table of the alignment features, most parsimonious tree statistics for the different data sets, and nucleotide evolution model selected. Al, alignment length; CD, number of ambiguous characters deleted from analyses; PI, number of parsimony-informative characters; Var, number of variable characters; NT, number of most parsimonious trees; L, length of the most parsimonious trees; CI, consistency index; RI, retention index.

	Al	CD	PI/Var	NT	L	CI	RI	Model Selected
cpDNAMatrix	4867	321	1236/1908	6	3696	0.6989	0.8152	GTR + I + G
26S	1762	12	139/251	107	521	0.5643	0.6856	GTR + I + G
5.8S/ITS2	451	115	90/133	10,000	427	0.5633	0.6574	K80 + I/GTR + I + G
rDNAMatrix	2213	127	229/384	10,000	974	0.537	0.6531	GTR + I + G/K80 + I

trnG intron, and *rps16* intron) and two from nuclear ribosomal DNA (partial sequence of *26S* gene and ITS region)] to construct a phylogeny of the entire subfamily Fumarioideae in order to establish the relationships between all its genera. Using this phylogeny we interpret their biogeographical patterns through the analyses of ancestral areas and dating methods.

2. Materials and methods

2.1. Plant material

This study included 58 species representative of all genera of subfamily Fumarioideae *sensu* Wang et al. (2009). For outgroup species, we used *Eschscholzia californica* Cham. as representatives of the subfamily Papaveroideae and *Euptelea pleiosperma* Hook.f. & Thomson and *Euptelea polyandra* Siebold & Zucc. as representatives of the family Eupteleaceae K.Wilh. (earliest-diverging lineage of Ranunculales; Kim et al., 2004; Worberg et al., 2007; Wang et al., 2009). Plant material was collected in the wild, from botanic gardens, and from herbarium material (Table S1, in on-line attachment).

2.2. DNA extraction, PCR amplification, and DNA sequencing

Total genomic DNA was extracted from fresh or dry leaves, and seeds following the CTAB method (Doyle and Doyle, 1987), and, using the NucleoSpin® Plant Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), from difficult, old material.

The plastid markers (*matK* gene, *trnL* intron, *trnL-F* intergenic spacer, *trnG* intron, and *rps16* intron) and nuclear ribosomal regions (partial *26S* gene and ITS region) were amplified by polymerase chain reaction (PCR). The PCR amplifications were performed with the following primer pairs: *trnK2R* (Johnson and Soltis, 1995), *matK1166* (5'>GGCTTACTAATGGGAT<3') and *matK192* (5'>CGGGTTGCAAMAATAAAGGA<3') for *matK* gene, primers C and F (Taberlet et al., 1991) were used to amplify the *trnL* intron and *trnL-F* spacer as a whole (hereafter *trnL-F* region), primers 3'*trnG* and 5'*trnG2G* (Shaw et al., 2005) for *trnG* intron, *rpS16F* and *rpS16R* (Shaw et al., 2005, modified from Oxelman et al., 1997) for the *rps16* intron, the set of primers N-nc26s10, 1229rev, 1839rev (Kuzoff et al., 1998) for *26S* gene, and finally the primers N-nc18s10 and C26A (Wen and Zimmer, 1996) were used for the ITS region. PCR reactions were made under standard conditions (Innis et al., 1990) for the nuclear markers and under the recommended conditions for plastid markers (Taberlet et al., 1991; Johnson and Soltis, 1995; Shaw et al., 2005). Automated sequencing of the purified PCR products was performed in both directions using the amplification primers on a 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, California, USA) in the Centro de Instrumentación Científica of the University of Granada (Spain).

All sequences used in Pérez-Gutiérrez et al. (2012) plus 19 sequences of Fumarioideae and outgroup species (four of *matK*, one of *trnL-F* region, seven of *rps16* intron, three of *26S* gene and four of ITS region) were taken from GenBank (Table S1). All

sequences generated as part of this study were deposited in the EMBL database (Table S1).

2.3. Phylogenetic analyses

Nucleotide sequences were edited and aligned with SEQMAN II version 3.61 and MEGALIN version 3.18 programs, respectively, from the DNASTAR software package (DNASTAR Madison, Wisconsin, USA) and then adjusted by eye. One (28pb), four (75pb), six (123pb), six (95pb), four (135pb), and two (12pb) regions of the *matK*, *trnG*, *trnL-F*, *rps16*, ITS, and *26S* aligned matrices, respectively, were ambiguous and excluded from analyses. Moreover, due to the high sequence variability shown by the ITS1, we excluded it from the analyses. To test the congruence between data sets, we performed the incongruence-length difference test (ILD; Farris et al., 1994). The ILD was implemented in PAUP* version 4.0b10 as the partition homogeneity test (Swofford, 2003), using 100 replicates with 1000 random addition sequences each.

Phylogenetic analyses of the whole group included two independent analyses, one with all plastid data combined, and one with *26S* and ITS region (*5.8S-ITS2*) combined. The number of sequences included in each data matrix varied due to the inability to determine some sequences for several taxa (20 sequences in total: four of *matK*, three of *rps16*, 10 of *26S*, and three of ITS; Table S1).

Phylogenetic relationships were inferred using Maximum Parsimony (MP) as implemented in PAUP* 4.0b10 and Bayesian Inference (BI) using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). Gaps were treated as missing data. The MP analyses used heuristic searches with 1000 replicates of random sequence addition using tree bisection–reconnection (TBR) branch swapping under the Fitch criterion (unordered states and equal weights). Only 10 trees were maintained at each step, to minimize the time the algorithms spent searching for trees on suboptimal islands. The starting tree was constructed by stepwise addition. Finally, 1000 bootstrap replicates (BS; Felsenstein, 1985) with 10 heuristic searches, as above, were performed to assess internal support for nodes. The amount of phylogenetic signal in the analyses was given by consistency index (CI; Kluge and Farris, 1969) and the retention index (RI; Swofford, 1993).

Bayesian analyses were implemented using the best-fit nucleotide substitution model for each data set: GTR + I + G (nst = 6; rates = invgamma; statefreqpr = dirichlet) for plastid, *26S* and ITS2, and K80 + I (nst = 2; rates = propinv; statefreqpr = fixed (equal)) for *5.8S*. These models were selected using MrModeltest version 2.3 (Nylander, 2004) and Akaike's information criterion (Akaike, 1973). A partitioned model was used for the combined analysis of *26S* and *5.8S*, which included that selected for the independent data sets. The analyses were based on 2 million generations with four simultaneous runs (16 Markov chain Monte Carlo chains) starting from random trees that were sampled every 100 generations. The stationary of the runs and the convergence between runs were checked with Tracer version 1.5 (Rambaut and Drummond, 2007). The initial 25% of the trees that resulted were discarded as

burn-in, and the remaining trees were used to build 50% majority-rule consensus trees.

2.4. Divergence-time estimation

To estimate the divergence times of the Fumarioideae lineages, we used a Bayesian relaxed-clock method as implemented in the BEAST 1.7.5 package (Drummond et al., 2012) for the chloroplast-combined datamatrix. For tree calibration, two fossils assigned to Papaveraceae were considered, but the ambiguity in their assignation precludes us from using them as calibration points. One of them, *Palaeoaster* Knowlton (Smith, 2001), an alleged member of Papaveraceae, was recently used by Valtueña et al. (2012) to date Papaveraceae in their population study on *Meconopsis cambrica* Vig.; however, that fossil has been recognized to be close to the order Bennettitales and not to Papaveraceae (Little et al., 2010; Manchester, personal communication). On the other hand, recently Jud and Hickey (2013) have published leaf fossils from the Lower Cretaceous (from Aptian sediments in Dutch Gap, Virginia, USA), which they assigned to the species *Potomacarpus apeleutheron* Jud & Hickey within the subfamily Fumarioideae. In the cladistic analysis performed by these authors, *P. apeleutheron* includes in the tribe Fumarieae. However, the authors on the basis of the age of the fossils and their fragmentary nature could not rule out the possibility of a phylogenetic position of *P. apeleutheron* as a ranunculalean-or eudicot-stem lineage instead of a Papaveraceae, preventing its use as phylogeny calibration point. Therefore, due to the lack of fossils for Fumarioideae, we followed a two-step strategy for tree calibration.

An initial analysis included sequences of external taxa belonging to all families of Ranunculales order and two species of Ceratophyllales. For this analysis the genes *matK* and *rbcL* were used due to its higher availability in the nucleotide database (Table S1). The inclusion of Ceratophyllales allows us to introduce as calibration point the split of Eudicots, while the Ranunculaceae Juss. and Menispermaceae Juss. representatives allow us to include the fossils both of Ranunculaceae stem lineage from the Early Cretaceous, *Leefructus mirus* Sun, Dilcher, Wang et Chen (Sun et al., 2011), and of the Menispermaceae stem lineage *Protinomisium vangerowii* Knobloch & Mai from the Late Cretaceous (Knobloch and Mai, 1986). Therefore three calibration points were used, the first one by constricting Ranunculales origin to the range 131–147 Ma (Wikström et al., 2001), and the other two through a minimum age for Ranunculaceae and Menispermaceae stems of 122.6–125.6 Ma and 91 Ma, respectively.

We used the Wikström et al. (2001) date, based in tricolpate pollen fossil, and no other dating for eudicots (Anderson et al., 2005; Bell et al., 2010) because these other dates are not consistent with the data of the recently discovered Ranunculaceae stem lineage fossil. The partitioned .xml file was made up in BEAUTY v1.7.5 (Drummond et al., 2012) by means the selection a GTR model and a four-categories-gamma-shape distribution with invariant sites for the datamatrix, an uncorrelated lognormal relaxed-clock model (Drummond et al., 2006) and a Yule speciation process as the tree priors. BEAST v1.7.5 (Drummond et al., 2012) was launched with 50 million generations sampling one tree and parameters every 1000 generations. Tracer v1.5 (Rambaut and Drummond, 2007) was employed to check chain convergence and effective sampling size of the parameters. The maximum clade credibility tree summarizing the estimated mean age and the 95% confidence intervals from post-burn-in (10%) trees was calculated with TreeAnnotator v1.7.5 (Drummond et al., 2012).

The second analysis was focused on the subfamily Fumarioideae using the chloroplast-combined matrix. For phylogeny calibration, we used the dates from the first analysis as

calibration points; the split between Eupteleaceae and rest of Ranunculales, and crown Papaveraceae were used according a normal distribution covering the standard deviation of the highest posterior densities previously found (HPD; 138.01–125.99 Ma and 116.14–99.27 Ma, respectively). BEAST package was used following the same procedure described above.

2.5. Biogeographical analysis

To reconstruct the biogeographical history of Fumarioideae, we used the dispersal–extinction–cladogenesis method (DEC) implemented in Lagrange v 2.0.1 (Ree et al., 2005; Ree and Smith, 2008). This method develops parametric likelihood analyses, and it allows data to be included both from dated phylogenies and from palaeogeographic models reflecting the history of the Earth in different time frames. Lagrange takes into account this information, estimating the dispersal and extinction indexes for the whole group and then it computing relative probabilities of inherited areas for each node on the topology. We used the maximum credibility tree derived from the BEAST analysis and performed two independent analyses: (i) one on the whole Fumarioideae subfamily, and (ii) other focused only on the Mediterranean lineages of the Fumarieae s.str.

To delimit the biogeographical areas, we defined regions in the framework of the current continents (Buerki et al., 2011), and subdivided these regions according to the current distribution of Fumarioideae taxa and its specific richness. For the biogeographical analysis of the entire subfamily Fumarioideae, we used 10 areas (Fig. 1c): (A) East Asia, including eastern Siberia, Manchuria, Korea, Japan and central and east of China, reaching the Verkhoyansk Range in the west, the Lake Baikal in the north, and the Gobi Desert in the south; (B) Indochina, including south and east of China, India and Indochina; (C) Himalayas, including the Qinghai-Tibetan Plateau (QTP) and all mountain areas that delimit it (Qilian and Kunlun in the north, Himalayan in the south, Karakorum in the west, and Hengduan in the east); (D) Central Asia, covering the region from Ural mountains in the west, to the contact zone of the Iranian Plateau and the Pamir Mountains in the south, and to the limits of regions A and C in the south-east and east, respectively; (E) Irano-Turanian, from the Anatolia Peninsula to the western Himalayas, covering the Caucasus, the Levant region, Iran and Iraq; (F) South Africa, from southern Namibia to the Lesotho region southwards; (G) the Mediterranean, including the Mediterranean basin and Canary Islands; (H) Central and Northern Europe, from the Cantabrian region of the Iberian Peninsula northwards and eastwards to the Ural mountains; (I) western North America, including the western half of North America and north-western Mexico; and (J) eastern North America, covering the eastern half of North America. Using these areas, we tested two different models in the DEC analysis: the first one (M0) without dispersal constraint between areas over time (with equal rates of dispersal among areas), and the second one (M1) for which a time stratification was defined along Fumarioideae history. Thus for M1 model, four time intervals were established (before 80 Ma, 80–35 Ma, 35–20 Ma, 20–0 Ma) following Buerki et al. (2011), and a specific dispersal-rate matrix was defined for each in order to reflect the main palaeogeographical connections between landmasses (Fig. S2, in on-line attachment). The dispersal-rate values among areas were inversely proportional to the number of areas and/or physical barriers (i.e. water masses) in between. The maximum number of permitted ancestral areas for the analysis was three, because most of the sampled species currently occur in no more than three areas. The Python scripts were completed in the online Lagrange configurator (<http://www.reelab.net/lagrange>). Taxa distribution was assigned following Lidén (1986), Stern (1997) and Zhang et al. (2008). Due to the high

number of species in *Corydalis* (c. 465; Zhang et al., 2008), and the low number included in our analysis, we repeated the analyses including the representatives of each *Corydalis* subgenus as placeholder for the distribution of its subgenera in order to provide a better generic-distribution representation. The subgenus distribution areas were established according to the distribution of all their species (ABCH for *Corydalis* subgenus *Corydalis*, CDE for *Corydalis* subgenus *Cremonocapnos* and ABC for *Corydalis* subgenus *Sophorocapnos*; the initial tree for launching Lagrange was accordingly modified). In this way, we could test whether the insufficient sampling of this genus could bias the result of the ancestral area reconstruction. With the original analyses, i.e. considering the distribution for each *Corydalis* species sampled, we were able to evaluate the North America/Asia connection shown by the subgenus *Corydalis* section *Archaeocapnos*.

With regard to the second biogeographical analysis focused on the Mediterranean region and adjacent territories, we defined the following operational areas on the basis of patterns of endemism among current taxa and maximizing congruence with the Mediterranean biogeographical pattern and palaeogeographic history (Fig. 1d): (A) the southern Mediterranean basin, including North Africa, the northern portion of the Sahara region and the Canary Islands; (B) Western Mediterranean, covering Mediterranean region of Iberian Peninsula, southern France, west and southern Italy and islands of the central Mediterranean; (C) Eastern Mediterranean, including the Balkans Peninsula, the Alps, and northern Italy; (D) Central and Northern Europe, from northern Spain; (E) Irano-Turanian, as previously defined; and (F) Central Asia, as previously defined. Two different models were also tested, an unconstrained model (MM0) and a second one with constrained dispersal rates stratified into three time intervals (MM1). For the latter the time intervals (35–25 Ma, 25–14 Ma and 14–0 Ma) were defined considering both the dates of the main diversifications of the Mediterranean representatives of Fumarioideae s.str., and the geological events described for this region (Rögl, 1999; Meulenkamp and Sissingh, 2003; Ree and Sanmartín, 2009). The exchange rates for each period were based on those in Ree and Sanmartín (2009) adjusted to our Mediterranean subdivision (see Fig. S3, in on-line attachment). Lagrange analysis was conducted as described above.

3. Results

3.1. Phylogenetic analyses

The only ILD test rejecting the data-set combination was the one performed on the combination of nuclear and plastid markers ($P = 0.002$); all remainder ILD test failed to reject the combinations tested. This result was consistent with the strong incongruences detected between the trees resulting from the nuclear and plastid independent analyses. Therefore, we did not combine the two data sets. The alignment features and tree statistics for parsimony analyses are shown in Table 1.

Bayesian and maximum-parsimony analyses of the chloroplast data set yielded almost the same topologies and same level of node support. Fig. 2 shows the Bayesian tree including the posterior probabilities (PP) and the bootstrap (BS) values. Almost all relationships were strongly supported ($>70\%$ BS; >0.90 PP). Only three differences were found between BI and MP analyses: (i) in the parsimony analysis *Pteridophyllum racemosum* Siebold & Zucc. form a weakly supported group (BS: 51) with the genus *Hypecoum* (taxa not grouped in the BI), (ii) *Rupicapnos* grouped, with low PP (0.61), with the clade *Cryptocapnos–Fumariola* in the Bayesian analysis; while in the parsimony analysis the relationships between *Cryptocapnos–Fumariola*, *Fumaria* L., and

Rupicapnos were not resolved (all three possible combinations resulted in the most-parsimonious trees); and (iii) *Sarcocapnos pulcherrima* C. Morales & Romero García and *S. speciosa* Boiss. were sister groups, and *S. crassifolia* DC. was sister to the group formed by the two of them in the Bayesian analysis; while in the parsimony analysis this relationship appeared in half of the six most parsimonious trees, with *S. crassifolia* and *S. integrifolia* (Boiss.) Cuatrec. being sister groups in the other three trees. All species were grouped by generic affinity, except those of *Cysticapnos* which appeared as paraphyletic. Relationship of *Pteridophyllum racemosum* to the remainder Fumarioideae subfamily was weakly supported, as also occurs with its relationship to *Hypecoum* (only supported by the parsimony analysis; BS: 51). Fumarioideae (excluding *Pteridophyllum*) appeared as a strongly supported monophyletic group (PP: 1; BS: 100 when analysis excluded *Pteridophyllum*, data not shown). The tree confirmed the paraphyly of the tribe Corydaleae *sensu* Lidén, since most of its genera appeared, forming a grade of basal Fumarioideae lineages (Fig. 2). The remaining genera formed a strongly supported clade (Core Fumarioideae), with well-resolved relationships inside, including *Capnoides*, *Dactylicapnos*, *Corydalis*, and all genera of Fumarioideae s.str. grouped in three clades according to the subtribal classification (Discocapninae, Fumariinae, and Sarcocapninae). All relationships were strongly supported except that of Sarcocapninae, which was weakly supported in the parsimony analysis (BS: 54%) but strongly supported in the Bayesian analysis (PP: 0.94). Generic relationships within Fumarioideae s.str. were all well resolved except those for subtribe Fumariinae (Fig. 2).

Phylogenies found from the parsimony and Bayesian analyses of the combined nuclear ribosomal markers were highly congruent, with only some occasional weakly-supported conflictive relationships inside the Core Fumarioideae clade (Fig. 3; Fig. S4, in on-line attachment). However, they differed in the support level of the internal nodes; parsimony bootstrap did not support any internal node, while Bayesian analysis strongly supported almost all internal nodes (Fig. 3; Fig. S4). In general, ribosomal phylogeny resolved much less than did the chloroplast one, especially within the Core Fumarioideae clade; here, several genera were not monophyletic (*Ceratocapnos* Dur., *Corydalis*, *Cysticapnos*, *Fumaria*, *Platycapnos* (DC.) Bernh.), and the generic relationships were not well defined. From the three subtribes defined within Fumarioideae s.str. in the chloroplast tree only Discocapninae (BS: 58, PP: 0.71) appears as monophyletic in the ribosomal phylogeny, while Sarcocapninae and Fumariinae were only partially recovered, excluding *Platycapnos* and *Pseudofumaria* Medik., and *Rupicapnos* respectively. *Dactylicapnos* was a monophyletic genus and sister to *Capnoides* (BS: 61; PP: 0.88). Outside the Core Fumarioideae, the ribosomal tree showed the same basal lineages of Fumarioideae as the chloroplast tree with few differences in the relationships between lineages (*Dicentra* and *Ehrendorferia* were sister groups, and *Pteridophyllum* was related to the representative of subfamily Papaveroideae; Figs. 2 and 3).

3.2. Divergence date estimates and biogeographical analyses

The first step in the divergence-time estimates resulted in the divergence of the family Eupteleaceae from the rest of Ranunculales lineages 130 Ma in the Hauterivian from the Early Cretaceous, with the split of the family Papaveraceae also in the Early Cretaceous (Barremian, 129 Ma) and its crown group originating 107 Ma in the Albian from the end of the Early Cretaceous (Fig. S5, in on-line attachment). Using the dates for the split of Eupteleaceae and for the crown Papaveraceae as secondary calibration points, and our chloroplast phylogeny, we dated all Fumarioideae nodes (Fig. 4a; Fig. S6, in-online attachment).

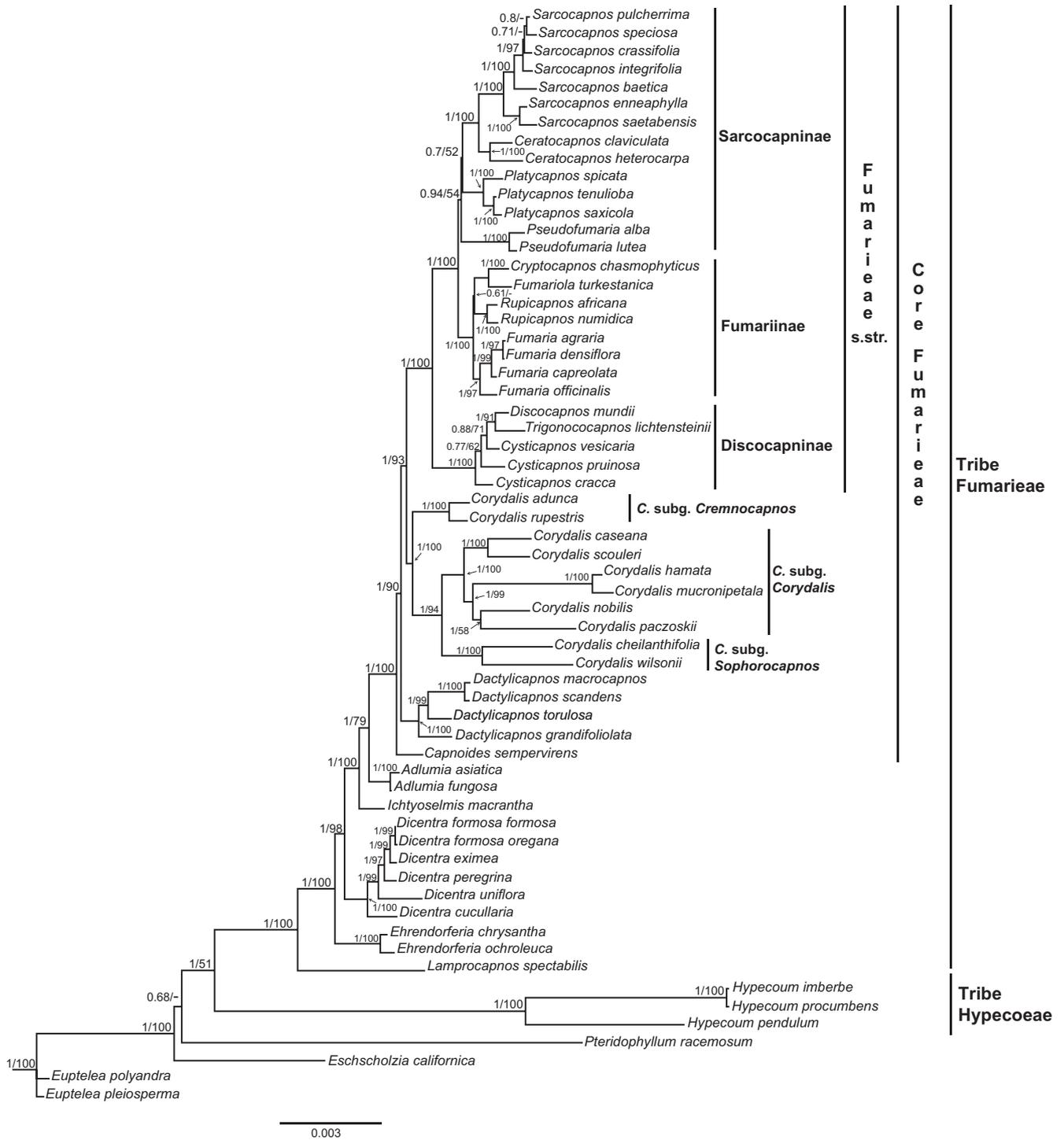


Fig. 2. Bayesian 50% majority-rule consensus tree inferred from the combined plastid data matrix. Posterior probabilities and bootstrap values are shown above branches (PP/BS). Hyphens show the branches collapsed in the strict consensus tree from the parsimony analysis. Classification and groupings are indicated on the right.

Unconstrained and constrained biogeographical analyses were congruent across all nodes, resulting in the same ancestral area reconstructions [Tables S7–S9, in on-line attachments]. In the same way, the analyses collapsing and not collapsing the *Corydalis* species by subgenera were congruent each other (Tables S7 and S8). The only difference between the unconstrained and constrained analyses of the whole subfamily was that the latter resolved the ancestral area for three (nodes 116, 115, 91; Fig. 4a) of the five ambiguous nodes obtained in the unconstrained analyses (nodes 117, 116, 115, 108, 91; Table S7). The areas and their relative

probabilities estimated in the constrained analysis are presented below and shown in Fig. 4a. The detailed biogeographical analysis of the subtribes Fumariinae and Sarcocapninae is shown in Fig. 4b and Table S9.

Our results show a complex biogeographical history as the result of multiple dispersal events both intercontinental and intra-continental in different directions and time periods (Figs. 4a,b and S6; Tables 2 and S7–S9). Four dispersals between East Asia and North America were detected (two from East Asia to North America and two in the opposite direction), four from western

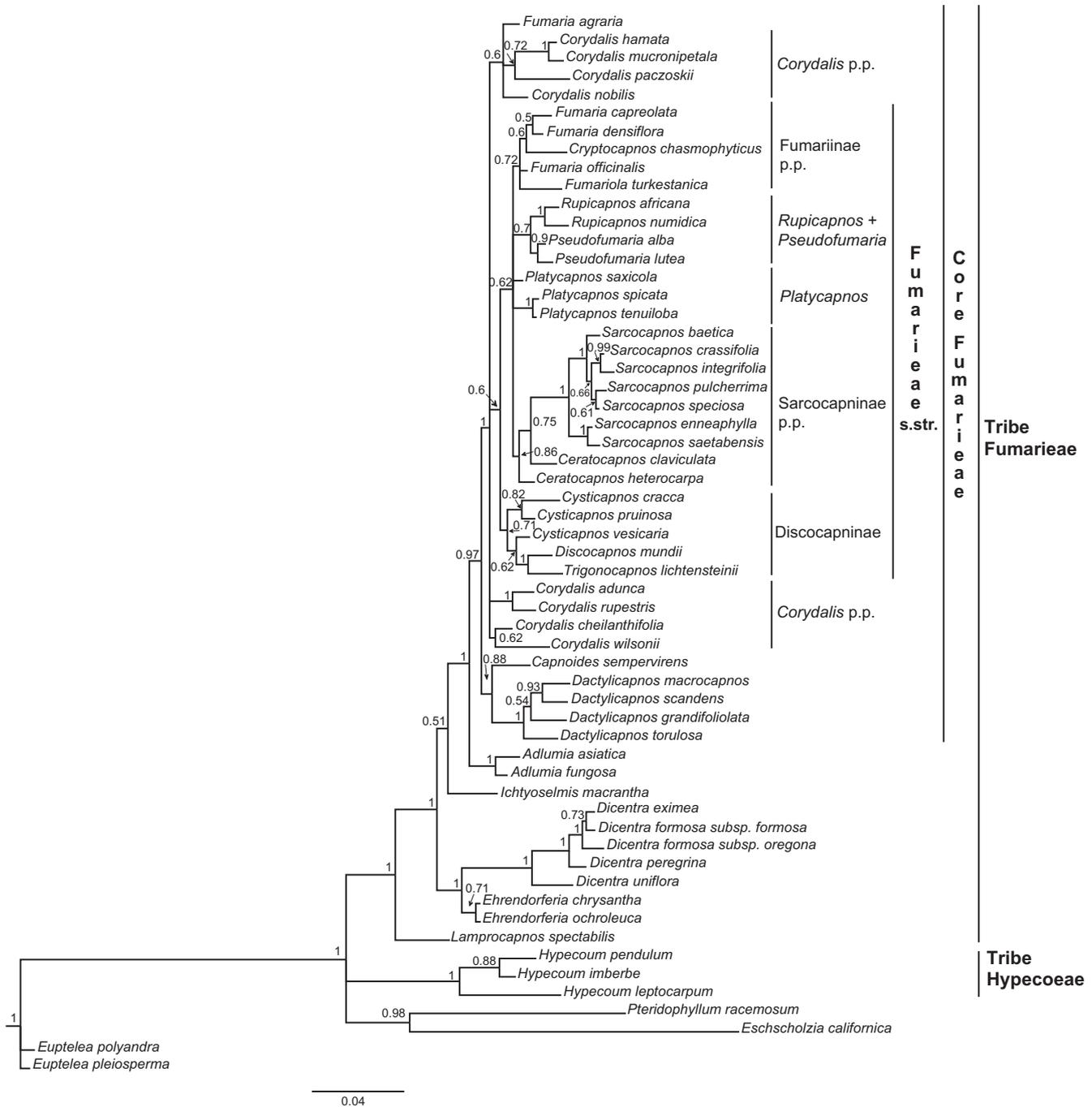


Fig. 3. Bayesian 50% majority-rule consensus tree inferred from the combined nuclear ribosomal datamatrix. Posterior probabilities are shown above branches. Classification and groupings are indicated on the right.

North America into eastern North America, at least eight dispersals between different regions of Asia (mainly from East Asia into other regions; without considering *Corydalis*), one from the Irano-Turanian region into South Africa, four dispersals between Mediterranean basin and other regions (Central Asia, Irano-Turanian, and North Europe), and multiple intra-Mediterranean dispersals.

3.2.1. Origin and initial diversification of Fumarioideae

Crown Fumarioideae (*Pteridophyllum* excluded) was dated during the transition between the Early and Late Cretaceous (96 Ma; Figs. 4a, S6, node 116). The origin of the Fumarioideae lineage was located in Asia, but with three alternative areas estimated as

possible: East Asia, East Asia + Central Asia, and East Asia + Central Asia + Irano-Turanian region. All three geographical alternatives are weakly supported; always being East Asia the ancestral area assigned the highest relative probability (Fig. 4a). The most probable origin of both Fumarioideae tribes was estimated in East Asia for Fumarioideae and Central Asia for Hypecoaceae (Fig. 4a, node 116). The latter implies the dispersion of the Fumarioideae ancestor from eastern to Central Asia followed by the split of its area into two in the second half of the Early Cretaceous, and resulting in the vicariance detected (Fig. 4a). Before the diversification of *Hypecoum* during the Eocene a range expansion into the Irano-Turanian and Mediterranean regions occurred in the stem lineage of the genus in the Late Cretaceous–Eocene (96–44 Ma; Fig. 4a;

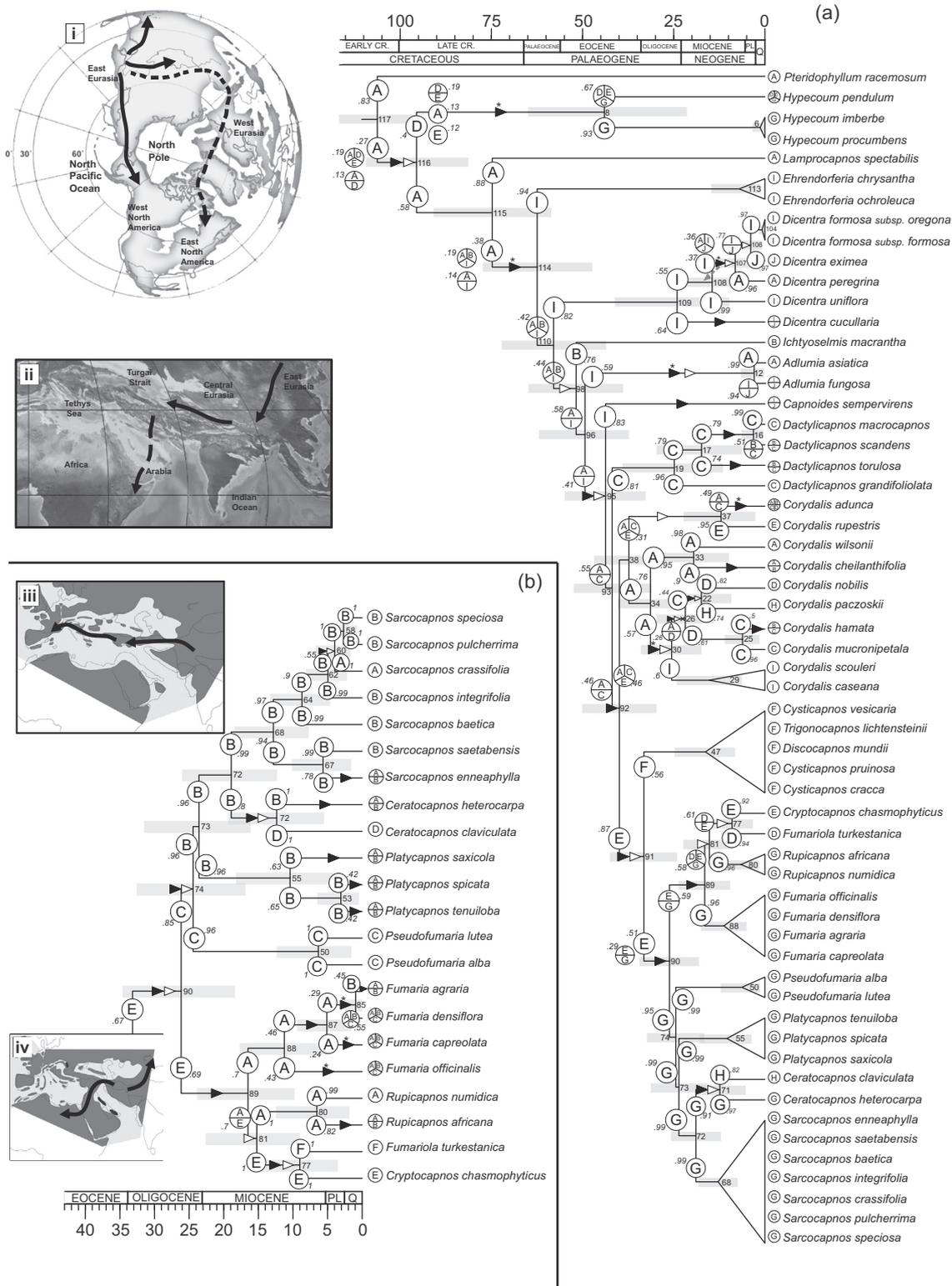


Fig. 4. Spatio-temporal reconstruction of subfamily Fumarioideae. Figure shows the maximum clade credibility tree (MCCT) from the BEAST analysis with the results of the ancestral-area reconstruction from the Lagrange analyses constrained for dispersal between areas over time for the whole subfamily (a), and for the partial analysis focused on the Mediterranean taxa of Fumarioideae s.str. (b). The outgroup species were pruned from the MCCT. Grey bars indicate the 95% highest posterior density of the divergence-time estimation. The current distribution areas of the species are shown beside each one, while the reconstructed areas are shown on the vertical branches linking the stem lineages [alternative areas for ambiguous nodes (117, 116, 115, 108, 91) are also shown]; both appear within circles. Clades with equally distributed species were condensed in the tree. The nodes are numbered according to the numbering generated by Lagrange. Values in italics represent the relative probability values for each reconstructed area. Each black arrowhead illustrates a dispersal event; a black arrowhead with an asterisk represents two or more dispersal events by the same lineage. White arrowheads illustrate vicariance events. Extinction events are represented by a black cross. The main dispersion events are illustrated on palaeo-maps to the left of the MCCT. Maps reflect: (i) the main Northern Hemisphere land connections during Late Cretaceous and Palaeocene, (ii) Eurasia and Africa continents during Eocene, (iii) Mediterranean during late Oligocene, and (iv) Mediterranean during Miocene. Maps were partially modified from Brikiatis (2014) and Rögl (1999) with permission from Wiley Online Library editorial and from Geologica Carpathica journal, respectively, and from the Global Palaeogeography Library (cpgeosystems.com/paleomaps.html).

Table 2

Biogeographical links between different regions as shown by the dispersal events that occurred during Fumarioideae evolution. Node labels correspond to Fig. 4; when superscript they refer to Fig. 4b. Direction reflects the path of the dispersal. Temporal range considers the mean age value between nodes for each stem lineage, as shown in Fig. S6. Biogeographical areas: CA, Central Asia; EA, East Asia; EM, eastern Mediterranean; ENA, eastern North America; Hi, Himalayas; IC, Indochina; IT, Irano-Turanian region; M, Mediterranean basin; NAF, North Africa; NE, Central and North Europe; SAF, South Africa; WM, western Mediterranean; WNA, western North America.

Biogeographical connection	Node label	Stem lineage	Direction	Time range
East Asia/North America	115–114	<i>Ehrendorferia</i> + rest tribe Fumarieae	EA > WNA	Late Cretaceous–Palaeocene (74.76–62.44 Ma)
	96–12	<i>Adlumia</i>	EA > WNA	Cenozoic (49.33–3.06 Ma)
	34–30	<i>Corydalis</i> sect. <i>Archaeocapnos</i>	WNA > EA	Eocene–Oligocene (31.37–25.56 Ma)
	108–107 (or 109–108)	<i>Dicentra peregrina</i> (or <i>D. uniflora</i>) + rest <i>Dicentra</i>	WNA > EA	Miocene (14.46 Ma–8.14 Ma) [or Oligocene–Miocene (24.07–14.46 Ma)]
Western North America/Eastern North America	96–12	<i>Adlumia</i>	WNA > ENA	Cenozoic (49.33–3.06 Ma)
	95	<i>Capnoides</i>	WNA > ENA	Cenozoic (43.69 Ma onwards)
	108–107 (or 109–108)	<i>Dicentra peregrina</i> (or <i>D. uniflora</i>) + rest <i>Dicentra</i>	WNA > ENA	Miocene (14.46 Ma–8.14 Ma) [or Oligocene–Miocene (24.07–14.46 Ma)]
	109	<i>Dicentra cucullaria</i>	WNA > ENA	Neogene (24.07 Ma onwards)
Intra-Asiatic	117–116	<i>Hypocoum</i> and Fumarieae	EA > CA	Middle Cretaceous (106.27–95.52 Ma)
	116–8	<i>Hypocoum</i>	CA > IT	Cretaceous–Palaeogene (95.52–44.02 Ma)
	115–114	<i>Ehrendorferia</i> + rest tribe Fumarieae	EA > IC	Late Cretaceous–Palaeocene (74.76–62.44 Ma)
	96–95	Core Fumarieae	EA > Hi	Middle Eocene (43.69–41.89 Ma)
	93–92	<i>Corydalis</i> + Fumarieae s.str.	Hi > IT	Middle Eocene (41.89–39.91 Ma)
	17–16	<i>Dactylicapnos macrocapnos</i> + <i>D. scandens</i>	Hi > IC	Miocene (17.37–3.13 Ma)
	17	<i>Dactylicapnos torulosa</i>	Hi > IC	Neogene (17.37 Ma onwards)
South African	37–36 ^a	<i>Cryptocapnos</i> – <i>Fumariola</i> group	IT > CA	Miocene (15.22–9.01 Ma)
	92–91	Fumarieae s.str.	IT > SAF	Eocene (39.91–33.17 Ma)
Mediterranean	116–8	<i>Hypocoum</i>	CA > M	Cretaceous–Palaeogene (95.52–44.02 Ma)
	91–90 ^a	Fumariinae + Sarcocapninae	IT > EM	Oligocene (33.17–26.15 Ma)
	90–74 ^a	Sarcocapninae	EM > WM	Oligocene (26.15–24.42 Ma)
	90–89 ^a	Fumariinae	IT > NAF	Oligocene–Miocene (26.15–16.5 Ma)
	72–71	<i>Ceratocapnos</i>	M > NE	Miocene (18.91–12.35 Ma)

Table 2). The crown group of the tribe Fumarieae dates back to 75 Ma in the Late Cretaceous (Fig. 4a, node 115).

3.2.2. Basal lineages of Fumarieae

Origins of the basal lineages occurred in a progressive way within a time window of around 26 Ma (75–49 Ma; Figs. 4a, S6, nodes 115–96). After the origin of the *Lamprocapnos* lineage, the ancestor of the remainder of Fumarieae expanded its range in two ways between 75 and 62 Ma (Fig. 4a, node 114; Table 2): to Indochina, and into western North America. It was in western North America where the ancestors of *Ehrendorferia* and *Dicentra* originated. The origin of *Ichtyoselmis* lineage was in Indochina 52 Ma after a split between this region and the East Asia/western North America area (Fig. 4a, node 98). The stem lineage of *Adlumia* was dated back 49 Ma in western North America (Figs. 4a, S6, node 96). *Dicentra* began its diversification in western North America during the Oligocene–Miocene transition (24 Ma). Two intra-continental (from western into eastern North America) and one inter-continental (from western North America into East Asia) dispersal events were detected in *Dicentra* (Fig. 4a; Table 2). Two range expansions toward eastern North America and East Asia were also detected in the stem lineage of *Adlumia* from western North America (Fig. 4a; Table 2). An interruption of the ancestral area of *Adlumia* between East Asia and North America was responsible of the diversification of this genus around 3 Ma (Fig. 4a).

3.2.3. Core Fumarieae

The ancestor of Core Fumarieae should occupy a wide area including East Asia and western North America (Fig. 4a, node 95). Diversification began in the Eocene (44 Ma; Figs. 4a, S6), resulting in the split of *Capnoides* lineage in western North America (after that, the stem lineage of this species dispersed into eastern North America; Fig. 4a; Table 2) and the ancestor of the remainder lineages occupying East Asia and Himalayas. Presence in the Himalayas of this latter implies a previous range expansion of the ancestor of

Fumarieae from East Asia (Fig. 4a, node 95; Table 2). After this initial split, the remaining main Fumarieae lineages appeared in a very short time window, around 4 million years in the middle Eocene (44–40 Ma; Figs. 4a, S6, nodes 95–92). Close to this period *Corydalis* also diversified (37 Ma; Figs. 4a, S6, node 38). The crown of the genus *Dactylicapnos* originated in the Himalayas 25 Ma ago (Fig. 4a, node 19); within this genus two independent range expansions into Indochina were detected (Fig. 4a; Table 2). The reconstructed ancestral areas for both the stem lineage of *Corydalis* (East Asia/Himalayas/Irano-Turanian) and Fumarieae s.str. (Irano-Turanian region; Fig. 4a, node 92) show the westward range expansion into the Irano-Turanian region of their most recent common ancestor (MRCA) (Fig. 4a, node 92; Table 2). After the diversification of *Corydalis* subgenus *Cremoncapnos*, the split between subgenera *Sophorocapnos* and *Corydalis* occurred 31 Ma ago; the crown group of subgenus *Corydalis* was dated back to 26 Ma. Within the genus *Corydalis*, different dispersal events have been detected, from East Asia into Indochina and into Central Asia, and from Central Asia into Northern Europe and into Himalayas, but highlights a dispersion of the ancestor of subgenus *Corydalis* from East Asia into western North America during the Oligocene (Figs. 4a, S6, nodes 34–30; Table 2), explaining the presence there of the section *Archaeocapnos*.

The ancestor of Fumarieae s.str. expanded its area from the Irano-Turanian region towards South Africa and also towards the Mediterranean basin (Fig. 4a, nodes 91 and 90). Split between the Irano-Turanian/Mediterranean and the South African lineages was dated at 33 Ma (Eocene–Oligocene transition; Figs. 4a, S6, node 91); while the diversification of the South African group (crown *Discocapninae*) took place approximately 17 million years afterwards, in the middle Miocene (Figs. 4a, S6, node 47).

3.2.4. Mediterranean Fumarieae

Ancestor of Mediterranean Fumarieae was distributed in the Irano-Turanian region during Oligocene (Fig. 4b). Split resulting in the Fumariinae and Sarcocapninae lineages was dated to 26 Ma ago (Figs. 4b, S6, node 90). While Fumariinae lineage inherited the

area of the ancestor, Sarcocapninae originated in the eastern Mediterranean, involving a previous westward expansion from the Irano-Turanian region through the north of the Mediterranean basin between 33–26 Ma (Fig. 4b, nodes 91–90; Table 2). From here, this latter ancestor reached the western Mediterranean basin in less than 2 million years (Figs. 4b, S6, nodes 90–74; Table 2); afterwards, the western Mediterranean lineages and the eastern Mediterranean lineage (stem lineage of *Pseudofumaria*) split by vicariance (Fig. 4b, node 74). The ancestor of *Platycapnos* also originated at the end of Oligocene (c. 24 Ma; Figs. 4b, S6, node 73), and the genus diversified in the western Mediterranean in the Miocene (10 Ma). According our results, three dispersal events into North Africa had to occur in *Platycapnos* (each *Platycapnos* species). The split between *Sarcocapnos* and *Ceratocapnos* lineages was dated 19 Ma during Miocene in western Mediterranean. Crown *Sarcocapnos* was dated around 13 Ma in the western Mediterranean and two independent range expansions into North Africa happened within this genus. Diversification of *Ceratocapnos* was by vicariance, after the range expansion of the ancestor into Northern Europe between 19 and 12 Ma (Figs. 4b, S6, nodes 72–71); a dispersal from the western Mediterranean into North Africa occurred in the stem lineage of *C. heterocarpa*.

Conversely to Sarcocapninae ancestor, that of Fumariinae colonized the Mediterranean basin from Irano-Turanian region by expansion westward through North Africa (26–17 Ma, Oligocene–Miocene; Figs. 4b, S6, nodes 90–89; Table 2). *Fumaria* diversified in North Africa during the Miocene (around 11 Ma ago), from where it expanded its range towards other Mediterranean areas (Fig. 4b, node 88). Lineage of chasmophytes originated in North Africa and Irano-Turanian region, and diversified 15 Ma by vicariance, resulting in the ancestor of *Rupicapnos* in North Africa and the ancestor of *Cryptocapnos* and *Fumariola* in the Irano-Turanian region (Fig. 4b, node 81). This latter expanded its range by dispersal into Central Asia (between 15 and 9 Ma) and originated the ancestors of *Cryptocapnos* and *Fumariola* by vicariance (Fig. 4b, node 77).

4. Discussion

Here, we present a phylogenetic analysis of the subfamily Fumarioideae *sensu* Wang et al. (2009), including all its genera; until now the most complete molecular phylogeny made for this plant group. Results found with chloroplast and nuclear markers were highly incongruent with regard to the relationships within Core Fumarieae, while they were almost congruent in the Fumarioideae basal lineages. Incongruence between chloroplast and nuclear Papaveraceae trees was also detected in Hoot et al. (1997). These cases of incongruence may come from both the low resolution of the 26S gene [few informative characters, 139 from 1762, and high homoplasy (CI: 0.56, RI: 0.69, RC: 0.39 for independent analysis)], and from the high saturation degree of the ITS region [133 characters variable from 336 used (115 excluded as ambiguous); CI: 0.56, RI: 0.66, RC: 0.37 for independent analysis] (Table 1). Therefore, the discussion presented below focuses mainly on the chloroplast analysis.

Our results enable us to evaluate the generic boundaries and to clarify the relationships among Fumarioideae genera. The phylogeny has provided a basis for a detailed biogeographical analysis of this widely distributed subfamily with noteworthy inter-continental disjunctions.

4.1. Relationships of *Pteridophyllum* with the rest of Fumarioideae

Pteridophyllum is a monotypic genus endemic from Japan and morphologically well differentiated, but with uncertain phylogenetic affinities within Papaveraceae. It has been considered (using

morphological and molecular markers) to be an independent lineage occupying the most basal position within Papaveraceae (Kadereit et al., 1994, 1995; Hoot and Crane, 1995; Hoot et al., 1997). However, when only molecular markers are used, this position is always poorly supported and thus unresolved (Hoot et al., 1997). The molecular phylogeny of Ranunculales also resulted in an inconclusive position of *Pteridophyllum* within Papaveraceae (Wang et al., 2009). Nevertheless, combining molecular data with morphology, these authors found a different position of *Pteridophyllum* with respect to that reported by Hoot et al. (1997), with *Pteridophyllum* as sister group to *Hypecoum* and related to Fumarioideae (Wang et al., 2009). Accordingly, Wang et al. (2009) proposed the inclusion of *Pteridophyllum* in the subfamily Fumarioideae.

Our molecular analysis was also inconclusive with regard to *Pteridophyllum* position. Chloroplast phylogeny relate *Pteridophyllum* to Fumarioideae (as a sister group of *Hypecoum* in the parsimony analysis), but without statistical support; while nuclear phylogeny relate it to Papaveroideae (as sister group of *Eschscholzia californica*) in the Bayesian analysis (Pp: 0.98), and to Fumarioideae (without BS support) in the parsimony analysis (Fig. S4). Both the *Pteridophyllum* and *Hypecoum* sequences showed a large number of changes with regard to the rest of taxa, resulting in long branch lengths on the trees; especially for chloroplast and ITS markers, much more variable than gene 26S (e.g. n° of changes for external branch of the chloroplast tree: *Pteridophyllum* = 302, *Hypecoum* = 249, *Lamprocapnos* = 241, *Ehrendorferia* = 97, *Dicentra* = 44, *Ichtyoselmis* = 43, *Adlumia* = 13, Core Fumarieae = 44). Long-branch attraction is a phenomenon affecting the topologies from phylogenetic inferences (Sanderson et al., 2000), especially when the parsimony criterion is used (Swofford et al., 2001). This may be responsible of the ambiguity in the *Pteridophyllum* position.

According to our findings, *Pteridophyllum* should be not considered as belonging to Fumarioideae until its position is resolved. We base this on the inconclusive position of *Pteridophyllum* in the molecular phylogenies, and that the inclusion of *Pteridophyllum* in Fumarioideae by Wang et al. (2009) is based only on two morphological characters, the interpretation of one of them (exine sculpturing of pollen grain) being wrong for Fumarioideae representatives [spinose exine according to Wang et al. (2009), when it is actually a non-spinose exine (Kalis, 1979; Blackmore et al., 1995; Pérez-Gutiérrez et al., unpubl. res.)]. More work is needed, including a much broader sampling of Papaveroideae and more definitive markers at this level, to elucidate the relationship of *Pteridophyllum* with the rest of Papaveraceae lineages. From here on, we will refer to the subfamily Fumarioideae excluding *Pteridophyllum*.

4.2. Phylogenetic relationships within subfamily Fumarioideae

Fumarioideae shown as a strongly supported natural group with two well-defined lineages, *Hypecoum* and the rest of Fumarioideae (Figs. 1 and 2; tribes Hypecoae and Fumarieae; Stevens, 2001 onwards).

Within Fumarieae, we have identified a grade of basal lineages with all taxa having bisymmetric and two-spurred flowers, and a group of genera with asymmetric and one-spurred flowers (except *Dactylicapnos*, bisymmetric and two-spurred flowers) that we term Core Fumarieae. The order of diversification of basal lineages largely coincides with that reported by Lidén et al. (1997) using the chloroplast gene *rps16* intron, with the exception of *Ichtyoselmis*. In that paper *Ichtyoselmis* grouped with *Dicentra*, while our results show *Ichtyoselmis* as diverging after *Dicentra* divergence (Figs. 2 and 3). The result obtained by Lidén et al. (1997) is explained by the use of an erroneous *Ichtyoselmis* sequence corresponding to

one species of *Dicentra* (Lidén, personal communication). Our results support the split of *Dicentra* (in its traditional concept) in *Lamprocapnos*, *Ehrendorferia*, *Dicentra* s.str., and *Ichtyoselmis* as proposed by Lidén et al. (1997).

One of the major controversies regarding intergeneric relationships within the Core Fumariaceae is the position of the North American *Capnoides sempervirens* (L.) Borckh. (zygomorphic and one-spurred flowers, unlike other species of tribe Corydaleae sensu Lidén, 1986, 1993a). Its position was not resolved in Lidén et al. (1997). Moreover, in the latter study the position of *Dactylicapnos* (bisymmetric and two-spurred flowers) was weakly supported as sister clade of *Corydalis*. All of the above has prevented the unambiguous reconstruction of the flower symmetry (and evolution of the genes involved) in the subfamily and of the other morphological characters (e.g. Lidén et al., 1997; Fukuhara, 1999; Damerval and Nadot, 2007; Damerval et al., 2007). Our results show a totally resolved phylogeny, offering a basis for morphological character reconstructions, with *Capnoides* as the basal lineage within Core Fumariaceae, followed by *Dactylicapnos*, and *Corydalis* (with three groups reflecting its subgeneric classification, Lidén et al., 1997) as the sister group to Fumariaceae s.str. (Fig. 2).

Within Core Fumariaceae, Fumariaceae s.str. forms a strongly supported clade, in which the same groupings as in Pérez-Gutiérrez et al. (2012) were recovered [see this study for a detailed discussion of this group (considered as tribe Fumariaceae)], the South African clade being the early divergent lineage. Unlike that of Pérez-Gutiérrez et al. (2012), our study includes the monotypic genus *Fumariola* (from Turkestan), which was included in the subtribe Fumariinae and sister to the monotypic *Cryptocapnos* (from south-central Afghanistan); however, as in the aforementioned study the relationships between *Fumaria*–*Rupicapnos*–*Cryptocapnos* + *Fumariola* were poorly resolved. According to Lidén (personal communication), this low resolution within Fumariinae might be due to possible reticulations (involving allopolyploidy) among these genera.

4.3. The origin of Fumarioideae and ancestral connections East Asia–North America–Indochina

Fumarioideae originated at the end of the Early Cretaceous in East Asia (Figs. 4a, S6), and the crown group began to diversify 96 Ma at the boundary between Early and Late Cretaceous. In our analyses East Asia + Central Asia + Irano-Turanian was an alternative to the ancestral range of the subfamily; however this wider region is meaningless because during the Late Cretaceous the Irano-Turanian region did not exist, but its origin dates from the Eocene onwards (Yin, 2010; Bosboom et al., 2011). An ancestral region formed by only East Asia + Central Asia was a much less probable alternative in our analyses (Fig. 4a; Table S7). Our results almost coincide with those of previous dating studies including the subfamily (Wikström et al., 2001; Anderson et al., 2005; Wang et al., 2012). The estimated ancestral geographical distribution agrees with the Asiatic origin of Fumarioideae proposed by Lidén (1986) and Dahl (1990). Climatic conditions proposed for East Asia during the Cretaceous involves a stable humid and warm climate from the Albian onwards (Hsü, 1983; Clarke and Jenkyns, 1999; Hasegawa, 2003; Morley, 2011), which favoured the development of megathermal vegetation (Golozoubov et al., 1999; Morley, 2011) and suggests the subfamily ancestor could occupy a forest-floor habitat. All basal lineages (except the tribe Hypecoae and *Ehrendorferia*, both growing in arid and open habitats) and *Capnoides*, *Dactylicapnos* and many *Corydalis* show forest-floor habitat (almost all taxa from Fumariaceae s.str. occupy arid and open habitat). Our results support the proposal by Kadereit et al. (1995; based on a phylogenetic analysis of morphological characters) that forest-floor habitat is basal for the whole Papaveraceae

family, and that transition from forest floor into open arid habitats took place a few times within Fumarioideae.

The diversification of the basal lineages of the subfamily occurs progressively within a large time frame (47 million years between the split of *Hypecoum*, node 116, and that of *Adlumia*, node 96; 26 million years for the tribe Fumariaceae, node 115; Fig. 4a). Similar origin patterns with slow initial diversification in the Late Cretaceous were identified also for sapindaceous lineages in South-east Asia (Buerki et al., 2013); and Mao et al. (2010) found a slow diversification within *Juniperus* L. during the climate-stable Oligocene. The latter authors propounded that diversification in *Juniperus* was suppressed by long periods of stable climate, as described for other Tertiary flora (Milne and Abbot, 2002). The Late Cretaceous is the best-known period of warm and equable climate during the Phanerozoic (e.g. Frakes et al., 1992), which could have discouraged a rapid early diversification in Fumarioideae. However, more studies are showing that Late Cretaceous climate was not as stable, having documented even short-term glaciation events (e.g. Barrera and Savin, 1999; Miller et al., 1999). This climatic variability was more pronounced in northern latitudes such as in the area of the Arctic Ocean (cf. Brikiatis, 2014), where the tribe Fumariaceae ancestor arrived on its way to North America. Together with the possible absence of climate stability, it also highlights the existence of long branch lengths for all subfamily basal lineages, leading to monotypic or few-species genera with crown groups dating very late (Miocene or Pliocene), suggesting an important role for extinction in the evolutionary history of the basal lineages of Fumarioideae.

Three main dispersal events occurred in the basal lineages of the subfamily (Fig. 4a). Firstly, the ancestor of the subfamily expanded its range towards Central Asia during the Early Cretaceous–Late Cretaceous transition, before the vicariance caused the split of the lineage of the tribe Hypecoae. Secondly, the MRCA of *Ehrendorferia* and remainder tribe Fumariaceae expanded its range northwards into western North America and also southwards into Indochina. These two latter dispersals would have occurred between Late Cretaceous (end of the Campanian) and the early Palaeocene (end of the Danian) (Fig. 4a).

Range expansions of the Fumarioideae ancestor could have been promoted by the global warming trends during Late Cretaceous, from the Albian to the Turonian, and the Palaeocene, from the end of the Maastrichtian (or beginning of the Palaeocene) to the Palaeocene–Eocene boundary or early Eocene. The westward and northward dispersal routes we suggested for Fumarioideae coincide with those proposed for the warm temperate Tsagayan flora that developed on the continental boundary of the western Pacific (Amur region and northern China). Akhmetiev and Beniamovski (2009), and Akhmetiev (2010) proposed that the global regression at the Maastrichtian–Danian transition provoked the desiccation of the Northern Central Eurasian epicontinental seas and straits, favouring the migration of this flora in a westward way during the Late Cretaceous–Palaeocene boundary. That migration occurred both along mid-latitude from the Amur River to south-eastern Kazakhstan, and at high latitudes along the northern boundary of the West Siberian plate to reach in the Danian the northern and middle Urals as well as the Arctic region. Moiseeva et al. (2009) showed the relationship between the Late Sagwon Flora of the northern slope of Alaska and the Tsagayan Flora, and suggested a northward migration of the latter to high latitudes of the Arctic Pacific via the Bering Land Bridge due to the progressively warming climate of the Palaeocene.

Given that the lineage of the tribe Hypecoae originated in the early Late Cretaceous in Central Asia (Fig. 4a), its westward migration should have been affected by the Late Cretaceous warming trend. Crown *Hypecoum* was dated at around 52 million years after the split of its stem lineage. *Hypecoum* grows in arid and open

habitats, so the transition from forest floor to the *Hypecoum* habitat had to occur during its long stem lineage. Ecology of *Hypecoum* agrees with the Palaeogene climatic environment in Central Asia, with a zonal climate pattern dominated by desert and steppe conditions, since a large expanse of the arid/semi-arid region was distributed between 20°N and 40°N palaeolatitude from west to east (Guo et al., 2008; Zhang et al., 2012). To the north of this region, a Mediterranean-like climate was situated in Central Asia (Zhang et al., 2012). Therefore, before the diversification of *Hypecoum* (44 Ma), Mediterranean and arid/semi-arid climates existed in Central Asia, fostering the adaptation of *Hypecoum* ancestor to these conditions. Our date for the *Hypecoum* ancestor coincides with the Lutetian (middle Eocene), when sclerophyllous flora distributed from northern Ukraine to eastern Kazakhstan (Akhmetiev, 2010). Recent biogeographical analyses of *Ruta* (Salvo et al., 2010) and *Haplophyllum* (Manafzadeh et al., 2014) proposed a Central Asia origin and initial diversification for these genera during the Eocene, and subsequent colonization of the Mediterranean during the Miocene. Our results show the Mediterranean arrival of *Hypecoum* in the middle Eocene but in view of the Mediterranean palaeogeography (Rögl, 1999), this arrival was probably delayed until Oligocene–Miocene boundary, as described for the Rutaceae genera. A more detailed study on *Hypecoum*, including more species, is necessary to decipher its Mediterranean colonization and diversification.

With regard to the North America dispersion of the Fumarioideae ancestor, three high-latitude land bridges connected Eurasia and North America during Late Cretaceous and Palaeocene (cf. Brikiatis, 2014): Beringia, connecting East Asia and western North America intermittently (100–c. 75 Ma, c. 65.5 Ma, 58 Ma); De Geer, connecting north-eastern North America–Greenland and Fennoscandia (71–63 Ma); and Thulean, connecting North America and Europe via Greenland (c. 56.8 Ma). If the dispersal is dated to between the end of Late Cretaceous and the early Palaeocene (75–62 Ma; Figs. 4a, S6, nodes 115–114; Table 2), both the De Geer and Beringia routes could be possible. During this time period, climatic conditions favoured the biotic exchange between Eurasia and North America in two time windows, c. 69 Ma and 65.5 Ma (cf. Brikiatis, 2014). While the De Geer route functioned in both time windows, Beringia did so only during the 65.5 Ma window. Connection between East Asia and North America through De Geer route was facilitated by the global regression at the Maastrichtian–Danian transition and the interruption of the Turgai strait (epicontinental sea from the Arctic Ocean to the Tethys Seaway, in existence from the Middle Jurassic until the early Oligocene; Tiffney, 1985; cf. Brikiatis, 2014). However, De Geer route connected East Asia and north-eastern North America, in disagreement with our results, since the North American ancestral area for Fumarioideae was western North America, supporting a Beringia route (Fig. 4a, nodes 115–114). During Late Cretaceous the Western Interior Sea divided North America into western and eastern regions (extending from the Gulf of Mexico through the western interior lowlands to the Arctic Ocean; Kauffman, 1984), preventing dispersal between the two (cf. Graham, 1993). The regression of the Western Interior Sea began at the end of the Cretaceous (cf. Graham, 1993), and then a passage from north-eastern to western North America could have existed during the early Palaeocene; and therefore De Geer route cannot be ruled out for the arrival of Fumarioideae to North America.

Western North America was a diversification and dispersal centre of the basal lineages of tribe Fumarieae (Fig. 4a). All North American genera diversified in the West, from where secondary dispersal into eastern North America and into East Asia took place (Table 2). It is noteworthy that diversification of these genera occurred long after the split of their stem lineages (Fig. 4a). These lineages were affected by climatic factors from the Eocene

onwards (cf. Graham, 1993), as the Palaeocene–Eocene Thermal Maximum, the abrupt cooling near the Eocene–Oligocene boundary (c. 33.7 Ma), the onset of a drier climate from the Miocene, or the Pleistocene glaciations, which could have led to extinction events along these lineages. The *Dicentra* crown group was dated to the Oligocene–Miocene transition, when drier climates and colder winters were established. As a consequence, North American megathermal vegetation shifted southwards and began its decline, while mesothermal broad-leaved temperate deciduous vegetation expanded at mid-latitudes (cf. Graham, 1993). The area of the southern Rocky Mountains and the Sierra Madre Occidental has been considered an important centre for the evolution of the Madro-Tertiary geoflora (Axelrod, 1958), and it could be the place where *Dicentra* diversified. Two independent eastward dispersals occurred in *Dicentra*, one of the stem lineage of *D. cucullaria* (L.) Bernh., and the other occurred during the Miocene; however the ambiguity in the ancestral area of the node 108 prevents ascertain whether the latter was by the MRCA of *D. formosa*, *D. eximea*, and *D. peregrina*, or by the MRCA of *D. uniflora* and the rest (Fig. 4a). One of these last two ancestors also expanded its range into East Asia by the Bering land bridge, the only active land bridge at that time (cf. Brikiatis, 2014). Crown *Ehrendorferia* was dated in the Miocene–Pliocene boundary. This genus is endemic to California (USA), reaching *E. chrysantha* (Hook. & Arn.) Rylander Baja California (Mexico). It grows in open and arid habitats from 15 to 2200 m (Stern, 1997), in agreement with the general cooling that occurred from the late Miocene and the drier conditions, especially at the southern end of the Rocky Mountains, where a sclerophyllous vegetation occurred. Finally, *Adlumia* is a classical example of East Asia/eastern North America disjunction (Kadereit et al., 1995). Our results show western North America as the ancestral area for the stem lineage, which expanded its range into East Asia and into eastern North America. The split between *Adlumia* species was dated to the end of the Pliocene (3.06 Ma), shortly after Bering land-bridge disruption (3.5 Ma; cf. Sanmartín et al., 2001). Currently *Adlumia fungosa* shows a very narrow distribution in western North America suggesting a past wider distribution which could have shrunk during the Pleistocene climatic oscillations; the effect of this climatic oscillations were milder in the eastern North America (Soltis et al., 2006), since *A. fungosa* is widely distributed.

In the same time period of the initial dispersion into western North America (second half of the Late Cretaceous and beginning of Palaeocene; Fig. 4a, nodes 115–114; Table 2) the Fumarieae lineage arrived to Indochina. South-East Asia has played important roles in the angiosperm evolution, representing a dispersal route between Northern and Southern Hemispheres during periods of climate change, and a refugium for tropical lineages (Buerki et al., 2014). Thus the climatic instability at the end of the Cretaceous, when the Northern Hemisphere humid environments suffered a significant decline, could promote the southward migration of the MRCA of *Ehrendorferia* and remainder tribe Fumarieae. Between 58 and 52 Ma (late Palaeocene–early Eocene) the continuous area occupied by the ancestor of *Dicentra* and the rest of Fumarieae is fragmented (western North America + East Asia/Indochina) leading to a vicariance and the origin of *Ichtyoselmis* lineage 52 Ma (Figs. 4a, S6). Split of *Ichtyoselmis* lineage coincides with the instauration of the zonal arid/semi-arid climate from western to eastern China in the early Eocene epoch (palaeolatitude 20°N–40°N), caused by the subtropical high-pressure belt in the Northern Hemisphere (Zhang et al., 2012). *Ichtyoselmis* inhabit the mountainous area in northern Myanmar and west part of south-eastern China, under humid conditions during the Palaeogene (Guo et al., 2008), and considered as refuge for several plants lineages (cf. Wen et al., 2014). It grows at middle and high elevations (1500–2000 m) under woods on humid well-drained

soils (Zhang et al., 2008). Its distribution and ecology, together it is a monotypic genus, suggest that this plant as a palaeoendemism.

4.4. Core Fumariaceae: Eocene Himalayas diversification and westward expansion

The ancestor of Core Fumariaceae expanded from East Asia into the Himalayas before the middle Eocene (Fig. 4a, nodes 96–95). Core Fumariaceae diversification began 44 Ma with the origin of the *Capnoides* lineage in western North America by vicariance (Fig. 4a, node 95). From this moment onwards acceleration occurred in the diversification rate, resulting in the origin of its basal lineages (*Capnoides*, *Dactylicapnos*, *Corydalis*) and the ancestor of Fumariaceae s.str. in only four million years (44–40 Ma; Figs. 4a, S6). Moreover, crown *Corydalis* was dated to only somewhat less three million years after the split of its stem lineage from Fumariaceae s.str. lineage; however, this latter did not begin diversification until seven million years afterwards in the Irano-Turanian region. The geography and time frame for these fast diversification events suggest that they might be related to the geological events that occurred in the Himalayan region during the late Eocene. The uplifts of the Qinghai-Tibetan Plateau (QTP) together the Palaeogene climate pattern dominated by desert and steppe climates have triggered and facilitated plant speciation and diversification (cf. Wen et al., 2014). According to our results these events also could have governed the diversification of the basal lineages of the Core Fumariaceae, and its westward range expansion.

During the Eocene, a strong fluctuation of climate conditions in North America and Asia has been documented (Woodburne et al., 2009; Zhang et al., 2012). Climatic deterioration (Bridgerian Crash) after the Early Eocene Climatic Optimum (EEO) from 50 to 47 Ma, resulted in a strong retreat from tropical climates to the increased seasonality and aridity. This could be a stimulus for southward migration of the Core Fumariaceae ancestor, responsible for the vicariance into Asian and western North American lineages and the arrival of the Asian lineage to the Himalayan region (Fig. 4a). A similar vicariance pattern across Beringia as result of the global cooling during Eocene was proposed by Chin et al. (2014) to explain the diversification of the North American and eastern Asian lineages of *Prunus* subgenus *Prunus*. North–South plant migrations into the Himalayan region through East Asia have been shown, since close biogeographical relationships can be found between the Hengduan–Himalayan forest regions and the Arcto-Tertiary floristic elements and relict taxa in eastern Asia and North America (cf. Wen et al., 2014).

The fast diversification of *Dactylicapnos* and *Corydalis* lineages (between the Himalayas and East Asia, 42 and 40 Ma), and the split of the basal subgenera of *Corydalis* (37 and 31 Ma) closely follow the end of the Indian–Asian collision in the easternmost part (41 Ma, Rowley, 1996) and then the first phase of the QTP uplift (Gangdese motion, 45–38 Ma). Palaeogeographic interpretations by Ding et al. (2014) of the Eocene Tibet suggest that relatively low elevation basin was sandwiched by two mountains in excess of 4500 m of Qiangtang to the north and Gangdese to the south.

Dactylicapnos originated and diversified in the Himalayas 25 Ma (Figs. 4a, S6, node 19), coinciding with the second uplift phase of Tibet, which occurred in the early Miocene (the Himalayan motion, 25–17 Ma; Shi et al., 1999); from where it expanded into Indochina. Diversification of many plant groups have been proposed as responses to the early Miocene uplift (cf. Zhang and Fritsch, 2010; cf. Wen et al., 2014); and several lineages originated on the QTP have been shown to have migrated into other regions (the out-of-QTP hypothesis), as in the case of Eurasia, Central Asia, and northern China (cf. Wen et al., 2014). *Dactylicapnos* represents a genus that originated on the QTP and that have migrated eastwards into South-east Asia. Our results show that this dispersal

route has been followed independently by different *Dactylicapnos* lineages from the mid-Miocene onwards (Fig. 4a). Most migrations of the QTP lineages have been related with the climatic oscillations that occurred from Pliocene (cf. Wen et al., 2014). This could also be the case of *Dactylicapnos*, which must have also undergone the possible influence of subsequent QTP uplift events.

Corydalis is a numerous (c. 465 species) and widely distributed genus, mainly through Northern Hemisphere, but especially well represented in China (357 species, 262 endemic; Zhang et al., 2008). Despite the bias in our biogeographical analysis with regard to *Corydalis* (see Materials and Methods), our results (considering the two analyses made to test the sampling bias; Fig. 4a; Table S8) suggest the Himalayas and a close part of East Asia as the ancestral area for diversification, which is coherent with that area being the most species-rich region; and also suggest that the section *Archaeocapnos* arrived to western North America during Oligocene (Figs. 4a, S6, nodes 34–30; Table 2), when Beringia was the only land bridge between Asia and North America. At least two more dispersion events into North America had to happen in *Corydalis*, one in subgenus *Corydalis* section *Dactylotuber*, and another in subgenus *Sophorocapnos*, section *Sophorocapnos*; these dispersals could not be evaluated in our analysis for lack of plant material. *Corydalis* is the only Fumariaceae genus for which the crown group is dated to the Palaeogene (Eocene). In terms of the subfamily basal lineages, only crown *Hypecoum* was dated to the Eocene, as other genera diversified during Neogene, and therefore all (including *Hypecoum*) showed very long branches for their stem lineages, implying a major role of extinction during their evolution. The high number of *Corydalis* species and its short stem lineage (somewhat less than 3 million years) suggest little impact of extinction on its evolution. In this sense, the high capacity of *Corydalis* species to adapt to very different habitats (forest, high mountain habitats, desert, rocky places, etc.; Zhang et al., 2008) may have prevented a high rate of extinction and favoured the great diversification and distribution of the genus. The extensive uplifts of the QTP have triggered rapid radiations in several plant groups (cf. Wen et al., 2014) and, according to our dating results, this seems also to be the case of *Corydalis*.

The MRCA of *Corydalis* and Fumariaceae s.str. arrived to the Irano-Turanian region during the late Eocene (42–40 Ma; Figs. 4a, S6, nodes 93–92; Table 2). Biogeographical connections between the floras of QTP and Central Asia, Minor Asia, or Mediterranean Eurasia have been reported for many plant lineages (cf. Wen et al., 2014); however, most of these connections are proposed to be post-Oligocene connections. Barres et al. (2013) proposed a late Eocene–early Oligocene expansion of the subtribe Carlininae ancestor (Asteraceae) from western Asia to Central Asia. Since in the early Eocene the Tethys Sea covered the western Asian portion of the Irano-Turanian region, the westward migration of the *Corydalis*-Fumariaceae s.str. ancestor should have occurred, coinciding with the vanishing of the Tethys Sea. By the end of the Eocene, the Tethys Sea had nearly disappeared as consequence of the collision of India with Eurasia, the Indian Ocean was born, and the western Tethys was reduced to the Mediterranean Sea (Rögl, 1999). When migration was dated (42–40 Ma) an elongated deep basin (from the Mediterranean eastwards) divided the Irano-Turanian region into two portions (western Asia and Central Asia), defining two possible migration routes from Himalayas, one to the north (from the North Caspian Sea) and one to the south (through the Iranian connection). Barres et al. (2013) proposed the northern route for the eastward migration of Carlininae. Considering the sea basin as a barrier for southward plant migrations (e.g. *Haplophyllum*; Manafzadeh et al., 2014), which was present at least until early Oligocene (Rögl, 1999), and that the South African dispersal from the Irano-Turanian region of the Fumariaceae s.str. ancestor had already happened in the early Oligocene (lineage of

Cysticapnos Clade originated in South Africa 33 Ma; Figs. 4a, S6), the most probable migration route from the Himalayas is the southern route. Therefore, the origin of the Fumariaceae s.str. lineage was probably the Iranian area. This result agrees with Pérez-Gutiérrez et al. (2012), which estimated the area for the Fumariaceae s.str. ancestor (tribe Fumariaceae in Pérez-Gutiérrez et al., 2012) to be a continuous area between western Eurasia and Africa.

During the late Eocene a vegetation belt extended along the southern areas of Eurasia and North America with sclerophyllous species adapted to warm temperate semi-arid habitats, the Madrean–Tethyan vegetation (Axelrod, 1975; Wen and Ickert-Bond, 2009). Kadereit et al. (1995) suggested that in Fumarioideae the transition from forest floor into arid and open habitats happened in Fumariaceae s.str. This transition could have occurred before the westward migration and may be related to the QTP uplift, since the high elevation of Gangdese Mountains may have contributed to the aridification of the inner plateau (Ding et al., 2014), probably promoting the adaptation of Fumariaceae s.str. ancestor to open dry habitats. In this sense, two of the three subgenera of *Corydalis* (subgenera *Cremoncapnos*, the earliest-divergent, and *Sophorocapnos*) are adapted to open-arid conditions, fact that might suggest the ancestor of this genus had already developed the adaptation to semi-arid habitats and that the subgenus *Corydalis* adapted during its evolution to woodlands ecologies.

4.5. The South African dispersal of Fumariaceae s.str

The beginning of the Fumariaceae s.str. diversification was around 33 Ma, with the split between Mediterranean groups (Fumariinae–Sarcocapninae subtribes) and South African ones (Discocapninae; Fig. 4a, node 91). The latter originated in South Africa after the disruption of the continuous area reached by the Fumariaceae s.str. ancestor after its dispersal from the Irano-Turanian region.

The dating methods in this study show that the time framework estimated does not coincide with our previous interpretation (Pérez-Gutiérrez et al., 2012). Thus, the Fumariaceae s.str. ancestor expanded its range from the Irano-Turanian region during late Eocene, and then a vicariance resulted in the split of the South Africa and Mediterranean (still in the Irano-Turanian region) lineages in the early Oligocene (Fig. 4a, node 91; Table 2); instead the diversification of the ancestor of the subtribe Discocapninae in the Irano-Turanian region before its Miocene dispersal (Pérez-Gutiérrez et al., 2012). Our new results suggest a late Eocene dispersal route for the Fumariaceae s.str. ancestor across north-eastern Africa. An alternative route would be across the north-western Africa via protomediterranean (area G in our analysis); however this alternative was always much less probable than the north-eastern dispersal route in our biogeographical analyses (Tables S7 and S8; node 91), even when a higher dispersal constraint was defined between Irano-Turanian (area E) and South Africa (area F) than between the areas E–G–F (M1 analysis, Fig. S2c). During late Eocene and until late Oligocene no land connection could favour the dispersal of the Fumariaceae s.str. ancestor from western Asia to north-eastern Africa, so that dispersal might have occurred via stepping-stones along the Tethyan coast (and migration along the African mountains to South Africa before the split of lineages in the early Oligocene), as it has been described for other plant groups such as for Tribe Cardueae, also during late Eocene (Barres et al., 2013). South African early Oligocene climate might have been similar to the modern climate (cf. Linder, 2005), which could allow the establishment of the arid-habitat-adapted lineages. Long-distance dispersal of lineages adapted to the Oligocene climates (temperate climates, low-nutrient soils, and seasonal drought) has been documented for the Cape flora, which

after climatic changes gave rise to additional Cape lineages (cf. Linder, 2005). Crown Discocapninae was dated in the mid-Miocene (16 Ma), coinciding with a more mesic South African climate (established from early Miocene) and before the beginning of the trend towards the modern seasonally arid conditions (14 Ma; Zachos et al., 2001). Climatic change from Oligocene to Miocene climates could have been responsible for the initial diversification of *Cysticapnos* by isolation of the ancestor populations in open and dry disjunct areas.

4.6. The Mediterranean colonization by Fumariaceae s.str

According to our results the arrival to the Mediterranean of Fumariaceae s.str. from the Irano-Turanian region occurred in two separate dispersion events following two different routes (Fig. 4b). One dispersal event was that of Sarcocapninae following a northern route during the early Oligocene (33–26 Ma); the other was that of Fumariinae through northern Africa between late Oligocene and middle Miocene (26–17 Ma). The Irano-Turanian region has been proposed as a geographical source for temperate Mediterranean flora (e.g., Quézel, 1985; Thompson, 2005); and this hypothesis has been shown by biogeographical studies of several plant groups (e.g. Araceae, Mansion et al., 2008; Ruta, Salvo et al., 2010; *Consolida* s.l., Jabbour and Renner, 2011; *Haplophyllum*, Manafzadeh et al., 2014), to which the Mediterranean Fumariaceae s.str. genera need to be added.

Two important tectonic events occurred between the late Eocene and the early Miocene, which could be involved in the expansion of Fumariaceae s.str. into the Mediterranean during the estimated dates. On one hand, the origin of the Paratethys Sea in the Eocene–Oligocene boundary (Rögl, 1999) and its isolation from a proto-Mediterranean Sea during the early Oligocene by the progressive accretion of the microplates located between them (Rögl, 1999; Meulenkaamp and Sissingh, 2003). This microplate accretion resulted in an elongated and more-or-less continuous landmass connecting the proto-Mediterranean basin with Asia Minor, and both two Mediterranean domains (western and eastern Mediterranean); and it allowed floristic exchanges between these areas (Salvo et al., 2010; Manafzadeh et al., 2014). According to our results, this land bridge would have allowed the invasion of the Mediterranean by the ancestor of Sarcocapninae from the Irano-Turanian region and its westward expansion towards the western Mediterranean (Fig. 4b). This eastern–western dispersal through the Mediterranean happened in 2 million years (26–24 Ma; Figs. 4b, S6). Relationships between the three main lineages of Sarcocapninae were not well supported (Fig. 2), and a rapid and simultaneous radiation from the common ancestor was proposed to explain this phylogenetic conflict and the incomplete lineage sorting of morphological characters (Pérez-Gutiérrez et al., 2012). Our results of dating and ancestral area reconstruction support this hypothesis, since the vicariance explaining the split of the eastern-Mediterranean (*Pseudofumaria*) and western-Mediterranean (*Platycapnos* and *Sarcocapnos*–*Ceratocapnos*) lineages in the Oligocene–Miocene boundary is compatible with the geological instability shown for the migratory route followed during this time (Rögl, 1999; Meulenkaamp and Sissingh, 2003). Thus, the land bridge connecting the western and eastern Mediterranean was repeatedly disrupted by cycles of marine transgression–regression between the Tethys and Paratethys seas (Rögl, 1999; Meulenkaamp and Sissingh, 2003), promoting east–west Mediterranean disjunctions, as that in Sarcocapninae (Quézel, 1985; Oosterbroek and Arntzen, 1992; Sanmartín, 2003; Mansion et al., 2008; Manafzadeh et al., 2014).

On the other hand, the interruption of the Tethys Sea by the collision of the Arabian plate with the Anatolian microplate around 20 Ma resulted in the formation of a land corridor between

western Irano-Turanian region and Africa across Arabia, the Gomphotherium landbridge (Rögl, 1999). The time of the appearance of this land corridor is consistent with our dating of the southern Mediterranean invasion by Fumariinae (26–17 Ma), and then it likely facilitated the arrival of the Fumariinae ancestor from Irano-Turanian region, through the Arabian plate, to North Africa (Fig. 4b). This migration route in the same time period was also proposed for the ancestor of *Ruta* (Salvo et al., 2010). The Fumariinae ancestor occupied a wide area from western Irano-Turanian region to western North Africa. Progress of the diversification of Fumariinae began with the split of *Fumaria* and quickly continued with a vicariance between *Rupicapnos* and *Cryptocapnos*–*Fumariola* lineages (Fig. 4b). However, relationships between these lineages were not well resolved in our phylogenies, since another hypothesis on this relationship involves *Rupicapnos* as sister group of *Fumaria*; and thus the first diversification event would have been a vicariance between North African lineages and the Irano-Turanian ancestor of *Cryptocapnos*–*Fumariola* (data not shown). The onset of diversification of Fumariinae coincided not only with the intense orogenic activity in western Asia during middle Miocene as consequence of the collision between Arabian and Eurasian plates, which has been proposed as responsible for the isolation of plant lineages and for promoting allopatric speciation (Sanmartín, 2003), but also with the emergence of intermittent seaways interrupting the Gomphotherium landbridge (Rögl, 1999). All these factors could have promoted the rapid radiation of the three lineages in the clade (*Fumaria*/*Fumariola*–*Cryptocapnos*/*Rupicapnos*), explaining the low resolution of the molecular markers to establish the relationships between lineages in the phylogenies. Finally, Fumariinae reached Central Asia by the dispersion of the ancestor of *Cryptocapnos* and *Fumariola* from the Irano-Turanian region during the late Miocene (Fig. 4b, Table 2).

The current North African-western Mediterranean distribution for several species of Fumariaceae s.str. genera can be explained by range expansion in both directions from the late Miocene, with a concentration of dispersal events from 6 Ma (Figs. 4b, S6). The coincidence of such dispersion with the closure of the western Mediterranean Sea from the Atlantic Ocean in the late Tortonian, increasing the aridity of western Mediterranean, and with the desiccation of the Mediterranean Sea (c. 6 Ma; Duggen et al., 2003) suggests the influence of these geological events on the exchange of Fumariaceae s.str. species between western Mediterranean and North Africa across the Strait of Gibraltar.

The Mediterranean lineages of Fumariaceae s.str. appeared and evolved *in situ* in the Mediterranean basin. The structure and composition of Mediterranean flora is believed to have been influenced both by the trend towards increasing aridification starting 9–8 Ma and the onset of the Mediterranean climate (3–2 Ma) (Suc, 1984; Thompson, 2005); however, in Fumariaceae s.str., neither the migration events (33–26 Ma and 26–17 Ma) nor the origin of major lineages (all between 24 and 16 Ma) were affected by such climatic regimes. On the contrary, most generic diversification was triggered during the climatic trend to aridity starting 9 Ma, but before the onset of the Mediterranean climate (9–6 Ma; split of the basal lineage of *Sarcocapnos* occurred 13 Ma, but the core *Sarcocapnos* diversified 9 Ma; Figs. 4b, S6). An important question arising from the above concerns one of the most characteristic and striking adaptations of Fumariaceae s.str., i.e. its perennial-chasmophytic habit. According to Pérez-Gutiérrez et al. (2012) the ancestor of Fumariaceae s.str. was annual and non-chasmophyte, and the perennial-chasmophytic habit was acquired independently in Fumariinae (by *Rupicapnos* and *Cryptocapnos*–*Fumariola*) and in *Sarcocapnos* (independently by *Pseudofumaria* and by *Sarcocapnos*). Annual lifespan (considered as an adaptation to open and dry habitats; Raunkiaer, 1918) was acquired by the Fumariaceae

s.str. ancestor (in the Irano-Turanian region or even in the Himalayas before its range expansion), while the perennial-chasmophytic habit was acquired in the stem lineages of those taxa, and therefore prior to the beginning of the aridification of the Mediterranean. Before 16 Ma the Mediterranean basin underwent subtropical conditions, with little seasonal change in temperature and relatively high levels of summer rainfall (Thompson, 2005). Therefore, acquisition of perennial-chasmophytic habit under these climate conditions suggests it was an adaptation to occupy the less-competitive open, dry niches of the cracks of the cliff.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jympev.2015.03.026>.

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