Reconstructing the history of Campanulaceae with a Bayesian approach to molecular dating and dispersal–vicariance analyses

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ARTICLE INFO

ABSTRACT

We reconstruct here the spatial and temporal evolution of the *Campanula* alliance in order to better understand its evolutionary history. To increase phylogenetic resolution among major groups (Wahlenbergieae–Campanuleae), new sequences from the *rbcL* region were added to the *trnL-F* dataset obtained in a previous study. These phylogenies were used to infer ancestral areas and divergence times in *Campanula* and related genera using a Bayesian approach to molecular dating and dispersal–vicariance analyses that takes into account phylogenetic uncertainty. The new phylogenetic analysis confirms Platycodoneae as the sister group of Wahlenbergieae–Campanuleae, the two last ones inter-graded into a well-supported clade. Biogeographic and dating analyses suggest that Western Asia and the Eastern Mediterranean have played a major role as centers of migration and diversification within the *Campanula* alliance, probably in relation to the intense orogenic activity that took place in this region during the Late Neogene, and that could have promoted isolation and allopatric speciation within lineages. Diversification rates within several *Campanula* lineages would have increased at the end of the Miocene, coinciding with the Messinian Stage. Strong selective pressures from climate changes and the expansion of mountainous regions during this period are suggested to explain the adaptation to drought, cold or disturbed environments observed in many *Campanula* species. Several independent long-distance dispersal events to North America are inferred within the *Rapunculus* clade, which seem to be related to high ploidy levels.

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1. Introduction

Phylogenetic reconstruction is nowadays an important tool for biologists to understand the processes governing organismal evolution. One aspect that has grown in importance is the reconstruction of divergence times and past biogeographical ranges. This is reflected in the plethora of new methods developed to infer the spatial and temporal evolution of organisms (Sanderson, 2002; Thorne and Kishino, 2002; Ree et al., 2005; Drummond et al., 2006). Dating methods have evolved from the strict-molecular clock (Zuckerkandl and Paulin, 1965) to more realistic methods that use a "relaxed clock" approach, which models the rate variation among lineages (e.g., Penalized Likelihood, Sanderson, 2002;

Multidivtime, Thorne and Kishino, 2002; Relaxed Phylogenetics, Drummond et al., 2006).

Similarly, new biogeographical methods that allow incorporating the errors usually associated with phylogenetic and ancestral area reconstruction into the biogeographic inference have been developed (e.g., Ree et al., 2005; Moore et al., in press; Ree and Smith, 2008; Sanmartín et al., 2008). Current analytical methods, including the widely popular dispersal–vicariance analysis method (DIVA, Ronquist, 1996, 1997), reconstruct biogeographic patterns on a fixed, fully resolved tree, thus ignoring the uncertainty in phylogenetic reconstruction (Ronquist, 2004). This requirement can be problematic, since phylogenetic hypotheses are usually not fully resolved (i.e. polytomies) and may contain nodes that are better supported than others.

Recently, Nylander et al. (2008) have proposed a new Bayesian approach to dispersal–vicariance analysis (Bayes-DIVA) that averages DIVA biogeographical reconstructions over a Bayesian sample of trees reflecting the relative confidence (credibility values) on the different clades in the phylogeny. This method has the advantage that it allows uncertainty in phylogenetic relationships to

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influence ancestral area reconstructions, sometimes helping to reduce biogeographic uncertainty by favoring some areas (with higher marginal probability) over others (Nylander et al., 2008). This is the first time that this approach is applied to a group of plants.

The Campanulaceae sensu stricto comprise about 600–950 species and 35–55 genera, and their allied families are the Lobeliaceae, Cyphiaceae, Cyphocarpaceae, Nemacladaceae, Pentaphragmataceae, and Sphenocleaceae (Cosner et al., 2004). All these families are grouped as Campanulaceae sensu lato by some authors. The Campanulaceae s. str. have a nearly cosmopolitan distribution (Fig. 1). *Campanula* L. is the largest genus of the family, with *c.* 350–500 species inhabiting a wide range of habitats in the Northern Hemisphere (Fedorov, 1957). It is mainly distributed in Eurasia, and poorly represented in North America and Africa. A large concentration of *Campanula* species is found in the Eastern Mediterranean region and the Caucasus (Fig. 1).

Taxonomic treatments within Campanulaceae differ depending on the author (see Cosner et al., 2004 for a review). Schönland (1889) defined three subtribes based on ovarian characters. Kovanda (1978) followed this classification. Later, Yeo (1993) upgraded these to tribal rank: Campanuleae, Wahlenbergieae and Platycodoneae. The tribe Campanuleae differs from tribes Wahlenbergieae and Platycodoneae in that the ovary is inferior and the capsule dehisces at the sides (indehiscent in few cases), while in Wahlenbergieae the capsule dehisces at the top. In Platycodoneae the carpels alternate with the sepals (Yeo, 1993). Following this classification, Platycodoneae is monotypic, including only *Platycodon*. However, Thulin (1975) suggested that all taxa with colporate or colpate grains related to *Campanula* should be included also in Platycodoneae: (*Campanumoea* Blume, *Canarina* L., *Codonopsis* Wall., *Cyananthus* Wall. ex Benth., *Leptocodon* Lem. and *Platycodon* A. DC.). Recent phylogenetic analyses based on ITS-DNA sequence data (Eddie et al., 2003) and cpDNA gene order (Cosner et al., 2004) have confirmed that taking into account the pollen pores characteristics for the tribal classification makes this more natural. These studies show that the tribe Platycodoneae (including all the genera with colpate or colporate pollen) is the sister group of the tribes Wahlenbergieae and Campanuleae, which constitute together a well-supported clade (Cosner et al., 2004). Recent works about the phylogenetic relationships of the genus *Campanula* and allied genera (Eddie et al., 2003; Cosner et al., 2004; Roquet et al., 2008) have shown that the tribe Campanuleae sensu Yeo (1993) should include also *Edraianthus* DC., which was originally placed in Wahlenbergieae (Schönland, 1889) because of its irregular fruit dehiscence. Following these studies, here we consider that Platycodoneae includes the genera with sepals alternating with carpels and colpate or colporate pollen, while Wahlenbergieae and Campanuleae show opposite sepals and porate pollen (Dunbar, 1975; Thulin, 1975; Yeo, 1993; Cosner et al., 2004).

According to previous studies (Eddie et al., 2003; Shulkina et al., 2003; Cosner et al., 2004; Roquet et al., 2008; Stefanovic et al., 2008), *Campanula* is paraphyletic in its current generic circumscription, with many satellite genera nested within it. *Campanula* appears divided into two main clades: a large core of *Campanula* species that includes related genera (*Adenophora* Fisch., *Asyneuma* Griseb. and Schenk, *Azorina* Feer, *Campanulastrum* Small, *Diosphaera* Feer, *Edraianthus*, *Githopsis* Nutt., *Hanabusaya* Nakai, *Heterocodon* Nutt., *Legousia* Durand, *Michauxia* L'Hér., *Petromarula* Vent. ex R. Hedw., *Physoplexis* Schur, *Phyteuma* L., *Trachelium* L. and *Triodanis* Raf.), plus a clade constituted by *Musschia* Dumort. and two *Campanula* species (Eddie et al., 2003; Roquet et al., 2008). Characters related to flower morphology have proven to be of little usefulness in the natural delimitation of the group, as they are probably under strong selective pressures from pollinators (Roquet et al., 2008). Fruit dehiscence has also been used as a diagnostic character by some authors, but Kolakovsky (1986) and Stefanovic et al. (2008) have shown that this character is homoplasic.

There has been considerable debate regarding the geographic origin of Campanulaceae. In their classic paper of angiosperm biogeography, Raven and Axelrod (1974) hypothesized an Old World
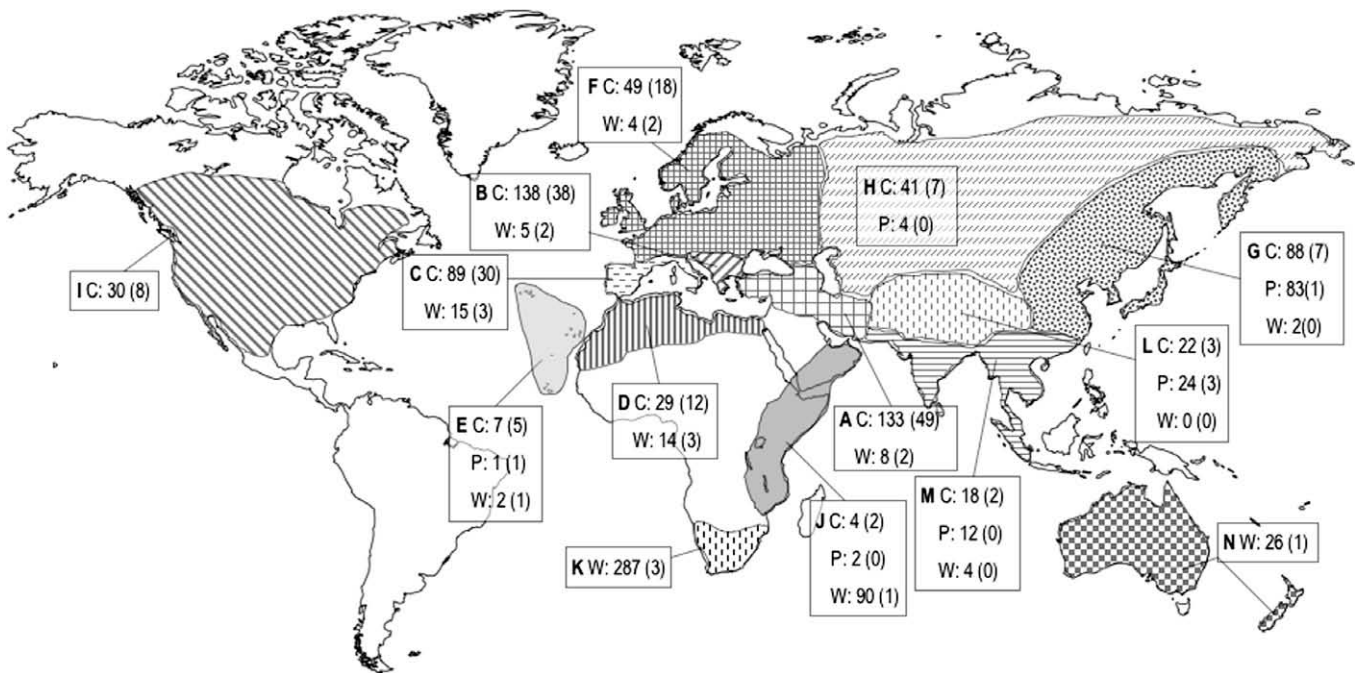
Fig. 1. Distribution map of Campanulaceae s. str. showing 14 areas of endemism: (A) Western Asia (from Anatolia to Iran, including the Caucasus); (B) Eastern Mediterranean Basin; (C) Western Mediterranean Basin; (D) North Africa; (E) Macaronesia; (F) North and Central Europe; (G) Eastern Asia; (H) Central Asia; (I) North America; (J) East Africa; (K) South Africa; (L) Himalayan range; (M): India (except the higher mountains area) and Indonesia; and (N) Australia and New Zealand. For each area of distribution, the number of species belonging to the tribes Campanuleae (C), Wahlenbergiae (W) and Platycodoneae (P) is given inside a box; the number between brackets indicates the number of species present in the area that have been sampled for this study.

origin during the Paleogene. Hong (1995) suggested East Asia as the center of origin of the family, with the Mediterranean region and South Africa as secondary centers of differentiation and diversification within the family starting no later than the Cretaceous. In contrast, Bremer and Gustaffsson (1997) argued for a Gondwana origin for the family based on the current Southern Hemisphere distribution of many early diverging genera. Similarly, Eddie and Cupido (2001) suggested an African origin based on the present distribution of the closely related family Lobeliaceae. Eddie et al. (2003) hypothesized that *Campanula* and related genera evolved in the Mediterranean region, from where they rapidly spread over other Northern Hemisphere landmasses during pre-glacial times. Regardless of the postulated geographic origin, the great variety of distributions, ecological habitats, morphological characters, reproductive systems, and chromosome numbers exhibited by *Campanula* and allied genera (Roquet et al., 2008) suggests a history of high and rapid diversification and a complex biogeographic pattern.

Until now, attempts to estimate divergence times in Campanulaceae have been limited. In a large-scale study of angiosperms divergence times, Wikström et al. (2001) included one *Campanula* and one *Codonopsis* species, and obtained a divergence date of 41 million years ago (mya) for the split of those two genera. *Codonopsis*, *Platycodon* and *Cyananthus* form a basal group within Campanulaceae (Cosner et al., 2004). A more specific study focused on the species of *Campanula* subsection *Isophylla* Damboldt (Park et al., 2006) dates the first diversification of the *Campanula garganica* Ten. complex within the Late Miocene. To date, no attempts have been made to date phylogenetic divergence within the main lineages of Campanulaceae or diversification events within *Campanula*.

Here, we explore the spatial and temporal evolution of the Campanulaceae s. str., and of the *Campanula* alliance in particular, by applying a Bayesian approach to molecular dating and dispersal–vicariance analysis that takes into account phylogenetic uncertainty: biogeographic and temporal reconstructions are averaged among a Bayesian distribution of trees weighted according to their posterior probability (Nylander et al., 2008).

Despite the analytical advances mentioned above, all current methods of biogeographic and temporal reconstruction still depend on the existence of a sound phylogenetic hypothesis for reliable and accurate inference of ancestral areas and divergence times. Previous phylogenetic hypotheses on Campanulaceae based on ITS (Eddie et al., 2003; Park et al., 2006; Roquet et al., 2008), *trnL-F* and combined data (Roquet et al., 2008) provided a framework for the classification of the genus, but left the relationships among major groups (e.g., Wahlenbergieae–Campanuleae) unresolved, and disagreed in the position of some key genera, such as the relationship of *Trachelium* with respect to *Campanula* (Roquet et al., 2008). To help resolve relationships within *Campanula* and to reconstruct the spatial and temporal evolution within *Campanula* and its close allies, we add here 78 new sequences from the *rbcL* region, 22 *rbcL* sequences obtained from GenBank and one new *trnL-F* sequence (of *Gadellia* Shulkina) to the dataset of Roquet et al. (2008) and increase the taxon sampling within major lineages in Platycodoneae and Wahlenbergieae.

2. Materials and methods

2.1. Taxon sampling

In order to help solve previously unresolved relationships and to obtain a consistent phylogenetic framework for dating and spatial reconstruction analyses, we sequenced the *rbcL* gene for *Campanula* species and related genera. Taxa were selected to represent the main lineages detected in previous molecular phylogenetic work based on less conserved markers (Eddie et al., 2003; Roquet et al., 2008), and to include genera not sampled before with chloroplast markers (*Merciera* A. DC., *Prismatocarpus* L'Hér.). We have also increased the sampling for some genera (*Wahlenbergia gloriosa* Lothian, *W. lobelioides* Link).

For this purpose, we conducted phylogenetic analyses of 100 *rbcL* sequences. A total of 78 new sequences were produced specifically for this study, to which we added 22 sequences obtained from GenBank. Three sequences from Lobeliaceae, a family closely related to Campanulaceae (Cosner et al., 2004), were included as outgroups. The 105 sequences of *trnL-F* produced in Roquet et al. (2008) plus one new sequence were also used to be combined with the *rbcL* data to conduct phylogenetic, dating and biogeographic analyses. Sources of material and location of vouchers are given in Table 1.

2.2. DNA extraction, PCR amplification and sequencing

DNA was extracted from herbarium material or, in few cases, from silica gel-dried plant tissue by the CTAB method (Doyle and Doyle, 1987) modified following suggestions by Culling (1992). We also used the "Dneasy® Mini Kit" (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions for difficult, old material.

The *rbcL* gene was amplified and sequenced in two overlapping fragments using the primers 1F/724 R (Olmstead et al., 1992) and 636F/1460R (Fay et al., 1997, 1998). PCR amplifications were performed with the thermocycler PTC-100™ Programmable Thermal Controller (MJ Research Inc.). The thermal cycling profile consisted of 1 min at 95 °C; 5 min at 80 °C, while DNA-polymerase (Ecotaq, Ecogen S. R. L., Barcelona, Spain) was added; 29 cycles of 1 min denaturing at 95 °C, 30 s annealing at 50 °C, and 1 min of extension at 72 °C; with final extension of 7 min at 72 °C. PCR products were cleaned using the "QIAQuick® DNA cleanup system" (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. DNA sequencing of PCR-purified templates was done using reactions based on chemistry of "Big Dye® Terminator v3.1" (Applied Biosystems, Foster City, CA) following the protocol recommended by the manufacturer. The products obtained were analyzed on an ABI Prism® 3730 PE Biosystems/Hitachi automated sequencer in the "Serveis Cientificotècnics de la Universitat de Barcelona", and the chromatograms were edited with Chromas 2.0 (Technelysium Pty Ltd, Tewantin, Australia).

2.3. Phylogenetic analyses

Sequences of *rbcL* gene were aligned by eye using the text editor TextPad® 4.7.3, with no indel/deletion events detected. Bases 1–30, which formed the primer-binding region of the 1F primer, were excluded. Phylogenetic analyses were performed using Maximum Parsimony (MP) and Bayesian Inference (BI). The new *trnL-F* sequence was aligned manually with the other *trnL-F* sequences obtained in Roquet et al. (2008). In order to investigate if there was significant incongruence between the *rbcL* and the *trnL-F* datasets, we performed a partition homogeneity test (Farris et al., 1994) with PAUP* 4.0b10 (Swofford, 2002) with 10 homogeneity replicates, 10 random-addition sequences, tree bisection–reconnection (TBR) branch-swapping on best trees and MulTrees option.

Phylogenetic analyses were conducted for three datasets: *rbcL*, *trnL-F* and the total-evidence combined matrix for both regions. Parsimony analyses involved heuristic searches conducted with PAUP* 4.0b10 (Swofford, 2002) with TBR branch swapping, MulTrees option in effect, 10 replicates of random addition-sequence and character states specified as unordered and unweighted. Bootstrap (BS) analyses were also performed to assess nodal support

Table 1
Origin of the materials, herbaria where the vouchers are deposited and GenBank accession numbers (new sequences indicated by bold type).

Species	Voucher (*rbcL*)	*rbcL* accession	Voucher (*trnL-F*)	*trnL-F* accession
Adenophora confusa Nannf.	Cosner et al. (2004)	AY655145	–	–
Adenophora remotiflora (Sich. & Zucc.) Miq.	Japan, Kawasaki: Honshu, *Estebánez 1511* (MA *s. n.*)	**EU643715**	Roquet et al. (2008)	EF088693
Asyneuma limonifolium Bornm.	Turkey: Erzurum, *Nisa 1006* (MA 689405)	**EU643704**	Roquet et al. (2008)	EF088694
Asyneuma lobelioides Hand.–Mazz.	Turkey: Ermenek, *Aldasoro 9157* et al. (MA *s. n.*)	**EU643733**	Roquet et al. (2008)	EF088695
Asyneuma virgatum Bornm.	Cosner et al. (2004)	AY655146	–	–
Azorina vidalii (Wats.) Feer	Portugal: Açores, *Sequeira 4493* (MA *s. n.*)	**EU643713**	Roquet et al. (2008)	EF088696
Campanula abietina Griseb. & Schenk	–	–	Roquet et al. (2008)	EF088697
Campanula affinis Roem. & Schult.	Spain, Barcelona: Montserrat, *Roquet V-2004* (BC)	**FJ587240**	Roquet et al. (2008)	EF088698
Campanula alliariifolia Willd.	Cultivated at Botanical Garden of Madrid (MA 688448)	**FJ587241**	Roquet et al. (2008)	EF088700
Campanula andrewsii DC.	–	–	Roquet et al. (2008)	EF088701
Campanula aparinoides Pursh	Finland, Palkane: Lake Tykolanjawi, *Nunmi s. n.* (MA 451610)	**EU643728**	Roquet et al. (2008)	EF088702
Campanula argaea Boiss. & Bal.	–	–	Roquet et al. (2008)	EF088703
Campanula armena Stev.	Armenia, Ashtarak: Mt. Arailer, *Vasak 15-VII-1975* (MA 642322)	**FJ587242**	Roquet et al. (2008)	EF088704
Campanula balfourii Wagner & Vierh.	Yemen, Socotra: Qalansiyah, *Thulin 8712* et al. (UPS 82575)	**FJ587243**	Roquet et al. (2008)	EF088705
Campanula bellidifolia Adams	Cultivated at Botanical Garden of Madrid, *Alarcón 230* (MA)	**FJ587244**	Roquet et al. (2008)	EF088706
Campanula betulifolia K. Koch	Turkey, Gümüshane, Tirebolu-Kürtün, *Herrero 1180* (MA 689193)	**FJ587245**	Roquet et al. (2008)	EF088707
Campanula chamissonis Fed.	Japan: Honshu, *Estebánez 1478* (MA *s. n.*)	**EU643724**	Roquet et al. (2008)	EF088709
Campanula cochleariifolia Lam.	Spain, Huesca: Bielsa, *Roquet 12-X-2004* (BC)	**FJ587247**	Roquet et al. (2008)	EF088710
Campanula collina M. Bieb.	Georgia, Javakhati: Mt. Taushan-Tagan, *Ketzkoveli 22-VII-80* (MA 575569)	**FJ587248**	Roquet et al. (2008)	EF088711
Campanula conferta DC.	Turkey, Sakaltutan Gecidi: Erzincan, *Aldasoro 2647* (MA 689787)	**FJ587249**	Roquet et al. (2008)	EF088712
Campanula coriacea Boiss. & Kotschy	–	–	Roquet et al. (2008)	EF088713
Campanula creutzburgii Greuter	Greece, Kreta: Dia, *Alpinar* (ISTE *s. n.*)	**EU643730**	Roquet et al. (2008)	EF088714
Campanula cymbalaria Sibth & Sm.	–	–	Roquet et al. (2008)	EF088715
Campanula decumbens DC.	Spain, Cuenca: Barajas de Melo, *Arán 30-V-98* et al. (MA 623787)	**FJ587250**	Roquet et al. (2008)	EF088716
Campanula dichotoma L.	Italy: Sicily, sine col. (MA 645874)	**FJ587251**	Roquet et al. (2008)	EF088717
Campanula dimorphantha Schwein.	Taiwan, Hualien: Hisiulin Hsiang, *Chih-Chia Wang 1353* (LE)	**FJ587246**	Roquet et al. (2008)	EF088708
Campanula divaricata Michx.	USA, Waterville: Pigeon River (MA 391570)	**EU643732**	Roquet et al. (2008)	EF088718
Campanula drabifolia Sibth. & Sm.	Greece, Peloponnese, Tolon: Argolida, *Buggenhourt 18481* (MA 625645)	**FJ587252**	Roquet et al. (2008)	EF088719
Campanula elatines Bout. ex Willk. & Lange	Cosner et al. (2004)	AY655147	Bremer et al. (2002)	AJ430970
Campanula erinus L.	Spain, Mallorca: Cova Negra, *Sáez 6135* (BCB)	**EU643734**	Roquet et al. (2008)	EF088720
Campanula fastigiata Dufour ex Schult.	Spain, Albacete, *Aedo 3937* (MA 591308)	**EU643727**	Roquet et al. (2008)	EF088721
Campanula filicaulis Dur.	Morocco, Middle Atlas: Midelt, *Jury 17866* (MA 616923)	**FJ587253**	Roquet et al. (2008)	EF088722
Campanula foliosa Ten.	Italy: Mt. Vigula, *Snogerup 15903* (UPS)	**FJ587254**	Roquet et al. (2008)	EF088723
Campanula fruticulosa (O. Schwarz & Davis) Damboldt	Turkey, Burdur, Dirmil: Masda Dagi, *Dumar 6279* (ISTE *s. n.*)	**EU643716**	Roquet et al. (2008)	EF088724
Campanula garganica Ten.	Italy: Foggia, *Aldobrandi 12-VII-96* et al. (MA 625685)	**FJ587255**	Roquet et al. (2008)	EF088725
Campanula haradjanii Rech. f.	–	–	Roquet et al. (2008)	EF088726
Campanula hofmannii (Pant.) Greuter & Burdet	Cosner et al. (2004)	AY655159	Roquet et al. (2008)	EF088727
Campanula incurva Aucher ex DC.	Cultivated at Botanic Institute of Barcelona, *Roquet s. n.* (BC)	**FJ587256**	Roquet et al. (2008)	EF088728
Campanula involucrata Aucher ex DC.	Turkey, Gümüshane: Yagmürdere, *Herrero 1453* (MA 687604)	**FJ587257**	Roquet et al. (2008)	EF088729
Campanula karakuschensis Grossh.	Iran: Ghogeh Dag, *Rechinger 44029* (MA 417801)	**FJ587258**	Roquet et al. (2008)	EF088730
Campanula lanata Friv.	Bulgary, Rila: Kostenec, *Frost-Olsen 484* (MA 463958)	**FJ587259**	Roquet et al. (2008)	**EF088731**
Campanula latifolia L.	Turkey, Trabzon: Sumelas, *Valcárcel 379* (MA 689767)	**FJ587260**	Roquet et al. (2008)	EF088732
Campanula lusitanica Loefl.	Spain, A Corunya: Carnota, *Louzan 1-VI-96* (MA 581374)	**EU643714**	Roquet et al. (2008)	EF088733
Campanula lyrata Lam.	–	–	Roquet et al. (2008)	**EF088734**
Campanula macrochlamys Boiss. & Huet	–	–	Roquet et al. (2008)	**EF088735**
Campanula macrostachya Willd.	–	–	Roquet et al. (2008)	**EF088736**
Campanula macrostyla Boiss. & Heldreich	Turkey: Ermenek, *Aldasoro 9135* et al. (MA *s. n.*)	**EU643722**	Roquet et al. (2008)	**EF088737**
Campanula medium L.	Cultivated at Botanical Garden of Madrid, *MLA0183* (MA)	**FJ587261**	Roquet et al. (2008)	EF088738
Campanula mollis L.	Spain, Almería: Gádor, *Borja, Navarro 1303* (MA 545932)	**EU643721**	Roquet et al. (2008)	**EF088739**
Campanula moravica (Spitzn.) Kovanda	Cultivated at Botanical Institute of Barcelona, *Roquet 5-V-2004* (BC)	**FJ587262**	Roquet et al. (2008)	**EF088740**
Campanula olympica Boiss.	Turkey, Çamlik: Rize, *Nisa 772* (MA)	**FJ587263**	Roquet et al. (2008)	EF088741
Campanula peregrina L.	Turkey, Alanya: Antalya, *Baytop 26-VII-57* (ISTE 5437)	**EU643719**	Roquet et al. (2008)	**EF088742**
Campanula persicifolia L.	Cultivated at Botanical Garden of Madrid, *MLA0179* (MA)	**FJ587264**	Roquet et al. (2008)	EF088743
Campanula pinatzii Greuter & Phitos	Greece, Dhodhekanisos: Kastello, *Raus 9666* (MA 464542)	**FJ587265**	Roquet et al. (2008)	**EF088744**
Campanula pinnatifida Hub.–Mor.	–	–	Roquet et al. (2008)	EF088745
Campanula polyclada Rech. f. & Schiman–Czieka	–	–	Roquet et al. (2008)	**EF088746**
Campanula poscharskyana Degen	Cultivated at Botanical Garden of Madrid, *Alarcón 178* (MA)	**FJ587266**	Roquet et al. (2008)	**EF088747**
Campanula prenanthoides Durand	–	–	Roquet et al. (2008)	**EF088748**
Campanula primulifolia Brot.	Portugal, Algarve: Foia, *Julin 26-VI-1974* (UPS *s. n.*)	**EU643718**	Roquet et al. (2008)	EF088699
Campanula propinqua Fisch. & C. A. Mey. (1)	Turkey, Gümüshane: Kurtun-Torul, *Herrero 1287* (MA 688027)	**FJ587267**	Roquet et al. (2008)	EF088749
Campanula propinqua Fisch. & C. A. Mey. (2)	–	–	Roquet et al. (2008)	EF088794
Campanula ptarmicifolia Lam. (1)	Turkey: Tunceli, *Davis 31233* et al. (ISTE 43633)	**EU643710**	Roquet et al. (2008)	**EF088750**
Campanula ptarmicifolia Lam. (2)	–	–	Roquet et al. (2008)	EF088775
Campanula pterocaula Hausskn.	Turkey: Bolu, *Nydegger 19005* (MA 367633)	**FJ587268**	Roquet et al. (2008)	**EF088751**
Campanula pubicalyx (Davis) Damboldt	Turkey, Konya: Ermenek, *Davis 16244* (ISTE 43630)	**EU643717**	Roquet et al. (2008)	EF088752
Campanula punctata Lam.	Japan: Honshu, *Estebánez 1508* (MA *s. n.*)	**EU643725**	Roquet et al. (2008)	EF088753
Campanula pyramidalis L.	–	–	Roquet et al. (2008)	EF088754
Campanula quercetorum Hub.–Mor. & C. Simon	Turkey, Evciler: Bayramiç, *Castroviejo 15236* (MA 644286)	**FJ587269**	Roquet et al. (2008)	EF088755
Campanula radula Fisch.	Turkey: Hakkari, *Archibald 8340* (ISTE)	**FJ587270**	Roquet et al. (2008)	**EF088756**
Campanula rapunculoides L.	Turkey, Rize, *Nisa 763* (MA 689073)	**FJ587271**	Roquet et al. (2008)	EF088757

Table 1. (continued)

Species	Voucher (*rbcL*)	*rbcL* accession	Voucher (*trnL-F*)	*trnL-F* accession
Campanula rapunculus L.	Spain, Barcelona, Viladrau, *Sáez 6121* (BCB)	**FJ587272**	Roquet et al. (2008)	EF088758
Campanula ramosissima Sibth. & Sm.	Michaels et al. (1993)	L13861	–	–
Campanula rotundifolia L.	Andorra, *Sáez 6134* (BCB)	**FJ587273**	Roquet et al. (2008)	EF088759
Campanula savalanica Fed.	–	–	Roquet et al. (2008)	EF088760
Campanula saxifraga M. Bieb. (1)	Armenia, Akhurian: Krashen, *?tul=0?>Oganessian 26-VI-2004* (ERE *154864*)	**FJ587274**	Roquet et al. (2008)	EF088761
Campanula saxifraga M. Bieb. (2)		–	Roquet et al. (2008)	EF088795
Campanula scheuchzeri A. Gray	Spain, Huesca: Bielsa, *Roquet 12-X-2004* (BC)	**FJ587275**	Roquet et al. (2008)	**EF088762**
Campanula sclerotricha Boiss.	Turkey, Van: Bahçesaray, *Baytop 19-IX-1978* (ISTE *30991*)	**FJ587276**	Roquet et al. (2008)	EF088763
Campanula scoparia (Boiss. & Hausskn.) Damboldt	Turkey: Hakkari, *Duncan 71 et al.* (ISTE)	**FJ587277**	Roquet et al. (2008)	EF088764
Campanula scutellata Griseb.	–	–	Roquet et al. (2008)	EF088765
Campanula semisecta Murb.	Spain: Cazorla, *Muñoz-Garmendia 16-VI-76 et al.* (MA *456218*)	**FJ587278**	Roquet et al. (2008)	EF088766
Campanula sibirica L.	Russia, Altai: Artishtu-Karatsu, *Castroviejo 14132* (MA *613903*)	**FJ587279**	Roquet et al. (2008)	EF088767
Campanula speciosa Pourr.	France, Ariège: Mijanes, *Montserrat 8-VI-1983 et al.* (MA *256533*)	**FJ587280**	Roquet et al. (2008)	EF088768
Campanula spicata L.	Italy, Teramo: Fondo de la Salsa, *Navarro 4323* (MA *699308*)	**FJ587281**	Roquet et al. (2008)	EF088769
Campanula stevenii subsp. *stevenii* M. Bieb.	Armenia: Vayk, *Oganessian* (ERE *154865*)	**FJ587282**	Roquet et al. (2008)	EF088770
Campanula stricta Labill.	Iran, Chadi: Kuh, *Renz 48987* (MA *420241*)	**FJ587283**	Roquet et al. (2008)	EF088771
Campanula strigosa Banks & Sol.	–	–	Roquet et al. (2008)	EF088772
Campanula subcapitata Popov	Turkey, Erzurum: Pasinler, *Herrero 1831* (MA *687545*)	**FJ587284**	Roquet et al. (2008)	EF088773
Campanula thyrsoides L.	Switzerland, Berne: Geintrisch, *Hedberg 4037* (UPS *s. n.*)	**EU643723**	–	–
Campanula trachelium L.	Spain, *Sáez 6133u* (BCB)	**FJ587285**	Roquet et al. (2008)	EF088774
Campanula tymphaea Haasskn.	–	–	Roquet et al. (2008)	EF088796
Campanulastrum americanum (L.) Small	USA, Nebraska: Seward Co., *Nieto-Feliner 2063* (MA *459958*)	**EU643729**	Roquet et al. (2008)	EF088776
Canarina canariensis (L.) Vatke	Spain, Gran Canaria: Teror, *Aldasoro 9106* (MA *s. n.*)	**EU643709**	Roquet et al. (2008)	EF088777
Codonopsis ovata Benth.	Cosner et al. (1994)	L18797	–	–
Codonopsis viridis Wall.	Cosner et al. (2004)	AY655148	–	–
Cyananthus lobatus Wall. ex Benth.	Cosner et al. (2004)	AY655149	–	–
Diosphaera rumeliana (Hampe) Bornm.	Macedonia, Kavala: Mt. Pangeo, *Greuter 16056* (MA *540729*)	**EU643726**	Roquet et al. (2008)	**EF088778**
Edraianthus graminifolius (L.) DC.	Italy, Sicily: Palermo *Herrero 888* (MA *646860*)	**EU643705**	Roquet et al. (2008)	**EF088779**
Feeria angustifolia (Schousb.) Buser	Morocco, Marrakech: High Atlas, Hir-n-Ifri, *Podlech 47779* (MA *472233*)	**EU643711**	Roquet et al. (2008)	EF088780
Gadellia lactiflora (M. Bieb.) Schulkina	Turkey, Rize, *Nisa 732* (MA *688458*)	**EU643703**	Turkey, Rize, *Nisa 732* (MA *688458*)	**FJ589212**
Jasione heldreichii Boiss. & Orph.	Cosner et al. (2004)	AY655151	Roquet et al. (2008)	EF088781
Jasione montana L.	Spain, Barcelona: Saulons d'en Deu, *Sáez 6218* (BCB *s. n.*)	**EU643731**	Roquet et al. (2008)	EF088782
Legousia falcata (Ten.) Fritsch	Cosner et al. (2004)	AY655152	–	–
Legousia hybrida (L.) Delarb.	Morocco: Atlas, Dayer Iffer, *Cirujano R10113 et al.*(BC *s. n.*)	**EU643706**	Roquet et al. (2008)	EF088783
Lobelia cardinallis L.	Kress & Erickson (2007)	DQ285285	–	–
Lobelia erinus L.	Michaels et al. (1993)	L13930	–	–
Lobelia nicotianifolia Heyne	Givnish et al. (unpublished)	AF042660	–	–
Michauxia tchihatchewii Fisch. & C. A. Mey.	Turkey: Ermenek, *Aldasoro 9138 et al.* (MA *s. n.*)	**EU643720**	Roquet et al. (2008)	EF088784
Musschia aurea Dumort.	Cosner et al. (2004)	AY655154	Roquet et al. (2008)	EF088785
Merciera tenuifolia DC.	Cosner et al. (2004)	AY655153	–	–
Petromarula pinnata (L.) DC.	Cosner et al. (2004)	AY655155	Roquet et al. (2008)	EF088786
Phyteuma spicatum L.	Spain, Barcelona: Aiguafreda, *Roquet 8-V-05* (BC *s. n.*)	**EU643712**	Roquet et al. (2008)	**EF088787**
Platycodon grandiflorum (Jacq.) DC.	Cosner et al. (2004)	AY655156	Roquet et al. (2008)	EF088788
Prismatocarpus diffuses DC.	Cosner et al. (2004)	AY655157	–	
Roella ciliata L.	Cosner et al. (2004)	AY655158	Roquet et al. (2008)	**EF088789**
Solenopsis laurentia (L.) C. Presl	–	–	Roquet et al. (2008)	**EF088790**
Trachelium caeruleum L.	Cosner et al. (1994)	L18793	Roquet et al. (2008)	**EF088791**
Triodanis perfoliata (Nutt.) Nieuwl.	Cosner et al. (2004)	AY655160	–	–
Wahlenbergia gloriosa Lothian	Cosner et al. (2004)	AY655161	–	–
Wahlenbergia hederacea L.	Spain, Oviedo: Cangas de Narcea, *Serra 6070* (MA *705618*)	**EU643708**	Roquet et al. (2008)	**EF088792**
Wahlenbergia lobelioides Link	Portugal: Madeira, *Sequeira 4597* (MA *s. n.*)	**EU643707**	Roquet et al. (2008)	EF088793

(Felsenstein, 1985). We followed a similar approach to Lidén et al. (1997), and carried out BS analyses with 1000 replicates, 10 random taxon addition with 10 replicates per replicate and no branch swapping. To explore the amount of phylogenetic signal, we calculated the Consistency Index (CI) (Kluge and Farris, 1969) and the Retention Index (RI) (Swofford, 2002).

Prior to the BI analyses, we determined the best-fitting model of evolution for the *rbcL*, *trnL-F* and combined data using the program MrModeltest 2.2 (Nylander, 2004) and the Akaike Information Criteria (AIC). The model selected for the three matrices was the General Time Reversible model with equal base frequencies with some sites assumed to be invariant and variable sites assumed to follow a gamma distribution (GTR + I + Γ, Rodríguez et al., 1990). The model and parameter estimates were then used in the analyses conducted with MrBayes 3.1 (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003). We run two independent analyses with 10^6 generations saving one of each 100 trees. The first 2000 trees were eliminated before summarizing the posterior distribution. Only the results from the combined matrix are shown here.

2.4. Dating analyses

Two relaxed-clock methods based on different statistical basis were used to reconstruct divergence times for the combined matrix: the Penalized Likelihood approach (PL, Sanderson, 2002) and the Bayesian Relaxed Clock (BRC, Kishino et al., 2001; Thorne and Kishino, 2002). These two methods seem to be the most successful at finding optimal levels of smoothing to correct for rate heteroge-

neity and they are also less sensitive to undersampling (Linder et al., 2005). We used simultaneously multiple constraints such as minimum age (fossil) or maximum age constraints (geological events and independent molecular results) for both methods.

Penalized Likelihood analyses were conducted with the r8s v.1.71 program (Sanderson, 2002). This program assumes the tree topology and branch lengths provided by the user, and it does not provide any confidence intervals on the parameters. To obtain these intervals and account for both branch length and topological uncertainties, we applied an abbreviation of the Bayesian approach suggested by Lopez-Vaamonde et al. (2006): we randomly selected 100 trees from the Bayesian MCMC posterior distribution and conducted PL analyses on all 100 trees, each with its specific smoothing value (calculated by cross-validation). Each analysis was perturbed and restarted up to three times to avoid getting stuck on local optima. To summarize the ages obtained for each node of interest, we calculated the mode (the most likely value) and 90% highest posterior density (HPD) limits (credibility interval) for each estimate (Table 2; Annex 1, Supplementary material). The modes and 90% HPD were obtained by local density estimation using the program LOCFIT (Loader, 1999), implemented in the "R" statistical package (Ihaka and Gentlemen, 1996; see script in Lopez-Vaamonde et al., 2006).

Bayesian dating analyses were performed with the PAML (Yang, 1997) and Multidivtime packages (v. 9/25/03; Kishino et al., 2001; Thorne and Kishino, 2002). We used three programs: Baseml (PAML, v.3.14; Yang, 1997), to estimate model parameters; Estbranches (v.8/5/03; Thorne et al., 1998), to estimate the maximum likelihood of the branch lengths and a variance–covariance matrix; and Multidivtime (Kishino et al., 2001; Thorne and Kishino, 2002), to perform a MCMC Bayesian analysis for approximating the posterior distributions of substitution rates and divergence times. To perform the MCMC analysis, we used the default settings of

Multidivtime as recommended by Rutschmann (2005). Multidivtime provides direct confidence intervals for all dated nodes. The 50% majority rule consensus trees obtained from the BI analyses of each marker were used for the Bayesian dating analyses.

2.5. Calibrations

We used four different calibration points to place minimal and maximal age constraints on internal nodes in the phylogeny. (1) A fossil seed found by Lancucka-Srodoniowa (1979) from the Early-Middle Miocene (c. 16 mya), described by the author as *Campanula* sp., and whose structure resembles those of *Campanula* and related genera such as *Adenophora*, *Jasione* L., *Phyteuma* and *Wahlenbergia* Schrad. ex Roth. The age of the fossil was used as a minimum age constraint for the node of the most recent common ancestor (mrca) to all the above-cited genera in the BRC analyses (node 95). For the PL analyses we used the fossil as a fixed age because otherwise the credibility intervals obtained were very large and the mean ages were unrealistically old in the deepest nodes, as already seen in the comparative study of Hug and Roger (2007). (2) The sub-aerial stage of the oldest island of the Azores, Punta Delgada, is dated as 8 mya (Abdel-Monem et al., 1975). This age estimate was used as an upper age constraint ("maximum age constraint") for the appearance of the endemic species *Azorina vidalii* (Wats.) Feer (node 59). (3) The age of the emerged part of Madeira (dated as being no more than 5.2 mya, Ferreira et al., 1988) was used as the upper limit for the appearance of *Musschia aurea* Dumort., endemic to Madeira (node 21). (4) A maximum age of 41 mya was set for the root node (node 96), based on the dating analyses of Wikström et al. (2001).

2.6. Biogeographic analyses

The operational areas used in the biogeographic analyses were defined on the basis of the presence of one or more endemic taxa. In all the analyses, fourteen areas were used: A, Western Asia (from Anatolia to Iran, including the Caucasus); B, Eastern Mediterranean Basin; C, Western Mediterranean Basin; D, North Africa; E, Macaronesia; F, North and Central Europe; G, Eastern Asia; H, Central Asia; I, North America; J, East Africa; K, South Africa; L, Himalayan range; M, India (except the higher mountains area) and Indonesia; and N, Australia and New Zealand (Fig. 1). The maximum number of ancestral areas was first unconstrained and then constrained to four (Ronquist, 1996), which is the maximum geographic range of the majority of the species in the analysis. The second type of analysis proved more effective to reduce uncertainty in the biogeographical reconstructions and it is the only one presented here: it is equivalent to assuming that ancestral ranges were not more widespread than those of their extant descendants (Sanmartín, 2003).

We used the recently developed Bayes-DIVA method (Nylander et al., 2008) to reconstruct ancestral distributions on the phylogeny of Campanulaceae and average DIVA parsimony-based biogeographical reconstructions over a Bayesian distribution of trees reflecting the relative confidence on the clades (Nylander et al., 2008). Using scripts by Johan Nylander (Florida State University, personal communication), we first ran DIVA analyses (Ronquist, 1996, 1997) on each individual tree from the posterior probability distribution of the Bayesian MCMC analysis and then averaged ancestral area reconstructions across all trees for each node in a "reference tree". Here, the reference tree was the majority rule consensus tree of the Bayesian MCMC stationary sample. When several equally parsimonious reconstructions at a given node (e.g., A/B/AB) were obtained, these were downweighted by $1/n$, where n was the total number of alternative reconstructions at the node. Topologies in the Bayesian stationary sample are weighted according to their posterior probability, but only the

Table 2
Estimated ages and standard deviation for the discussed nodes in the Campanulaceae s. str. phylogeny using Penalized Likelihood (r8s) and Bayesian Relaxed Molecular Clock (Multidivtime) with multiple calibrations for combined data. The node numbers correspond to the ones given in the chronogram (Fig. 3). sd, standard deviation; LHPD, 90% lower highest posterior density limit; UHPD, 90% upper highest posterior density limit. [*] Indicates age constrained in this node, [**] indicates age fixed in this node.

Nodes	r8s mode (LHPD–UHPD)	Multidivtime (sd)
17 – Separation of the ancestor of *Platycodon–Canarina* from the ancestor of *Codonopsis–Cyananthus*	26.3 (19.9–29.7)	15.8 (3.5)
23 – Divergence of *Musschia* clade	12.6 (10.1–14)	10.8 (1.5)
31 – Diversification of *Campanula* s. str. clade 2	9.8 (7.9–11.8)	7.3 (1.4)
51 – Diversification of *Campanula* s. str. clade 1	9.6 (8.7–10.9)	5.1 (1)
60 – Diversification of *Campanula* s. str. clade 3	9.2 (8.6–11.2)	7.5 (1.5)
61 – Split of *Campanula* s. str. clade in three lineages plus the *Diosphaera* branch	12.6 (11.3–14.1)	10.6 (1.4)
67 – Split of *Legousia* from the ancestor of *Campanulastrum* and *Triodanis*	3.8 (3.3–5.2)	4.6 (1.3)
71 – Separation of *Asyneuma, Petromarula, Phyteuma* and the ancestor of *Campanulastrum, Legousia* and *Triodanis*.	7.2 (6–8.4)	7 (1.4)
77 – Separation of *C. rotundifolia*	0.3 (0.1–1.1)	0.5 (0.4)
80 – Separation of *C. divaricata*	4.5 (2.9–7.2)	3.7 (1.2)
91 – Split of *Rapunculus* clade in two main lineages (*Rapunculus* 1 and 2) plus the *Campanula cymbalaria* branch	11.5 (9.9–12.4)	10.8 (1.4)
93 – Beginning of diversification of the *Campanula* core	14.7 (13.5–15.1)	13.7 (1.1)
95 – Divergence of Campanuleae and Wahlenbergieae	16[**]	16.7 (0.7)[*]
96 – Split between Platycodoneae and the ancestor of Campanuleae and Wahlenbergieae	37.4 (36.1–41)[*]	23.5 (3.2)[*]
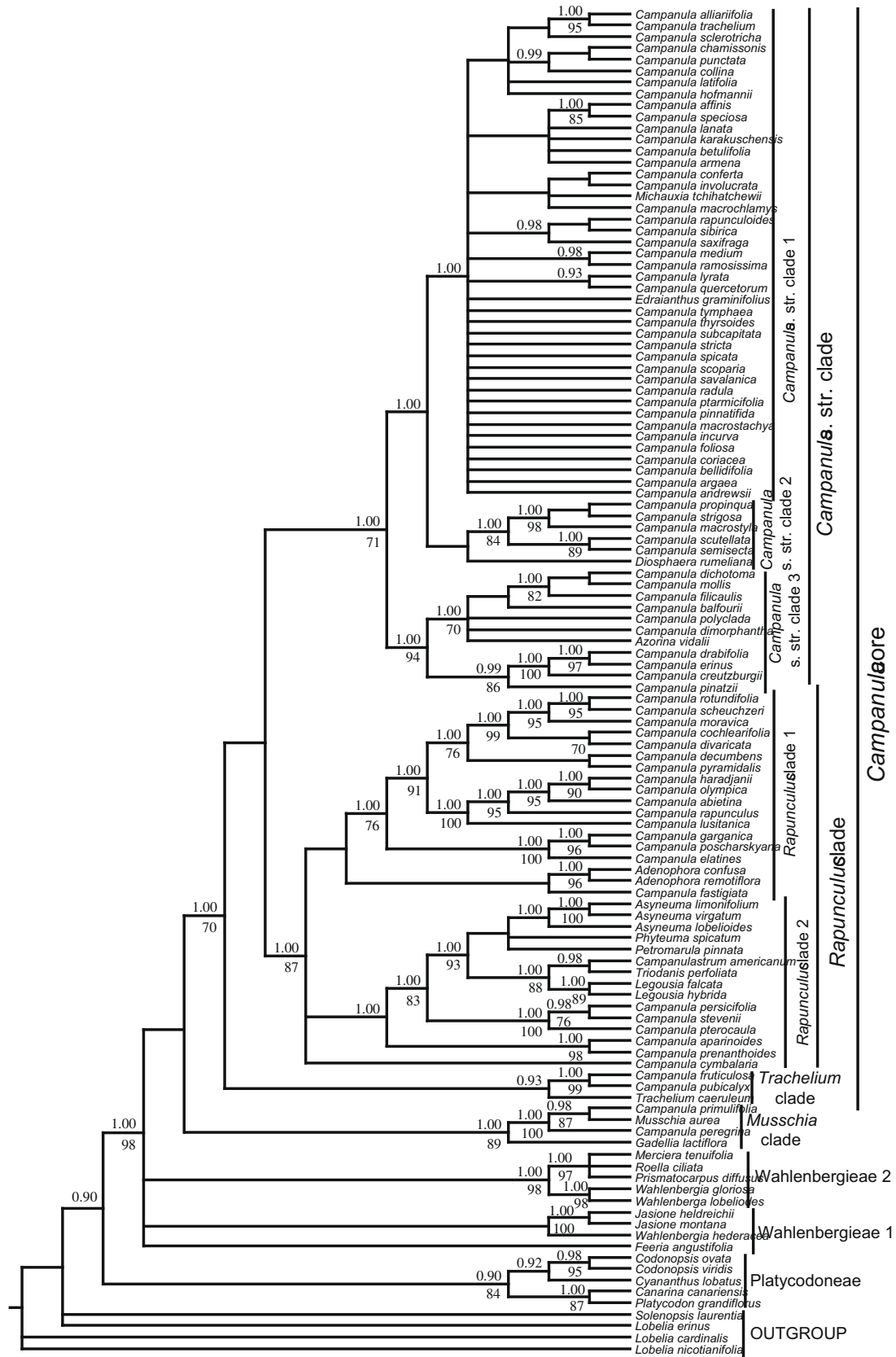
Fig. 2. Majority-rule consensus tree obtained from MCMC Bayesian analysis of the combined matrix (*rbcL* plus *trnL-F* data). Numbers above branches indicate Bayesian-credibility values (PP) (indicated only if greater than 0.90); numbers below branches indicate parsimony BS (indicated only if greater than 70%).

trees in the Bayesian sample where the node is present are used in the summary reconstructions. This means that Bayes-DIVA recon-struction (i.e. pie charts in Fig. 3) conducts a "node-by-node recon-struction" (Nylander et al., 2008).

3. Results

3.1. Phylogenetic results of rbcL, trnL-F and combined data

Bayesian and Maximum Parsimony analyses yielded the same topology in the sense that relationships that were strongly supported (>70% BS; >0.90 PP) in one analysis were also found in the other. Independent and combined matrices yielded totally congruent results. Moreover, no significant incongruence between the two datasets was detected with the partition homogeneity test (P = 0.60). Fig. 2 shows the topology of the majority rule consensus tree from the Bayesian analysis of the combined matrix. Branch support (BS and PP) is also shown in Fig. 2. Numeric results of the analyses of the combined matrix are summarized in Table 3. Our results confirm previous relationships within Campanulaceae based on cpDNA gene order (Cosner et al., 2004), ITS (Eddie et al., 2003; Park et al., 2006; Roquet et al., 2008), trnL-F and ITS plus trnL-F combined data (Roquet et al., 2008). Campanulaceae s. str. (sensu Kovanda, 1978; Cosner et al., 2004) is confirmed as a natural, monophyletic group, formed by all genera traditionally ascribed to tribes Platycodoneae, Campanuleae and Wahlenbergieae.

The tribe Platycodoneae appears as the sister-clade to the remaining Campanulaceae (Campanuleae and Wahlenbergieae), which constitute a monophyletic group (1.0 PP, 98% BS, Fig. 2), in agreement with cpDNA rearrangement data (Cosner et al., 2004). The analyses also show a monophyletic group within Campanuleae, the Campanula core (1.0 PP, 70% BS, Fig. 2), which is divided mainly into the Rapunculus clade and the Campanula s. str. clade plus the Trachelium clade. However, the analyses failed to recognize the monophyly of the Campanuleae and of the Wahlenbergieae, with the two tribes appearing inter-graded. The Wahlenbergieae are divided into two main branches that form a polytomy with the Campanula core and the Musschia clade: the branch of Jasione and Wahlenbergia hederacea plus Feeria Buser and the clade formed by Wahlenbergia gloriosa, W. lobelioides, Roella L., Prismatocarpus and Merciera (Fig. 2).

The Campanula core appears divided into the Campanula s. str. clade (1.0 PP, 71% BS, Fig. 2) and the Rapunculus clade (1.0 PP, 87% BS, Fig. 2). Unfortunately, the combination of rbcL and trnL-F could not resolve the position of the Trachelium clade with respect to Campanula: there is no bootstrap support for its placement in the phylogeny in respect to the two main clades of Campanula (Fig. 2).

The Rapunculus clade includes two main subclades. The first one, the Rapunculus clade 1, includes a central core of species and one basal clade, which corresponds to two species belonging to the genus Adenophora and Campanula fastigiata Dufour ex Schult. The core is formed by the assembly of the subsection Isophylla and the sections Heterophylla (Witas.) Fed. and Rapunculus (Fourr.) Boisier (1.0 PP, 76% BS, Fig. 2). The Rapunculus clade 2 shows a heterogeneous core (1.0 PP, 76% BS, Fig. 2) including taxa

distinct in morphology, distribution and habitat: Asyneuma, Petromarula, Phyteuma, Legousia, Triodanis and Campanulastrum. The groups of Campanula prenanthoides Durand, C. aparinoides Pursh and C. stevenii M. Bieb. branch off sister to these taxa (Fig. 2).

The Campanula s. str. clade has good support (1.0 PP, 71% BS, Fig. 2) and includes Azorina, Diosphaera, Edraianthus, Michauxia and many species of Campanula. It shows three main subclades: the large Campanula s. str. clade 1 including the core of Campanula s. str. and the genera Edraianthus and Michauxia (1.0 PP, Fig. 2); a second subclade (Campanula s. str. clade 2) formed by some species of the subgenus Megalocalyx plus the monotypic subgenus Sicyocodon (Feer) Damboldt (1.0 PP, 84% BS, Fig. 2) and the branch of Diosphaera rumeliana (Hampe) Bornm.; and a third subclade (Campanula s. str. clade 3) formed by Azorina, a part of Campanula subgenus Megalocalyx Damboldt, the subgenus Roucela (Feer) Damboldt, the group of C. mollis L. plus C. dimorphantha Schweinf. and C. polyclada Rech. f. & Schiman–Czieka (1.0 PP, 94% BS, Fig. 2).

3.2. Biogeographical and dating analyses

The ages estimated from combined data with PL and BRC yielded similar results with few exceptions, and credibility intervals largely overlap. All calibrations produced almost coincident results except for the Platycodoneae clade (Canarina, Codonopsis, Cyananthus and Platycodon) and the root node (see Table 2). Optimal smoothing levels (λ) obtained with the PL approach for 100 different Bayesian trees varied considerably, from 0.032 to 320, but most of them (80%) were intermediate (between 0.32 and 3.2), suggesting a moderate rate heterogeneity among lineages (Sanderson, 2002). Mean age estimates and their credibility intervals for each node and dating method are given in Annex 1 (Supplementary material). Table 2 reports dating results for the nodes discussed in the text.

The Bayes-DIVA analysis suggests a complex biogeographical history involving several events of intercontinental dispersal and a history of rapid diversification in the Mediterranean Basin (Fig. 3). Bayes-DIVA analysis (Fig. 3) indicates considerable ambiguity regarding the origin of the Campanulaceae. Basal diversification (the split between Platycodoneae and Campanuleae–Wahlenbergieae) is dated around the Late Oligocene–Early Miocene (37.4–23.5 mya, node 96, Fig. 3, Table 2). The ancestral area of the Platycodoneae is reconstructed as Asia or Asia plus Macaronesia, with the African–Macaronesian genera Canarina and the Eastern Asian Platycodon as the sister group of the Central Asian Cyananthus and Codonopsis. Diversification within this tribe is dated as starting c. 26.3–15.8 mya (node 17, Fig. 3, Table 2).

According to our reconstruction, the ancestor of Campanuleae and Wahlenbergieae could have originated around the Middle Miocene (16–16.7 mya; node 95, Fig. 3, Table 2), in the Western Mediterranean region or in Western Mediterranean and Anatolia (Fig. 3).

Ancestral reconstructions for the Musschia clade point to an Anatolian ancestor that extended its distribution to the whole Mediterranean and Macaronesian areas between the late Miocene and the Pliocene (node 23, Fig. 3, Table 2). Ancestral area reconstructions for the Campanula core suggest also a Mediterranean ancestor, with only Western Asia or Western Asia combined with the whole Mediterranean and North Africa as the most likely ancestral areas (Fig. 3). Divergence times place the start of the diversification within the Campanula core (i.e. the separation of the Trachelium, Campanula s. str. and Rapunculus clades) during the Middle Miocene (14.7–13.7 mya, node 93, Fig. 3, Table 2).

The ancestor of the Trachelium clade is reconstructed as distributed in two or more Mediterranean areas, with first diversification in the Middle Miocene (Fig. 3, Table 2). This genus is found in the Western Mediterranean and North Africa. Biogeographic analyses

Table 3
Numeric results from combined data. Consistency and retention indices and divergence were calculated excluding non-informative characters. ∗ RAM limit computer was reached at this value.

Data set	Combined (rbcL + trnL-F)
Total characters	2442
Informative characters	556
Number of taxa	118
Number of MPTs found	109,000
Number of steps	1735
Consistency index	0.5572
Retention index	0.7654
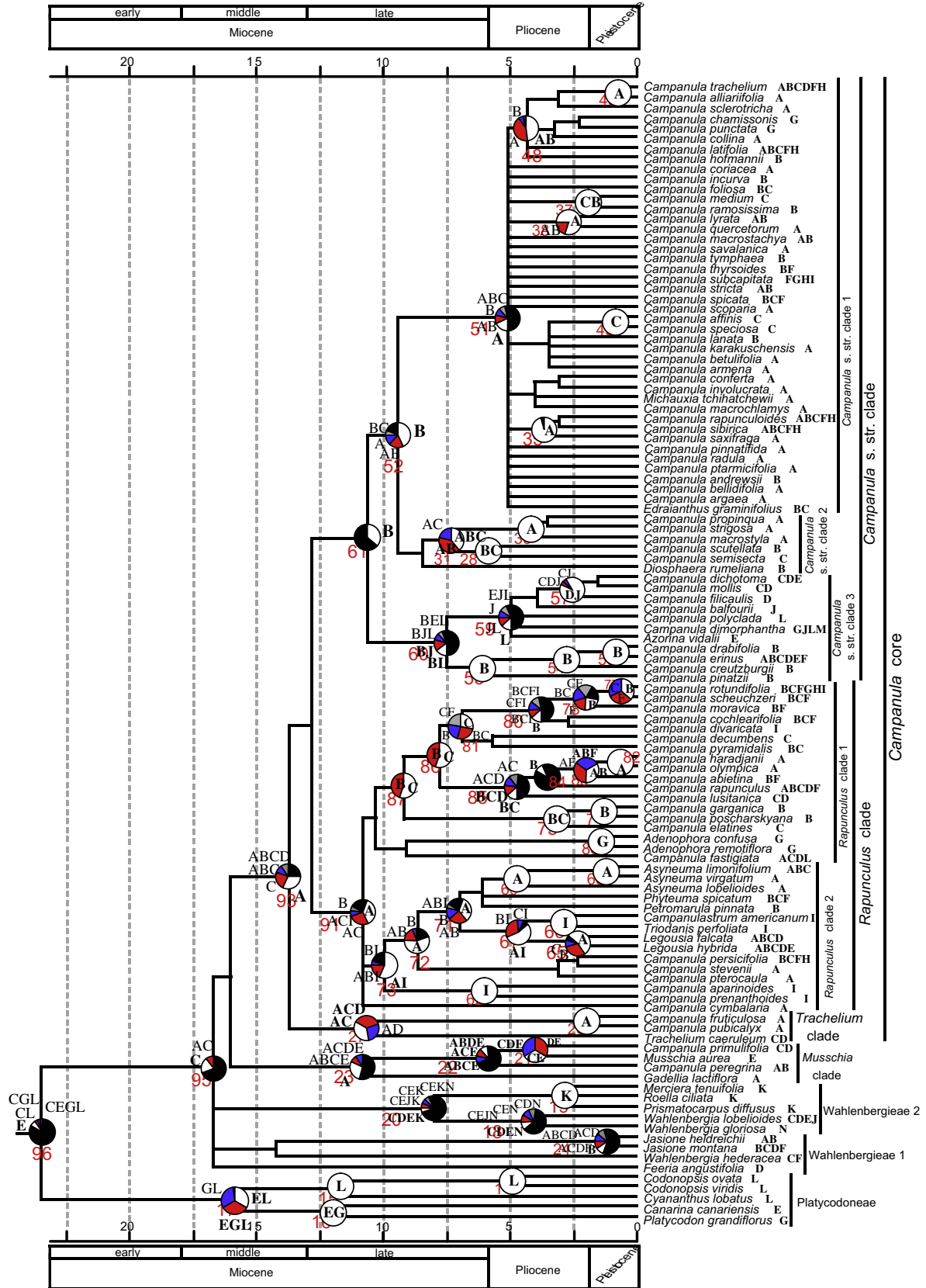
Fig. 3. Chronogram obtained with Bayesian Relaxed Clock (BRC) dating of the combined data. The topology corresponds to the majority rule consensus tree of the Bayesian stationary sample. Numbers in red below branches indicate the node number; they refer to Table 2. The pie charts represent the relative probability of ancestral areas reconstructed for each node over a Bayesian stationary sample of trees. The first four areas with highest probability are colored according to relative probability in the following order: white > red > blue > gray; and the black portion represents reconstructions with a probability <0.10. Letters next to pie charts and terminals correspond to areas of distribution with codes as in Fig. 1. Ancestral area reconstructions are only indicated for nodes with phylogenetic support (PP equal or greater than 0.90, and/or BS equal or greater than 70%).

suggest an origin in Western Asia combined with Western Mediterranean and/or North Africa. However, depending on the molecular marker used, *Trachelium* is situated in very different positions: basal to the *Rapunculus* clade (*rbcL*), sister to the *Campanula* s. str. clade (ITS, Roquet et al., 2008); or as the sister group of both clades *Rapunculus* and *Campanula* s. str. (*trnL-F*; *trnL-F* plus *rbcL* combined, Figs. 2 and 3). The incongruent phylogenetic signal between the three markers could be attributed to past hybridization events between species belonging to the two main *Campanula* clades.

The Eastern Mediterranean region is the most probable ancestral area for the *Campanula* s. str. clade (node 61, Fig. 3), whereas the most favored reconstruction for the *Rapunculus* clade (node 91 Fig. 3) is Western Asia. Main diversification within these subclades started in the Late Miocene (10.6–12.6 mya for the *Campanula* s. str. clade, node 61, Fig. 3, Table 2; 11.5–10.8 mya for the *Rapunculus* clade, node 91, Fig. 3, Table 2).

The *Campanula* s. str. clade 1 is assigned to Western Asia as the most probable area, followed by Eastern Mediterranean plus Western Asia; only Eastern Mediterranean; and the three areas combined as other possible ancestral distributions (Fig. 3). Divergence times place the start of this radiation in the Late Miocene–Pliocene (5.1–9.6 mya, node 51, Fig. 3, Table 2). The other two smaller clades of *Campanula* s. str. are assigned to a large combination of areas: clade 2 is reconstructed as having a Mediterranean origin (node 31, Fig. 3), whereas clade 3 shows a possible vicariance between Eastern Mediterranean and Eastern Africa and/or Central Asia (node 60, Fig. 3). Within *Rapunculus*, the origin of clade 1 is assigned to Western or Eastern Mediterranean, whereas the most probable ancestral area reconstruction indicates an Anatolian ancestor that reached North America.

It is interesting to note that several taxa within the *Rapunculus* clade 2 exhibit large differences in flower morphology (*Asyneuma*, *Campanulastrum*, *Legousia*, *Petromarula* and *Phyteuma*). The separation of these taxa is dated as 8.7–9.3 mya (node 71, Fig. 3, Table 2), whereas the split between the ancestor of *Campanulastrum*, *Triodanis* and *Legousia* is dated as 3.8–4.6 mya, (node 67, Fig. 3, Table 2).

4. Discussion

4.1. Origin of Platycodoneae, Wahlenbergieae and Campanuleae

Previous molecular phylogenies of the Asterales (e.g., Cosner et al., 1994; Gustafsson and Bremer, 1995; Bremer and Gustaffsson, 1997; Lundberg and Bremer, 2003) indicate that this order is composed of several families/subfamilies including Campanulaceae, Stylidiaceae and Lobeliaceae, many of which have a Southern Hemisphere distribution. The apparently African origin (Eddie et al., 2003) of the Lobeliaceae (included within Campanulales), as well as the mainly Southern Hemisphere distribution of the tribe Wahlenbergieae, have led some authors to hypothesize that the Southern Hemisphere is also the ancestral area for the Asterales (Bremer and Gustaffsson, 1997).

Our phylogenetic hypothesis confirms that the Platycodoneae (including all taxa with colporate or colpate pollen grains, i.e. *Canarina*, *Codonopsis*, *Cyananthus*, and *Platycodon*) are sister to the rest of Campanulaceae in agreement with molecular data (Eddie et al., 2003; Cosner et al., 2004) and morphological data (Thulin, 1975). This tribe is distributed in two main areas: (1) Eastern Africa and Macaronesia (*Canarina*) and (2) Central and Eastern Asia (*Codonopsis*, *Cyananthus* and *Platycodon*). Cosner et al. (2004) proposed an Asian origin for the Platycodoneae based on the position of the genus *Platycodon* within the tribe. Despite some ambiguity, our results suggest also an Asian origin for the tribe, with possible dispersal to the African region in *Canarina*, or an Asian–African

origin. The small genus *Canarina* is distributed in Eastern Africa (*C. abyssinica* Engl., *C. eminii* Asch. & Schweinf.) and Macaronesia [*C. canariensis* (L.) Vatke]. Although no African species of *Canarina* could be included in this work, a new molecular study (Olessen, unpublished) suggests that the two African species are the sister group of the Macaronesian *C. canariensis*. Considering geographic distance and the recent origin of the Macaronesia archipelago (maximum 20 mya, Abdel-Monem et al., 1971, 1975; Ferreira et al., 1988) it seems more plausible to hypothesize that *Canarina* originated in Africa (from Asian ancestors) and later dispersed to Macaronesia. Alternatively, *Canarina canariensis* can be a relict derived from an extinct species from a continuous, cross African flora.

The combined tree shows the Wahlenbergieae and Campanuleae inter-graded (see also Cosner et al., 2004). According to Eddie et al. (2003), Campanuleae should include genera of the Northern Hemisphere, while Wahlenbergieae should contain mainly Southern Hemisphere taxa. Thulin's definition (1975) of Wahlenbergieae includes the genera *Merciera*, *Microcodon* A. DC., *Prismatocarpus*, *Rhigiophyllum* Hochst., *Roella* and *Treichelia* Vatke (all endemic to South Africa), *Heterochaenia* A. DC. (Mascarene Islands), *Gunillaea* Thulin (tropical Africa and Madagascar), *Craterocapsa* Hilliard and B.L. Burtt (SE tropical Africa) and *Wahlenbergia*, with 81% of species in Africa, 13% in Australasia, and the rest in Southern China, Japan, India, South America and a few Pacific islands (Lammers, 1992). Other taxa sometimes included in Wahlebergieae (Kovanda, 1978; Yeo, 1993) are: *Jasione* (circum-Mediterranean), *Feeria* (North-Western Africa) and *Wahlenbergia hederacea* L. (which does not form a monophyletic group with the other *Wahlenbergia* species included in this study). Our combined phylogeny shows Wahlenbergieae divided into two main clades: the branch formed by *Wahlenbergia lobelioides*, *W. gloriosa*, *Roella*, *Merciera* and *Prismatocarpus*, mainly distributed in the Southern Hemisphere, and a clade comprising *Jasione* and *Wahlenbergia hederacea*, plus the branch of *Feeria*, which are mainly distributed in the Mediterranean Basin. Bayes-DIVA reconstructions for these two clades are ambiguous, but in general the ancestral area reconstructions for the *Jasione* clade favors the whole Mediterranean Basin as the ancestral area, whereas all possible reconstructions for the *Wahlenbergia* clade include South Africa as part of the ancestral area – the genus *Wahlenbergia* is specially rich in species in this area (150 species, Fig. 1). The ancestor of Wahlenbergieae and Campanuleae is most likely reconstructed as being of Western Mediterranean or Western Mediterranean plus Western Asian origin, and the first split between Wahlenbergieae and Campanuleae would have occurred in the Early–Middle Miocene (node 95, Fig. 3, Table 2).

According to the geographic distributions of Platycodoneae, Wahlenbergieae and Campanuleae, the ancestor of the family Campanulaceae could have originated in Africa and migrated to Asia, or diverged first in the Asian continent, dispersing later to Africa and the Mediterranean region. Similar movements between Asia and Africa, generally involving the Middle East and Levante regions, have been found in many other groups of organisms, including plants (Mummenhoff et al., 2001; Oberprieler, 2005; Inda et al., 2008; Mansion et al., 2008) mammals (Vrba, 1993; Cox and Moore, 2005) and passerine birds (Voelker, 1999; Nylander et al., 2008). These dispersal events have been attributed to the formation of a new dispersal route between Eurasia and Africa across the Levante region following the Mid-Miocene collision (16 mya) of the Arabian Plate with Eurasia (Sanmartín, 2003; Oberprieler, 2005). They could also have resulted from the cooling and drying trends in the climate during the Late Neogene that led to the development of open, steppe habitats in South-Western Asia and Eastern Africa (Axelrod and Raven, 1972, 1978; Retallack et al., 1990; Vrba, 1993; Jacobs, 1999; Fernandes et al., 2006) similar to those where many *Campanula* species are found today. Thus, early diversification in Campanulaceae (the split between Platycodoneae and

Wahlenbergieae–Campanuleae is dated as 37.4–23.5 mya, node 96, Fig. 3, Table 2) may have been triggered by geographic expansion into new areas during the Neogene, favored in turn by the availability of a new dispersal route between Africa and Eurasia via Arabia and the expansion of grassland biomes in these regions from the Miocene onwards.

4.2. Diversification within the Campanula core

The split of the three main clades in the *Campanula* core (*Campanula* s. str., *Rapunculus* and *Trachelium*) is dated as Middle Miocene (14.7–13.7 mya), coincident with the gradual cooling of the climate that began 15 mya and lasted until 13 mya (Flower and Kennett, 1994). These cooling trends led to an important sea level fall 14.8–11.2 mya associated to the rapid expansion of the Antarctic Ice sheet (Rögl and Steininger, 1984). This fall closed the Tethyan seaway, connecting the current Mediterranean Sea and the Indian Ocean, increasing the land in the Eastern Mediterranean and connecting areas previously separated in the Mediterranean, the Caucasus and the Western and Central Asia. The rising of mountains in these new continental sheets may have favored the diversification and expansion of the ancestors of *Campanula*. Increasingly cooler climates from the Middle Miocene onwards may also have favored diversification within *Campanula*, a cold-adapted genus whose highest species richness occurs in high steppes and mountain ranges (Fedorov, 1957).

The main clades of *Campanula*, *Rapunculus* and *Campanula* s. str., seem to have originated and evolved in the Eastern part of the Mediterranean Basin – the Balkan Peninsula in the case of *Campanula* s. str. and the Anatolian region in the case of *Rapunculus* – around the same period (10.6–12.6 mya, node 61; 10.8–11.5 mya, node 91; Fig. 3, Table 2), these two areas sharing today a high number of Campanuleae species (Fig. 1). The main subclade in *Campanula* s. str., a large non-resolved polytomy of perennial and monocarpic species, also originated in Western Asia and/or the Balkan Peninsula during the Late Miocene (9.6–5.1 mya, node 51, Fig. 3, Table 2), from where it dispersed to other regions. Two reasons may have favored diversification in this area: (1) The strategic position of Anatolia between Europe and Asia has probably made this area a crossroad from which species could migrate to North Africa, Central-Northern Europe and Asia. (2) The intense orogenic activity that took place in Western Asia during the Late Miocene onwards – as a result of the collision of the Arabian Plate with Eurasia – would have favored isolation of lineages promoting allopatric speciation (Sanmartín, 2003). Moreover, the fact that Pleistocene glaciations affected Western Asia to a limited extent could have favored Anatolia as a refugial area for many temperate organisms (Davis, 1965).

The low phylogenetic resolution of *Campanula* s. str. clade seems not to be due to too slow-evolving markers, as Roquet et al. (2008) obtained a similar topology with the fast-evolving region ITS. The big polytomy in *Campanula* s. str. clade 1 can be explained by a rapid radiation in Western Asia, from 5 to 10 mya, a time with intense orogenic activity, due to the movements of the Arabian plate (Quennell, 1984; Steininger and Rögl, 1984). Climate changing trends might have affected insect communities, which seem to have exercised a strong selective pressure in floral aspects of *Campanula* (Roquet et al., 2008). Pollinator availability (e.g., butterflies, bumblebees, sirphid flies) would have selected in favor of different flower morphologies in the three main clades: tubular corollas in the *Trachelium* clade; hanging-campanulate corollas, predominant in the *Campanula* s. str. clade; and the heterogeneous shaped-corollas found in the *Rapunculus* clade (Roquet et al., 2008).

The diversification of the clades *Campanula* s. str. 2 and 3 dates back to the late Miocene (nodes 31 and 60, Fig. 3, Table 2). Most species included in the clade 3 are dry-tolerant annuals and

short-lived perennials, and some of them (the species from the subgenus *Roucela*: *C. creutzburgii* Greuter, *C. drabifolia* Sibth. and Sm., *C. erinus* L. and *C. pinatzii* Greuter and Phitos) are endemic to the Aegean islands and the Greek and Turkish coasts. Apart from that, clade 3 includes two pairs of East–West disjunct taxa such as *Campanula polyclada* (Iran-Pakistan), sister to *Azorina vidalii* (Azores), and *C. balfourii* Wagner and Vierh. (Socotra) sister to the clade of *C. mollis*, *C. filicaulis* Dur. and *C. dichotoma* L. (North-Western Africa and South-Western Europe). These patterns can indicate that these species are derived from a widespread ancestor that disappeared from North Africa due to climatic fluctuations in this area. As indicated before, *Canarina*, with two species in Eastern Africa and one in Macaronesia, presents a similar disjunct pattern. There are at least 15 genera or sections of angiosperms that exhibit similar geographical disjunctions between Macaronesia/Mediterranean Basin and East/South Africa/Southern Arabia (reviewed by Andrus et al., 2004). The increase in precipitation during certain periods of the Pliocene would have wiped out a great part of the desert and permitted the exchange and expansion of species along areas now isolated by the Sahara (Quézel, 1978).

4.3. Migration to North America

At least four arrivals to North America are inferred within the *Rapunculus* clade. One concerns the ancestor of *Campanula prenanthoides* and *C. aparinoides*: the first is native to slopes near coniferous forests of Western North America and the second is found in wetlands of the Northern Great Plains. These two sister-species are placed basally within the *Rapunculus* clade 2, whose ancestor is reconstructed as already widespread in Western Asia and North America 10.8–11.5 mya (node 91, Fig. 3, Table 2). Migration between Asia and North America could have been possible via the Beringian Land Bridge (Wolfe, 1975; Tiffney, 1985; Sanmartín et al., 2001), which began to cool down significantly from the Middle Miocene (15 mya) onwards (Milne, 2006). Many *Campanula* taxa are adapted to cool and dry conditions. Increasing cooling of the Beringian Land Bridge in the Late Miocene would have lead to vicariance speciation of Old and New World populations of the *Rapunculus* clade 2 around 10–11 mya, coinciding with the most prominent temperature decrease of the Miocene (Mosbrugger et al., 2005).

A recent example of dispersal to North America is the circumpolar *Campanula rotundifolia* L. (node 77, Fig. 3), a very polymorphic species characterized by polyploidy superimposed on segmental chromosome rearrangements and with numerous infraspecific taxa (Bocher, 1960), which may have been promoted by glacial–interglacial cycles, when population underwent episodes of isolation in small enclaves during periods of extremely adverse conditions (Murray, 1995).

At least one long-distance dispersal event from the Mediterranean area to North America seems to have occurred in the *Rapunculus* clade 2: the ancestor of the Mediterranean–Asian *Legousia*, and the North-American endemics *Triodanis* and *Campanulastrum* would have dispersed from the Mediterranean region to North America. *Legousia* is a drought-tolerant annual, with rotate autogamous flowers which presents a large distribution in Eurasia and Africa. The separation of *Legousia* from the ancestor of *Campanulastrum* and *Triodanis* is dated here as 3.8–4.6 mya (node 67, Fig. 3, Table 2), when the terrestrial connection between Asia and North America did not existed anymore (Milne, 2006). Similarly, the Eastern North America distribution of *Campanula divaricata* Michx. and its recent age of origin (3.7–4.5 mya, node 80, Fig. 3, Table 2) suggests another long-distance dispersal event within the *Rapuculus* clade.

Only plant species bearing seeds with very low falling velocity (e.g., plumed seeds and dust-like seeds) are dispersed over long

distances in appreciable numbers (Tackenberg et al., 2003). The seeds of bellflowers (Campanulaceae) seem to be streamlined to facilitate wind dispersal (Emig and Leins, 1996; Maier et al., 1999; Kuss et al., 2007). They are dust-like seeds, egg or spindle-shaped, somewhat compressed and in some taxa with a narrow wing. They are released from capsules by wind under dry conditions, and a part of them fall on leaves, bracts or sepals if the wind speed and turbulence are weak.

Although seeds are very similar in all Campanula species (Shetler and Morin, 1986), only members of the Rapunculus clade are present nowadays in North-America. This clade presents much more heterogeneity in chromosome numbers, basic numbers and ploidy levels than Campanula s. str. clade (Gadella, 1964). Stebbins (1966) pointed out that aneuploid reduction in chromosome number and polyploidy are characteristic of many species groups which occupy pioneer habitats. These characteristics might be one of the factors involved in the success of this group to settle several times in the New World.

5. Conclusions

Biogeographic and dating analyses suggest that Western Asia and the Eastern Mediterranean region (Anatolia, Balkans) played a major role as centers of migration and diversification within the Campanula alliance. This is probably explained by the intense orogenic activity that took place in this region during the Late Neogene, and which could have promoted isolation and allopatric speciation within/among lineages. This result confirms similar trends found in other plant groups (e.g., Oberprieler, 2005; Fiz et al., 2008; Inda et al., 2008; Mansion et al., 2008) pointing out the role of SW Asia and the Eastern Mediterranean Basin as evolutionary cradles for lineage diversification.

Rates of species diversification within four of the five Campanula clades (Campanula s. str. clades 1 and 3, and Rapunculus clades 1 and 2) seem to have increased during the Messinian salinity crisis. During this period, drought and erosion were more intense and may have promoted diversification in annual, xeromorphic and other pioneer lineages (Bocquet et al., 1978; Kellogg, 2001). These climate changes and the expansion of mountainous regions probably would have led the ancestors of several Campanula lineages to adapt to disturbed, dry or cold environments.

Finally, our study confirms a complex biogeographic history of Campanula, with repeated events of allopatric diversification, followed by range expansion and posterior isolation to give rise to new endemisms, as well as several long-distance (independent) dispersal events to Macaronesia and North America, the latter restricted to the Rapunculus clade and species with high ploidy levels.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.05.014.

References

Abdel-Monem, A.A., Watkins, N.D., Gast, P.W., 1971. Potassium–argon ages, volcanic stratigraphy, and geomagnetic polarity history of the Canary Islands; Lanzarote, Fuerteventura, Gran Canaria, and La Gomera. Am. J. Sci. 271, 490–521.

Abdel-Monem, A.A., Fernández, L.A., Boone, G.M., 1975. K–Ar ages from the eastern Azores group (Santa Maria, São Miguel and the Formigas Islands). Lithos 8, 247–254.

Andrus, N., Trusty, J., Santos-Guerra, A., Jansen, R.K., Francisco-Ortega, J., 2004. Using molecular phylogenies to test phytogeographical links between East/South Africa–Southern Arabia and the Macaronesian islands: a review, and the case of Vierea and Pulicaria section Vieraeopsis (Asteraceae). Taxon 53, 333–346.

Axelrod, D.I., Raven, P.H., 1972. Evolutionary biogeography viewed from plate tectonic theory. In: Behnke, J.A. (Ed.), Future Directions in the Life Sciences. American Institute of Biological Sciences, Washington, DC, pp. 218–236.

Axelrod, D.I., Raven, P.H., 1978. Late Cretaceous and Tertiary vegetation history of Africa. In: Werger, M.J.A. (Ed.), Biogeography and Ecology of Southern Africa, vol. 1. Junk Publishers, The Hague, pp. 77–130.

Bocher, T.W., 1960. Experimental and cytological studies on plant species. V. The Campanula rotundifolia complex. K. Dan. Vidensk. Selsk. Biol. Skr. 11, 1–69.

Bocquet, G., Widler, B., Kiefer, H., 1978. The Messinian Model. A new outlook for the floristics and systematics of the Mediterranean area. Candollea 33, 269–287.

Bremer, K., Gustaffsson, M.H.G., 1997. East Gondwana ancestry of the sunflower alliance of families. Proc. Natl. Acad. Sci. USA 94, 9188–9190.

Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R.G., Anderberg, A.A., Kallersjo, M., Barkhordarian, E., 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. Mol. Phylogenet. Evol. 24, 274–301.

Cosner, M.E., Jansen, R.K., Lammers, T.G., 1994. Phylogenetic relationships in the Campanulales based on rbcL sequences. Plant Syst. Evol. 190, 79–95.

Cosner, M.E., Raubeson, L.A., Jansen, R.K., 2004. Chloroplast DNA rearrangements in Campanulaceae: phylogenetic utility of highly rearranged genomes. BMC Evol. Biol. 4, 1471–2148.

Cox, B.C., Moore, P.D., 2005. Biogeography: An Ecological and Evolutionary Approach. Blackwell Science, London.

Culling, K.W., 1992. Design and testing of plant-specific PCR primer for ecological and evolutionary studies. Mol. Ecol. 1, 223–240.

Davis, P.H., 1965. Flora of Turkey and East Aegean Islands, vol. 1. Edinburgh Univ. Press, Edinburgh.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, 699–710.

Dunbar, A., 1975. On pollen of Campanulaceae and related families with special reference to the surface ultrastructure. I. Campanulaceae subfam. Campanuloideae. Bot. Not. 128, 73–101.

Eddie, W.M., Cupido, C.N., 2001. Some observations on the reproductive morphology of the wahlenbergioid genera of the family Campanulaceae s. str. from the fynbos vegetation of South Africa. In: Botany 2001 Abstracts. Botanical Society of America, Albuquerque, p. 111.

Eddie, W.M., Shulkina, T., Gaskin, J., Haberle, R.C., Jansen, R.K., 2003. Phylogeny of Campanulaceae s. str. inferred from ITS sequences of nuclear ribosomal DNA. Ann. Mo. Bot. Gard. 90, 334–375.

Emig, W., Leins, P., 1996. Ausbreitungsbiologische Untersuchungen in der Gattung Campanula L. I. Vergleichende Windkanalexperimente zur Samenportionierung bei C. trachelium, C. sibirica und C. glomerata. Bot. Jahrb. Syst. Pflanzengesch. Pflanzengeogr. 116, 243–257.

Farris, J.S., Källersjö, M., Kluge, A.M., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.

Fay, M.F., Swensen, S.M., Chase, M.W., 1997. Taxonomic affinities of Medusagyne oppositifolia (Medusagynaceae). Kew Bull. 52, 111–120.

Fay, M.F., Bayer, C., Alverson, S., de Bruijn, A.Y., Chase, M.W., 1998. Plastid rbcL sequence data indicate a close affinity between Diegodendron and Bixa. Taxon 47, 43–50.

Fedorov, A.A., 1957. Campanulaceae. In: Shishkin, B.K. (Ed.), Flora SSSR, vol. 24. Akademii Nauk SSSR, Moscow, pp. 126–450.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Fernandes, C.A., Rohling, E.J., Siddall, M., 2006. Absence of post-Miocene Red Sea land bridges: biogeographic implications. J. Biogeogr. 33, 961–966.

Ferreira, M., Macedo, C., Ferreira, J., 1988. K–Ar geochronology in the Selvagens, Porto Santo and Madeira islands (Eastern Central Atlantic): a 30 m.y. spectrum of submarine volcanism. Lunar Planet. Sci. (Abstr.) 19, 325–326.

Fiz, O., Valcárcel, V., Vargas, P., 2008. Phylogenetic position of Mediterranean Astereae and character evolution of daisies (Bellis, Asteraceae) inferred from nrDNA ITS sequences. Mol. Phylogenet. Evol. 25, 157–171.

Flower, B.P., Kennett, J.P., 1994. The middle Miocene climatic transition: East Antarctic ice sheet development, deep ocean circulation and global carbon cycling. Palaeogeogr. Palaeoclimatol. Palaeoecol. 108, 537–555.

Gadella, T.W.J., 1964. Cytotaxonomic studies in the genus Campanula. Wentia 11, 1–104.

Gustafsson, M.H.G., Bremer, K., 1995. Morphology and phylogenetic interrelationships of the Asteraceae, Calyceraceae, Campanulaceae, Goodeniaceae, and related families (Asterales). Am. J. Bot. 82, 250–265.

Hong, D.-Y., 1995. The geography of the Campanulaceae: on the distribution centers. Acta Phytotaxonom. Sinica 33, 521–536.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294, 2310–2314.

Hug, L.A., Roger, A.J., 2007. The impact of fossils and taxon sampling on ancient molecular dating analyses. Mol. Biol. Evol. 24, 1889–1897.

Ihaka, R., Gentlemen, R., 1996. R: a language for data analysis and graphics. J. Comp. Graph. Stat. 5, 299–314.

Inda, L.A., Segarra-Moragues, J.G., Müller, J., Peterson, P.M., Catalán, P., 2008. Dated historical biogeography of the temperate Loliinae (Poaceae, Pooideae) grasses in the northern and southern hemispheres. Mol. Phylogenet. Evol. 46, 932–957.

Jacobs, B.F., 1999. Estimation of rainfall variables from leaf characters in tropical Africa. Palaeogeogr. Palaeoclimatol. Palaeoecol. 145, 231–250.

Kellogg, E.A., 2001. Evolutionary history of the grasses. Plant Physiol. 125, 1198–1205.

Kishino, H., Thorne, J.L., Bruno, W.J., 2001. Performance of a divergence time estimation method under of probabilistic model of rate evolution. Mol. Biol. Evol. 18, 352–361.

Kluge, A.G., Farris, J.S., 1969. Quantitative phyletics and the evolution of Anurans. Syst. Zool. 40, 315–328.

Kolakovsky, A.A., 1986. Carpology of the Campanulaceae and problems in their taxonomy. Bot. Zhur. 71, 1155–1166.

Kovanda, M., 1978. Campanulaceae. In: Heywood, V. (Ed.), Flowering Plants of the World. Oxford University Press, Oxford, pp. 254–256.

Kress, W.J., Erickson, D.L., 2007. A two-locus global DNA barcode for Land Plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. PLoS Biol. 2, e508.

Kuss, P.H., Aegisdottir, H., Stockling, J., 2007. The biological flora of central Europe: Campanula thyrsoides L. Perspect. Plant Ecol. Evol. Syst. 9, 37–51.

Lammers, T.G., 1992. Circumscription and phylogeny of the Campanulales. Ann. Mo. Bot. Gard. 79, 388–413.

Lancucka-Srodoniowa, M., 1979. Macroscopic plant remains from the freshwater Miocene of the Nowy Sacz Basin (West Carpathians, Poland). Acta Palaeobot. 20, 74–75.

Lidén, M., Fukuhara, T., Rylander, J., Oxelman, B., 1997. Phylogeny and classification of Fumariaceae, with emphasis on Dicentra s. l., based on the plastid gene rps16 intron. Plant Syst. Evol. 206, 411–420.

Linder, H.P., Hardy, C.R., Rutschmann, F., 2005. Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. Mol. Phylogenet. Evol. 35, 569–582.

Loader, C., 1999. Local Regression and Likelihood. Springer Mathematics, New York.

Lopez-Vaamonde, C., Wikström, N., Labandeira, C., Godfray, H.C.J., Goodman, S.J., Cook, J.M., 2006. Fossil-calibrated molecular phylogenies reveal that leaf-mining moths radiated several million years after their host plants. J. Evol. Biol. 19, 1314–1326.

Lundberg, J., Bremer, K., 2003. A phylogenetic study of the order Asterales using one morphological and three molecular data sets. Int. J. Plant. Sci. 164, 553–578.

Maier, A., Emig, W., Leins, P., 1999. Dispersal patterns of some Phyteuma species (Campanulaceae). Plant Biol. 1, 408–417.

Mansion, G., Rosenbaum, G., Schoenenberger, N., Bacchetta, G., Rosselló, J.A., Conti, E., 2008. Phylogenetic analysis informed by geological history supports multiple, sequential invasions of the mediterranean basin by the angiosperm family Araceae. Syst. Biol. 57, 269–285.

Michaels, H.J., Scott, K.M., Olmstead, R.G., Szaro, T., Jansen, R.K., Palmer, J.D., 1993. Interfamilial relationships of the Asteraceae: insights from rbcL sequence variation. Ann. Mo. Bot. Gard. 80, 742–751.

Milne, R.I., 2006. Northern hemisphere plant disjunctions: a window on Tertiary land bridges and climate change? Ann. Bot. 98, 465–472.

Moore, B.R., Smith, S.A., Ree, R.H., Donoghue, M.J., in press. Incorporating fossil data in biogeographic inference: a likelihood approach. Evolution.

Mosbrugger, V., Utescher, T., Dilcher, D.L., 2005. Cenozoic continental climatic evolution of Central Europe. Proc. Natl. Acad. Sci. USA 102, 14964–14969.

Mummenhoff, K., Brüggemann, H., Bowman, J.L., 2001. Chloroplast DNA phylogeny and biogeography of Lepidium (Brassicaceae). Am. J. Bot. 88, 2051–2063.

Murray, D.F., 1995. Causes of arctic plant diversity: origin and evolution. In: Chapin, F.S., III, Körner, C. (Eds.), Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences. Springer, Heidelberg, pp. 21–32.

Nylander, J.A.A., 2004. MrModeltest v2. Program distributed by the author. Uppsala University, Uppsala. Available from: <http://www.ebc.uu.se/systzoo/staff/nylander.html>.

Nylander, J.A.A., Olsson, O., Alström, P., Sanmartín, I., 2008. Accounting for phylogenetic uncertainty in biogeography: a Bayesian approach to dispersal–vicariance analysis of the thrushes (Aves: Turdus). Syst. Biol. 57, 257–268.

Oberprieler, C., 2005. Temporal and spatial diversification of circum-Mediterranean Compositae–Anthemidae. Taxon 54, 951–966.

Olmstead, R.G., Michaels, H.J., Scott, K.M., Palmer, J.D., 1992. Monophyly of the Asteridae and identification of major lineages inferred from DNA sequences of rbcL. Ann. Mo. Bot. Gard. 79, 249–265.

Park, J.-M., Kovacic, S., Liber, Z., Eddie, W.M.M., Schneeweiis, G.M., 2006. Phylogeny and biogeography of isophyllous species of Campanula (Campanulaceae) in the Mediterranean area. Syst. Bot. 31, 862–880.

Quennell, A.M., 1984. The western Arabia rift system. In: Dixon, J.E., Robertson, A.H.F. (Eds.), The Geological Evolution of the Eastern Mediterranean, vol. 17. Blackwell Scientific, Oxford, pp. 775–778.

Quézel, P., 1978. Analysis of the flora of Mediterranean and Saharan Africa. Ann. Mo. Bot. Gard. 65, 479–534.

Raven, P.H., Axelrod, D.I., 1974. Angiosperm biogeography and past continental movements. Ann. Mo. Bot. Gard. 61, 539–673.

Ree, R.H., Smith, S.A., 2008. A maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14.

Ree, R.H., Moore, B.R., Webb, C.O., Donoghue, M.J., 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59, 2299–2311.

Retallack, G.J., Dugas, D.P., Bestland, E.A., 1990. Fossil soils and grasses of a middle Miocene East African Grassland. Science 247, 1325–1328.

Rodríguez, F., Oliver, J.L., Marín, A., Medina, J.R., 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142, 485–501.

Rögl, F., Steininger, F.F., 1984. Neogene Paratethys, Mediterranean and Indo-Pacific Seaways. In: Brenchley, P.J. (Ed.), Fossils and Climate. Wiley, New York, pp. 171–200.

Ronquist, F., 1996. DIVA ver. 1.1. Computer Program and Manual. Uppsala, University Uppsala. Available from: <http://www.systbot.uu.se/personel/f_ronquist.html>.

Ronquist, F., 1997. Dispersal–vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203.

Ronquist, F., 2004. Bayesian inference of character evolution. Trends Ecol. Evol. 19, 475–481.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Roquet, C., Sáez, L., Aldasoro, J.J., Susanna, A., Alarcón, M.L., Garcia-Jacas, N., 2008. Natural delineation, molecular phylogeny and floral evolution in Campanula L. Syst. Bot. 33, 203–217.

Rutschmann, F., 2005. Bayesian molecular dating using PAML/multidivtime. A Step-by-Step Manual. University of Zurich, Switzerland. Available from: <http://www.plant.ch>.

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19, 101–109.

Sanmartín, I., 2003. Dispersal vs. vicariance in the Mediterranean: historical biogeography of the Palearctic Pachydeminae (Coleoptera, Scarabaeoidea). J. Biogeogr. 30, 1883–1897.

Sanmartín, I., Enghoff, H., Ronquist, F., 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. Biol. J. Linn. Soc. 73, 345–390.

Sanmartín, I., van der Mark, P., Ronquist, F., 2008. Inferring dispersal: a Bayesian, phylogeny-based approach to island biogeography, with special reference to the Canary Islands. J. Biogeogr. 35, 428–449.

Schönland, S., 1889. Campanulaceae. In: Engler, A., Prantl, K. (Eds.), Die Natürlichen Pflanzenfamilien, Teil IV, Abteilung 5. Wilhelm Engelmann, Leipzig, pp. 40–70.

Shetler, S.G., Morin, N., 1986. Seed morphology in North American Campanulaceae. Ann. Miss. Bot. Gard. 73, 653–688.

Shulkina, T.V., Gaskin, J.F., Eddie, W.M., 2003. Morphological studies toward an improved classification of Campanulaceae s. str. Ann. Mo. Bot. Gard. 90, 576–591.

Stebbins, G.L., 1966. Chromosomal variation and evolution. Science 152, 1463–1469.

Stefanovic, S., Lakusic, D., Kuzmina, M., Mededovic, S., Tan, K., Stevanovic, V., 2008. Molecular phylogeny of Edraianthus (Grassy Bells; Campanulaceae) based on non-coding plastid DNA sequences. Taxon 57, 452–475.

Steininger, F.F., Rögl, F., 1984. Paleogeography and palinspatic reconstruction of the Neogene of the Mediterranean and Paratethys. In: Dixon, J.E., Robertson, A.H.F. (Eds.), The Geological Evolution of the Eastern Mediterranean. Blackwell Scientific, Oxford, pp. 659–668.

Swofford, D.L., 2002. PAUP∗ Phylogenetic Analysis using Parsimony (∗ and other methods), v.4.0 beta 10. Sinauer Associates, Sunderland.

Tackenberg, O., Poschlod, P., Bonn, S., 2003. Assessment of wind dispersal potential in plant species. Ecol. Monogr. 73, 191–205.

Thorne, J.L., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51, 689–702.

Thorne, J.L., Kishino, H., Painter, I.S., 1998. Estimating the rate of evolution of the rate of molecular evolution. Mol. Biol. Evol. 15, 1647–1657.

Thulin, M., 1975. The genus Wahlenbergia s. lat. (Campanulaceae) in tropical Africa and Madagascar. Acta Univ. Ups. Symb. Bot. Ups. 21, 1–223.

Tiffney, B.H., 1985. The Eocene North Atlantic land bridge: its importance in Tertiary and modern phytogeography of the northern hemisphere. J. Arnold Arbor. 66, 243–273.

Voelker, G., 1999. Molecular evolutionary relationships in the avian genus Anthus (Pipits: Motacillidae). Mol. Phylogenet. Evol. 11, 84–94.

Vrba, E.S., 1993. Mammal evolution in the African neogene and a new look at the great American interchange. In: Goldblatt, P. (Ed.), Biological Relationships between Africa and South America. Yale University Press, New Haven, pp. 393–432.

Wikström, N., Savolainen, V., Chase, M., 2001. Evolution of the angiosperms: calibrating the family tree. Proc. R. Soc. Biol. Sci. Ser. B 268, 2211–2220.

Wolfe, J.A., 1975. Some aspects of plant geography of the Northern Hemisphere during the late Cretaceous and Tertiary. Ann. Mo. Bot. Gard. 62, 264–279.

Yang, Z., 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. Cabios Appl. Notes 13, 555–556.

Yeo, P.F., 1993. Secondary pollen presentation. Form, function and evolution. Plant Syst. Evol. Suppl. 6, 111–129.

Zuckerkandl, E., Paulin, L., 1965. Evolutionary divergence and convergence in proteins. In: Bryson, V., Vogel, H.J. (Eds.), Evolving Genes and Proteins. Academic Press, New York, pp. 97–166.