ORIGINAL ARTICLE

Phylogenetic assessment and biogeographic analyses of tribe *Peracarpeae* (Campanulaceae)

Zhuo Zhou · Jun Wen · Guodong Li · Hang Sun

Abstract *Peracarpeae* is a small tribe consisting of three genera: *Homocodon*, *Heterocodon* and *Peracarpa*, with a disjunct distribution between eastern Asia and western North America. *Homocodon* is endemic to southwestern China and was previously placed in the western North American genus *Heterocodon*. Our phylogenetic analysis using four plastid markers (*mat*K, *atp*B, *rbc*L and *trn*L-F) suggests the polyphyly of *Peracarpeae*. *Homocodon* is sister to a clade consisting of the eastern Asian *Adenophora*, *Hanabusaya* and species of *Campanula* from the Mediterranean region and North America, rather than forming a clade with *Heterocodon*. *Homocodon* and its Eurasia relatives are estimated to have diverged in the early Miocene (16.84 mya, 95% HPD 13.35–21.45 mya). The eastern Asian *Peracarpa* constitutes a clade with the North American *Heterocodon*, *Githopsis* and three species of *Campanula*, supporting a disjunction between eastern Asia and North America in Campanulaceae. The Asian-North American disjunct lineages diverged in the early Miocene (16.17 mya, 95% HPD 13.12–20.9 mya). The biogeographic analyses suggest that *Homocodon* may be a relict of an early radiation in eastern Asia, and that *Peracarpa* and its closest North American relatives most likely originated from a Eurasian ancestor.

Introduction

The disjunct distributions of closely related plants between eastern Asia and North America have attracted much attention from botanists and biogeographers since the Linnaean era (Li 1952; Tiffney 1985a, b; Wen 1999). Recent molecular phylogenetic studies have focused on many disjunct plant groups distributed between eastern Asia and eastern North America. This well-known biogeographic disjunction has been explained mostly by the fragmentation of the more widely distributed mesophytic forests in the Northern Hemisphere during the mid-Tertiary (Wood 1972; Axelrod 1975; Tiffney 1985b; Collinson 1994; Manchester 1999; Wen 1999; Tiffney and Manchester 2001; Weber 2003). Both the Bering and the North Atlantic land bridges were hypothesized to have contributed to the widespread distribution of the mesophytic forests (Tiffney 1985a, b; Nie et al. 2006). The forests became disrupted in response to (1) the climatic cooling in the Northern Hemisphere with heavy impact of glaciations in Europe and other regions of higher northern latitudes in the late Tertiary and the Quaternary, and (2) the Tertiary orogenies and subsequent development of the xeric and higher elevation coniferous flora in western North America. These events led to extinctions of many plants, especially those in Europe and central and western North America, but some plants survived and became relicts in widely discontinuous areas (Graham 1972; Wolfe 1972; Hoey and Parks 1991; Wen 1999; Wen et al. 2010).

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In contrast to the strong research interests on the disjunction between eastern Asia and eastern North America, the disjunction between eastern Asia and western North America (EAs-WNa) remains poorly understood in plants. This may be due to the fact that the EAs-WNa pattern is less common in plants in spite of their closer connection via the Bering land bridge (Wu 1983; Hong 1993; Donoghue and Smith 2004). At present, only a few genera distributed between eastern Asia and western North America have been studied phylogenetically. These groups include *Pseudotsuga* Carriere of Pinaceae (Wei et al. 2010), *Lysichiton* Schott of Araceae (Nie et al. 2006), *Kelloggia* Torr. ex Hook.f. of Rubiaceae (Nie et al. 2005), *Oplopanax* Miq. of Araliaceae (Lee and Wen 2002) and *Glehnia* F. Schmidt of Apiaceae (Sun et al. 2004). The first four genera were each shown to be monophyletic, and were estimated to have diverged between the two continents ca. 20.26 ± 5.84 (*Pseudotsuga*), 7.18 ± 4.33 (*Lysichiton*), 5.42 ± 2.32 (*Kelloggia*) and 5.6 mya (*Oplopanax*) in the Miocene to early Pliocene, while the two species of *Glehnia* were found to be non-monophyletic. More taxa with an eastern Asian and western North American disjunction need to be studied to enhance our understanding of this biogeographic pattern.

Homocodon D.Y. Hong from eastern Asia and *Heterocodon* Nutt. from western North America are presumed to be closely related members of Campanulaceae (Hong 1980; Hong et al. 1983; Fig. 1). While most disjunct taxa between eastern Asia and North America are two or more species from the same genus, a few disjuncts are at the infraspecific level or between closely related genera of the same family such as *Weigela* Thunb. (from eastern Asia, Caprifoliaceae) and *Diervilla* Mill. (from eastern North America) (see Kim and Kim 1999; Wen 1999).

Homocodon and *Heterocodon* represent another example of two presumably closely related genera disjunct between the two continents.

Homocodon (Campanulaceae) consists of two species, *H. brevipes* (Hemsl.) D.Y. Hong, widely distributed in SW China (Yunnan Province, W Sichuan Province and SW Guizhou Province), and *H. pedicellatus* D.Y. Hong and L.M. Ma, a narrow endemic to the Luding area of Sichuan province, China, in the Gongga Mountains. *Homocodon brevipes* was described by Hemsley (1903) as a species of *Wahlenbergia* Schrad ex Roth. Handel-Mazzetti (1936) argued that this species was most closely related to the monotypic western North American *Heterocodon* (*H. rariflorum* Nutt.) and the monotypic eastern Asian *Peracarpa* Hook.f. & Thomson (*P. carnosa* Hook.f. & Thomson). They transferred the species from *Wahlenbergia* to *Heterocodon*, making *Heterocodon* a genus discontinuously distributed between SW China and western North America. Hong (1980) compared the morphology of this species with that of *Wahlenbergia, Heterocodon, Peracarpa* and other genera in the family, and established the genus *Homocodon*. The dehiscence of capsules in *Homocodon* is by irregular tearings, unlike that in *Wahlenbergia* and the Wahlenbergieae by apical pores or valves. *Heterocodon, Homocodon* and *Peracarpa* are contrasted in Table 1. Hong and Ma (1991) published the second species of the genus, *H. pedicellatus*, for its obviously visible peduncle from the Gongga Mountains in Sichuan. Hong (1980) also pointed out these three genera were more closely related to each other in Campanulaceae based on their fruit dehiscence, and he defined tribe Peracarpeae as consisting of these three genera (Hong et al. 1983). This tribe is characterized by the thin, membranous pericarps, absence of tuberous roots, and capsule dehiscence by irregular

Fig. 1 Distribution of tribe Peracarpeae (*Heterocodon, Homocodon* and *Peracarpa*) of Campanulaceae showing the presumed disjunction between eastern Asia and western North America
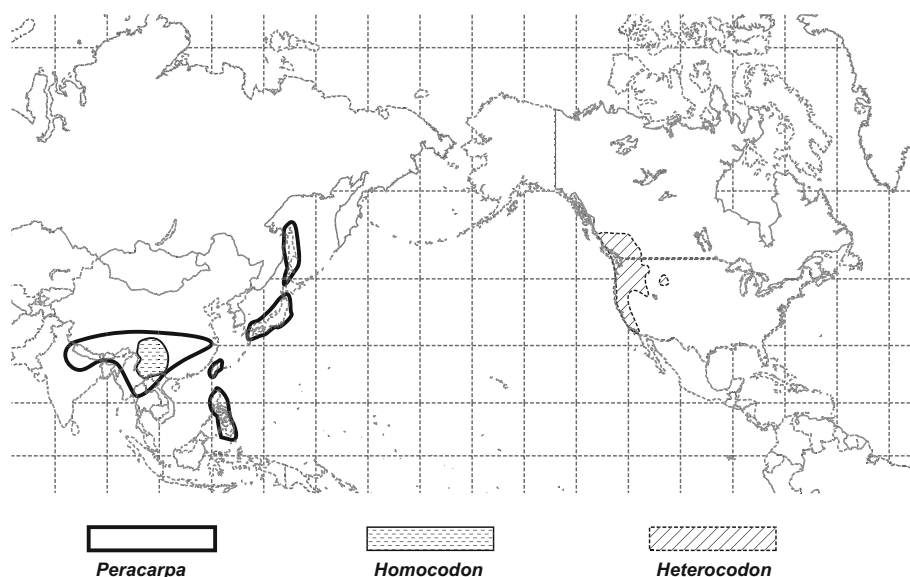
Table 1 Differences among *Heterocodon*, *Peracarpa* and *Homocodon*

	Heterocodon	Peracarpa	Homocodon
Duration	Annual	Perennial	Annual
Stem	Erect	Creeping	Creeping
Flower aggregation	Solitary	Solitary	1–3 flowers
Flower position	Opposite to a bract	In leaf axils	Aggregated flowers on terminal on highly shortened and leafy lateral branches
Peduncle	Absent	Long and slender	Absent or short
Calyx lobes	Broad with a pair of broad teeth	Narrow and entire	Narrowly triangular, with a pair of pointed and long teeth
Corolla	Dimorphic/heterogeneous; those on flowers of the lower part of stem not developed; those on upper part of stem well developed	Homogeneous; all developed	Homogeneous; all developed
Seed morphology	Ribbed, with smooth surface	Not ribbed, with smooth surface	Not ribbed, with reticulate surface

tearings. The distribution pattern of this tribe is shown in Fig. 1. Wang (1992) supported the close relationship between *Peracarpa* and *Homocodon*, and hypothesized that the two genera might have originated in the Yunnan-Guizhou Plateau in SW China. Wu et al. (2003) examined *Heterocodon* and *Homocodon*, and proposed that the two genera might have become isolated at the early expansion period of the Pacific Ocean and then differentiated respectively in the two continents, implying a very ancient origin of the intercontinental disjunction.

Recent cytological and molecular phylogenetic studies of Campanulaceae have contributed greatly toward the classification and the understanding of relationships within the family. However, these analyses so far have not evaluated the three genera of *Peracarpeae*. Lü et al. (2007) reported the chromosome number of *Homocodon brevipes* as $2n = 68$ and suggested that the species may be a tetraploid. He also proposed that *Homocodon* may be most closely related to *Adenophora* Fisch., which has the chromosome number $x = 17$. Recent molecular analyses (Eddie et al. 2003; Cosner et al. 2004; Haberle et al. 2009; Roquet et al. 2009) have supported two major clades in Campanulaceae: the platycodonoids sister to a clade that includes the walenbergioids and campanuloids. The smaller platycodonoid clade includes taxa with colporate/colpate pollen grains, and the clade includes *Codonopsis* Wall., *Platycodon* A.DC., and several other eastern Asian genera. The larger clade consists of taxa with porate pollen grains and includes wahlenbergioids (*Wahlenbergia* and allied taxa) and campanuloids (*Campanula* L. and many segregate genera). The campanuloids comprised the bulk of the family, which were further divided into two major clades. One clade is the "*Campanula* s.str." group consisting of taxa possessing calyx appendages (the c-1 clade in Haberle et al. 2009); the other clade is the "*Rapunculus*"

clade (the c-2 clade in Haberle et al. 2009) with taxa lacking calyx appendages. "*Campanula* s.str." and "*Rapunculus*" were initially used for designating the two sections of the genus *Campanula* by Boissier (1875) and Fedorov (1957). They are better applied in grouping the campanuloids. *Heterocodon* was shown to form a clade with the western North American endemic genus *Githopsis* Nutt. and three North American species of *Campanula*. This small clade of *Heterocodon-Githopsis* subset of three *Campanula* species is then sister to a diverse clade of *Campanula*, *Asyneuma* Grisb. & Schenk, *Petromarula* Vent. ex R.Hedw., *Physoplexis* Schur, *Phyteuma* L., *Campanulastrum* Small, *Triodanis* Raf. and *Legousia* Durand containing taxa from Eurasia, the Mediterranean region and North America (Haberle et al. 2009). Yet previous phylogenetic studies have never sampled the Asian *Homocodon* and *Peracarpa*, and their systematic positions still remain unclear. Overall the disjunction between Eurasia and North America is poorly represented in Campanulaceae. Except for the example demonstrated in the current study, some *Campanula* species also have this disjunct pattern. *Campanula chamissonis* Federov and *C. lasiocarpa* Cham. are distributed across the Beringia from Alaska, the Aleutian Islands to the Russian Far East and Japan; *C. rotundifolia* L. and *C. uniflora* L. have a circumpolar distribution in Eurasia and the Arctic North America (Fedorov 1957; Welsh 1974). The trans-Beringia distributions have been explained by the Beringia serving as a glacial refugium, or the Bering land bridge as a dispersal highway, or relicts that were more widely distributed in the past or recent long-distance dispersals (see review by Ickert-Bond et al. 2009). The circumpolar *Campanula rotundifolia* was supposed to have dispersed from Eurasia to the Arctic North America (Roquet et al. 2009). Nevertheless, our focus in the present study is to evaluate the

disjunction between eastern Asia and western North America, and we will not discuss the cases of trans-Beringian and circumpolar distributions here.

The objectives of this study are to (1) assess the phylogenetic positions of *Heterocodon*, *Homocodon* and *Peracarpa*; and (2) estimate the divergence times of the biogeographically disjunct lineages and construct the biogeographic diversification history of the disjuct taxa between eastern Asia and western North America.

Materials and methods

Plant material, DNA extraction, amplification and sequencing

We sampled all four species of the three genera in *Peracarpeae*. Representative species of *Adenophora*, *Codonopsis*, *Cyananthus* Wall ex. Benth. and *Leptocodon* Lem. were also sequenced (Table 3 of "Appendix"). All other sequences of Campanulaceae were downloaded from GenBank (Table 4 of "Appendix"). Total genomic DNA was extracted from silica-dried leaf material, using Axygen kits (Axygen Biosciences, Union City, CA, USA). Three chloroplast coding regions (*mat*K, *atp*B and *rbc*L) and the noncoding *trn*L-F intergenic spacer were amplified via polymerase chain reaction (PCR). All PCR reactions were conducted in a 25-μl volume consisting of 1–2 μl sample DNA ($\approx$1–10 ng), 2.5 μl 10× buffer, 1 μl MgCl$_2$ (25 mM stock), 2.5 μl dNTPs, 0.6 μl of 10 μM stock of each primer and 0.2 μl *Taq* polymerase, brought up to 25 μl with ddH$_2$O. The *trn*L-F region was amplified using primers "c" and "f" or in two shorter fragments using primers of "c" + "d" and "e" + "f" as described in Taberlet et al. (1991). Amplification protocols and Campanulaceae-specific primers for amplifying *mat*K, *atp*B and *rbc*L genes followed Haberle et al. (2009). A new internal sequencing primer for *atp*B gene was designed in this study (atpB_C811R 5′-CGG AAG ATA TTG TCG ATA AAG-3′). The amplified products were then purified with the polyethylene glycol (PEG) precipitation procedure (Hiraishi et al. 1995). The cycle sequencing reactions were conducted in 10 μl volumes that contained 0.25 μl BigDye 3.1, 0.5 μl primers, 2.0 μl purified PCR products and 1.75 μl sequencing buffer. The sequencing reactions were run on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

Sequence alignment and phylogenetic analyses

The program Sequencher 4.5 (Gene Codes Corp. 2005) was used to evaluate chromatograms and edit contiguous sequences. Sequences were aligned with ClustalX (Thompson et al. 1997) and manually adjusted with Se-Al v2.0a11 (Rambaut 2007).

Phylogenetic analyses were performed with PAUP4.0b10 (Swofford 2003) using maximum parsimony (MP). The MP analysis used heuristic searches with 100 replicates of random stepwise addition sequences, tree bisection-reconnection (TBR) branch swapping, MulTrees option in effect and character state changes equally weighted. The gaps of the sequence matrix were treated as missing data. Bootstrap probabilities (BP) were calculated from 1,000 replicates using a heuristic search with simple addition with the TBR and MULPARS options implemented (Felsenstein 1985).

Modeltest 3.7 (Posada and Crandall 1998; Posada and Buckley 2004) was used to determine the optimal model of molecular evolution and gamma rate heterogeneity using the Akaike information criterion (AIC). Bayesian inference (Rannala and Yang 1996) was implemented in MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003) with the model estimated above. The Markov chain Monte Carlo (MCMC) algorithm was run for 20,000,000 generations with one cold and three heated chains, starting from random trees. Runs were repeated twice. The resulting log likelihood and number of generations were plotted to determine the point after which the log likelihoods had stabilized by using the "sum parameters" commands. The halfcompat consensus tree obtaining posterior probabilities of the clades was generated from the last 18,001 out of the 20,001 trees sampled. The first 2,000 trees were discarded as burn-in to avoid trees that have been sampled prior to convergence of the Markov chains. Internodes with posterior probabilities >95% were considered statistically significant. Maximum likelihood (ML) trees were generated using the program GARLI version 0.951 (Zwickl 2006) starting from random trees and using 10,000,000 generations per search with the model estimated above. Two independent searches were repeated to avoid spurious results. Maximum likelihood bootstrap values (MB) were estimated from 100 bootstrap replicates in GARLI.

Bayesian dating analyses

We used a Bayesian dating method based on a relaxed clock model to estimate lineage divergence times as implemented in the program BEAST version 1.5.4 (Drummond and Rambaut 2007) and based on the combined four plastid markers. The program BEAUti was used to set criteria for the analysis, in which we applied a General Time Reversible (GTR) nucleotide-substitution model with Gamma + Invariant sites, gamma shape distribution (with four categories) and proportion of invariant sites. An uncorrelated log-normal relaxed molecular clock model and the Birth-Death prior tree topology were used to estimate the lineage divergence times and 95% HD credibility intervals. Posterior

distributions of parameters were made using MCMC analysis of 40,000,000 generations with 10% burn-in. The log file was then checked for convergence of the chains by using Tracer 1.3 (Rambaut and Drummond 2007). The samples from posterior were summarized on the maximum clade credibility (MCC) tree, which has the maximum sum of posterior probabilities on its internal nodes (Drummond et al. 2007) using TreeAnnotator v1.5.4 (Drummond and Rambaut 2007) with the posterior probability limit set to 0.5 summarizing mean node heights. The MCC tree was visualized using FigTree v.1.3.1, and the means and 95% higher posterior densities (HPD) could be obtained from it. The 95% HPD represents the shortest interval that contains 95% of the sampled values from the posterior (Drummond et al. 2007).

The sole fossil record of Campanulaceae is *Campanula* seeds from the Miocene (16.5–17.5 mya) in southern Poland (Lancucka-Srodoniowa 1979; Cellinese et al. 2009). These fossil seeds have been ascribed to *Campanula paleopyramidalis* Lanc-Srod., as they closely resemble those of *C. pyramidalis* L., an Italian-Balkan extant species in the *Rapunculus* clade (the c-2 clade in Haberle et al. 2009), although some of the seed characteristics appear in other species of this clade (Haberle et al. 2009). With the difficulty in placing the fossils with confidence in the large polyphyletic genus *Campanula* (Haberle et al. 2009; Roquet et al. 2009), Roquet et al. (2009) adopted a conservative approach and used these fossils as a minimum age constraint for the whole campanuloids. Cellinese et al. (2009) and Frajman and Schneeweiss (2009) used the same fossil record to date the stem age of *C. pyramidalis* and its sister species *C. carpatica* Jacq. Considering the certainty of this fossil taxon in the *Rapunculus* clade but the uncertainty concerning the specific affinity with extant species, we placed it as a minimum age constraint for the crown age of the *Rapunculus* clade (node 6 in Fig. 3, >16.5 mya). A lognormal prior of 16.1 was therefore taken as zero offset; the default lognormal mean of 1 and default lognormal standard deviation of 1 were used to constrain this node, giving a 95% confidence interval of 16.5–35.4 mya.

The divergence time between *Lobelia* and the Campanulaceae sensu stricto (Cosner et al. 2004) was estimated to be 49–56 mya based on the broad dating analyses of angiosperm families by Wikström et al. (2001). We used this age to calibrate the root age. A normal distribution was taken with a mean at 52.5 mya, the midpoint of Wikström et al.'s (2001) dates, and a standard deviation of 4.5, giving a 95% confidence interval of 45.1–59.9 mya, as our prior for the root node (node 7 in Fig. 3).

Biogeographic analyses

We employed a maximum likelihood based method (Ree et al. 2005; Ree and Smith 2008) to reconstruct the biogeographic history of *Peracarpeae*. The program LAGRANGE (Ree and Smith 2008) was used to run the analysis with a simple model of one rate of dispersal and extinction constant over time and among lineages. This program not only reconstructs the most likely ancestral area at each internal node, but also estimates the probability of range inheritance scenarios (i.e., the split of the ancestral area between the two descendant lineages).

Three areas of endemism were circumscribed: (A) western Eurasia (including western Asia, the Caucasus mountains, Mediterranean Europe and North Africa), (B) eastern Asia and (C) North America. The ML tree was used for the biogeographic analyses.

Results

The aligned matrix of the combined cpDNA data set had 5,392 characters, of which 3,313 were constant, 850 were variables but parsimony uninformative, and 1,229 were parsimony-informative. Modeltest suggested that the transversion model (TVM + I + G) was the best fitted model (-ln likelihood score = 36,625.2148).

A total of 83 taxa from all three major clades of the family are selected for the phylogenetic analysis (Tables 3, 4 of "Appendix"). Treating gaps as missing data, with *Lobelia cardinalis* L. as the outgroup, the maximum parsimony analysis produced 5,184 maximally parsimonious trees (MPTs) of 4,844 steps, with a consistency index (CI) of 0.60 CI, excluding uninformative characters of 0.50, and a retention index (RI) of 0.74. All three genera in tribe *Peracarpeae* are in the *Rapunculus* clade (the c-2 clade in Haberle et al. 2009) (Fig. 2). The topology of the *Rapunculus* clade in the 50% majority-rule consensus of 18,001 trees (20,001 trees minus 2,000 burn-in trees) from the Bayesian (BI) analysis was largely congruent with that of the MPTs. They only differed in that *Campanula aizoides* Zaffran. formed a clade with five other species of *Campanula* within the c-2a clade (as in Haberle et al. 2009) in the BI analysis, while this node collapsed in the MP strict consensus tree.

The MP and the Bayesian analyses of the combined plastid data suggested the following relationships concerning taxa of tribe *Peracarpeae*. *Homocodon* formed a clade with the eastern Asian *Adenophora* and *Hanabusaya* Nakai, and some North American and European species of *Campanula* (the *Homocodon-Adenophora* clade) with strong support (BS 94%, PP 1.00). *Peracarpa* and the western North American *Heterocodon*, *Githopsis*, and three species in *Campanula* from North America formed another clade (the *Peracarpa-Heterocodon-Githopsis* clade) with weak support (BS 56%, PP 0.75). These two clades were sister to each other (Fig. 2, only showing specific in *Rapunculus* clade).

Fig. 2 Strict consensus tree from maximum parsimony analysis of combined *rbc*L, *mat*K, *atp*B and *trn*L-F data. *Numbers above branches* indicate bootstrap values; *numbers below branches* are Bayesian posterior probabilities. Only topology of the *Rapunculus* clade (c-2 clade) is shown. Taxa in *Peracarpeae* are boldfaced. Geographic distribution of each taxon (except *Campanula* and *Homocodon*) is labeled to represent the area for the entire genus. ME, EA, NA, ENA and WNA stand for Mediterranean Europe, eastern Asia, North America, eastern North America and western North America, respectively
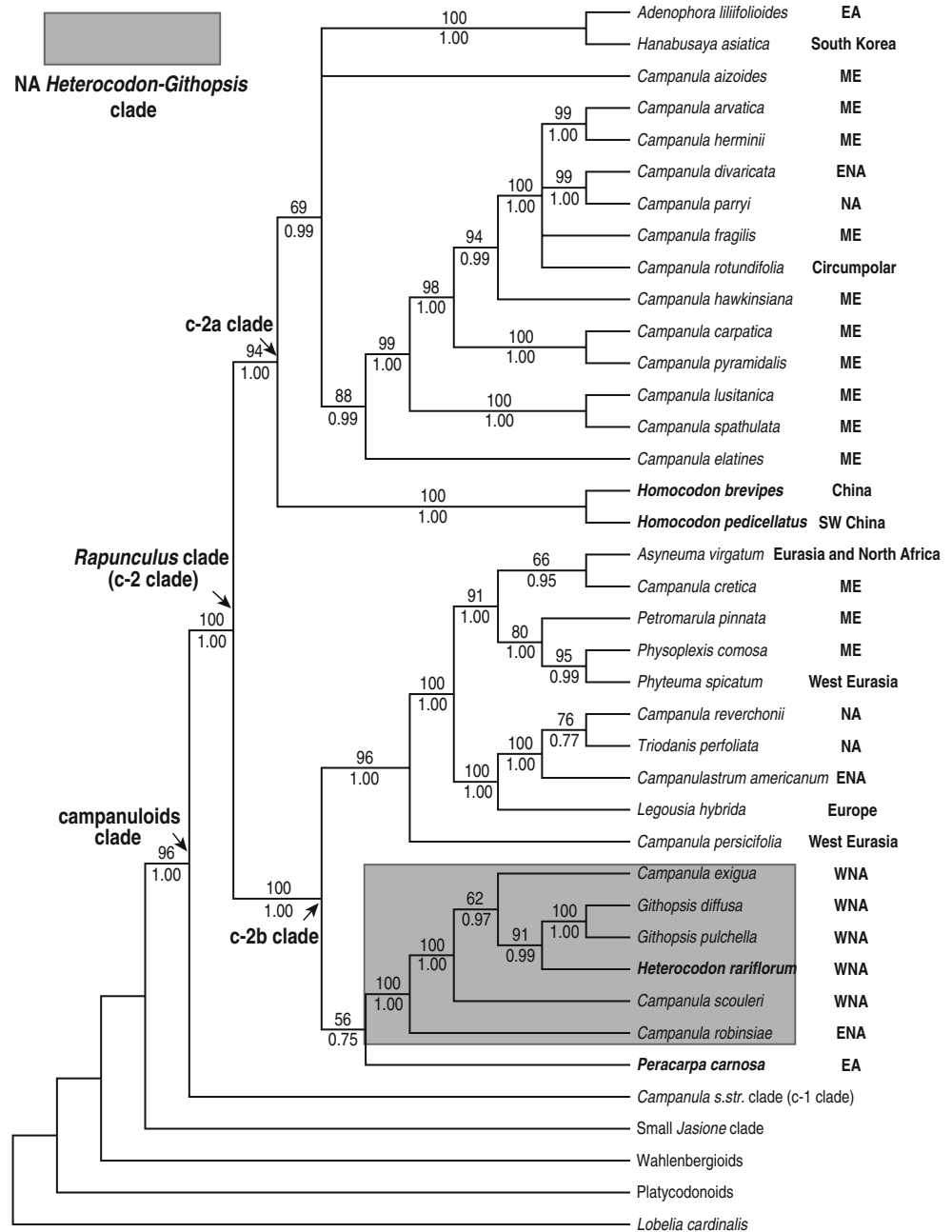
The topology of the *Rapunculus* clade in the maximum clade credibility (MCC) tree (Fig. 3) derived from the BEAST analysis is identical with that of the halfcompat consensus tree from MrBayes. Confidence intervals (95% HPD bars) are shown on the tree (Fig. 3). Lineage divergence times estimated for the nodes of interest are shown in Table 2. The divergence of the Azorean endemic species *Azorina vidalii* (H. C. Watson) Feer (node 8 in Fig. 3; Table 2) was estimated to be 5.15 mya, which is within the range of the age of the sub-aerial stage of the oldest island of the Azores according to geological reconstructions (8 mya, Abdel-Monem et al. 1975). The divergence time of *Musschia aurea* Dumort., a species endemic to Madeira,

with its closest relative *Campanula primulifolia* Brot. (node 9 in Fig. 3) was estimated to 4.90 mya (95% HPD 2.17–8.09), which corresponds to the geological age of Madeira island, which emerged no more than 5.2 mya (Ferreira et al. 1988). Our estimates are thus consistent with the geological evidence.

The LAGRANGE analysis was based on the maximum likelihood tree. Our emphasis is on the *Rapunculus* clade (the c-2 clade in Haberle et al. 2009). With taxa in *Campanula* s.str. clade (the c-1 clade in Haberle et al. 2009) all distributed in western Eurasia (A), we selected only one representative of the c-1 clade, but all taxa of c-2 clade in the LAGRANGE analysis. The topology of the c-2 clade
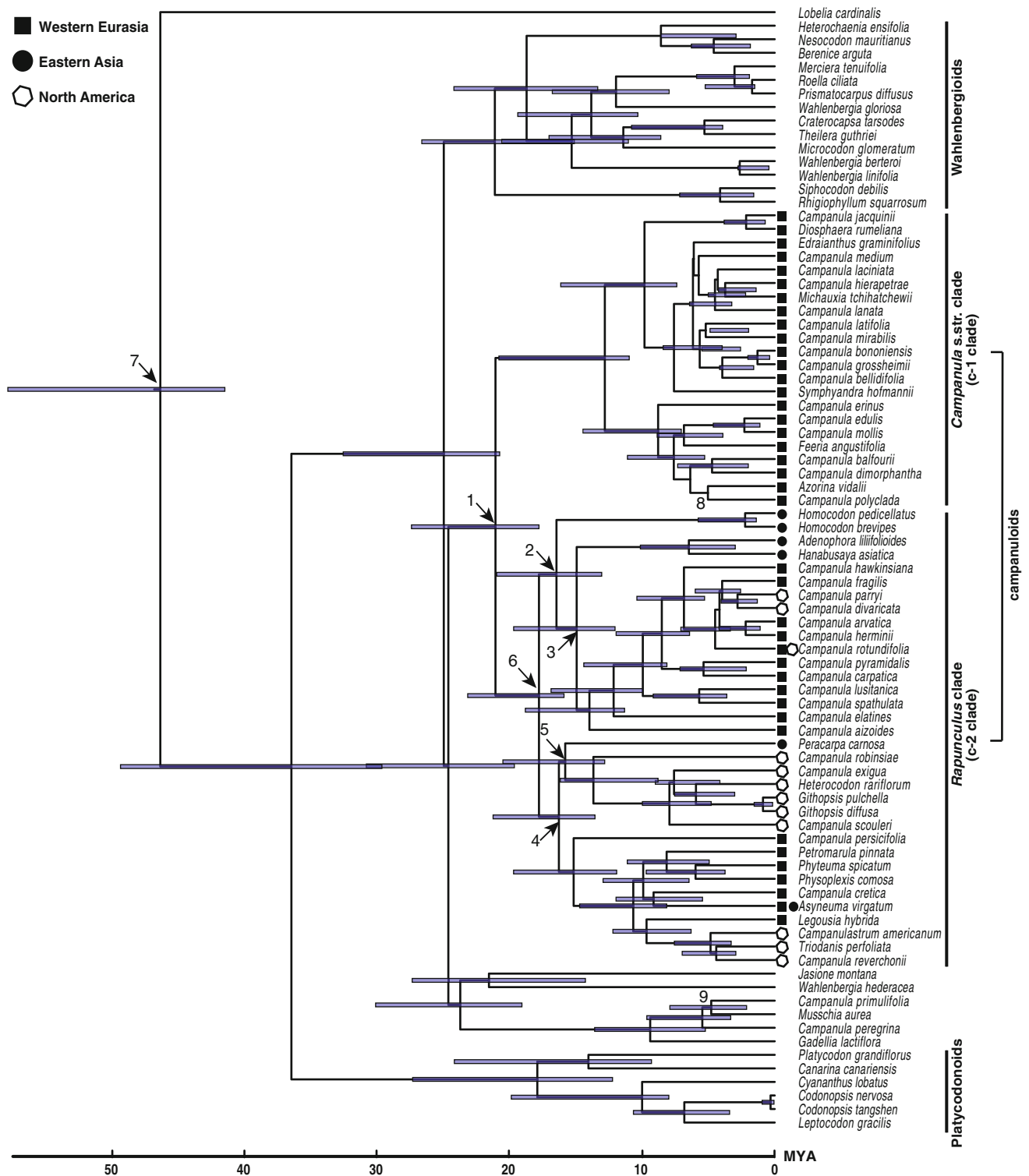
Fig. 3 Maximum clade credibility (MCC) tree from the BEAST analysis showing mean heights (ages). The *blue bars* on the nodes indicate 95% posterior credibility intervals. Fossil calibration is set in node 6 (>16.5 mya), and the root age is calibrated to be 52.5 ± 4.5 mya (node 7). The divergence time and ancestral distribution of the nodes of interest (*all marked*) are shown in Table 2

from the ML analysis is identical with that of the BI and the MCC trees. We presented our LAGRANGE and the dating results in Fig. 3; Table 2. The ancestral area of the c-2 clade

and the c-2a clade (including *Homocodon*) was inferred to be Eurasia (AB) or western Eurasia (A) (node 1 and 6, respectively, in Fig. 3; Table 2). The ancestral node of c-2b

Table 2 Lineage divergence times and range inheritance scenarios for some nodes of interest (Fig. 3) as estimated from BEAST and Lagrange, respectively

Node	Description	Mean age (95% HPD) (mya)	ML range inheritance scenario (likelihood probabilities)
1	Crown of campanuloids clade	21.54 (18.19–28.02)	A–AB (0.42)/A–A (0.38)
2	Crown of c-2a clade	16.84 (13.35–21.45)	B–AB (0.82)
3	Divergence between eastern Asian taxa and other taxa in c-2a clade	15.28 (12.32–20.14)	B–A (0.91)
4	Crown of c-2b clade	16.65 (13.86–21.73)	A–A (0.27)/C–A (0.24)
5	Divergence between *Peracarpa* and North American *Heterocodon–Githopsis* clade	16.17 (13.12–20.97)	C–C (0.37)/B–C (0.18)
6	Crown of *Rapunculus* (c-2) clade	18.19 (16.26–23.69)	AB–A (0.35)/A–A (0.25)
7	Root node	47.40 (42.42–59.16)	–
8	Divergence between *Azorina* and its closest relative *Campanula polyclada*	5.15	–
9	Divergence between *Musschia* and its closest relative *Campanula primulifolia*	4.90 (2.17–8.09)	–

Nodes are as labeled in Fig. 3

A dash in the result of LAGRANGE indicates the split of the ancestral distribution between the two descendant lineages

A Western Eurasia (including west Asia, Caucasus mountains, Mediterranean Europe and North Africa); *B* eastern Asia; *C* North America

(including *Heterocodon* and *Peracarpa*) was inferred to be western Eurasia (node 6 in Fig. 3; Table 2). The most likely root area of the c-2 clade was western Eurasia.

Discussion

Homocodon as a distinct genus endemic to eastern Asia and polyphyly of tribe *Peracarpeae*

Our results based on the combined analysis of four plastid markers support the monophyly of *Homocodon* as defined by Hong (1980) and Hong and Ma (1991). *Homocodon* sensu Hong and Ma (1991) consists of two species: *H. brevipes* widely distributed in SW China and *H. pedicellatus*, a narrow endemic of the Gongga Mountains in Sichuan, China. The two species of *Homocodon* are morphologically similar. They differ in that *H. pedicellatus* has an obviously visible peduncle (vs. only a short peduncle in *H. brevipes*).

Homocodon does not form a clade with *Heterocodon* from western North America in any of the analyses. *Homocodon* is instead part of a clade containing the primarily eastern Asian *Adenophora* (65 species with only one species in western Europe), the monotypic genus *Hanabusaya* from South Korea, and several species of the large and polyphyletic genus *Campanula* from North America and southern Europe. This clade was referred to as the c-2a clade in the "*Rapunculus*" group in campanuloids by Haberle et al. (2009). Both *Homocodon* and *Heterocodon* possess small flowers, entirely inferior ovaries, three-lobed stigmas, thin fruit walls and irregular tearing dehiscence of capsules. However, the two genera can be easily distinguished by the procumbent

stem, 1–3 flowers on the top of an extremely shortened and leafy lateral branch, and a homogeneous corolla in *Homocodon* (vs. erect stem, solitary flowers opposite to a bract, and a heterogeneous corolla in *Heterocodon*).

Our results clearly suggest that tribe *Peracarpeae* as defined by Hong et al. (1983) and containing these three genera (*Heterocodon*, *Homocodon* and *Peracarpa*) is not monophyletic (Fig. 2). *Peracarpa* and *Heterocodon* form a clade with a few North American taxa of *Githopsis* and *Campanula*. This clade of *Peracarpa*, *Heterocodon* and the few North American taxa is sister to a morphologically diverse clade consisting of North American and European species of *Asyneuma*, *Campanula*, *Campanulastrum*, *Legousia*, *Petromarula*, *Physoplexis*, *Phyteuma* and *Triodanis* (the c-2b clade in Haberle et al. 2009). Tribe *Peracarpeae* was first established by Fedorov (1957), who proposed a system of Campanulaceae genera distributed in the former Soviet Union. The tribe then consisted of only one genus, *Peracarpa*. Fedorov's system was modified by Hong et al. (1983), who added *Homocodon* and *Heterocodon* to the tribe. Kolakovskii (1987) also recognized tribe *Peracarpeae*, but he treated only the Old World genera without covering the North American taxa in his system. Takhtajan (1997) included *Homocodon* and *Peracarpa* as members of *Peracarpeae*, but placed the western North American *Heterocodon* and *Githopsis* in tribe Phyteumateae together with some SE European genera including *Asyneuma Phyteuma* and *Physoplexis*. The latter three genera have corolla deeply divided nearly to the base and lobes much longer than the tube (vs. lobes shorter than or about equal to the tube in *Heterocodon* and *Githopsis*). The capsule dehiscence is also different between the North American *Heterocodon/Githopsis* taxa and the core Phyteumateae genera.

Adenophora, *Hanabusaya* and *Campanula* have been placed in the tribe Campanuleae (Hong et al. 1983; Kolakovskii 1987; Hong 1995; Takhtajan 1997). *Campanula* has been shown to be polyphyletic (Eddie et al. 2003; Haberle et al. 2009; Roquet et al. 2009). Nevertheless, *Campanula* and its close allies, including *Adenophora*, *Hanabusaya* and *Zeugandra* P.H. Davis (not sampled), may still form a relatively natural group based on the following shared characters: a lobed corolla, capsules dehiscent by lateral pores, a cymose inflorescence and the perennial erect habit (Hong et al. 1983; Hong 1995). *Homocodon* does not possess these characteristics, and its phylogenetic position seems puzzling. It is morphologically similar to *Peracarpa* and *Heterocodon* in the thin fruit wall, the irregular tearing dehiscence of capsules and the entirely inferior ovary. Yet it is more closely related to the *Campanula* group in our molecular analyses. Our phylogenetic analysis is also consistent with cytological results. The chromosome number is $x = 17$ in *Homocodon*, *Adenophora* and the majority of *Campanula*, whereas *Peracarpa* has $x = 15$ (Lü et al. 2007). *Homocodon* thus seems to be a relatively isolated taxon in Campanuleae.

Biogeographic history of eastern Asian *Homocodon* and *Peracarpa*

Taxa of campanuloids in North America were hypothesized to have migrated from Eurasia (Haberle et al. 2009; Roquet et al. 2009). Our biogeographic analyses using LAGRANGE suggest the ancestral area of the *Rapunculus* clade (the c-2 clade in Haberle et al. 2009) and the c-2a clade (Fig. 2, including *Homocodon*), are either Eurasia (AB) or western Eurasia (A) (node 1 and node 6, respectively, in Table 2; Fig. 3). The ancestral area of the c-2b clade (Fig. 2, including *Peracarpa*) is western Eurasia (see node 6 in Table 2; Fig. 3). The results thus support the migration of the campanuloids clade from Eurasia to North America (Fig. 3).

The c-2a clade includes three eastern Asian genera (*Adenophora, Hanabusaya* and *Homocodon*), several Mediterranean *Campanula* species, two North American *Campanula* species and *Campanula rotundifolia* with circumpolar distribution (Fig. 2). With the only circumpolar and the two North American *Campanula* species apparently migrated from Eurasia (Fig. 3), the c-2a clade shows a discontinuous distribution within Eurasia. This western-eastern Eurasian disjunction has been reported in many plant genera (Sun 2002; Wu 2004). Vicariance triggered by the retreat of the Tethys Sea before Tertiary and the uplift of the Qinghai–Tibet Plateau (QTP) in the Tertiary has been recognized as the major factor on the formation of this disjunct pattern (Axelrod et al. 1998; Sun 2002; Zhang et al. 2006; Tu et al. 2010). This hypothesis has been corroborated by molecular phylogenetic studies of several plant groups, e.g.,

Helloborus L. (Ranunculaceae) (Sun et al. 2001), *Cedrus* Trew (Pinaceae) (Qiao et al. 2007) and *Mandragora* L. (Solanaceae) (Tu et al. 2010). The divergence times between the two corresponding geographic lineages were estimated as 23 mya in *Helloborus*, or after Miocene in *Cedrus* or 9.82 mya (95% HPD 4.40–16.18 mya) in *Mandragora*. These estimates support the multiple vicariance events within Eurasia due to the main uplifts of QTP since the early Miocene (Harrison et al. 1992). *Homocodon* diverged from its relatives in Eurasia in the early Miocene [16.84 mya (95% HPD 13.35–21.45 mya)], and an eastern Asian lineage diverged from the Mediterranean-North America lineage within the c-2a clade at 15.28 mya (95% HPD 12.32–20.14 mya) (node 2 and node 3, respectively, in Fig. 3; Table 2). With the ancestral area of the c-2a clade either in Eurasia (AB) or only in Mediterranean Europe (A), *Homocodon* may be an isolated relict of an early vicariance from a wide-spread Tethyan ancestor, or a relict of an early radiation dispersed from the Mediterranean region.

In the c-2b clade, the sister relationship between eastern Asian *Peracarpa* and a North American clade is fascinating. The two lineages are estimated to have diverged at 16.17 mya (95% HPD 13.12–20.97 mya) (node 5 in Table 2; Fig. 3). *Peracarpa* is widely distributed in Asia (southern China, Indochina, the Philippines, Japan and the Russian Far East) (Hong et al. 1983; Barnesky and Lammers 1997; Fig. 1). The North American clade sister to *Peracarapa* contains species of three genera distributed from California to Alaska with one *Campanula* species (*C. robinsiae* Small) from Florida of eastern North America. Nevertheless, this *Peracarpa-Heterocodon–Githopsis* clade (with a few *Campanula* species) is only weakly supported (BS 56%, PP 0.75; see Fig. 2). We thus discuss the origin of the distribution pattern in a broader context of the highly supported c-2b clade (BS 100%, PP 1.00). With most taxa of the c-2b clade from Mediterranean Eurasia and North America, this distribution pattern may represent a case of the Madrean-Tethyan disjunction (Wen and Ickert-Bond 2009). The Madrean-Tethyan disjunction refers to the discontinuous distribution of closely related plants between Mediterranean Eurasia and western North America, which adapted to a Mediterranean-type climate characterized by hot, dry summers and cool, moist winters (Wen and Ickert-Bond 2009). The North Atlantic land bridges have often been used to explain the origin of this type of disjunction (Wen and Ickert-Bond 2009). Most Eurasian taxa (*Asyneuma, Legousia, Petromarula* and *Physoplexis*) of the c-2b clade of our analyses are distributed around the Mediterranean basin in southern Europe (also see Haberle et al. 2009), and *Githopsis* endemic to western North America is a genus of small, slender annuals of generally xeric habitats similar to the Mediterranean-type climate. Morin (1983) discussed the distribution and evolution of *Githopsis* in the

context of the Madro-Tertiary sclerophyllous forest present in California by the Miocene. Axelrod (1973, 1975) proposed a nearly continuous trans-Atlantic belt of Madrean-Tethyan broadleaf evergreen sclerophyllous vegetation from western North America to central Asia in the early Tertiary. Axelrod (1975) also suggested the age of the disjunct elements as about 20–25 mya. The divergence time between the *Peracarpa-Heterocodon-Githopsis* clade and the sister Mediterranean clade was dated to 16.65 mya (95% HPD 13.86–21.73 mya) in the early Miocene (node 4 in Table 2; Fig. 3). Though the North Atlantic land bridges were no longer directly viable through Neogene, several molecular studies (Hoey and Parks 1991; Wen 1998, 1999; Wen et al. 2010) supported that some exchanges of temperate taxa may have continued. Eddie et al. (2003) recognized that the *Rapunculus* clade (the c-2 clade) was most diverse in the Mediterranean basin, and they hypothesized that *Campanula* and its closely related genera originated in the Mediterranean region, and then they rapidly expanded into other Northern Hemisphere regions during pre-glacial times. They also mentioned that the western North American endemic taxa such as *Githopsis*, *Heterocodon* and some Asian genera such as *Homocodon* and *Peracarpa* may have all derived from the main rapunculoid radiation in Eurasia (also see Shetler 1979). Our biogeographic analysis supports the hypothesis suggested by Eddie et al. (2003). Based on the LAGRANGE analyses using three areas of endemism, our results strongly support the ancestral distribution of the c-2b clade in western Eurasia (especially in the Mediterranean region). The eastern Asian *Peracarpa* and

the North American sister group may have originated from a common ancestor distributed in Mediterranean Europe. Morphologically *Peracarpa* is highly distinct from taxa in the North American *Heterocodon-Githopsis* clade. *Peracarpa carnosa* is a delicate stoloniferous perennial herb with petiolate leaves and a strikingly long and slender peduncle, whereas taxa in the *Heterocodon-Githopsis* clade are annual herbaceous plants with a more or less erect stem, sessile leaves, and sessile or short-pedicellate flowers (Lammers 2007; also see Table 1). With only the weak support of the *Peracarpa-Heterocodon-Githopsis* clade and their distinct morphological differences, *Peracarpa* may not be the direct sister to the North American *Heterocodon-Githopsis* clade (Fig. 2). They likely represent relics after the radiation and intercontinental migration in the early Miocene from the Mediterranean ancestor.

Appendix

Tables 3, 4

Table 3 New sequences for samples of *Peracarpeae* and other taxa of Campanulaceae used in the study with voucher information and GenBank accession numbers

Taxon	Voucher	Location	*mat*K	*rbc*L	*atp*B	*trn*L-F
Adenophora liliifolioides Pax & K.Hoffm.	*Sun H-YN11235* (KUN)	China: Yunnan, Dongchuan Co., 26°14′52″N, 103°00′18″E, 3,900 m	JN851163	JN851172	JN851154	JN851181
Codonopsis nervosa Nannf.	*Sun H-SC0056* (KUN)	China: Sichuan, Muli Co., 28°00′14″N, 101°20′51″E, 3,996 m	JN851164	JN851173	JN851155	JN851186
Codonopsis tangshen Oliv.	*Sun H-QH11182* (KUN)	China: Qinghai, Yushu Co., 3,900 m	JN851165	JN851174	JN851156	JN851187
Cyananthus lobatus Wall.	*Nie ZL-772* (KUN)	China: Xizang, Linzhi Co., 29°38′05″N, 94°42′50.2″E, 4,115 m	JN851166	JN851175	JN851157	JN851188
Heterocodon rariflorum Nutt.	*Janeway L.s.n.* (US)	USA: California	JN851167	JN851176	JN851158	JN851182
Homocodon brevipes (Hemsl.) Hong	*Zhou Z-003* (KUN)	China: Yunnan, Shilin Co., Guishan, 1,800 m	JN851168	JN851177	JN851159	JN851183
Homocodon pedicellatus Hong & Ma	*Sun H-SC11189* (KUN)	China: Sichuan, Luding Co., Gongga Mountains 29°36′19″N, 102°07′09.2″E, 1,823 m	JN851169	JN851178	JN851160	JN851184
Leptocodon gracilis Lem.	*Nie ZL-798* (KUN)	China: Xizang, Cuona Co., 27°50′54″N, 91°46′33″E, 2,604 m	JN851170	JN851179	JN851161	–
Peracarpa carnosa Hook.f. & Thomson	*GBCWS-942* (KUN)	China: Yunnan, Jinping Co., Fengshuiling 22°52′09″N,103°14′13″E, 2,394 m	JN851171	JN851180	JN851162	JN851185

Table 4 Accessions of taxa in Campanulaceae from GenBank used in the study with source information

Taxon	*atp*B	*mat*K	*rbc*L	*trn*L-F	Source
Asyneuma virgatum	EU437665	EU713332	EU713439	–	Haberle et al. (2009)
Azorina vidalii	EU437601	EU713266	EU713373	EF088696	Haberle et al. (2009); Roquet et al. (2009)
Berenice arguta	EU437622	EU713339	EU713446	–	Haberle et al. (2009)
Campanula aizoides	EU437662	EU713329	EU713436	–	Haberle et al. (2009)
Campanula arvatica	EU437677	EU713344	EU713451	–	Haberle et al. (2009)
Campanula balfourii	–	–	FJ587243	EF088705	Roquet et al. (2009)
Campanula bellidifolia	EU437575	EU713240	EU713347	EF088706	Haberle et al. (2009); Roquet et al. (2009)
Campanula bononiensis	EU437609	EU713274	EU713381	–	Haberle et al. (2009)
Campanula carpatica	–	EU713303	EU713410	–	Haberle et al. (2009)
Campanula cretica	EU437663	EU713330	EU713437	–	Haberle et al. (2009)
Campanula dimorphantha	–	–	FJ587246	EF088708	Roquet et al. (2009)
Campanula divaricata	EU437676	EU713343	EU713450	EF088718	Haberle et al. (2009); Roquet et al. (2009)
Campanula edulis	EU437602	EU713267	EU713374	–	Haberle et al. (2009)
Campanula elatines	EU437664	EU713331	EU713438	AJ430970	Haberle et al. (2009); Roquet et al. (2009)
Campanula erinus	EU437626	EU713291	EU713398	EF088720	Haberle et al. (2009); Roquet et al. (2009)
Campanula exigua	EU437643	EU713309	EU713416	–	Haberle et al.(2009)
Campanula fragilis	EU437655	EU713321	EU713428	FJ426580	Frajman and Schneeweiss (2009); Haberle et al. (2009)
Campanula grossheimii	EU437621	EU713286	EU713393	–	Haberle et al. (2009)
Campanula hawkinsiana	EU437671	EU713338	EU713445	EF213146	Stefanovic et al. (2008); Haberle et al. (2009)
Campanula herminii	EU437673	EU713340	EU713447	–	Haberle et al. (2009)
Campanula hierapetrae	EU437623	EU713288	EU713395	–	Haberle et al. (2009)
Campanula jacquinii	EU437674	EU713341	EU713448	–	Haberle et al. (2009)
Campanula laciniata	EU437579	EU713244	EU713351	–	Haberle et al. (2009)
Campanula lanata	EU437610	EU713275	EU713382	EF088731	Haberle et al. (2009); Roquet et al. (2009)
Campanula latifolia	EU437606	EU713271	EU713378	EF088732	Haberle et al. (2009); Roquet et al. (2009)
Campanula lusitanica	EU437667	EU713334	EU713441	EF088733	Haberle et al. (2009); Roquet et al. (2009)
Campanula medium	EU437607	EU713272	EU713379	EF088738	Haberle et al. (2009); Roquet et al. (2009)
Campanula mirabilis	EU437612	EU713277	EU713384	–	Haberle et al. (2009)
Campanula mollis	EU437603	EU713268	EU713375	EF088739	Haberle et al. (2009); Roquet et al. (2009)
Campanula parryi	EU437675	EU713342	EU713449	EF213147	Stefanovic et al. (2008); Haberle et al. (2009)
Campanula peregrina	EU437654	EU713320	EU713427	EF088742	Haberle et al. (2009); Roquet et al. (2009)
Campanula persicifolia	EU437657	EU713324	EU713431	FJ426573	Frajman and Schneeweiss (2009); Haberle et al. 2009)
Campanula polyclada	–	–	–	EF088746	Roquet et al. (2009)
Campanula primulifolia	–	–	EU643718	EF088699	Roquet et al. (2009)
Campanula pyramidalis	–	EU713322	EU713429	EF088754	Haberle et al. (2009); Roquet et al. (2009)
Campanula reverchonii	EU437594	EU713259	EU713366	–	Haberle et al. (2009)
Campanula robinsiae	EU437642	EU713308	EU713415	–	Haberle et al. (2009)
Campanula rotundifolia	EU437668	EU713336	EU713443	EF213153	Haberle et al. (2009); Roquet et al. (2009)
Campanula scouleri	EU437678	EU713345	EU713452	–	Haberle et al. (2009)
Campanula spathulata	EU437670	EU713337	EU713444	–	Haberle et al. (2009)
Campanulastrum americanum	EU437646	EU713312	EU713419	EF088776	Haberle et al. (2009); Roquet et al. (2009)
Canarina canariensis	EU437581	EU713246	EU713353	EF088777	Haberle et al. (2009); Roquet et al. 2009)
Craterocapsa tarsodes	EU437636	EU713301	EU713408	–	Haberle et al. (2009)
Diosphaera rumeliana	EU437619	EU713284	EU713391	EF088778	Haberle et al. (2009); Roquet et al. (2009)
Edraianthus graminifolius	EU437608	EU713273	EU713380	EF088779	Haberle et al. (2009); Roquet et al. (2009)
Feeria angustifolia	EU437622	EU713287	EU713394	EF088780	Haberle et al. (2009); Roquet et al. (2009)
Gadellia lactiflora	EU437652	EU713318	EU713425	FJ589212	Haberle et al. (2009); Roquet et al. (2009)
Githopsis diffusa	EU437644	EU713310	EU713417	–	Haberle et al. (2009)

Table 4 continued

Taxon	*atp*B	*mat*K	*rbc*L	*trn*L-F	Source
Githopsis pulchella	EU437647	EU713313	EU713420	–	Haberle et al. (2009)
Hanabusaya asiatica	EU437658	EU713325	EU713432	–	Haberle et al. (2009)
Heterochaenia ensifolia	EU437666	EU713333	EU713440	–	Haberle et al. (2009)
Jasione montana	EU437582	EU713247	EU713354	EF088782	Haberle et al. (2009); Roquet et al. (2009)
Legousia hybrida	EU437660	EU713327	EU713434	EF088783	Haberle et al. (2009); Roquet et al. (2009)
Lobelia cardinalis	EU437598	EU713263	EU713370	DQ356231	Antonell (2008); Haberle et al. (2009)
Merciera tenuifolia	EU437630	EU713295	EU713402	–	Haberle et al. (2009)
Michauxia tchihatchewii	EU437574	EU713239	EU713346	EF088784	Haberle et al. (2009); Roquet et al. (2009)
Microcodon glomeratum	EU437627	EU713292	EU713399	–	Haberle et al. (2009)
Musschia aurea	EU437638	EU713304	EU713411	EF088785	Haberle et al. (2009); Roquet et al. (2009)
Nescodon mauritianus	EU437648	EU773314	EU713421	–	Haberle et al. (2009)
Peteromarula pinnata	EU437659	EU713326	EU713433	EF088786	Haberle et al. (2009); Roquet et al. (2009)
Physoplexis comosa	EU437590	EU713255	EU713362	FJ426586	Frajman and Schneeweiss (2009); Haberle et al. (2009)
Phyteuma spicatum	EU437589	EU713254	EU713361	EF088787	Haberle et al. (2009); Roquet et al. (2009)
Platycodon grandiflorus	EU437586	EU713251	EU713358	EF088788	Haberle et al. (2009); Roquet et al. (2009)
Prismatocarpus diffusus	EU437629	EU713294	EU713401	–	Haberle et al. (2009)
Rhigiophyllum squarrosum	EU437653	EU713319	EU713426	–	Haberle et al. (2009)
Roella ciliata	EU437633	EU713298	EU713405	EF088789	Haberle et al. (2009); Roquet et al. (2009)
Siphocodon debilis	EU437640	EU713306	EU713413	–	Haberle et al. (2009)
Symphyandra hofmannii	EU437605	EU713270	EU713377	GQ254931	Haberle et al. (2009); Stefanovic and Lakusic (2009)
Theilera guthriei	EU437637	EU713302	EU713409	–	Haberle et al. (2009)
Triodanis perfoliata	EU437591	EU713256	EU713363	–	Haberle et al. (2009)
Wahlenbergia berteroi	EU437650	EU713316	EU713423	–	Haberle et al. (2009)
Wahlenbergia gloriosa	EU437635	EU713300	EU713407	–	Haberle et al. (2009)
Wahlenbergia hederacea	EU437628	EU713293	EU713400	EF088792	Haberle et al. (2009); Roquet et al. (2009)
Wahlenbergia linifolia	EU437651	EU713317	EU713424	–	Haberle et al. (2009)

-, missing data

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