



Historical biogeography of the endemic Campanulaceae of Crete

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ABSTRACT

Aim The clade Campanulaceae in the Cretan area is rich in endemics, with c. 50% of its species having restricted distributions. These species are analysed in the context of a larger phylogeny of the Campanulaceae. Divergence times are calculated and hypotheses of vicariance and dispersal are tested with the aim of understanding whether Cretan lineages represent remnants of an older continental flora.

Location The Cretan area: Crete and the Karpathos Islands (Greece).

Methods We obtained chloroplast DNA sequence data from *rbcL*, *atpB* and *matK* genes for 102 ingroup taxa, of which 18 are from the Cretan area, 11 are endemics, and two have disjunct, bi-regional distributions. We analysed the data using BEAST, a Bayesian approach that simultaneously infers the phylogeny and divergence times. We calibrated the tree by placing a seed fossil in the phylogeny, and used published age estimates as a prior for the root.

Results The phylogenetic reconstruction shows that all *Campanula* species fall within a well-supported campanuloid clade; however, *Campanula* is highly polyphyletic. The Cretan endemics do not form a monophyletic group, and species are scattered throughout the campanuloid clade. Therefore, the Cretan taxa did not evolve following a single vicariance or dispersal event. Most Cretan lineages represent remnants of an older continental flora, with the exception of one clade that radiated *in situ* after island isolation, and one lineage that appears to have arrived by dispersal.

Main conclusions Most Cretan species were present in the islands at the time of their isolation, and very little long-distance dispersal to Crete and diversification within Crete has occurred since then. Endemism is probably driven by loss of species on the mainland after island isolation. Species on the islands may have been more widespread in the past, but they are now restricted to often inaccessible areas, probably as a result of human pressure.

Keywords

BEAST, Campanulaceae, Crete, dating, dispersal, endemics, historical biogeography, molecular phylogeny, relicts, vicariance.

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INTRODUCTION

The Cretan area lies in the southernmost part of the Aegean Sea (Fig. 1). This large section of the Mediterranean extends from Crete northwards to the shores of Macedonia and Thrace, connecting with the Black Sea and the Sea of Marmara through the Dardanelles and Bosphorus straits. The Aegean islands represent geological extensions of the mountains in

Greece that once formed a land-bridge from the mainland to Asia Minor (Le Pichon *et al.*, 1981).

The Cretan area includes Crete and the Karpathos Islands, which lie c. 50 km north-east of Crete (Karpathos, Kasos and Saria). Located c. 75 km south-east of the easternmost peninsula of the Peloponnese, Crete is the largest island in Greece and the fifth largest in the Mediterranean Sea, at 243 km in length and 12–56 km in width. The topography is

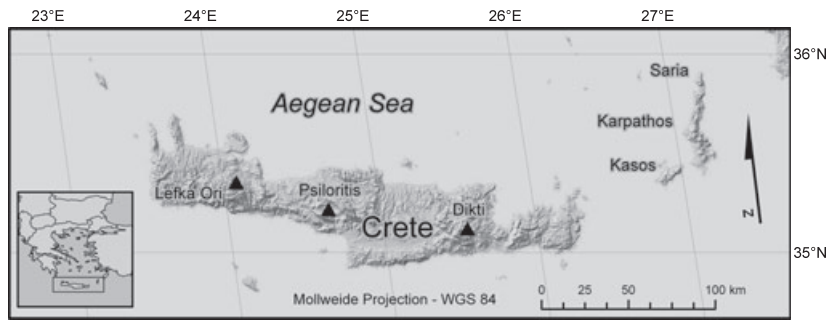


Figure 1 Map of Crete and the Karpathos Islands. The inset shows the Aegean area.

dominated by rugged, mountainous terrain that forms a rocky coastline in the south and more moderate slopes in the north, characterized by coastal plain and natural harbours. Three groups of mountain ranges define the island: the White Mountains in western Crete (Lefka Ori, 2452 m), the Psiloritis range in the centre, with Mt Ida being the highest peak of the island at 2456 m, and the Dikti Mountains (2148 m) in eastern Crete. These mountains provide the island with fertile plateaus, gorges and caves, important refuges for many taxa.

Since the plate tectonic scenario was developed by Creutzburg (1958), there has been an ever-growing interest in the complex historical evolution of the Aegean region. The Cretan area was once part of an Aegean landmass (Aegea) that formed some time after the Lower Oligocene and before the Serravallian (Upper Miocene, 13 Ma) (Meulenkamp, 1971, 1985; Le Pichon *et al.*, 1981). Since Serravallian–Upper Tortonian times, Aegea has been subject to multiple stresses caused by tectonic movements and crustal stretching as a direct response to the African subduction under the south-eastern European plate (Angelier *et al.*, 1982; Mercier *et al.*, 1987; Meulenkamp *et al.*, 1988; Fassoulas, 2001). These geodynamic events caused the Aegean landmass to expand gravitationally (Le Pichon & Angelier, 1979), initiating major palaeogeographical changes. The progressive fragmentation of the Aegean landmass allowed the sea to invade Aegea, forming a mosaic of small-sized horsts and grabens (Meulenkamp, 1985). Submergence in the island of Crete began in the Middle Miocene, starting from the east (Ierapetra) (Meulenkamp, 1971) and causing the formation of several basins (Meulenkamp *et al.*, 1979), while the land connection to the Balkans started to lower (Dermitzakis & Papanikolaou, 1981; Meulenkamp, 1985). In contrast, the Karpathos Islands and even Rhodes probably did not submerge before the Late Pleistocene (Meulenkamp, 1971).

During the Late Miocene (Messinian), open marine conditions started to disappear in various parts of the Cretan area (and everywhere in the Mediterranean basin), the sea level changed multiple times, and many thick evaporites were laid down (Meulenkamp *et al.*, 1979). This period marked the beginning of the dramatic events during the Cenozoic era known as the Messinian Salinity Crisis (MSC) (Hsu *et al.*, 1973). The MSC took place between 5.96 and 5.33 Ma (Krijgsman *et al.*, 1999), and the isolation of the Mediterranean from the Atlantic Ocean provides an obvious explanation for the desiccation of the Mediterranean basin. The cause of

the closure of the marine gateway in southern Spain and northern Morocco is still unclear, and there is a large body of literature, often controversial, that provides causal explanations for this major event (Clauzon *et al.*, 1996; Krijgsman *et al.*, 1999; Duggen *et al.*, 2003; Fauquette *et al.*, 2006; Jolivet *et al.*, 2006; Rouchy *et al.*, 2006; Gargani & Rigollet, 2007). In short, the available literature suggests that the MSC was driven by a complex combination of tectonic and glacio-eustatic processes that progressively isolated the Mediterranean Sea from the open ocean (Krijgsman *et al.*, 1999). An interesting study based on pollen records suggests that no significant climatic changes occurred during this period, and that plant migration and regional extinction were driven exclusively by the increased salinity of the environment caused by the desiccation of the Mediterranean basin (Fauquette *et al.*, 2006). In Crete, the MSC resulted in a short interruption of the gradual subsidence of the island and in a tectonic reorganization that facilitated the reconnection of previously isolated areas (Meulenkamp *et al.*, 1979; Meulenkamp, 1985).

At the beginning of the Pliocene (5.3 Ma), the sea level rose again and covered most of Crete, giving the island a highly fragmented appearance (Meulenkamp, 1971, 1985; Dermitzakis & Papanikolaou, 1981). Studies of fossil vertebrates in the Aegean area clearly suggest that Crete was partially connected to the mainland for most of the Miocene, whereas it was mostly isolated during the Pliocene; on the other hand, the Karpathos island group was still connected with the mainland during the Lower Pliocene (Daams & Van Der Weerd, 1980; Dermitzakis, 1990). During the Middle Pliocene, parts of Crete started to experience uplift but the sea level continued to alternate until the Upper Pliocene–Lower Pleistocene (Dermitzakis & Papanikolaou, 1981). During this time, tectonic movements resulted in an overall regression and the emergence of the entire island (Meulenkamp, 1985). The present configuration of the Cretan area was reached during the Pleistocene (Meulenkamp, 1971, 1985; Meulenkamp *et al.*, 1979; Dermitzakis & Papanikolaou, 1981; Le Pichon *et al.*, 1981; Dermitzakis, 1990).

The family Campanulaceae *sensu stricto* (Shetler & Morin, 1986; Kolakovskii, 1994; Takhtajan, 1997) comprises 50 genera and c. 1046 species, 96% of which occur in the Old World (Lammers, 2007). Over half of the species belong to *Campanula* (421 species) and *Wahlenbergia* (260 species). The paraphyly of these two groups has contributed to the historical

disagreement among various authors on the intrafamilial classification of the Campanulaceae (de Candolle, 1839; Schönland, 1889; Fedorov, 1957; Kovanda, 1978; Kolakovskii, 1987; Takhtajan, 1987).

Most Campanulaceae are herbaceous perennials, and less often annual or biennial. The exstipulate leaves are simple, alternate, rarely opposite or whorled. Flowers are tetracyclic, usually pentamerous, characterized by protandry and various secondary pollen presentation mechanisms (Erbar & Leins, 1989, 1995; Leins & Erbar, 1990, 1997). Lammers (2007) provides a comprehensive taxonomic synopsis of the Campanuloideae, Campanulaceae *sensu lato*, which include the Lobelioideae, Nemacladoideae, Cyphocarpoideae and Cyphioideae.

The Campanulaceae *sensu stricto* in the Cretan area comprises four genera and 21 species, 11 of which are strictly endemic to Crete and/or the Karpathos Islands, and two of which have a localized disjunct, bi-regional distribution in Crete and the Cyclades archipelago and Mt Helmos (Peloponnesus). Several species are chasmophytes (see Table 1), with their distribution restricted to calcareous cliffs exposed to drought and elevated temperatures. In a few cases they are facultative chasmophytes and able to compete in less harsh habitats (e.g. *Petromarula pinnata*). Calcareous cliffs are home to the only communities that have not been dramatically

affected and changed by over 6000 years of human presence on the island. The phrygana, which in Greek means dry firewood or twigs, is a typical Mediterranean habitat characterized by very dry conditions and dwarf, hemispherical, often aromatic and spiny shrubs, resistant to extreme drought and grazing. A few *Campanula* species thrive in phrygana or on rocky walls along the roadsides in this habitat. However, in a few localities, even phrygana has been subject to overgrazing and reduced to desert-like landscapes characterized by the spiny *Sarcopoterium spinosum* growing in association with *Campanula spatulata* subsp. *filicaulis*. A few *Campanula* endemics occur at higher elevations, on rocky mountain slopes and cliffs, and limestone or dolomite substrates, sometimes well above the timberline (e.g. *Campanula jacquinii*). With the exception of the ubiquitous *Petromarula pinnata*, the distribution of most endemic Campanulaceae is very much restricted to one or a few localities and specialized habitats. They often grow in sparse populations composed of one or a few individuals (e.g. *Campanula laciniata*). Human pressure and overgrazing has probably contributed to the present distribution of many of these species.

The islands of the Cretan area represent subcontinental systems in which most taxa were once found in a much larger continent, and therefore have evolved from a balanced continental flora and fauna (Greuter, 1972). As subcontinental

Table 1 List of Cretan taxa used in the phylogenetic analyses showing distribution, elevation and habitat information.

Taxon	Location	Elevation (m)	Habitat
<i>Campanula aizoides</i> Zaffran ex Greuter	Endemic to W. Crete (Lefka Ori) and Mt Helmos (N. Peloponnesus)	1800–2300	Calcareous cliffs and rock crevices
<i>Campanula carpatha</i> Halácsy	Endemic to the Karpathos Islands	0–1200	Cliffs and rock crevices
<i>Campanula cretica</i> (A. DC.) D. Dietr.	Endemic to W. Crete	50–1700	Cliffs and rock crevices
<i>Campanula creutzburgii</i> Greuter	Endemic to Crete	0–100	Calcareous cliffs and phrygana
<i>Campanula erinus</i> L.	W. Crete, Karpathos Islands, and widespread in S. Europe	0–600	Calcareous rocks, abandoned fields
<i>Campanula hierapetrae</i> Rech. f.	Endemic to E. Crete (Afendis Kavousi)	1000–1450	Calcareous rocks
<i>Campanula jacquinii</i> (Sieber) A. DC.	Endemic to Crete	1100–2200	Calcareous cliffs
<i>Campanula laciniata</i> L.	W. and E. Crete, Karpathos, and the Cyclades	0–550	Calcareous cliffs
<i>Campanula pelviformis</i> Lam.	Endemic to E. Crete	0–950	Calcareous cliffs, rocky slopes, roadside banks
<i>Campanula pinatzii</i> Greuter & Phitos	Endemic to the Karpathos Islands	0–800	Calcareous cliffs
<i>Campanula saxatilis</i> L.	Endemic to W. Crete	0–300	Calcareous cliffs, rocky places and slopes
<i>Campanula spatulata</i> subsp. <i>filicaulis</i> (Halácsy) Phitos	Endemic to C. and E. Crete	100–1600	Overgrazed phrygana, rocky places
<i>Campanula tubulosa</i> Lam.	Endemic to W. and C. Crete	100–800	Cliffs and rocky walls
<i>Legousia falcata</i> (Ten.) Fritsch	Crete, Karpathos Island, and widespread in the Mediterranean region	100–1200	Open fields, abandoned land
<i>Legousia hybrida</i> L.	W. and C. Crete, Karpathos Island, and W. and S. Europe	100–1400	Cultivated and abandoned land
<i>Legousia pentagonia</i> L.	Crete, Karpathos, and Aegean Region	50–600	Calcareous cliffs, rocky walls, terraces
<i>Legousia speculum-veneris</i> (L.) Chaix	W. Crete, Karpathos Island, and widespread in S. and C. Europe	100–1300	Calcareous open fields
<i>Petromarula pinnata</i> (L.) A. DC.	Endemic to Crete (widespread)	0–1200	Calcareous cliffs, rocks, phrygana, disturbed habitats

fragments, the islands have remained in complete isolation at least since the Pliocene, and they are presently characterized by a high degree of endemism amounting to 10% of the native vascular flora (Turland *et al.*, 1993). In the case of the Campanulaceae, 50% of the species are strictly endemic, not including the two species with restricted bi-regional distributions (*C. aizoides* and *C. laciniata*). Given the complex historical biogeography of the area, at least some of these endemics may represent relicts of an older continental flora that went extinct elsewhere. Therefore, a wide phylogeny of the Campanulaceae becomes necessary to determine: (1) the phylogenetic relationships of the Cretan lineages, and (2) whether the Cretan Campanulaceae are the result of vicariance or dispersal events. We dated the inferred phylogeny to test whether the Cretan lineages were present in the islands before they were isolated from the continental landmass. Lineages that were present in the islands before isolation (5.3 Ma) are the product of vicariance events (for example, taxa become isolated owing to the formation of a barrier); lineages that appeared in the islands after isolation are more likely to represent the product of dispersal and *in situ* radiation.

MATERIALS AND METHODS

Taxon sampling

Our analyses included 102 ingroup taxa and four outgroup taxa representing three clades closely allied to the Campanulaceae *sensu stricto* in other published phylogenies, and often included in the Campanulaceae *sensu lato* (Lammers, 2007): Nemacladaceae (*Pseudonemacladus oppositifolius*), Lobeliaceae (*Solenopsis minuta*, *Lobelia cardinalis*) and Cyphiaceae (*Cyphia elata*) (Gustafsson & Bremer, 1995; Lundberg & Bremer, 2003). We included 18 taxa from the Cretan area and all of the endemic species (Table 1). Our Cretan sample is missing *Asyneuma giganteum*, which occurs in Karpathos and other eastern Aegean islands; *Campanula delicatula*, found from the Karpathos group to the Cyclades and Southwest Asia and potentially a close relative of *C. erinus*, *C. pinatzii* and *C. creutzburgii* (all included in the analysis); and *Campanula trichocalycina*, which is fairly widespread from central and southern Italy to the Balkans and has been confirmed to belong to the genus *Asyneuma* (Lakušić & Conti, 2004), although it is often still referred to by its basionym.

This is the first study of phylogeny and biogeography in the Campanulaceae that includes all Cretan endemics, and one of the very few available on the historical biogeography of continental islands based on plant data. Our taxon sample includes many Mediterranean close relatives, although many more are missing as a result of lack of data. However, although our results are based only on the available dataset, we recover a pattern that is congruent with several other studies carried out in this region (de Vries, 1985; Douris *et al.*, 1998; Welter-Schultes & Williams, 1999; Fattorini, 2002; Marcussen, 2003; Poulakakis *et al.*, 2003; Parmakelis *et al.*, 2006).

DNA extraction, amplification and sequencing

Sequences for 87 taxa were provided by Haberle *et al.* (in press). We generated additional new sequences for 18 Cretan taxa that had mostly never been included in previous analyses of the Campanulaceae, and sequences of *Campanula rotundifolia*. Total genomic DNA was isolated from dried leaf tissue using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). We amplified three chloroplast gene regions using either standard primers that are commonly used in plant phylogenetic studies for *rbcl* (Bousquet *et al.*, 1992), or newly designed primers optimized for the Campanulaceae for *matK* and *atpB* (Haberle *et al.*, in press). Fragments were amplified in 25- μ L reactions [1–2 μ L genomic DNA, 0.2 μ L Amplitaq polymerase (Applied Biosystems, Foster City, CA, USA), 25 nmol total dNTPs, 2.5 μ L 10 \times buffer, 25 nmol MgCl₂, 25 pmol primer, 1–2 μ L BSA, and 9.5–11.5 μ L ddH₂O] using an automated thermal cycler and standard polymerase chain reaction (PCR) protocols, with annealing temperatures varying from 45 to 50°C. PCR products were cleaned using a PCR purification kit (Qiagen), and directly sequenced. Dye terminator cycle sequencing reactions for all regions were performed in 20- μ L volumes, cleaned using EdgeBiosystem plates (Baithersburg, MD, USA), and run on an ABI Prism 3700 automated sequencer (Applied Biosystems). Contiguous sequences were constructed and edited using SEQUENCHER ver. 4.2 (Gene Codes Corp., Ann Arbor, MI, USA). Sampled species, voucher specimen information and GenBank accession numbers for all sequences are listed in Table 2.

In the *atpB* dataset, sequences for *Campanula carpatica* and *C. pyramidalis* were missing. Sequences for each group were aligned using MUSCLE (Edgar, 2004) and adjusted manually.

Fossils and tree calibration

The fossil record for the Campanulaceae is extremely poor, and the only available and suitable material for tree calibration are seeds of *Campanula palaeopyramidalis* (Łańcucka-Środoniowa, 1977, 1979). These seeds were described from the Miocene of the Nowy Sacz Basin, in the West Carpathians (southern Poland). The age of these sediments is Upper Karpathian (Ewa Zastawniak, personal communication to Steven Manchester), 16.5–17.5 Ma (Nemcok *et al.*, 1998). We used this age range to calibrate an internal node of the tree.

The reticulate surface is strikingly similar to that of seeds of *C. pyramidalis*. Reticulate seed surfaces in the Campanulaceae are not as common as smooth and striate surfaces, and after close comparison with seeds of extant taxa, Łańcucka-Środoniowa (1977) concluded that the fossil represents a Tertiary relative of the extant *C. pyramidalis*.

Work on seed morphology in the Campanulaceae is not extensive, especially within *Campanula* and close relatives (Belyaev, 1984a,b, 1985, 1986; Shetler & Morin, 1986). However, the available literature shows that when seed size, shape and surface morphology are considered, *C. carpatica* is the only other taxon that could have possibly been determined

Table 2 List of sequenced taxa, voucher specimens and genes (with GenBank numbers) used in the phylogenetic analyses.

Taxon name	Voucher	<i>atpB</i>	<i>matK</i>	<i>rbcL</i>
<i>Adenophora divaricata</i> Franch. & Sav.	Eddie 96086 (EGHB)	EU437656	EU713323	EU713430
<i>Asyneuma virgatum</i> (Labill.) Bornm.	Berlin-Dahlem 0104 (OS)	EU437665	EU713332	EU713439
<i>Azorina vidalii</i> (H. C. Wats.) Feer	Eddie 454840814 (EGHB)	EU437601	EU713266	EU713373
<i>Berenice arguta</i> Tul.	Strasberg 10102000 (EGHB)	EU437672	EU713339	EU713446
<i>Campanula aizoides</i> Zaffran ex Greuter	Edwards 34 (YU)	EU437662	EU713329	EU713436
<i>Campanula alliariifolia</i> Willd.	Cosner s.n. (OS)	EU437604	EU713269	EU713376
<i>Campanula arvatica</i> Lag.	NCC 94003 (EGHB)	EU437677	EU713344	EU713451
<i>Campanula aucheri</i> A. DC.	Eddie 95010 (EGHB)	EU437576	EU713241	EU713348
<i>Campanula bellidifolia</i> Adams	Gaskin 115 (MO)	EU437575	EU713240	EU713347
<i>Campanula bononiensis</i> L.	Cosner 1003 (OS)	EU437609	EU713274	EU713381
<i>Campanula carpatha</i> Halácsy	M. Avramakis s.n.	EU437596	EU713261	EU713368
<i>Campanula carpatica</i> Jacq.	Gaskin 121 (MO)		EU713303	EU713410
<i>Campanula cretica</i> (A. DC.) D. Dietr.	Edwards 50 (YU)	EU437663	EU713330	EU713437
<i>Campanula creutzburgii</i> Greuter	Cellinese 1003 (YU)	EU437625	EU713290	EU713397
<i>Campanula divaricata</i> Michx.	Haberle 150 (TEX)	EU437676	EU713343	EU713450
<i>Campanula edulis</i> Forssk.	S. Collette 8782 (TEX)	EU437602	EU713267	EU713374
<i>Campanula elatines</i> L.	Ayers 88-287 (BH)	EU437664	EU713331	EU713438
<i>Campanula erinus</i> L.	Cellinese 1006 (YU)	EU437626	EU713291	EU713398
<i>Campanula exigua</i> Rattan	Haberle 148 (TEX)	EU437643	EU713309	EU713416
<i>Campanula fragilis</i> Cirillo	Eddie 98014 (EGHB)	EU437655	EU713321	EU713428
<i>Campanula grossheimii</i> Kharadze	Gaskin 206 (MO)	EU437621	EU713286	EU713393
<i>Campanula hawkinsiana</i> Hausskn. & Heldreich ex Hausskn.	Eddie 94002 (EGHB)	EU437671	EU713338	EU713445
<i>Campanula herminii</i> Hoffmans. & Link	Neves 227 (TEX)	EU437673	EU713340	EU713447
<i>Campanula hierapetrae</i> Rech. f.	Edwards 27 (YU)	EU437623	EU713288	EU713395
<i>Campanula jacquini</i> (Sieber) A. DC.	Edwards 41 (YU)	EU437674	EU713341	EU713448
<i>Campanula laciniata</i> L.	Cellinese 1004 (YU)	EU437579	EU713244	EU713351
<i>Campanula lanata</i> Friv.	Eddie 96051 (EGHB)	EU437610	EU713275	EU713382
<i>Campanula latifolia</i> L.	Gaskin 405 (MO)	EU437606	EU713271	EU713378
<i>Campanula lusitanica</i> Loeffl.	Neves 226 (TEX)	EU437667	EU713334	EU713441
<i>Campanula medium</i> L.	Lammers 8768 (OSH)	EU437607	EU713272	EU713379
<i>Campanula mirabilis</i> Albov	Eddie 96056 (EGHB)	EU437612	EU713277	EU713384
<i>Campanula mollis</i> L.	Neves 230 (TEX)	EU437603	EU713268	EU713375
<i>Campanula parryi</i> A. Gray	Chumley 7365 (TEX)	EU437675	EU713342	EU713449
<i>Campanula pelviformis</i> Lam.	Cellinese 1010 (YU)	EU437578	EU713243	EU713350
<i>Campanula peregrina</i> L.	Eddie 95007 (TEX)	EU437654	EU713320	EU713427
<i>Campanula persicifolia</i> L.	Eddie 95027 (EGHB)	EU437657	EU713324	EU713431
<i>Campanula pinatzii</i> Greuter & Phitos	M. Avramakis s.n.	EU437624	EU713289	EU713396
<i>Campanula pyramidalis</i> L.	Eddie 96089 (EGHB)		EU713322	EU713429
<i>Campanula rapunculoides</i> L.	Jansen 8/00-007 (TEX)	EU437620	EU713285	EU713392
<i>Campanula reverchonii</i> A. Gray	Eddie 00004 (TEX)	EU437594	EU713259	EU713366
<i>Campanula robbinsiae</i> Small	C. Peterson s.n. (TEX)	EU437642	EU713308	EU713415
<i>Campanula rotundifolia</i> L.	Edwards 71 (YU)	EU437668	EU713335	EU713442
<i>Campanula sarmatica</i> Ker Gawl.	Gaskin 458 (MO)	EU437614	EU713279	EU713386
<i>Campanula saxatilis</i> L.	Cellinese 1007 (YU)	EU437577	EU713242	EU713349
<i>Campanula scouleri</i> Hook. ex A. DC.	Haberle ex Hogue (no voucher)	EU437678	EU713345	EU713452
<i>Campanula spatulata</i> Sibth. & Sm. subsp. <i>filicaulis</i> (Halácsy) Phitos	Cellinese 1005 (YU)	EU437670	EU713337	EU713444
<i>Campanula tubulosa</i> Lam.	Cellinese 1012 (YU)	EU437580	EU713245	EU713352
<i>Campanulastrum americanum</i> (L.) Small	Eddie 96050 (TEX)	EU437646	EU713312	EU713419
<i>Canarina canariensis</i> (L.) Vatke	Eddie 96048 (EGHB)	EU437581	EU713246	EU713353
<i>Codonopsis dicentrifolia</i> (C. B. Clarke) W.W. Sm.	Eddie 95022 (EGHB)	EU437585	EU713250	EU713357
<i>Codonopsis kawakamii</i> Hayata	Lammers 8439 (F)	EU437588	EU713253	EU713360
<i>Codonopsis lanceolata</i> (Siebold & Zucc.) Trautv.	Eddie 95023 (EGHB)	EU437583	EU713248	EU713355
<i>Codonopsis viridis</i> (Spreng.) Wall.	T. Ayers 88-229 (BH)	EU437584	EU713249	EU713356
<i>Craterocapsa tarsodes</i> Hilliard & B. L. Burt	Eddie 01022 (EGHB)	EU437636	EU713301	EU713408
<i>Cyananthus lobatus</i> Wall. ex Benth.	Cosner 179 (OS)	EU437587	EU713252	EU713359
<i>Cyphia elata</i> Harv.	Cosner s.n. (OS)	EU437599	EU713264	EU713371

Table 2 (Continued).

Taxon name	Voucher	<i>atpB</i>	<i>matK</i>	<i>rbcL</i>
<i>Diosphaera rumeliana</i> (Hampe) Bornm.	Eddie 95405 (EGHB)	EU437619	EU713284	EU713391
<i>Feeria angustifolia</i> (Schousb.) Buser	Jury <i>et al.</i> 17429 (TEX)	EU437622	EU713287	EU713394
<i>Gadellia lactiflora</i> (M. Bieb.) Shulkina	Eddie 95009 (EGHB)	EU437652	EU713318	EU713425
<i>Githopsis diffusa</i> A. Gray	Morin s.n. (OS)	EU437644	EU713310	EU713417
<i>Githopsis pulchella</i> Vatke	Morin s.n. (OS)	EU437647	EU713313	EU713420
<i>Hanabusaya asiatica</i> (Nakai) Nakai	Eddie 95018 (EGHB)	EU437658	EU713325	EU713432
<i>Heterochaenia ensifolia</i> (Lam.) A. DC.	Strasberg 12172000 (EGHB)	EU437666	EU713333	EU713440
<i>Heterocodon rariflorum</i> Nutt.	Haberle 149 (TEX)	EU437641	EU713307	EU713414
<i>Jasione crispa</i> (Pourr.) Samp.	Eddie 95083 (EGHB)	EU437618	EU713283	EU713390
<i>Jasione heldreichii</i> Boiss. & Orph.	T. Ayers 88-208 (BH)	EU437616	EU713281	EU713388
<i>Jasione laevis</i> Lam.	Eddie 95035 (EGHB)	EU437615	EU713280	EU713387
<i>Jasione montana</i> L.	Sales and Hedge 9898 (EGHB)	EU437582	EU713247	EU713354
<i>Legousia falcata</i> (Ten.) Fritsch	Cosner 143 (OS)	EU437645	EU713311	EU713418
<i>Legousia hybrida</i> L.	M. Avramakis s.n.	EU437660	EU713327	EU713434
<i>Legousia pentagonia</i> L.	Cellinese 1013 (YU)	EU437595	EU713260	EU713367
<i>Legousia speculum-veneris</i> (L.) Chaix	Eddie 95034 (EGHB)	EU437593	EU713258	EU713365
<i>Leptocodon gracilis</i> (Hook. f.) Lem.	Eddie 95021 (EGHB)	EU437617	EU713282	EU713389
<i>Lobelia cardinalis</i> L.	Cosner s.n. (OS)	EU437598	EU713263	EU713370
<i>Merciera tenuifolia</i> A. DC.	K. Steiner 2445 (OS)	EU437630	EU713295	EU713402
<i>Michauxia tchihatchewii</i> Fisch. & C. A. Mey.	Eddie 2449 (EGHB)	EU437574	EU713239	EU713346
<i>Microcodon glomeratum</i> A. DC.	Cupido & Eddie 1007 (TEX)	EU437627	EU713292	EU713399
<i>Musschia aurea</i> (L.) Dum.	T. Ayers 88-274 (BH)	EU437638	EU713304	EU713411
<i>Nesocodon mauritianus</i> (I. B. K. Richardson) Thulin	Eddie 95042 (EGHB)	EU437648	EU713314	EU713421
<i>Petromarula pinnata</i> (L.) A. DC.	Cellinese 1008 (YU)	EU437659	EU713326	EU713433
<i>Physoplexis comosa</i> (L.) Schur	Eddie 95008 (EGHB)	EU437590	EU713255	EU713362
<i>Phyteuma spicatum</i> L.	Eddie 96090 (EGHB)	EU437589	EU713254	EU713361
<i>Platycodon grandiflorus</i> (Jacq.) A. DC.	T. Ayers 88-216 (BH)	EU437586	EU713251	EU713358
<i>Prismatocarpus diffusus</i> (L.) A. DC.	Eddie 01006 (TEX)	EU437629	EU713294	EU713401
<i>Prismatocarpus fruticosus</i> L'Hér.	Eddie 01004 (TEX)	EU437634	EU713299	EU713406
<i>Prismatocarpus schlechteri</i> Adamson	Eddie 010033 (TEX)	EU437632	EU713297	EU713404
<i>Prismatocarpus sessilis</i> Eckl. ex A. DC.	Eddie 01018 (TEX)	EU437631	EU713296	EU713403
<i>Pseudonemacladus oppositifolius</i> (B. L. Rob.) McVaugh	Cosner s.n. (OS)	EU437600	EU713265	EU713372
<i>Rhigiophyllum squarrosum</i> Hochst.	Eddie 01015 (TEX)	EU437653	EU713319	EU713426
<i>Roella ciliata</i> L.	Ayers s.n. (B.H.)	EU437633	EU713298	EU713405
<i>Siphocodon debilis</i> Schltr.	Eddie 01016 (TEX)	EU437649	EU713315	EU713422
<i>Siphocodon spartioides</i> Turcz.	Eddie 01017 (TEX)	EU437640	EU713306	EU713413
<i>Solenopsis minuta</i> L.	Cellinese 1011 (YU)	EU437597	EU713262	EU713369
<i>Symphyandra armena</i> (Stev.) A. DC.	Eddie 760258 (EGHB)	EU437611	EU713276	EU713383
<i>Symphyandra hoffmanni</i> Pant.	Eddie 750893A (EGHB)	EU437605	EU713270	EU713377
<i>Symphyandra pendula</i> (M. Bieb.) A. DC.	Gaskin 255 (MO)	EU437613	EU713278	EU713385
<i>Theilera guthriei</i> (L. Bolus) E. Phillips	Eddie 01019 (TEX)	EU437637	EU713302	EU713409
<i>Trachelium caeruleum</i> L.	Cosner 173 (OS)	EU437661	EU713328	EU713435
<i>Triodanis coloradoensis</i> (Buckley) McVaugh	Haberle 141 (TEX)	EU437592	EU713257	EU713364
<i>Triodanis perfoliata</i> (L.) Nieuwl.	Cosner 178 (OS)	EU437591	EU713256	EU713363
<i>Wahlenbergia angustifolia</i> (Roxb.) A. DC.	Eddie/C.-Wicks s.n.	EU437639	EU713305	EU713412
<i>Wahlenbergia berteroi</i> Hook. & Arn.	Anderson s.n. (CONN)	EU437650	EU713316	EU713423
<i>Wahlenbergia gloriosa</i> Lothian	Ayers 88-217 (BH)	EU437635	EU713300	EU713407
<i>Wahlenbergia hederaceae</i> (L.) Rchb.	Eddie 98004W (EGHB)	EU437628	EU713293	EU713400
<i>Wahlenbergia linifolia</i> (Roxb.) A. DC.	Eddie/C.-Wicks 98005 (EGHB)	EU437651	EU713317	EU713424

as the closest relative of *C. palaeopyramidalis* (Belyaev, 1984a,b). In analyses of the individual genes (*matK* and *rbcL*) and combined analyses, *C. carpatica* is the strongly supported sister taxon of *C. pyramidalis*. This sister relationship is recovered in other analyses as well (Eddie *et al.*, 2003; Roquet *et al.*, 2008).

Although we are confident about the correct fossil identification, and favour its placement on the crown node, we also analysed the data with the fossil placed on the stem lineage node. Both positions need to be considered, and neither is more conservative. The phylogenetic placement of the fossil on the crown node implies that the split between *C. pyramidalis*

Table 3 Divergence-time estimates calculated by placing the fossil on the stem lineage node of the *pyramidalis/carpatica* clade.

Lineage	Divergence-time estimate (Ma)
<i>Campanula spatulata</i>	12.2 ± 4.7
<i>Campanula aizoides</i>	27.5 ± 5.6
<i>Campanula pinatzii</i>	15 ± 6
<i>Campanula creutzburgii</i>	1.8 ± 1.4
<i>Campanula carpatha</i>	3 ± 1.6
<i>carpatha-laciniata</i> clade	7.3 ± 2.7
<i>Campanula hierapetræ</i>	5.7 ± 2.8
<i>Campanula jacquinii</i>	5.9 ± 3.7
<i>Petromarula pinnata</i>	15.8 ± 6.3
<i>Campanula cretica</i>	18 ± 6.7

and *C. carpatha* occurred at least by the time of the age of the fossil; placing the fossil on the node subtending the stem lineage implies that the split between the *carpatha/pyramidalis* clade and its sister clade occurred at least by the time of the age of the fossil. Divergence times calculated by placing the fossil on the crown clade node recovered older ages with larger error margins. Calibration on the stem lineage node generated divergence times that were somewhat younger with smaller error margins (see Table 3). Overall, we recovered divergence time estimates that were insignificantly different and our conclusions were not affected. For the purpose of our discussion in this paper, we show the placement of the fossil on the crown clade node.

We calibrated the root of the Campanulaceae *sensu stricto* tree using 45 Ma as the minimum age of Campanulaceae inferred by a study of divergence times among angiosperm families (Wikström *et al.*, 2003). This age represents the split between the Campanulaceae and Lobeliaceae. The age of the split between the Rousseeaceae and the lineage that leads to the Campanulaceae and Lobeliaceae is 80 Ma. The relationship between the Rousseeaceae plus Campanulaceae and Lobeliaceae is strongly supported by a recent study on campanulid phylogeny (Winkworth *et al.*, 2008). Therefore, we set the prior of the root of the Campanulaceae to be between 45 and 80 Ma, which places the majority of the prior probability on the younger age (45 Ma) estimated by Wikström *et al.* (2003), but allows for ages as old as the estimated date of the split between the Rousseeaceae and Campanulaceae/Lobeliaceae clade (80 Ma).

Phylogenetic analyses and divergence time estimation

We conducted a series of analyses to estimate phylogenetic relationships and divergence times in Campanulaceae *sensu stricto*. Model selection for each data partition was based on Akaike scores for the set of substitution models evaluated by MODELTEST ver. 3.7 (Posada & Crandall, 1998). TVM + I + G was the model selected for *atpB*, GTR + G that for *matK*, and GTR + I + G that for *rbcl*. Partition homogeneity tests were conducted using PAUP* ver. 4.0b10 to determine congruence

between datasets. We ran 100 replicates with maxtrees = 100, and congruence was rejected at 0.01.

The posterior probability distribution of trees using the combined dataset from all genes was approximated using the Metropolis-coupled Markov chain Monte Carlo (MC³) algorithm implemented in MRBAYES ver. 3.1.2 (Ronquist & Huelsenbeck, 2003). Each MC³ analysis employed partition-specific substitution models (GTR + I + G for *atpB* and *rbcl*; GTR + G for *matK*) with parameters unlinked across each gene. The chain was run for 10⁷ generations, sampling every 10³ steps. Four independent analyses were performed to verify that the run had reached stationarity. We removed the first 10⁶ generations of all MC³ analyses as burn-in. Examination of plots for likelihood scores and parameter estimates in TRACER ver. 1.4 revealed that each converged on the same topology (Drummond, 2007). Therefore, the post-burn-in samples from each analysis were combined and summarized in a majority rule consensus (Fig. 2).

Using the likelihood ratio tests (Felsenstein, 1981), each individual partition and combined dataset rejected the molecular clock. Therefore, divergence times were estimated under a relaxed molecular clock using BEAST ver. 1.4.6 (Drummond & Rambaut, 2007). We used the uncorrelated lognormal model to estimate divergence times, which does not assume the autocorrelation of molecular rate variation between ancestral and descendant lineages. BEAST allows for age-calibration points in the tree to be defined by prior distributions instead of by point estimates. We constrained two nodes with these prior distributions as described above.

The posterior probabilities of divergence times were estimated using the models selected for the previous phylogenetic analyses and assuming the uncorrelated lognormal model for rates. We confirmed the analysis with three independent runs, which converged on the same posterior distributions. We ran the chains for 10⁸ generations, sampling every 10⁴ steps. The first 10⁷ generations were removed as burn-in, and the posterior probability density was summarized using TREEANNOTATOR ver. 1.4.1 (Drummond & Rambaut, 2003).

RESULTS AND DISCUSSION

Phylogenetic analysis

The combined phylogenetic analysis recovered three well-supported clades: the platycodonoids as sister to the wahlenbergioids plus the campanuloids (Fig. 2). Relationships between and within these major clades are discussed in detail by Haberle *et al.* (in press). The individual gene analyses generated broadly congruent trees, apart from a few taxon shifts in some clades. The phylogenetic reconstruction obtained with BEAST ver. 1.4.6 is congruent with the reconstruction generated by MRBAYES ver. 3.1.2. The tree topology is also congruent with the bootstrap tree generated by parsimony and maximum likelihood (results not shown). Our results show that many genera within the Campanulaceae are paraphyletic or polyphyletic (Haberle *et al.*, in press). All

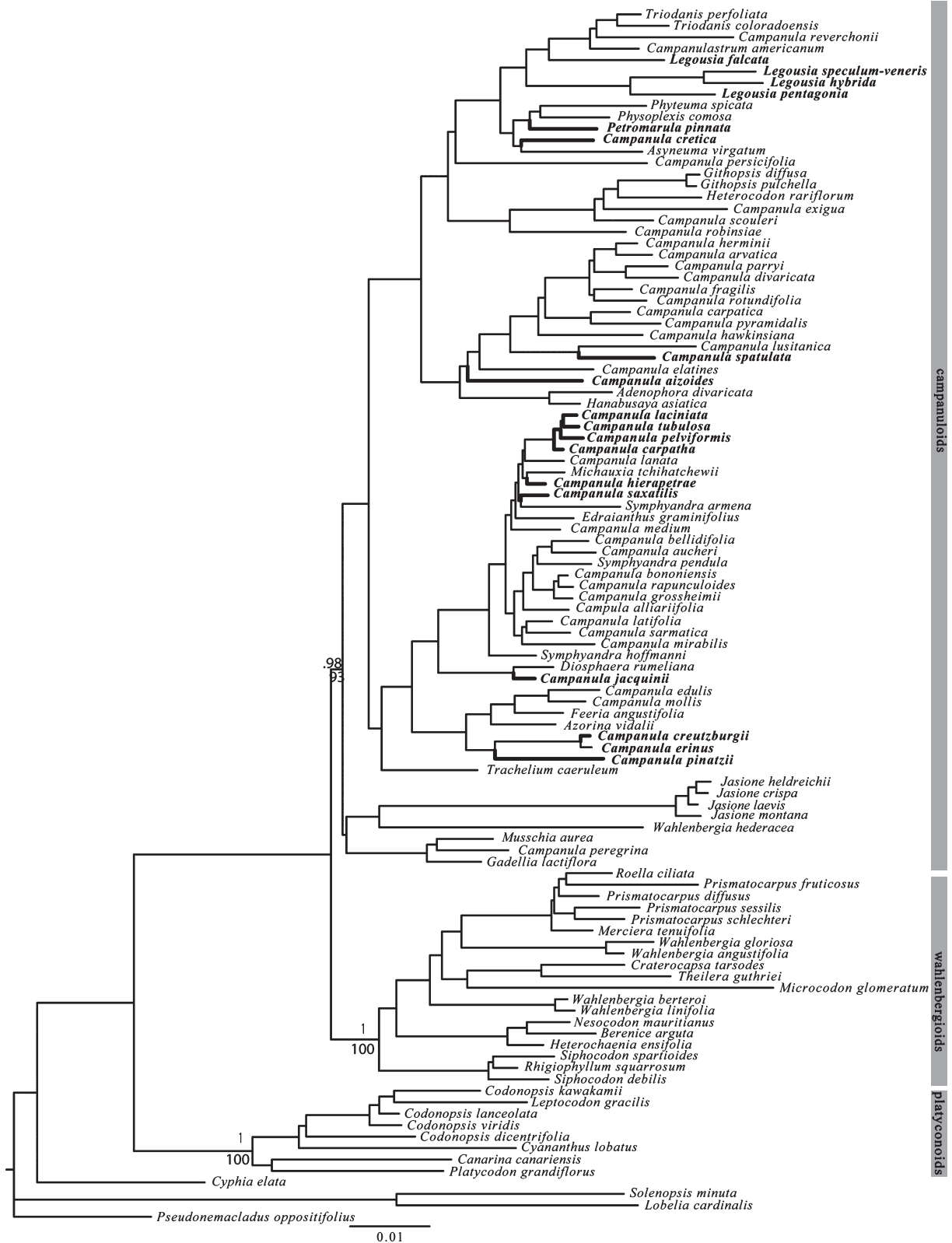


Figure 2 Majority rule consensus phylogram generated by Bayesian analysis of combined *rbcl*, *matk* and *atpB* sequence data, and showing major clades recovered by the analyses. Numbers above branches are posterior probability values. Numbers below branches are maximum likelihood bootstrap support values. Cretan species are in bold. Lineages in bold represent Cretan endemics.

Campanula species fall within a well-supported campanuloid clade, although the group is highly polyphyletic (Fig. 3). The inclusion of the clade containing *Jasione*, *Wahlenbergia hederacea*, *Campanula peregrina* and *Musschia* is not supported, and further discussion is found in Haberle *et al.* (in press). However, we note that the inflorescence and flower morphology of *Jasione* resembles that of *Phyteuma*, characterized by a deeply lobed corolla with narrowly linear lobes, and a densely contracted inflorescence that in both *Jasione* and *Phyteuma* is subtended by bracts and appears capitulate. In *Phyteuma*, the corolla lobes take an active role in pollen deposition and release. The lobes remain apically coherent throughout anthesis to force the brushing mechanism between the dehiscing anthers and the hairy portion of the style (Erbar & Leins, 1995). Subsequently, the corolla lobes separate and spread. In *Jasione*, the mechanism of secondary pollen presentation is similar to that in *Campanula* species and occurs by simple deposition of the pollen onto the stylar hairs (Erbar & Leins, 1989). The position of *Jasione* remains controversial (Eddie *et al.*, 2003) and more investigation is clearly needed, but morphological characters may provide some additional indication of its correct placement.

Within the campanuloids, two major clades, A and B, are recovered, and clade B is divided into subclades C and D (Fig. 3). Clades A and B very roughly correspond to section *Campanula sensu stricto* and section *Rapunculus*, respectively (Fedorov, 1957). In the traditional classification, taxa in section *Campanula sensu stricto* are characterized by the presence of calyx appendages and basal capsule dehiscence; but taxa in section *Rapunculus* have no calyx appendages and apical capsule dehiscence. However, several species in Clade A lack calyx appendages and/or have apical dehiscence, and *vice versa* in Clade B. Overall, significant morphological diversity and homoplasy are shown across these clades. These results are generally congruent with previous analyses of the campanuloids (Eddie *et al.*, 2003), and are discussed in much more detail in Haberle *et al.* (in press).

Position and history of Cretan lineages

The Cretan taxa are scattered throughout the campanuloid clade and nested in every subclade, indicating that they do not form a monophyletic group. Therefore, it appears that the Cretan taxa did not evolve following a single vicariance or dispersal event. Oceanic islands have long been studied as evolutionary laboratories in which newcomers colonize different ecological zones and radiate to become endemic lineages (Francisco-Ortega *et al.*, 1997; Baldwin *et al.*, 1998). However, for continental island systems we lack the body of literature that is available for explaining plant diversity in oceanic islands. The process of isolation in the Cretan area started c. 10 Ma and stabilized c. 5.3 Ma, long enough to expect considerable diversification in the area.

Several non-molecular floristic, faunistic and palaeogeographical studies in the Aegean area have suggested that island lineages arose as the result of taxon range fragmentation

caused by tectonic processes and change in sea level since the Tortonian (Snogerup, 1967; Sondaar, 1971; Greuter, 1972, 1975; Stork, 1972; Dermitzakis, 1990). In view of the results of our phylogenetic analyses, two alternative hypotheses can be generated. The first scenario implies that all endemic lineages represent relicts of a continental flora that existed in the Mediterranean basin before the Pliocene. The submersion of the Aegean landmass resulted in taxon range fragmentation and subsequent isolation, and this process may have occurred as many times as the various continental fragments reconnected to the mainland, or to each other, as a result of sea-level changes. At the same time, dispersal events from mainland or other continental fragments may have also occurred by island hopping (Lieberman, 2005). Essentially, small corridors may have formed as a result of sea-level changes and tectonic processes throughout the Miocene, especially during the Messinian Salinity Crisis. Moreover, the entire Hellenic Arc has expanded through time, and we know that islands were once much closer to each other and to the mainland than they are at present (Angelier *et al.*, 1982; Schellart & Lister, 2004). In any case, after the last isolation in the Pliocene, Crete and adjacent islands had a well-established flora with taxa that subsequently may have become extinct on the islands and elsewhere.

An alternative scenario implies that a series of dispersal events followed by divergence occurred at various times after the Pliocene. Therefore, it is possible that no endemic lineages were present on the islands prior to tectonic isolation.

Fig. 4 shows a chronogram of divergence times determined by using one internal calibration point set at 16.5–17.5 Ma, and a prior for the root of the Campanulaceae *sensu stricto* set between 45 and 80 Ma (see Methods). Divergence-time estimates represent the minimum ages of clade divergence. The grey vertical bar indicates 5.3 Ma, the time when Crete was last isolated. Results and discussion on relevant clades follow.

Clade 1

Campanula pinatzii is sister to a clade containing *C. erinus* and *C. creutzburgii*. *Campanula pinatzii* and *C. creutzburgii*, endemic to the Karpathos group and Crete, respectively, are treated as subspecies of *C. drabifolia* in *Flora Europaea* (Fedorov & Kovanda, 1976). *Campanula erinus* is widespread in southern Europe and Asia Minor. These three species are spreading, hispid, annual herbs and are similar in habit and gross morphology. The flower of *C. erinus* is very inconspicuous and sessile, whereas *C. creutzburgii* and *C. pinatzii* have more conspicuous flowers on short pedicels. They grow on calcareous, rocky soils and phrygana, from low to mid-elevations, not exceeding 800 m. The split of *C. pinatzii* and its sister clade dates to 20.5 (\pm 9) Ma, suggesting that the *C. pinatzii* lineage was present in the Karpathos Islands at least during the Middle Miocene. However, the split between *C. erinus* and *C. creutzburgii* is very recent, at 2.5 (\pm 2) Ma. This appears to be the result of a dispersal event from the mainland to Crete during the Pleistocene, after the isolation of Crete.

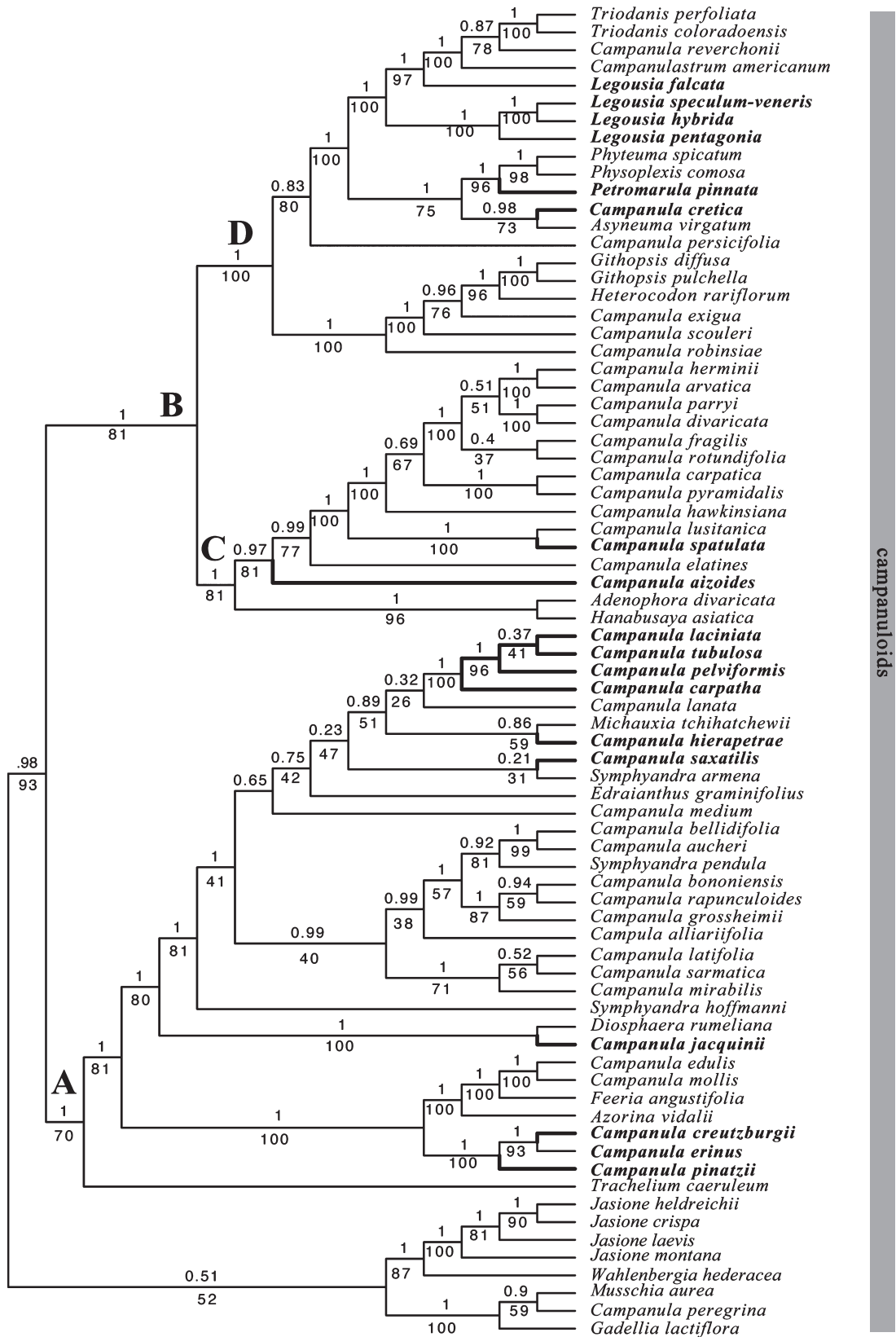


Figure 3 Majority rule consensus tree generated by Bayesian analysis of combined *rbcl*, *matK* and *atpB* sequence data showing only the campanuloid clade. Numbers above branches are posterior probability values. Numbers below branches are maximum likelihood bootstrap support values. Names in bold represent all Cretan species. Lineages in bold represent Cretan endemics. Letters refer to clades discussed in the text.

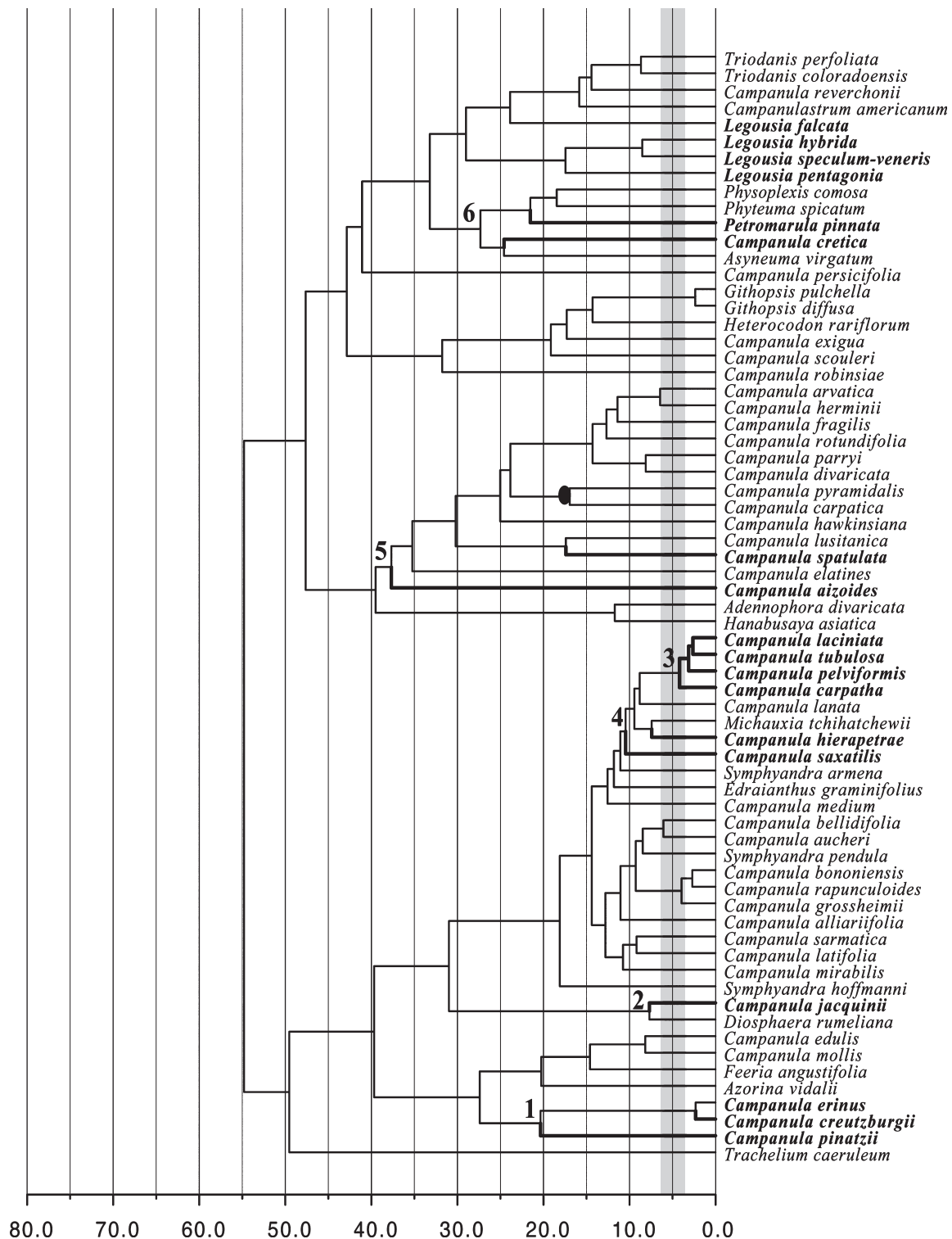


Figure 4 Chronogram of divergence times determined by using one internal calibration point set at 16.5–17.5 Ma, and a prior for the root of the Campanulaceae set between 45 and 80 Ma. The circle shows fossil placement. The grey vertical bar indicates c. 5.3 Ma, the time when Crete was last isolated. Names in bold represent all Cretan species. Lineages in bold represent Cretan endemics. Numbered clades are discussed in the text.

Campanula pinatzii, *C. erinus* and *C. creutzburgii* belong to the *drabifolia* species complex, which includes 12 species (Carlström, 1986). These taxa are morphologically very similar

and distributed in the Aegean area, often in very restricted localities. The inclusion of these additional species in the taxon sample would possibly shed more light on the origin of

C. pinatzii and *C. creutzburgii*. However, none of these 12 species is found in the Cretan area apart from *C. delicatula*, occurring from the Karpathos Islands to the Cyclades and into Southwest Asia.

Clade 2

The Cretan mountain flora is renowned for containing a few relict species, mainly endemic or with a disjunct distribution (Greuter, 1972). *Campanula jacquinii* is known from a few, high-elevation localities (1100–2200 m) within Crete, specifically on major mountain peaks (Lefka Ori, Psiloritis, Kofina and Dikti) that were isolated islands during the Early Pliocene (Meulenkamp, 1971, 1985). A perennial herb, this species is distinct in having a stout root stock, mostly sessile, coriaceous leaves, and flowers arranged in dense, terminal, highly contracted corymbose inflorescences. The corolla is tubular with the tube as long as the lobes. *Campanula jacquinii* is sister to the morphologically similar *Diosphaera rumeliana* that occurs in southern Bulgaria, mainland Greece and the Sporades Islands in the Aegean Sea, growing at elevations not exceeding 1200 m, but in a similar habitat. The split between *C. jacquinii* and *D. rumeliana* is dated at 8.3 (± 5.5) Ma. In view of the small population size and the fragmented, restricted distribution of *C. jacquinii*, which is found only in a few mountainous, isolated localities that correspond to past islands, we favour the hypothesis that *C. jacquinii* may represent one of the few Cretan endemics that is a Tertiary relict. Both *C. jacquinii* and *Diosphaera rumeliana* were treated as subspecies of *Trachelium jacquinii* in *Flora Europaea* (Fedorov & Kovanda, 1976), and were later placed in the genus *Campanula*, with *D. rumeliana* sometimes treated as a subspecies of *C. jacquinii*. Our analyses clearly support a close relationship between *C. jacquinii* and *D. rumeliana*.

Clade 3

The position of *Campanula lanata*, a native of the southern Balkans, is poorly resolved as sister to a strongly supported clade that includes *C. laciniata*, *C. tubulosa*, *C. pelviformis* and *C. carpatha*. They are all endemic to the Cretan area, with *C. carpatha* found in the Karpathos islands, *C. tubulosa* and *C. pelviformis* in Crete, and *C. laciniata* with a bi-regional distribution in the Cretan area (Crete and Karpathos) and the Cyclades, in the Aegean Sea. These species are among the most attractive campanulas in the region, with large, showy, broadly campanulate to urceolate and funnel-form flowers. *Campanula laciniata* and *C. carpatha* are restricted to often inaccessible calcareous cliffs, growing in scattered, small populations, with one to a few individuals. *Campanula tubulosa* and *C. pelviformis* are more common and are also found growing along steep, rocky, roadside banks. The split between *C. carpatha* and its sister clade dates to 4.5 (± 2) Ma, but the stem lineage of the *carpatha*–*laciniata* clade dates to 9 (± 3) Ma, suggesting that the ancestors of this clade may have been present on the islands before the Pliocene. Lack of

support within the *laciniata*–*carpatha* clade prevents us from stating whether diversification occurred first in the Karpathos groups with dispersal into Crete, or *vice versa*. In any case, this clade seems to represent the only *in situ* radiation event in the Cretan area. It is possible that this radiation resulted from the presence of a distinct ecological niche. However, this hypothesis is hard to test because of the significant human and animal pressure exerted in these islands for more than 6000 years (Evans, 1971; Broodbank & Strasser, 1991), which has dramatically shaped their biodiversity and species distribution.

Clade 4

The *lanata*–*laciniata* clade is sister to a clade that includes *Campanula hierapetrae* and *Michauxia tchihatchewii*. *Campanula hierapetrae* is a localized endemic, found on Mt Thripti in eastern Crete. It is a perennial herb with a stout, ascending or decumbent stem that grows at mid-elevations between 1000 and 1500 m. Its relationship to *M. tchihatchewii* is only weakly supported. *Michauxia* represents an eastern Mediterranean group of about seven species, mostly endemic to Asia Minor. This robust, biennial herb differs in having larger flowers with a corolla that is split nearly to the base, and lobes that are completely reflexed. This atypical campanulaceous flower with strongly reflexed lobes is found only in *Petromarula pinnata*. Therefore, our analysis suggests that this character has evolved independently. The *C. hierapetrae* lineage is 7.5 (± 4) Ma, and, given the generally low support values and significant error associated with the age estimate, its origin remains unclear. However, its scattered distribution and restricted localized occurrence in a palaeoarea that remained isolated as an island throughout the Early Pliocene favours the hypothesis that *C. hierapetrae* is a continental remnant. The inclusion of potentially close relatives, such as the Aegean endemics *C. amorgina* and *C. heterophylla* found on nearby islands, would possibly better resolve the position of *C. hierapetrae*, and further support the hypothesis that all these narrowly localized taxa scattered throughout the Aegean region also represent palaeoendemics.

We find *Campanula saxatilis* subsp. *saxatilis* to be sister to the *lanata*–*hierapetrae* clade. Endemic to a few localities in western Crete, this dwarf chasmophyte is characterized by having spatulate leaves and a velutinous, narrowly tubular corolla. *Campanula saxatilis* subsp. *cytherea*, endemic to the Aegean islands of Kythira and Andikythira (NW of Crete), differs in having oblanceolate leaves and a broadly tubular corolla. This Cretan lineage dates to 10.5 (± 4) Ma and probably represents an example of taxon range fragmentation that may have occurred before the Pliocene.

Clade 5

Campanula aizoides and *C. spatulata* are nested in a large, well-supported clade containing taxa that are mostly native to southern Europe and the Balkans. *Campanula aizoides* is a

high-elevation species (1800–2400 m) with a bi-regional, disjunct distribution in a restricted area of western Crete (Lefka Ori) and on Mt Helmos, in the northern region of the Peloponnese. This species is characterized by a thyrsoid inflorescence and large, tubular-campanulate flowers. *Campanula aizoides* is another example of a mountain endemic for which isolation of disjunct populations has not driven adaptive radiation and species differentiation. The divergence age estimate at its node is 38 (\pm 10) Ma, indicating that this lineage was present in Crete long before the Pliocene and represents a palaeo-subendemic. The populations on both Lefka Ori and Mt Helmos may have avoided Quaternary extinction by adaptation to orophytic habitats, and/or by gradually taking refugia in these less competitive, species-impooverished areas.

Campanula spatulata subsp. *filicaulis* is a delicate, filiform, one-flowered perennial that is endemic to central and eastern Crete. It grows in phrygana and rocky places, but most often thrives in overgrazed habitats, growing exclusively within the branches of the dwarf, spiny shrub *Sarcopoterium spinosum* (Rosaceae). In such open and degraded habitats, where few delicate herbs would survive overgrazing, the association with *Sarcopoterium spinosum* has proved to be very successful. *Campanula spatulata* subsp. *filicaulis* is sister to *C. lusitanica*, an annual found in Spain and Portugal with one-to-few-flowered inflorescences and similar infundibuliform-campanulate flowers with filiform pedicels. This well-supported split dates to 17 (\pm 8) Ma, suggesting that the ancestor of this lineage was present in Crete long before its isolation. *Campanula spatulata* subsp. *filicaulis* is part of a small complex that includes the highland subsp. *spatulata* and the lowland subsp. *spruneriana*, two morphologically and ecologically well-defined taxa that are found in Greece and the southern Balkans (Blionis & Vokou, 2002). This small complex is clearly the result of geological isolation and subsequent divergence.

Clade 6

With moderate support, *Campanula cretica* and *Petromarula pinnata* are nested in a clade that includes exclusively eastern Mediterranean and European taxa. *Campanula cretica* is a perennial with pendant, tubular flowers arranged in a distinct, secund raceme. It is endemic to western Crete and appears as sister to *Asyneuma virgatum*, although support is not strong. *Asyneuma* represents a group of usually robust, erect herbs found from south-east Europe to eastern Asia, and similar in habit to *Petromarula* and *Michauxia*, with distinct, paniculate inflorescences and corolla lobes deeply divided nearly to the base, although not reflexed. The putative age of the *C. cretica* lineage is 24 (\pm 10) Ma, which suggests that this species represents another continental remnant that has not diversified in isolation.

Petromarula pinnata is the only endemic in Campanulaceae that is widespread across the island of Crete. Among the Cretan Campanulaceae, it is very distinct for its robust habit,

flower morphology, and ability to thrive in less harsh, more competitive habitats. *Petromarula* was observed (personal observation) rooting high on the trunk of *Phoenix theophrastii* (Arecaceae), and it may represent a case of facultative epiphytism. Epiphytism has not previously been recorded in any of the campanuloids and wahlenbergioids. It occurs in one species of *Canarina* (platycodonoids) (Lankester, 1922; Tweedie, 1976; Benzing, 1990), but is more common in Neotropical lobelioids (*Burmeistera*, *Centropogon*, *Clermontia*, and *Cyanea*). Therefore, epiphytism seems to have evolved independently in the Campanulaceae *sensu lato*, and at least twice in the Campanulaceae *sensu stricto*.

With little obvious affinity to other Mediterranean species, the monotypic *Petromarula* has traditionally been viewed as an ancient taxon with no close relatives (Barclay, 1986). In our analyses, *Petromarula* is sister to a clade that includes *Physoplexis comosa* and *Phyteuma spicatum*. However, the topology of this clade is not congruent across the individual gene trees. In the tree generated by *matK*, *Petromarula* and *Asyneuma* are sisters to a clade that includes *C. cretica*, *Phyteuma spicatum* and *Physoplexis comosa*. In the tree generated by *rbcl* data, *C. cretica* is related to a clade that includes *Petromarula* as sister to a much larger group. A clade including *Petromarula*, *Physoplexis*, *Phyteuma* and *Asyneuma* was also recovered in a phylogenetic analysis based on ITS sequences (Eddie *et al.*, 2003) that did not include *C. cretica* in the taxon sample. The *Petromarula* lineage dates to 21 (\pm 9) Ma, suggesting that this is a relictual species.

Our analyses represent the first attempt to understand the origin of Cretan endemics in view of the complex geographical history of the Aegean area and new phylogenetic data. Our taxon sample is clearly missing many species that occur in the eastern Mediterranean region, including several endemics to neighbouring islands. Nevertheless, based on our results, we can clearly identify lineages that pre-date island isolation and probably represent palaeoendemics. Similar studies analysing a variety of zoological data in the Aegean area, including the island of Crete, have also concluded that many narrowly distributed endemics represent relicts of an older continental fauna that has failed to diversify (de Vries, 1985; Douris *et al.*, 1998; Welter-Schultes & Williams, 1999; Fattorini, 2002; Poulakakis *et al.*, 2003; Parmakelis *et al.*, 2006). In addition, it has been shown that limited over-sea dispersal is the rule rather than the exception in the Aegean archipelago, as the sea presents a severe barrier for many taxa (Runemark, 1969; Bittkau & Comes, 2005). For example, *Klasea cretica* (Asteraceae–Cardueae) is the only species in the genus *Klasea* occurring in the Aegean region, despite the presence of many different species in the west (Spain, Italy and North Africa) and the east (Anatolia); yet, the Asteraceae are renowned for their ability to disperse easily (Martins, 2006). In the specific case of the Campanulaceae, the lack of diversification in the Cretan area is similar to patterns found on oceanic islands (e.g. Macaronesia), where endemic species show extraordinary morphological variation but limited adaptive radiation (Haberle *et al.*, in press).

CONCLUDING REMARKS

Our results suggest that most Campanulaceae taxa were present on the islands at the time of their physical isolation, and that they have been there for quite a long time. It appears that very little long-distance dispersal and diversification has occurred since then. In general, long-distance dispersal in terrestrial plants is favoured by anemochory and zoochory (Cain *et al.*, 2000). These Campanulaceae are characterized by capsular fruits that are not adapted to either of these seed dispersal mechanisms; rather, dispersal depends largely on gravity. In the case of populations growing on steep cliffs and slopes, rainstorms and wind may play some role in population expansion. However, in general, offspring tend to grow near their parents. From the perspective of their reproductive biology, it is perhaps not surprising that dispersal appears to have played a minor role in the assembly of the Cretan Campanulaceae.

What is perhaps more intriguing is the lack of significant diversification within the Cretan area after millions of years of isolation. This may be a result of the lack of opportunities for radiation in these continental island systems, where many niches have long been occupied. We find that *C. carpatha*, *C. pelviformis*, *C. laciniata* and *C. tubulosa* form a well-supported clade that is probably the result of speciation in the islands after isolation, and *C. creutzburgii* may represent a case of divergence after dispersal. However, for the most part, Cretan Campanulaceae are remnants of a continental flora that is no longer present on the mainland, and have not diversified significantly. The high degree of endemism is therefore probably the result of the loss of species on the mainland after isolation. Species differentiation took place before island isolation, and subsequently these lineages have been maintained in their environments. This is also probably true of the non-endemic taxa. For example, *Legousia*, which is fairly widespread on the mainland, may have been present in the Cretan area before isolation.

We note that, although some apparent relicts have small, isolated populations, such as *C. hierapetrae* and *C. jacquinii*, this is not always the case. Conversely, isolation and restricted distributions may not always reflect the relict nature of taxa, as previously suggested (Davis, 1951; Runemark, 1971). For example, Davis (1951) argued that *C. laciniata* is a relict species on the grounds of its disrupted, bi-regional distribution. However, our results indicate that *C. laciniata* is part of a clade that has radiated on the island more recently.

During the past 6000 years the islands have been under great human pressure, making it very difficult to reconstruct the past distributions of species. Neolithic settlers brought with them a full range of domesticated animals, including sheep and goats (Evans, 1971; Broodbank & Strasser, 1991). *Olea* (olive) pollen records recovered from sediments in western Crete suggest that human manipulation of the local vegetation started at least by 4750 BC (Moody *et al.*, 1996), and possibly even earlier, given that *Olea* pollen dating to 7000 BC has been found in nearby Peloponnesus (Kraft *et al.*, 1980). Today, at least 50% of the

land surface is used for grazing sheep and goats, and most of the rest is dedicated to agriculture and tourism.

In a paper by Runemark (1971), Greuter, as part of a discussion at the end of the paper, contended that very few cliff-loving species are obligate chasmophytes. These species lack adaptive specialization, and in the absence of pressure from grazing and competition they would successfully grow in other habitats. There are a few cases among the Cretan campanulas for which species are able to switch from phrygana and roadside banks to calcareous cliffs, and *vice versa* (e.g. *Petromarula pinnata*, *C. pelviformis*, *C. creutzburgii*). A striking example is provided by *C. spatulata* subsp. *filicaulis*. This delicate herb normally grows in phrygana and rocky places. However, its close association with the spiny *S. spinosum*, which offers protection from predators, allows it to survive in desert-like habitats that are constantly under pressure from overgrazing. Such shifts support the hypothesis that many of these species may have been more widespread on the mainland but are now restricted by environmental pressure rather than specific adaptations.

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BIOSKETCH

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