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Phylogenetic Analysis Informed by Geological History Supports Multiple, Sequential Invasions of the Mediterranean Basin by the Angiosperm Family Araceae

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Abstract.—Despite the remarkable species richness of the Mediterranean flora and its well-known geological history, few studies have investigated its temporal and spatial origins. Most importantly, the relative contribution of geological processes and long-distance dispersal to the composition of contemporary Mediterranean biotas remains largely unknown. We used phylogenetic analyses of sequences from six chloroplast DNA markers, Bayesian dating methods, and ancestral area reconstructions, in combination with paleogeographic, paleoclimatic, and ecological evidence, to elucidate the time frame and biogeographic events associated with the diversification of Araceae in the Mediterranean Basin. We focused on the origin of four species, *Ambrosina bassii*, *Biarum dispar*, *Helicodiceros muscivorus*, *Arum pictum*, subendemic or endemic to Corsica, Sardinia, and the Balearic Archipelago. The results support two main invasions of the Mediterranean Basin by the Araceae, one from an area connecting North America and Eurasia in the Late Cretaceous and one from the Anatolian microplate in western Asia during the Late Eocene, thus confirming the proposed heterogeneous origins of the Mediterranean flora. The subendemic *Ambrosina bassii* and *Biarum dispar* likely diverged sympatrically from their widespread Mediterranean sister clades in the Early-Middle Eocene and Early-Middle Miocene, respectively. Combined evidence corroborates a relictual origin for the endemic *Helicodiceros muscivorus* and *Arum pictum*, the former apparently representing the first documented case of vicariance driven by the initial splitting of the Hercynian belt in the Early Oligocene. A recurrent theme emerging from our analyses is that land connections and interruptions, caused by repeated cycles of marine transgressions-regressions between the Tethys and Paratethys, favored geodispersalist expansion of biotic ranges from western Asia into the western Mediterranean Basin and subsequent allopatric speciation at different points in time from the Late Eocene to the Late Oligocene. [Araceae; biogeography; DIVA; endemism; fossil calibration; Mediterranean Basin; molecular dating; paleomap.]

A key idea in biogeography is that geological and climatic changes profoundly affect biotic distributions. For example, the separation of landmasses caused by tectonic events divides previously continuous species ranges, enabling the origin of vicariant taxa (Nelson and Platnick, 1981). Alternatively, geological dynamics can reduce or even eliminate physical barriers to range expansion, as when tectonic plates come into closer proximity and even contact, sometimes through the formation of temporary land corridors. Under these circumstances, species can extend their range via overland migration, in a process termed "geodispersal" by Lieberman (2000). Range expansion can also occur via long-distance dispersal (or LDD) involving movement beyond existing geographic barriers (Lomolino et al., 2006). Understanding the relative contribution of vicariance, geodispersal, and LDD to the composition of contemporary biotas is one of the most challenging goals of biogeography (Lomolino et al., 2006). If congruence between past geological connections and species origins can be demonstrated at the temporal and spatial levels, then vicariant and/or geodispersalist explanations for current distributions can be accepted. However, if congruence at either level is lacking, LDD over existing barriers has to be invoked.

Multiple lines of evidence are necessary to discern among the above-mentioned processes, including paleogeographic and paleoclimatic reconstructions, paleontological record, and estimation of the phylogenetic relationships, ancestral areas of distribution, and ages of selected groups. In this paper, we follow a multifaceted, integrative approach to infer the biogeographic history of Araceae in the Mediterranean Basin. This region represents an ideal case study to elucidate the role of earth-driven processes and LDD in shaping contemporary biogeographic patterns, because detailed reconstructions have been recently developed for its geological history, characterized by horizontal movements of microplates and formation of land connections at different points in time and space (Biju-Duval et al., 1977; Dercourt et al., 1986; Meulenkamp and Sissingh, 2003; Rosenbaum et al., 2002a, 2002b; Steininger and Rögl, 1984; see below). Furthermore, hypotheses on the geographic sources of the Mediterranean flora are available, although they were derived from qualitative assessments of similarities with other floras worldwide, rather than explicit estimations of areas and times of origin for the Mediterranean taxa or a detailed understanding of the paleogeology of the basin. The existing hypotheses posit that the tropical elements (e.g., Araceae, Oleaceae, Vitaceae) of the Mediterranean flora evolved from African, Asian, and American lineages, while its temperate elements (e.g., Boraginaceae, Platanaceae) evolved either from Irano-Turanian (i.e., Anatolian, Iranian, and Eurasiatic) lineages or diversified in situ (e.g., *Arbutus*, *Lavatera*; Quézel, 1978, 1985; Raven, 1973; Thompson, 2005). The current Mediterranean vegetation thus may have highly heterogeneous roots.

Climatically defined by hot, dry summers and cool, wet winters, and floristically distinguished by sclerophyllous vegetation (Daget, 1977; Quézel, 1985), the Mediterranean is home to 24,000 plant species (approximately 10% of known seed plants; Greuter, 1991), among which 13,000 are endemic (Quézel, 1978, 1985; Suc, 1984). The region, listed among the earth's hotspots of biodiversity (Myers et al., 2000), contains 10 areas characterized by high species richness, including Corsica, Sardinia, and the Balearic Islands, with approximately 6000 plant species (Médail and Quézel, 1997), of which 10% to 12% are endemic (Bacchetta and Pontecorvo, 2005; Contandriopolous, 1990; Gamisan and Jeanmonod, 1995; Mariotti, 1990; Médail and Quézel, 1997). Available biogeographic hypotheses propose that a majority of taxa endemic to the mentioned islands developed in situ or came from Europe and to a lesser extent North Africa, whereas a minority originated in the eastern Mediterranean (Cardona and Contandripoulos, 1977, 1979; Contandriopolous, 1990; Contandriopoulos and Cardona, 1984; Gamisan and Jeanmonod, 1995).

The Mediterranean Basin consists of two distinct domains. In the eastern part, the Ionian, Herodotus, and Levant basins represent remnants of the Early Mesozoic Neotethys Ocean (Garfunkel, 2004). The western Mediterranean Basin, including the Tyrrhenian, Ligurian, Provencal, Algerian, and Alboran basins, started to form during the Oligocene (ca. 34 to 30 million years ago or mega annum [Ma]) in an overall convergence setting between the African and Eurasian plates (Dercourt et al., 1986; Dewey et al., 1973; Krijgsman, 2002). The formation of these basins has been attributed to extension in a back-arc region controlled by backward migration of subduction zones (Biju-Duval et al., 1977; Gueguen et al., 1998; Rosenbaum et al., 2002a). Similar processes also occurred in the Aegean Sea, which formed as a back-arc basin since the Middle Miocene (ca. 16 Ma; Lepichon and Angelier, 1979), whereas the other eastern Mediterranean basins have been subjected to subduction and collision processes (Robertson et al., 1991; Stampfli et al., 1991; Steininger and Rögl, 1984). During the Late Miocene (ca. 8 to 6 Ma), subduction processes in the westernmost Mediterranean led to the closure of marine gateways between the Atlantic Ocean and the Mediterranean Sea and triggered the desiccation of the Mediterranean Sea (ca. 6 Ma; Duggen et al., 2003; Hsü, 1972). This Messinian event was followed by the onset of the Mediterranean climatic regime during the Pliocene (ca. 3 Ma) and the alternation of warmer and colder conditions during the glacial cycles of the Pleistocene (1.8 to 0.01 Ma; Hugues et al., 2006; Suc, 1984). Overall, the complex geological history and paleoclimatology of the Mediterranean Basin likely drove biotas to fragment and merge as dispersal barriers appeared and disappeared through time.

Within the western Mediterranean, Corsica, Sardinia, the Balearic Islands (off the coast of Spain), the internal parts of the Betic Mountains of Spain and the Rif Mountains of Morocco, the Kabylies (in the Atlas mountains of Algeria), and Calabria (in the southern tip of the Italian peninsula) are all remnants of a Paleozoic mountain chain (the Hercynian belt) that was situated in Iberia and southern Europe during the Early Oligocene (Fig. 1a). Corsica, Sardinia, and the Balearic Islands are referred here as the "Hercynian Islands," reflecting their geological origin, and their endemic species are designated as "Hercynian endemics." According to tectonic reconstructions, the Balearic-Kabylies microplate and the Corsica-Sardinia-Calabria microplate rifted off from Iberia and southern Europe at ca. 30 to 28 Ma (Alvarez et al., 1974; Rosenbaum et al., 2002a). From around 25 Ma,

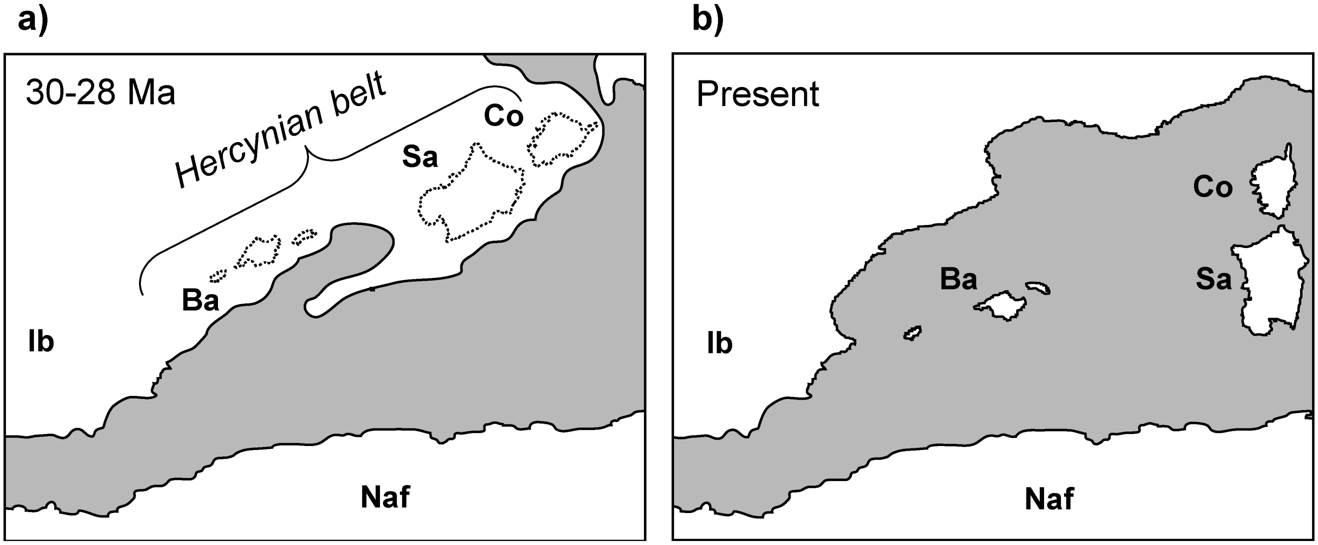


FIGURE 1. (a) Early Oligocene geography of the western Mediterranean Basin. Corsica, Sardinia, and the Balearic Islands were part of a continuous geological entity—the Hercynian belt—located in the Iberian microplate (redrawn after: Meulenkamp and Sissingh, 2003; Roca, 2002; Rosenbaum et al., 2002a). (b) Present-day geography of the western Mediterranean Basin (redrawn after: Schettino and Turco, 2006). Ma, mega annum (million years ago). Area designations as in Figure 3.

TABLE 1. Land connections and disconnections among western Mediterranean microplates since the Oligocene, according to the geological model of Alvarez (1972), Alvarez et al. (1974), and Rosenbaum et al. (2002). Ma, mega annum (million years ago).

Epoch	Time frame (Ma)	Geologic event
Early Oligocene	Until 30	Western Mediterranean terranes are connected to the Hercynian basement in the Iberian microplate.
Early to Middle Oligocene	30 to 28	The Corsica-Sardinia-Calabria and Balearic-Kabylies microplates break off from the Iberian microplate.
Late Oligocene/ Early Miocene	20 to 16	Corsica breaks off from Sardinia. The Sardinia-Calabria block is connected to Eurasia via central Apulia to the east.
Late Oligocene/ Early Miocene	25 to 21	The Balearic-Kabylies microplate rotates from Iberia to Africa; the Balearic Islands split off from the Kabylies and reach their current position.
Late Miocene (Messinian)	5.96 to 5.36	Some connections exist between North Africa, Corsica, Sardinia and Eurasia, and between the Balearic Islands and Iberia during the Messinian Salinity Crisis.
Pliocene/Pleistocene	5 to present	Calabria separates from Sardinia and collides with southern Italy.

the Balearic-Kabylies microplate rotated clockwise until the Balearic archipelago reached its current position (ca. 21 Ma) and separated from the Kabylies terrane. At the same time, the Corsica-Sardinia-Calabria microplate rotated southeastwards until it collided with the central part of the Apulian microplate (corresponding to the current Italian peninsula). Corsica reached its current position at about 16 Ma (Speranza et al., 2002), achieving final separation from the Sardinia-Calabria microplate. After splitting off from Sardinia approximately 5 Ma, Calabria collided with the southern part of Apulia by the Late Pliocene (Fig. 1b, Table 1).

In spite of the complex, yet well-investigated geology of the Mediterranean Basin in general and the Hercynian Islands in particular, biogeographic studies of Mediterranean taxa based on the integration of evidence from phylogeny, molecular dating, ancestral area reconstruction, paleoclimatology, paleogeography, and paleontology remain rare (Comes, 2004); published articles are mostly based on subsets of the mentioned approaches, in both animals (e.g., Caccone and Sbordoni, 2001; Fromhage et al., 2004; Ketmaier et al., 2003; Sanmartin, 2003) and plants (e.g., Comes and Abbott, 2001; Oberprieler, 2005). Indeed, the mentioned studies did not incorporate knowledge from the most recent paleogeological models or phylogeny-based estimation of temporal and geographic origins. In this paper, we use evidence from all the mentioned disciplines to elucidate whether the geological history of the Mediterranean Basin left detectable traces in the current distribution of Araceae, focusing especially on the Hercynian endemics.

The primarily tropical Araceae (3300 species and 100 genera; Mayo et al., 1997) are represented in the Mediterranean Basin by *Arum*, *Biarum*, *Dracunculus*, *Eminium*, *Helicodiceros* (Areae), *Ambrosina* (Ambrosineae), and *Arisarum* (Arisareae), with four species either endemic or subendemic to the Hercynian Islands: *Helicodiceros muscivorus* and *Arum pictum* restricted to Corsica, Sardinia, and the Balearic archipelago, *Biarum dispar* in southern Sardinia, southeastern Spain, and northwestern Africa, and *Ambrosina bassii* in southern Corsica, Sardinia, Sicily, southern Italy, and northwestern Africa. According to available hypotheses, both *Helicodiceros muscivorus* and *Arum pictum* would represent relictual elements having possible affinities with central or eastern Mediterranean Araceae (Boyce, 1994; Contandriopoulos, 1981; Contandriopoulos and Cardona, 1984; Hruby, 1912); *Biarum dispar* shows karyological similarities with extant Spanish species (Talavera, 1976); and *Ambrosina bassii* is closely related to the Mediterranean *Arisarum* and the North American *Peltandra*, as demonstrated by recent molecular and morphological studies (Barabé et al., 2004a, 2004b). The occurrence of reliable fossils (Bogner et al., 2005; Friis et al., 2004; Smith and Stockey, 2003; Wilde et al., 2005; Zetter et al., 2001), fundamental to generate an absolute time frame for the origin of the Mediterranean endemics through molecular dating analysis, and the lack of obvious adaptations to wind dispersal make Araceae especially suitable for an integrative biogeographic study.

Using techniques of molecular dating and reconstruction of ancestral areas of distribution, it is now possible to assign specific age intervals to the phylogenetic separation of the selected Hercynian endemics from their sister taxa and infer the regions where the divergence occurred. If the estimated times and areas are congruent with known paleogeological connections of the Hercynian Islands in the Mediterranean Basin (see Table 1), it is parsimonious to conclude that earth-driven processes possibly played an important role in the origin of the studied endemics. Obviously, the availability of land corridors for biotic migration does not preclude the possibility of LDD, but the latter modality of range expansion cannot be tested directly, because no specific time frame can be proposed for it. The hypothesis of colonization of the Hercynian islands via LDD must be invoked when either the time or place estimated for the origin of the selected endemics is incompatible with geologic reconstructions. In the study presented here, we asked the following specific questions: (1) Did the Araceae colonize the Mediterranean Basin from Asia, Africa, or America (Quézel, 1978, 1985; Raven, 1973; Thompson, 2005)? (2) When and where did the species endemic to Corsica, Sardinia, and the Balearic Islands diverge from their sister clades? (3) Are the inferred temporal and spatial origins of the investigated species congruent with the reconstructed geological connections of the Hercynian Islands with Iberia until the Early to Middle Oligocene, Apulia during the Late Oligocene to Early Miocene, and North Africa and Eurasia during the Late Miocene? (4) Are our results congruent with previous hypotheses on the origins of Hercynian endemics? (5) Can we identify repeated biogeographic patterns and processes among the selected taxa?

MATERIAL AND METHODS

Taxon and cpDNA Sampling

Sequences were generated from a total of 88 accessions, targeting the following six chloroplast DNA (cpDNA) regions: (i) the *trn*L intron and (ii) the *trn*L-*trn*F intergenic spacer, included in the tRNA^{LEU} intron/spacer region; (iii) part of the *trn*K intron and (iv) the *mat*K gene, included in the *mat*K region; (v) the *rbc*L gene; and (vi) the *rps*16 intron. Plant samples were either collected in the field (36) or obtained from botanical gardens and herbaria (52). A list of collected accessions and species authority names is shown in online Appendix 1, available at www.systematicbiology.org.

Taxon sampling included representatives of all seven genera (*Arum*, *Biarum*, *Dracunculus*, *Eminium*, *Helicodiceros*, *Sauromatum*, and *Typhonium*) ascribed to Areae, the single genus (*Ambrosina*) ascribed to the Ambrosineae, and the single genus (*Arisarum*) ascribed to the Arisareae (Boyce, 1993; Mayo et al., 1997). More intensive sampling focused on the Mediterranean genera, including 19 of the 35 species/subspecies ascribed to *Arum* and eight of the 26 species/subspecies ascribed to *Biarum*, representing all sections recognized in these two large genera (Boyce, 1993; Riedl, 1980), both species of *Dracunculus*, one of the seven species of *Eminium*, the single species of *Helicodiceros*, the single species of *Ambrosina*, and one of the three species of *Arisarum* (Boyce, 1990, 1994; Lobin and Boyce, 1991; Quilichini et al., 2005). When possible, the Hercynian endemics were represented by multiple accessions within their geographic range: *Arum pictum* (13 accessions) and *Helicodiceros muscivorus* (7 accessions) were sampled in Corsica, Sardinia, and the Balearic Islands; *Biarum dispar* (5 accessions) in Sardinia and Spain; and *Ambrosina bassii* (1 accession) in Sardinia (see online Appendix 1). Based on published phylogenetic analyses of Araceae (Barabé et al., 2004a; French et al., 1995; Nie et al., 2006; Renner and Zhang, 2004; Tam et al., 2004), accessions from the closely related Arisaemateae (2), Colocasieae (5), Peltandreae (2), and Pistieae (1) were also included in the study and *Calla* (Calloideae), *Amorphophallus* (Thomsonieae), *Caladium*, and *Xanthosoma* (Caladieae) were used as outgroups (Fig. 2).

DNA Extraction, PCR Parameters, and Sequencing

Total DNA was extracted from silica gel–dried samples or from herbarium specimens using the DNeasy Plant Mini Kit (Qiagen, Hombrechtikon, Switzerland). Double-stranded DNA was amplified by polymerase chain reactions (PCR) performed in 20-μL volumes containing 1× buffer, 2 mM $MgCl_2$, 200 μM dNTPs, 0.2 μM of each primer, and one unit of Taq polymerase (Sigma, Buchs, Switzerland) in a TGradient thermocycler (Biometra, Goettingen, Germany). PCR amplifications included 1 cycle of 3 to 5 min denaturation at 94°C, followed by 30 cycles of 30 s denaturation at 94°C, 1 min annealing at 52°C to 56°C, and 1 to 2 min elongation at 72°C, finishing with a 5- to 10-min elongation step at 72°C to complete primer extension. The main characteristics of the primers used for DNA amplification and sequencing are given in Table 2. The PCR products were then run on a 1% agarose gel stained with ethidium bromide to evaluate the quality (absence of contamination) and length (by comparison with a molecular weight marker) of amplicons and finally purified using the GFX PCR DNA and Gel Band purification Kit (Bioscience Amersham, Otelfingen, Switzerland). Cycle sequencing reactions for both DNA strands of each locus were performed with the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Sequencing reactions were then purified on 96-well multiscreen filtration plates (Millipore, Billirica) and loaded on an ABI Prism 3100 Genetic Analyzer. New sequences were carefully checked, edited, and assembled using Sequencher 4.2 (Gene Code, Ann Arbor, MI), and submitted to GenBank (see online Appendix 1).

Phylogenetic Inference

DNA sequences were aligned manually using Se-Al (Rambaut, 1996) and combined in a matrix of 88 accessions and 4498 aligned characters (88-taxon matrix). Difficult-to-align regions (i.e., poly-AT regions ranging from 50 base pairs in *Calla palustris* to ca. 190 base pairs in *Arum pictum*), situated between positions 253 and 254 of the final alignment, were excluded from the analysis. Gaps were either treated as missing data or coded with Gapcoder (Young and Healy, 1987) and added as binary states at the end of the matrix. The final matrix was submitted to TreeBASE (study accession number SN3636).

The data set was analyzed using Bayesian inference, with a selected model of sequence evolution for each of the six partitions (i.e., *trn*L intron, *trn*L-*trn*F intergenic spacer, *trn*K intron, *mat*K gene, *rbc*L gene, *rps*16 intron). The best respective models were chosen using the Akaike information criterion, as implemented in MrModelTest (Nylander, 2004). A binary model (Lset coding = variable) was applied to the coded gaps. Bayesian runs were performed with MrBayes 3.1 (Huelsenbeck and Ronquist, 2001), using one cold and three heated Markov chain Monte Carlo (MCMC) chains run for 5×10^6 cycles, sampling trees every 100 generations, and with a default temperature parameter value of 0.2. The MCMC runs were repeated twice, and the first 400 trees were discarded as burn-in after checking for (i) stationarity on the log-likelihood curves; (ii) similarity of the majority-rule topologies and final likelihood scores; (iii) the values of standard deviation of split frequencies (<0.001); and (iv) the value of the potential scale reduction factor (close to 1). The remaining trees were used to produce a majority-rule consensus tree and to calculate the posterior probability (pp) values.

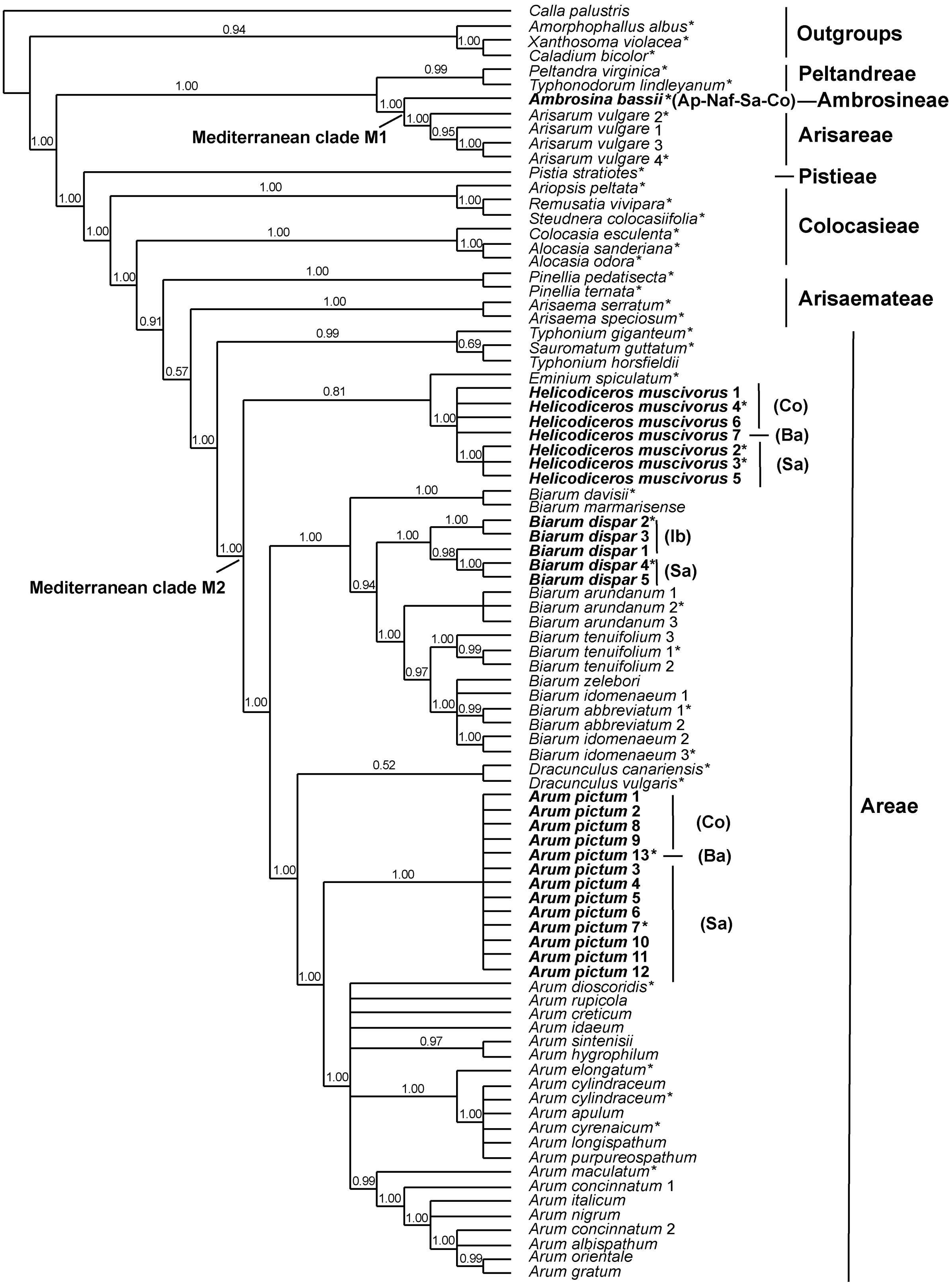


FIGURE 2. MrBayes 50% majority-rule consensus tree inferred from the combined 88-taxon data set (4553 aligned positions). Posterior probabilities, ranging from 0 to 1, are reported above the branches. Tribal ranks and distribution of the Hercynian endemics (highlighted in bold) are reported to the right of the tree. Asterisks indicate the taxa selected for biogeographic and dating analyses. Area designations as in Figure 3.

TABLE 2. General information for primers used in this study. F = forward; R = reverse; A = amplification by PCR; S = sequencing. References: [1]Taberlet et al., 1991; [2]Sang et al., 1997; [3]Winkworth et al., 2002; [4]Johnson and Soltis, 1995; [5]Asmussen and Chase, 2001; [6]Oxelman et al., 1997.

Name	Sequence (5′ • 3′)	Direction	Use	cpDNA region	Reference
trnLc	CGAAATCGGTAGACGCTACG	F	A, S	tRNA^{LEU} intron/spacer	1
*trn*Ld	GGGGATAGAGGGACTTGAAC	R	S	tRNA^{LEU} intron/spacer	1
*trn*Le	GGTTCAAGTCCCTCTATCCC	F	S	tRNA^{LEU} intron/spacer	1
*trn*Lf	ATTTGAACTGGTGCAACGAG	R	A, S	tRNA^{LEU} intron/spacer	1
*mat*k-F1	ACTGTATCGCACTATGTATCA	F	A, S	*mat*K gene	2
*mat*K-3AR	CGTACASTACTTTTGTGTTTMCG	R	A, S	*mat*K gene	3
tk3-MY2F	CAATCAAAATCTTCTGGAATC	F	A, S	*mat*K gene	3
*trn*K-2R	AACTAGTCGGATGGAGTAG	R	A, S	*mat*K gene	4
*rbc*L-1F	ATGTCACCACAAACAGAAAC	F	A, S	*rbc*L gene	5
*rbc*L-739R	CCGTTAAGTAGTCGTGCA	R	A, S	*rbc*L gene	5
*rbc*L-636F	CGAAATCGGTAGACGCTACG	F	A, S	*rbc*L gene	5
*rbc*L-Rev	TCCTTTTAGTAAAAGATTGGGCCGAG	R	A, S	*rbc*L gene	5
*rps*16-F	GTGGTAGAAAGCAACGTGCGACTT	F	A, S	*rps*16 intron	6
*rps*16-R2	TCGGGATCGAACATCAATTGCAAC	R	A, S	*rps*16 intron	6

For the molecular dating analysis and biogeographic reconstruction, both requiring fully resolved trees, the initial data set of 88 accessions was reduced by pruning similar accessions and keeping representatives of all major clades recovered in the broader analysis (see Figs. 2, 3). The reduced data set, comprising 42 accessions (42-taxon data set), was analyzed using Bayesian inference with the same settings as above. A list of selected taxa with their current distribution is shown in online Appendix 2 (available at www.systematicbiology.org).

Divergence Time Estimation

To evaluate whether the sequences in the 42-taxon data set evolved under constant rates, a likelihood-ratio (LR) test was performed by comparing the likelihood scores of the trees resulting from Bayesian analysis with and without a clock (Felsenstein, 1981). Because rate constancy was rejected ($LR = 216$, $df = 39$, $P < 0.01$), we used the Bayesian approach implemented in Multidivtime (Kishino et al., 2001; Thorne and Kishino, 2002; Thorne et al., 1998) to estimate divergence times at the nodes of interest. This program assumes autocorrelation of substitution rates between ancestral and descendant nodes. Branch lengths and their variance-covariance matrix were first estimated on the 42-taxon cladogram using the BASEML and ESTBRANCHES programs in PAML version 3.14 (Thorne et al., 1998; Yang, 2000) under the F84+G model of substitution. Output branch lengths were then used as priors in MCMC searches in Multidivtime (Thorne and Kishino, 2002), to find the most likely model of rate change given the sequence data, the chosen topology, node constraints, and a Brownian motion parameter nu that determines the permitted rate change between an ancestral and a descendant node. Mean values for four prior distributions and their respective standard deviation (SD) were specified in units of 10 Ma and determined following Thornes' protocol. (1) The prior of the age of the ingroup (rttm) was set to 10 units (SD = 1) because the Peltandreae stem lineage, well embedded in our ingroup (Tam et al., 2004), was recently estimated to be ca. 9 units old (Renner and Zhang, 2004). (2) The prior of the rate at root node (rrate) was set to 0.0023 unit (SD = 0.0023), this value being calculated by dividing the median distance between the ingroup root and the tips by rttm. (3) The prior of the Brownian motion parameter nu (brownmean) was set to 0.1 (SD = 0.1) substitutions per site per million years, so that brownmean x rttm = 1. (4) Finally, prior of the age of a larger clade to which the group belongs (bigtime) was set to 11 units, an age corresponding to the oldest known fossil for the Araceae and, indeed, the entire Monocots (Friis et al., 2004). This fossil, represented by pollen grains of *Mayoa* sp. from the Barremian-Aptian zones of the western Portugal basin, was assigned to the Spathiphylleae (Friis et al., 2004), a tribe included in the Monsteroideae-Pothoideae clade that diverged early in a global phylogenetic analysis of Araceae encompassing 45 genera (Barabé et al., 2004a). The Markov chain was run for 100,000 cycles and sampled every 100 cycles, after an initial burn-in period of 10,000 cycles. The MultiDivTime analysis was performed at least twice with different initial conditions and the output files checked for convergence of the Markov chain.

To check for robustness of the results obtained with Bayesian dating, divergence times were also estimated by using penalized likelihood (PL) implemented in r8s (Sanderson, 2002, 2003). The PL method was applied to the same 42-taxon ML phylogram used for Bayesian dating analyses to estimate divergence times for nodes 1 through 10 (see Fig. 3). Application of the cross-validation procedure determined the optimal smoothing value to be 0.0025. This value was used to calculate nodal ages from (i) the ML phylogram and (ii) 1000 phylograms generated from bootstrap resampling in PAUP (Swofford, 2002). Nodal ages obtained from the 1000 phylograms were summarized (using the "profile" command in r8s) and the resulting standard deviations for nodal ages were used to derive 95% confidence intervals for the point estimates obtained using the ML phylogram.

In both dating analyses, the transformation of relative ages into absolute ages was performed using two fossil constraints on nodes F1 (Peltandreae crown node) and F2 (Colocasieae crown node; Fig. 3a; online Appendix 3

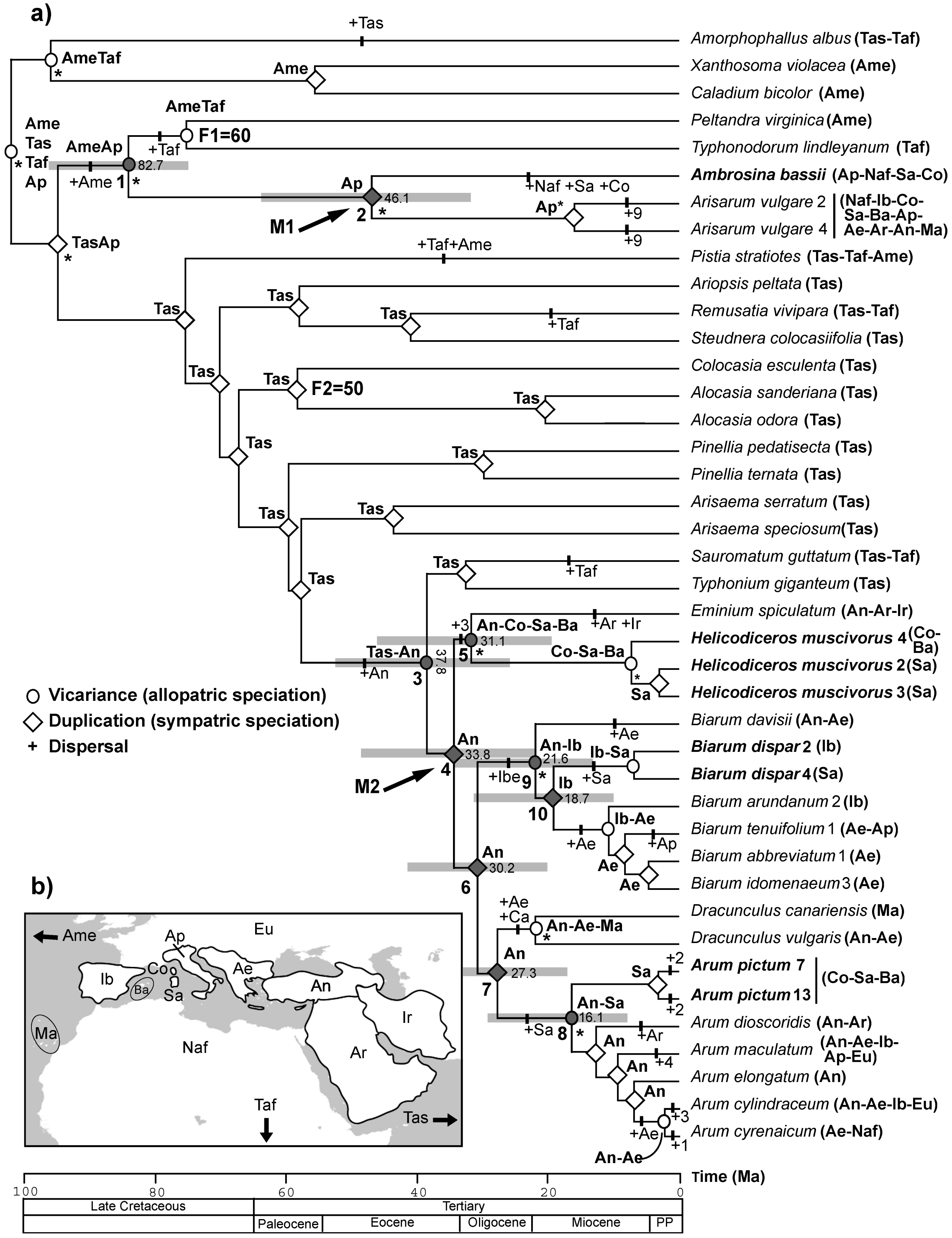


FIGURE 3. (a) Chronogram inferred from Bayesian dating analysis of the 42-taxon data set. F1 and F2 indicate fossil constraints at 60 Ma and 50 Ma, respectively. Labeled nodes (1 to 10), with mean age estimates and 95% confidence intervals (grey bars), obtained with MULTIDIVTIME (Thorne and Kishino, 2002), are discussed in the text. Contemporary areas of distribution are reported to the right of the tree (Naf, North Africa; Ib, Iberian microplate; Co, Corsica; Sa, Sardinia; Ba, Balearic Islands; Ap, Apulian microplate; Ae, Aegean microplate; Ar, Arabian plate; An, Anatolian plate; Ir, Iranian plate; Eu, Eurasian plate; Ma, Macaronesian Islands; Taf, tropical Africa; Tas, tropical Asia; and Ame, American plate). Nodes with multiple optimal reconstructions are marked by an asterisk (1: AmeAp/AmeNaf/AmeSa/AmCo/AmeNafTaf/AmeSaTaf/AmeCoTaf/AmeApTaf/NafTaF/SaTaf/CoTaf/ApTaf; 2: Ap/Naf/Sa/Co; 5: AnCoSaBa/AnCo/AnSa/AnCoSa/AnBa/AnCoBa/AnSaBa/AnCoAr/AnArSa/AnArCoSa/AnArBa/AnArCoBa/AnArSaBa/AnIrCo/AnIrSa/AnIrCoSa/AnIrBa/AnIrCoBa/AnIrSaBa/AnArIrCo/AnArIrSa/AnArIrBa; 8: SaAn/CoAn/BaAn; 9: AnIb/AnAeIb; alternative reconstructions are separated by a slash). The ancestral areas listed at the nodes were selected based on congruence between the inferred nodal ages and paleogeological models. Circle = vicariance; diamond = duplication; + = dispersal; PP, Pliocene and Pleistocene; Ma, mega annum (million years ago). (b) Map showing the areas used for biogeographic reconstructions.

[available at www.systematicbiology.org]). The fossil assigned to node F1 is characterized by leaf fragments of *Nitophyllites zaisanicus* from the Paleocene formation of the Zaisan Basin of Kazakhstan (ca. 60 Ma; Wilde et al., 2005), whereas the fossil assigned to node F2 is represented by leaf prints of *Caladiosoma messelense*, found in the Messel formation of Germany (ca. 50 Ma; Wilde et al., 2005; see online Appendix 3 for details). Because the goal of our study was to check the congruence between geological and biological history in the Mediterranean, we used only paleobotanical constraints to avoid potential pitfalls of circularity with geological calibration.

Biogeographic Analyses

We used the dispersal vicariance method (Ronquist, 1997), implemented in the software DIVA (Ronquist, 1996), to reconstruct ancestral areas of distribution. Unlike "pattern-based methods," which rely on hierarchical area cladograms (van Veller and Brooks, 2001), DIVA reconstructs ancestral distributions for a given phylogeny without a priori assuming any specific area relationships ("event-based methods"; Ronquist, 1997). This strategy allows for the reconstruction of reticulate biogeographic scenarios in regions where past tectonic events have permitted repeated contacts between different microplates, as in the case of the Mediterranean Basin (Oberprieler, 2005; Sanmartin, 2003). When multiple area reconstructions were possible, the equivocal nodes were marked by an asterisk and the ancestral areas selected at these nodes were based on geographic contiguity with areas at adjacent nodes during the relevant geological periods (Figs. 3a, 4), thus assuming, when possible, continuous range expansion.

DIVA analyses were performed on a fully resolved Bayesian cladogram generated from the 42-taxon data set (Fig. 3a). *Calla palustris*, used as the dating outgroup in Multidivtime (Thorne and Kishino, 2002) analyses, was not included in the DIVA reconstructions. As recommended by Ronquist (1996, 1997), we restricted the number of areas inferred at internal nodes by using the "maxareas" option (e.g., Donoghue et al., 2003; Jonsson and Fjeldsa, 2006; Oberprieler, 2005). The maximum number of possible areas at each node was set to four units ("maxareas" = 4) because (i) all but two of the sampled species now occur in no more than four areas; (ii) the two species with a more widespread distribution (*Arum maculatum*, seven areas; *Arisarum vulgare*, 10 areas) are common in three to four areas and rare in the remaining ones (Boyce, 1993; Maire, 1957; Moggi, 1959). To check the sensitivity of the ancestral area reconstruction to the assumption of ancestral species inhabiting at most four areas, another optimization was also performed without constraints of unit areas for each ancestral node. Finally, areas where species occurrence was linked with human introduction were excluded from the analysis. The areas assigned to the taxa used in the dated phylogeny (Fig. 3b) are described in detail in online Appendix 4 (available at www.systematicbiology.org).

RESULTS

Phylogenetic Reconstructions

The larger cpDNA matrix consisted of 88 taxa and 4553 aligned positions from the following partitions: *trn*L intron = 444 bp; *trn*L-*trn*F intergenic spacer = 453 bp; *trn*K intron = 230 bp; *mat*K gene = 1392 bp; *rbc*L gene = 1264 bp; *rps*16 intron = 712 bp; unambiguous coded gaps = 56 binary characters. The reduced cpDNA matrix, used for subsequent dating and biogeographic analyses, consisted of 42 taxa and 4508 aligned positions from the same partitions listed above. Independent Bayesian analyses of the two matrices, performed under specific models of nucleotide substitution assigned to each of the seven partitions (online Appendix 5; available at www.systematicbiology.org), yielded congruent topologies and similar pp values for the resulting 50% majority-rule consensus trees (Figs. 2, 3a).

Both the 88-taxon and the 42-taxon consensus trees (Figs. 2, 3a) strongly support (pp = 1.00) two independent origins for the Mediterranean Araceae. The Mediterranean clade M1, comprising the Hercynian subendemic *Ambrosina* and the widespread Mediterranean *Arisarum*, is sister to the geographically disjunct, tropical/temperate Peltandreae (pp = 1.00). The Mediterranean clade M2, comprising *Arum*, *Dracunculus*, *Biarum*, *Helicodiceros*, and *Eminium*, is sister to the clade formed by the tropical *Typhonium* and *Sauromatum* (pp = 1.00), all seven genera being included in Areae (Fig. 2). Within M2, the Hercynian endemic *Helicodiceros* forms a moderately supported clade (pp = 0.81) with the eastern Mediterranean *Eminium.* The Sardinian accessions of *Helicodiceros* (2, 3, and 5) form a well-supported clade (pp = 1.00), whereas those from Corsica (1, 4, and 6) and the Balearic Islands (7) remain unresolved. The *Biarum* clade is sister to the *Dracunculus-Arum* clade (pp = 1.00). Two Sardinian accessions (4 and 5) of the Hercynian subendemic *Biarum dispar* form a sister clade with one Spanish accession (1) from Murcia (pp = 0.98). The two remaining accessions from Spain (2, 3) are sister to the Sardinia-Murcia clade (pp = 1.00). Lastly, an unresolved clade comprising all accessions of the Hercynian endemic *Arum pictum* (pp = 1.00) from Corsica, Sardinia, and the Balearic Islands is sister to a clade formed by the remaining accessions of *Arum* (pp = 1.00).

Divergence Time Estimations for the Mediterranean Araceae

Multidivtime (Thorne and Kishino, 2002) dating analyses on the fully resolved 42-taxon tree (Fig. 3a) produced estimated ages of 82.7 ± 6.8 Ma (Late Cretaceous) and 46.1 ± 9.0 Ma (Middle Eocene) for the stem and crown nodes of M1, respectively, and of 37.8 ± 7.2 Ma (Late Eocene) and 33.8 ± 6.9 Ma (Early Oligocene) for the stem and crown nodes of M2, respectively (nodes 1 to 4; Fig. 3a). Within M1, the age of the crown node corresponds to the age of the Hercynian subendemic *Ambrosina bassii* (node 2 = 46.1 ± 9.0 Ma; Middle Eocene; Fig. 3a). We should notice here some discrepancy with the mean age inferred with the PL method (node 2 = 62.7 ±

8.1 Ma; Early Paleocene; online Appendix 6 [available at www.systematicbiology.org]). Within M2, the Hercynian endemics *Helicodiceros muscivorus* and *Arum pictum* diverged from their sister clades in the Early Oligocene (node 5 = 31.1 ± 6.7 Ma; Fig. 3a) and Middle Miocene (node 8 = 16.1 ± 5.8 Ma; Fig. 3a), respectively, whereas the subendemic *Biarum dispar* diverged in the Early Miocene (node 10 = 18.7 ± 5.8 Ma; Fig. 3a). For all the mean nodal ages estimated by penalized likelihood (r8s; Sanderson, 2002, 2003) were all included within the confidence intervals calculated by Bayesian dating (see online Appendix 6), we will mainly refer to the Bayesian dating results in the Discussion, unless otherwise stated.

Biogeographic Reconstructions

The constrained DIVA (Ronquist, 1996) analysis produced 22,784 equally parsimonious reconstructions, each requiring a total cost of 56 dispersal, 13 vicariance, and 27 duplication events. In the optimization shown on the chronogram of Figure 3a, the nodes numbered 1 to 10 correspond to the origin and diversification of the Mediterranean clades M1 and M2 and to the divergence of the four Hercynian endemic or subendemic *Ambrosina bassii, Helicodiceros muscivorus, Arum pictum*, and *Biarum dispar* from their respective sister clades. In this preferred reconstruction, compatible with the assumption that contiguous range expansion is favored when possible, duplication events were inferred for nodes 2, 4, 6, 7, 10, whereas vicariance events were inferred for nodes 1, 3, 5, 8, 9, described below (Fig. 3a).

Among the 12 possible reconstructions for the equivocal node 1, dated at 82.7 ± 6.8 Ma (Fig. 3a), the America-Apulia ancestral area was selected, based on paleomaps showing the continuity of these ancestral ranges and, thus, possible expansion of plant populations in the respective areas (see Discussion; Fig. 4a). Of the four reconstructions inferred by DIVA (Ronquist, 1996) for node 2, dated at 46.1 ± 9.0 Ma (Fig. 3a), Apulia was selected as the most likely ancestral area based on paleobotanical and ecological evidence (see Discussion; Fig. 4b). All 22 reconstructions proposed for node 5, dated at 31.1 ± 6.7, involved vicariance between Anatolia and at least one of the Hercynian Islands (Fig. 3a). Vicariance events between Anatolia and the Hercynian microplates were also inferred for node 8, dated at 16.1 ± 5.8 Ma, and node 9, dated at 21.6 ± 6.2 Ma (Fig. 3a). The unconstrained DIVA analysis (favoring total vicariance) gave identical results (not shown), except for the deepest nodes 1 (one widespread ancestral range encompassing 12 areas), 2 (one widespread ancestral range encompassing 10 areas), 4 (one widespread ancestral range encompassing 11 areas), and 5 (13 alternative solutions, also involving the Anatolian and Hercynian microplates).

DISCUSSION

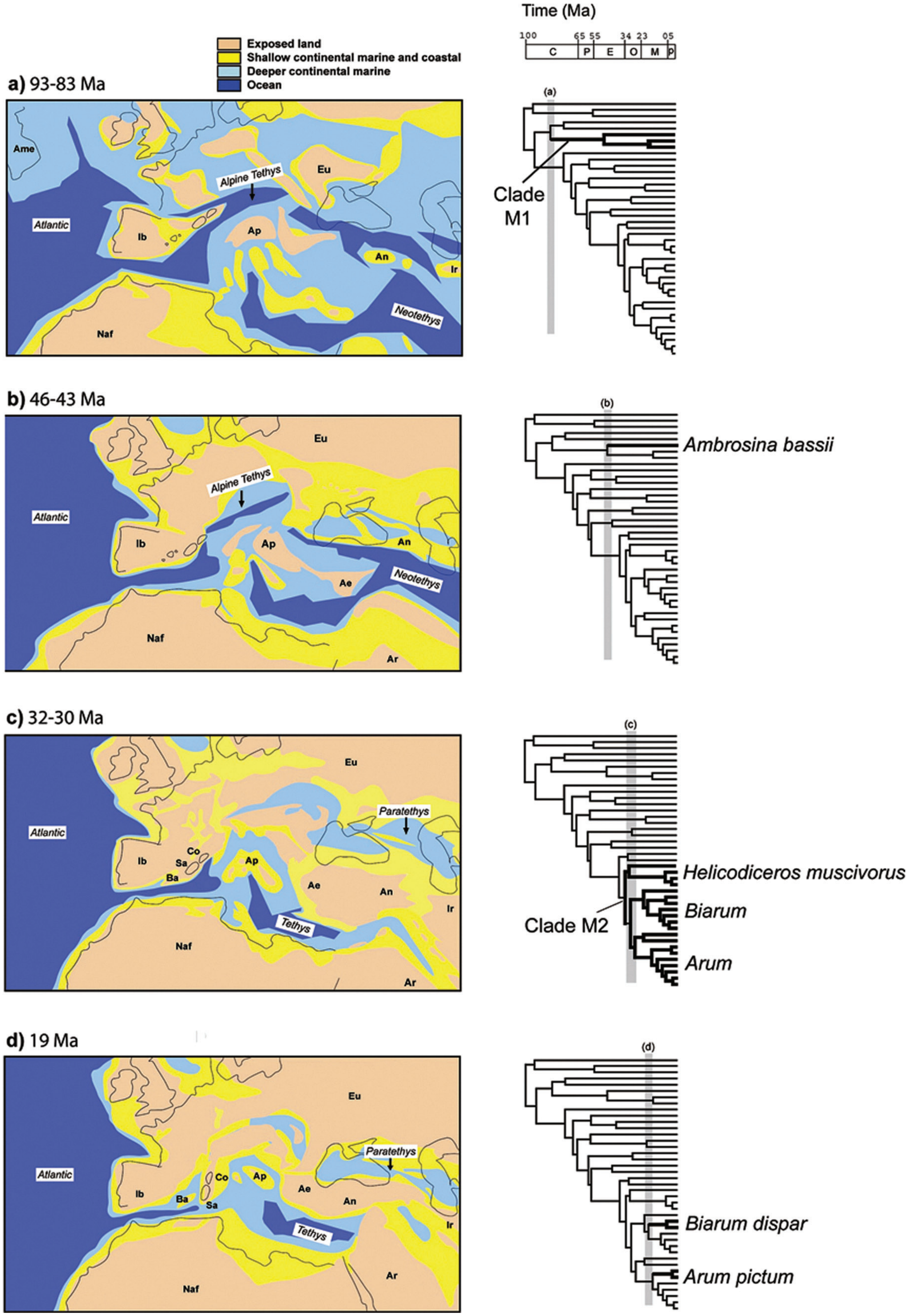
Overall, the study presented here underlines that an integrated approach, including phylogenetic hypotheses, lineage age estimates, ancestral area reconstructions, paleogeological models, and, where available, paleontological, paleoclimatic, and ecological evidence, is indispensable to improving our understanding of the relative contribution of earth-driven processes and LDD to current biogeographic patterns. Keeping in mind that the ages estimated in this study are minimum ages with sometimes large confidence intervals, we cautiously provide in the following discussion tentative hypotheses concerning the origin of the Mediterranean clades M1 and M2 and their respective endemics.

Origin of the Mediterranean Clade M1

The Mediterranean clade M1, formed by *Ambrosina* and *Arisarum*, likely diverged from its temperate-subtropical sister clade, comprising the American *Peltandra* and the African *Typhonodorum* (both assigned to Peltandreae), around 82.7 Ma (node 1; Fig. 3a), in an area that connected North America and Eurasia, including the Apulian microplate, during the Late Cretaceous (Dercourt et al., 1993; Fig. 4a). The presence of Peltandreae fossils, specifically leaf fragments and seeds, in the Late Cretaceous/Early Tertiary formations of North Bohemia and Kazakhstan (Wilde et al., 2005) corroborates the choice of this ancestral area for node 1. The inferred allopatric divergence between the Peltandreae and Ambrosineae-Arisareae clades might have been associated with range fragmentation caused by the progressive closure of the Alpine Tethys—an ancient ocean separating the Eurasian plate and the Apulian microplate throughout the Early Cretaceous—and the subsequent Alpine orogeny (Stampfli et al., 2002). Alternative processes of geodispersal, associated with episodes of marine transgression-regression, or short distance dispersal by island hopping between the respective landmasses, cannot be excluded.

Our analyses support sympatric divergence between the Hercynian subendemic *Ambrosina bassii* and *Arisarum* in the Middle Eocene (node 2 = 46.1 ± 9.0 Ma; Fig. 3a), or in the Early Paleocene (node 2 = 62.7 ± 8.1; r8s estimation in online Appendix 6), either in Apulia, Corsica, North Africa, or Sardinia (Fig. 3a). Ecological and paleobotanical factors are instrumental in choosing among these putative ancestral areas. *Ambrosina* and *Arisarum*, which colonize stony grounds in the understory of the maquis, form starchy dormant tubers during the dry season and resume their vegetative growth with the onset of the rainy season (Mayo et al., 1997), a life cycle commonly viewed as an adaptation to the marked seasonality typical of the Mediterranean climate (Thompson, 2005). Considering these ecological traits, the fact that the sclerophyllous vegetation typical of the Mediterranean Basin started to develop north of the Tethys during the Early Eocene (Palamarev, 1987), and the retrieval of Oligo-Miocene fossils of sclerophyllous vegetation in Italy, our combined results suggest that the initial, sympatric diversification of clade M1 likely occurred in Apulia, rather than in Corsica, North Africa, or Sardinia (Fig. 4b).

Subsequently, the *Arisarum* lineage expanded throughout the Mediterranean, whereas the *Ambrosina*


Exposed land
Shallow continental marine and coastal
Deeper continental marine
Ocean
Time (Ma)
100 65 55 34 23 05
C P E O M P
a) 93-83 Ma
Ame
Atlantic
Alpine Tethys
Eu
Ib
Ap
An
Ir
Neotethys
Naf
(a)
Clade M1
b) 46-43 Ma
Eu
Atlantic
Alpine Tethys
Ib
Ap
An
Ae
Neotethys
Naf
Ar
(b)
Ambrosina bassii
c) 32-30 Ma
Eu
Atlantic
Paratethys
Ib
Co
Sa
Ba
Ap
Ae
An
Ir
Tethys
Naf
Ar
(c)
Helicodiceros muscivorus
Biarum
Clade M2
Arum
d) 19 Ma
Eu
Atlantic
Paratethys
Ib
Ba
Co
Sa
Ap
Ae
An
Ir
Tethys
Naf
Ar
(d)
Biarum dispar
Arum pictum

lineage dispersed into North Africa, Sardinia, and Corsica. Migration via land from Apulia into North Africa was possible until the Middle Eocene (Figs. 4a, b), when the former was connected to the latter (Dercourt et al., 1986), whereas Corsica and Sardinia were still connected to the Hercynian basement of the Iberian microplate (Alvarez, 1972; Bellon et al., 1977). Direct expansion from Apulia into Sardinia and Corsica became possible at different times during the Miocene, according to tectonic models (Table 1; Alvarez, 1972; Alvarez et al., 1974; Rosenbaum et al., 2002a), but our current sampling does not allow us to make any specific hypothesis about the most likely migration route of *Ambrosina* towards these two islands.

Origin of the Mediterranean Clade M2

Combined evidence from DIVA (Ronquist, 1996) and dating analyses indicates that the stem lineage of the Mediterranean clade M2 (Fig. 3a), comprising *Eminium, Helicodiceros, Biarum, Dracunculus*, and *Arum* and included in Areae (Fig. 2), diverged allopatrically from a tropical, primarily Asian sister clade, formed by *Sauromatum guttatum* and *Typhonium giganteum* (the latter genus being paraphyletic; Renner and Zhang, 2004), in a region encompassing Tropical Asia and Anatolia during the Late Eocene (node 3 = 37.8 ± 7.2 Ma; Fig. 3a). The vicariant hypothesis for the origin of M2 and its estimated age seem congruent with paleoreconstructions suggesting that geological continuity between Anatolia and the current tropical Asian region was disrupted by the uplift of the Tibetan plateau following plate collision between the Indian margin and Eurasia during the Middle to the Late Eocene (44 to 36 Ma; Patriat and Achacha, 1984; Ruddiman and Kutzbach, 1991).

M2 then diversified sympatrically on the Anatolian microplate at the Eocene-Oligocene boundary (node 4 = 33.8 ± 6.9 Ma; Fig. 3a), giving origin to a clade formed by the eastern Mediterranean/western Asian *Eminium* and the Hercynian endemic *Helicodiceros muscivorus*, and a clade formed by the disjunct eastern-western Mediterranean *Biarum*, the peri-Mediterranean *Arum*, and the disjunct eastern Mediterranean-Macaronesian *Dracunculus* (Mayo et al., 1997). The repeated cycles of marine transgression-regression between Tethys and Paratethys during the Oligocene (Jolivet et al., 2006; Rögl, 1999) likely enabled speciation and range expansion by both vicariant and geodispersalist processes on terranes that were part of the current Anatolian microplate (Fig. 4c).

The east-west migratory route inferred for the origin of clade M2 has also been suggested for other Mediterranean taxa, including (i) species of the steppic flora of Spain that are related to taxa found only in the Middle East and Central Asia (Polunin and Smithies, 1973); (ii) some Fabaceae with an east-west disjunct distribution; e.g., *Anthyllis hermanniae,* occurring in Anatolia and Aegean Islands, Balkan Peninsula, South Italy, Sicily, Malta, Corsica, and Sardinia (Brullo and Giusso del Galdo, 2006), and *Genista* section *Acanthospartum* occurring in Greece, Crete, Sardinia, and Majorca (Contandriopoulos and Cardona, 1984); and (iii) several groups of animals; e.g., insects (Sanmartin, 2003), scorpions, frogs, and newts (Oosterbroek and Arntzen, 1992).

Origin of the Hercynian endemic Helicodiceros muscivorus *(L. fil.) Engler*

Ancestral area reconstructions suggest that the common ancestor of *Eminium* and *Helicodiceros* originated in the Anatolian plate (node 4; Fig. 3a), then dispersed to the western Mediterranean and split allopatrically into an eastern Mediterranean lineage and a Hercynian lineage, respectively (node 5; Fig. 3a). The vicariant hypothesis for the divergence between *Eminium* and *Helicodiceros muscivorus* and the inferred age of the event (node 5 = 31.1 ± 6.7 Ma; Fig. 3a) are compatible with geological reconstructions, suggesting that (i) during the Early Oligocene, the Anatolian microplate was alternatively connected and disconnected from the Iberian microplate via intermediate microplates in the forming Mediterranean Basin, due to cycles of marine transgression-regression between the Tethys Sea to the south and the Paratethys Sea to the north (Jolivet et al., 2006; Rögl, 1999); and (ii) Corsica, Sardinia, and the Balearic Islands were part of the Iberian microplate until they started to separate from the Hercynian basement around 30 to 28 Ma (Table 1; Alvarez, 1972; Alvarez et al., 1974; Rosenbaum et al., 2002a).

The inferred origin of *H. muscivorus* by vicariance, rather than over-water dispersal, is also congruent with the low dispersability of its fruits, which consist in orange, ovoid berries, generally containing two seeds (Mayo et al., 1997). The only available evidence on dispersal vectors concerns the lizard *Podarcis lilfordii*, endemic to the Balearic islets, which has been reported to consume the fruits of *H. muscivorus* and disperse its seeds (Pérez-Mellado et al., 2000; Valentin Perez-Mellado, Universtity of La Rioja, Spain, March 2007, personal communication). Seed dispersal by lizards, also reported in

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FIGURE 4. Paleomaps of the developing Mediterranean region from the Late Cretaceous to the Early Miocene. Each time frame is reported on the chronogram (see Fig. 3a) to the right of the respective paleomap and the corresponding clade is bolded. (a) Origin of the M1 stem lineage during the Late Cretaceous (node 1 = 82.7 ± 6.8 Ma); map redrawn after Philip et al. (1993, 2000) and Stampfli (2000). (b) Origin of *Ambrosina* during the Early Eocene (node 2 = 46.1 ± 9.0 Ma); map redrawn after Meulenkamp and Sissingh (2003) and Butterlin et al. (1993). (c) Origin of M2, *Helicodiceros*, *Biarum*, and *Arum* stem lineages during the Late Eocene to Middle Oligocene (node 3 = 37.8 ± 7.2 Ma; node 5 = 31.1 ± 6.7 Ma; node 6 = 30.2 ± 6.7 Ma; node 7 = 27.3 ± 6.5 Ma); map redrawn after Lorenz et al. (1993) and Meulenkamp et al. (2000b). (d) Origin of *Arum pictum* and *Biarum dispar* during the Middle Miocene (node 8 = 16.1 ± 5.8 Ma; node 10 = 18.7 ± 5.8 Ma); map redrawn after Meulenkamp et al. (2000a) and Rosenbaum et al. (2002a). Area designations as in Figure 3. C, Cretaceous; P, Paleocene; E, Eocene; O, Oligocene; M, Miocene; P, Pliocene and Pleistocene. Ma, mega annum (million years ago).

other island systems for different plants, e.g., *Coprosma propinqua* (Rubiaceae; Wotton, 2002) or *Withania aristata* (Solanaceae; Valido and Nogales, 1994), can only reach small distances, apparently contradicting a hypothetical origin of *H. muscivorus* by LDD.

Previous studies suggested that approximately 3% of the Corsican endemics have an eastern Mediterranean origin (Contandriopoulos, 1981; Gamisan and Jeanmonod, 1995) and that *H. muscivorus* might represent a paleoendemic (Contandriopoulos and Cardona, 1984). Our phylogeny-based results provide the first explicit evidence supporting the ancient origin of a Hercynian endemic from the eastern Mediterranean. Available investigations on other proposed Hercynian paleoendemics (Contandriopoulos and Cardona, 1984) were unable to conclusively demonstrate their relictual nature. For example, the putative paleoendemic *Nananthea perpusilla* (Asteraceae) was found to diverge from the mainly eastern Mediterranean *Tripleurospermum* in the Late Miocene (ca. 8.5 Ma; Oberprieler, 2005), thus not conforming to the Early Oligocene origin that would be congruent with the geological splitting of the Hercynian basement. A vicariant origin was also proposed to explain the current disjunct distribution of *Cyclamen balearicum* (Myrsinaceae) in the Balearic Islands and southern France, although no time frame or biogeographic reconstructions were generated to support the claim (Gielly et al., 2001). Similarly, no explicit temporal and biogeographic analyses have been generated to test the paleoendemic hypothesis for the origin of *Helichrysum frigidum* (Asteraceae; Galbany-Casals et al., 2004), *Hypericum balearicum* (Hypericaceae; Crockett et al., 2004), and *Naufraga balearica* (Apiaceae; Fridlender and Boisselier-Dubayle, 2000). In animals, the split of the Corso-Sardinian block from the Hercynian basement of the Iberian microplate was assumed, rather than tested, to estimate rates of substitution in mitochondrial DNA and infer biogeographic scenarios in newts (Caccone et al., 1994, 1997), snails (Ketmaier et al., 2003), lizards (Oliverio et al., 2000), and insects (Caccone and Sbordoni, 2001; Fochetti et al., 2004).

In summary, the Early Oligocene origin of *H. muscivorus* from a widespread eastern-western Mediterranean ancestor (Fig. 3a), the low levels of infraspecific morphological (Fridlender, 2000; Rosselló and Saez, 1997) and genetic variation (ranging from zero to one nucleotide differences among the eight accessions from the three island systems; Fig. 2), and its taxonomic isolation in a monospecific genus (Boyce, 1994) are congruent with the criteria commonly used to define paleoendemics, which emphasize their ancient age; the absence of their closest relatives from the same or adjacent geographical areas; their relictual nature, as the only survivors of a once more widely distributed ancestor; the lack of morphological and genetic variation, interpreted as the product of a long process of adaptation in isolation to the conditions of the refugial area; and their taxonomic/phylogenetic isolation, due to the extinction of the closest relatives (Bramwell, 1972; Favarger and Contandriopolous, 1961; Greuter, 1972; Lomolino et al., 2006; Major, 1988).

Origin of the Hercynian Endemic Arum pictum *L. fil.*

DIVA (Ronquist, 1996) and molecular dating estimates suggest that the common ancestor of *Biarum*, *Dracunculus*, and *Arum* originated in Anatolia during the Early Oligocene (node 6 = 30.2 ± 6.7 Ma; Fig. 3a), subsequently generating the *Arum-Dracunculus* lineage on the same microplate (node 7 = 27.3 ± 6.5 Ma; Fig. 3a). As already remarked above for M2 and *Helicodiceros* (nodes 3 and 5; Fig. 3a), in the Early Oligocene (Jolivet et al., 2006; Rögl, 1999) extensive land connections allowed for biotic movement from Anatolia into Iberia during episodes of marine regression between the Tethys and Paratethys Seas, whereas oceanic barriers favored allopatric speciation between eastern and western Mediterranean microplates during episodes of marine transgression (Fig. 4c).

Later, the *Arum* lineage expanded its range into the western Mediterranean, until the ancestor of the Hercynian endemic *Arum pictum* diverged allopatrically from the remaining species during the Miocene (node 8 = 16.1 ± 5.8 Ma; Fig. 3a). Migration of *A. pictum* from Anatolia into Sardinia (node 8; Fig. 3a) would have been possible between the Early and Middle Miocene via land connection with Eurasia through the Apulia microplate (Fig. 4d), with final separation of Sardinia, still attached to the Calabria block, around 16 Ma (Table 1; Alvarez, 1972; Alvarez et al., 1974; Rosenbaum et al., 2002a). However, the geological connections between Sardinia and neighboring areas are still debated (e.g., Meulenkamp and Sissingh, 2003; Roca, 2002), and the allopatric origin of *A. pictum* might have also been achieved via LDD.

The greater or lesser ability of fruits and seeds to disperse over long distances provides another important line of evidence to discern between over-land migration and dispersal over existing barriers (Bohning-Gaese et al., 2006). The red, fleshy fruits of *A. pictum*, about 4 to 5 mm long and containing one to two spherical, reticulate, brown seeds (Boyce, 1988), are generally transported by short-distance dispersers such as ants (Boyce, 1988), snakes (Gianni Bacchetta, University of Cagliari, Italy, March 2007, personal observation) and lizards, which enhance seed germination by ingestion (Valentin Perez-Mellado, Universtity of La Rioja, Spain, March 2007, personal communication). Furthermore, the demonstrated toxicity for birds of the calcium oxalate crystals abundant in the fruits and leaves of most Araceae (Arai et al., 1992), including *A. pictum* (Boyce, 1993), does not favor LDD by such vectors. Therefore, the low dispersibility of the propagules seems to be more congruent with a geodispersalist hypothesis for the diversification of *A. pictum* than with LDD by birds. In conclusion, as seen for *Helicodiceros muscivorus*, our phylogeny-based evidence appears consistent with published scenarios on the eastern Mediterranean origin of some Hercynian endemics (Cardona and Contandripoulos, 1979; Contandriopoulos, 1981; Gamisan and Jeanmonod, 1995).

Origin of the Sardinian Subendemic Biarum dispar *(Schott) Tavalera*

Molecular dating estimates and reconstruction of ancestral areas suggest that the ancestor of *Biarum* diverged from its sister clade during the Early Oligocene in Anatolia (node 6 = 30.2 ± 6.7 Ma; Fig. 3a), from where it dispersed into Iberia. In the Early Miocene, the *Biarum* lineage split allopatrically into an eastern Mediterranean lineage, represented by *B. davisii*, and a widespread Mediterranean clade that includes the subendemic *B. dispar* (node 9 = 21.6 ± 6.2 Ma; Fig. 3a), which diverged sympatrically from its sister clade on the Iberian peninsula (node 10 = 18.7 ± 5.8 Ma; Fig. 3a), expanding into Sardinia later in the Miocene.

As seen above, terrestrial connections separating the Tethys and Paratethys Seas during the Miocene (Fig. 4d; Jolivet et al., 2006; Rögl, 1999) might have allowed for biotic exchanges between eastern and western Mediterranean. Furthermore, the formation of water barriers during periods of marine transgression might have enabled allopatric speciation. The east-west pattern of migration inferred for the expansion of *Biarum* is congruent with trans-Tethys biotic exchanges proposed for several groups of animals (Oosterbroek and Arntzen, 1992) and plants, including the Hercynian endemic *Nananthea* (Asteraceae; Oberprieler, 2005).

The ecological characteristics of *Biarum* seem compatible with a history of sympatric speciation and range expansion when geological connections were available, and allopatric speciation when geological connections were interrupted. Indeed, most extant *Biarum* species are geocarpic, i.e., bear their fruits below the ground (Mayo et al., 1997), and are generally dispersed by ants (Boyce, 1993), thus allowing for delayed seed dispersal, partitioned germination through time, and survival in inhospitable habitats (Ellner and Shmida, 1981).

One limit of our investigation is that the method used for ancestral area reconstruction (DIVA; Ronquist, 1996) does not incorporate any knowledge of past and present connections among the areas of distribution defined at the tips of the tree, thus leading to many alternative optimizations at the nodes that are not compatible with geological models. Selection of the most likely ancestral area, therefore, must be performed a posteriori, by comparing the equally optimal solutions for ancestral areas (Fig. 3a) with available geological reconstructions (Table 1; Fig. 4), introducing a subjective element in the inference of integrative biogeographic scenarios. Furthermore, by favoring a priori vicariance and range expansion against dispersal, DIVA assumes continuity in all ancestral ranges, overall making ancestral reconstruction particularly equivocal. A promising analytical development incorporates geological models into the reconstruction of ancestral areas a priori, thus helping to achieve a sounder basis for the estimation of the relative roles of long-distance and earth-driven processes (Ree et al., 2005). Ideally, further progress in this direction will allow for the widespread application of this novel approach to cases with very complex geological histories, including the Mediterranean Basin.

Despite the above-mentioned caveats and the breadth of the inferred time intervals for the divergence of the selected taxa, the phylogenetic results on Araceae allowed us to make at least some progress towards two goals: (i) the assessment of congruence between the estimated ages and geographic sources of the Mediterranean endemics with independently derived paleogeological models; (ii) the evaluation of previous, intuitive hypotheses about the origin of the Mediterranean flora in general and the Hercynian endemics in particular. Increased precision of the estimated time frames, coupled with improved methods of ancestral area inference (Ree et al., 2005) and the explicit incorporation of paleoclimatic models and ecological parameters (Jakob et al., 2007; Wiens and Donoghue, 2004; Yesson and Culham, 2006), will be necessary to further refine hypotheses about the most likely biogeographic history of the examined Mediterranean endemics.

CONCLUSIONS

Our results from phylogenetic, molecular dating, and ancestral area analyses, combined with geological, paleobotanical, paleoclimatic, and ecological evidence, support two main invasions of the Mediterranean region by the Araceae: one from an area that connected North America and Eurasia during the Late Cretaceous (clade M1) and one from the Anatolian plate in western Asia during the Late Eocene (clade M2; Figs. 3, 4). The inferred invasions are congruent with the diverse biogeographic origins postulated for the heterogeneous Mediterranean flora (Quézel, 1985). The stem lineage of the M1 clade might have been part of an ancestral subtropical flora likely widespread in the Tethyan region before the progressive opening of the North Atlantic from the Late Cretaceous to the Early Eocene (Quézel, 1985; Raven, 1973; Scotese, 2004). On the other hand, the stem lineage of M2 appears to have been an element of the tropical Irano-Turanian flora that presumably invaded the Mediterranean from the East multiple times in the Tertiary (Quézel, 1985), mainly during episodes associated with the geological evolution of the Tethys and Paratethys Seas. Indeed, a recurrent theme that emerges from our analyses is that land connections and interruptions caused by repeated cycles of marine transgression-regression between the Tethys and Paratethys (Meulenkamp and Sissingh, 2003; Oosterbroek and Arntzen, 1992; Popov et al., 2006; Quézel, 1985; Rögl, 1999; Sanmartin, 2003; Stampfli et al., 1991; Steininger and Rögl, 1984) favored geodispersalist expansion of biotic ranges from western Asia into the western Mediterranean Basin and subsequent allopatric speciation at different points in time, thus providing a geological framework for the origin of M2, *Helicodiceros muscivorus*, *Arum pictum*, and *Biarum dispar* from the Late Eocene to the Late Oligocene (Figs. 3, 4c, d).

It has been hypothesized that islands formed through fragmentation of continental plates (fragment islands: Gillespie and Roderick, 2002; continental islands: Lomolino et al., 2006) should be characterized by a high proportion of relictual, paleoendemic elements from the original continental biota, while oceanic islands of volcanic origin (Darwinian islands: Gillespie and Roderick, 2002) would include a large number of neoendemics derived from colonization of empty ecological niches. The integrative evidence summarized above supports a relictual origin for the Hercynian endemics *Helicodiceros muscivorus* and *Arum pictum*, a conclusion that is congruent with the suggested high representation of paleoendemics on islands that were connected with continental plates for at least part of their geological history, as in the case of Corsica, Sardinia, and the Balearic Islands (Table 1).

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REFERENCES

Alvarez, W. 1972. Rotation of Corsica-Sardinia microplate. Nature 235:103–105.

Alvarez, W., T. Cocozza, and F. C. Wezel. 1974. Fragmentation of Alpine orogenic belt by microplate dispersal. Nature 248:309–314.

Arai, M., E. Stauber, and C. M. Shropshire. 1992. Evaluation of selected plants for their toxic effects in canaries. J. Am. Vet. Med. Assoc. 200:1329–1331.

Asmussen, C. B., and M. W. Chase. 2001. Coding and noncoding plastid DNA in palm systematics. Am. J. Bot. 88:1103–1117.

Bacchetta, G., and C. Pontecorvo. 2005. Contribution to the knowledge of the endemic vascular flora of Iglesiente (SW Sardinia-Italy). Candollea 60:481–501.

Barabé, D., C. Lacroix, A. Bruneau, A. Archambault, and M. Gibernau. 2004a. Floral development and phylogenetic position of *Schismatoglottis* (Araceae). Int. J. Plant Sci. 165:173–189.

Barabé, D., C. Lacroix, and M. Gibernau. 2004b. Aspects of floral morphology in *Ambrosina* and *Arisarum* (Araceae). Can. J. Bot. 82:282–289.

Bellon, H., C. Coulon, and J. B. Edel. 1977. Le déplacement de la Sardaigne: Synthèse des données géochronologiques, magmatiques et paléomagnétiques. Bull. Soc. Géol. Fr. 7:825–831.

Biju-Duval, B., J. Dercourt, and X. Le Pichon. 1977. From the Tethys ocean to the Mediterranean seas: A plate tectonic model of the evolution of the western Alpine system. Pages 143–164 *in* Structural history of the Mediterranean Basin (B. Biju-Duval, and L. Montadert, eds.). Technip, Paris.

Bogner, J., G. L. Hoffman, and K. R. Aulenback. 2005. A fossilized aroid infructescence, *Albertarum pueri* gen.nov. et sp.nov., of Late Cretaceous (Late Campanian) age from the Horseshoe Canyon Formation of southern Alberta, Canada. Can. J. Bot. 83:591–598.

Bohning-Gaese, K., T. Caprano, K. van Ewijk, and M. Veith. 2006. Range size: Disentangling current traits and phylogenetic and biogeographic factors. Am. Nat. 167:555–567.

Boyce, P. C. 1988. Plant portrait: *Arum pictum*. Kew Magazine 5:72–76.

Boyce, P. C. 1990. Friar's cowls and mouse plants. Kew Magazine 7:14–21.

Boyce, P. C. 1993. The genus *Arum*. Royal Botanic Gardens, Kew.

Boyce, P. C. 1994. The genera *Dracunculus* and *Helicodiceros* (Araceae: Aroideae). Thaiszia 4:175–182.

Bramwell, D. 1972. Endemism in the flora of the Canary Islands. Pages 141–159 *in* Taxonomy, phytogeography and evolution (D. H. Valentine, ed.). Academic Press, London.

Brullo, S., and G. Giusso del Galdo. 2006. Taxonomic remarks on the *Anthyllis hermanniae* L. (Fabaceae, Faboideae) species complex of the Mediterranean flora. Novon 16:304–314.

Butterlin, J., B. Vrielynck, R. Guiraud, G. Bignot, M. Colchen, J. Clermonte, P. Andreieff, Y. Bellion, J. Benkhelil, C. Cavelier, J. J. Cornee, B. Mercier De Lepinay, C. Montenat, C. Moreau, A. Poisson, and J. M. Vila. 1993. Lutetian palaeoenvironments (46–40 Ma). Pages 197–210 *in* Atlas Tethys palaeoenvironmental maps (J. Dercourt, L. E. Ricou, and B. Vrielynck, eds.). BEICIP-FRANLAB, Rueil-Malmaison.

Caccone, A., M. C. Milinkovitch, V. Sbordoni, and J. R. Powell. 1994. Using the Corsica-Sardinia microplate disjunction to calibrate mitochondrial rDNA evolutionary rates in mountain newts (*Euproctus*). J. Evol. Biol. 7:227–245.

Caccone, A., M. C. Milinkovitch, V. Sbordoni, and J. R. Powell. 1997. Mitochondrial DNA rates and biogeography in European newts (genus *Euproctus*). Syst. Biol. 46:126–144.

Caccone, A., and V. Sbordoni. 2001. Molecular biogeography of cave life: A study using mitochondrial DNA from Bathysciine beetles. Evolution 55:122–130.

Cardona, M. A., and J. Contandripoulos. 1977. L'endémisme dans les flores insulaires méditerranéennes. Mediterranea 2:49–77.

Cardona, M. A., and J. Contandripoulos. 1979. Endemism and evolution in the islands of the Western Mediterranean. Pages 133–169 *in* Plants and islands (D. Bramwell, ed.). Academic Press, London.

Comes, H. P. 2004. The Mediterranean region—A hotspot for plant biogeographic research. New Phytologist 164:11–14.

Comes, H. P., and R. J. Abbott. 2001. Molecular phylogeography, reticulation, and lineage sorting in Mediterranean *Senecio* sect. *Senecio* (Asteraceae). Evolution 55:1943–1952.

Contandriopoulos, J. 1981. Endémisme et origine de la flore de la Corse: Mise au point des connaissances actuelles. Boll. Soc.Sarda Sci. Nat. 20:187–230.

Contandriopolous, J. 1990. Spécificité de l'endémisme corse. Atti dei Convegni Lincei 85:393–416.

Contandriopoulos, J., and M. A. Cardona. 1984. Original character of the endemic flora in the Balearic Islands. Bot. Helvet. 94:101–132.

Crockett, S. L., A. W. Douglas, B. E. Scheffler, and I. A. Khan. 2004. Genetic profiling of *Hypericum* (St. John's Wort) species by nuclear ribosomal ITS sequence analysis. Planta Med. 70:929–935.

Daget, P. 1977. Mediterranean bioclimate—General characteristics and modes of definition. Vegetatio 34:1–20.

Dercourt, J., L. P. Zonenshain, L. E. Ricou, V. G. Kazmin, X. Lepichon, A. L. Knipper, C. Grandjacquet, I. M. Sbortshikov, J. Geyssant, C. Lepvrier, D. H. Pechersky, J. Boulin, J. C. Sibuet, L. A. Savostin, O. Sorokhtin, M. Westphal, M. L. Bazhenov, J. P. Lauer, and B. Bijuduval. 1986. Geological evolution of the Tethys Belt from the Atlantic to the Pamirs since the Lias. Tectonophysics 123:241–315.

Dewey, J. F., W. C. Pitman, W. B. F. Ryan, and J. Bonnin. 1973. Plate tectonics and evolution of Alpine system. Geol. Soc. Am. Bull. 84:3137–3180.

Donoghue, M. J., C. D. Bell, and R. C. Winkworth. 2003. The evolution of reproductive characters in Dipsacales. Int. J. Plant Sci. 164:S453–S464.

Duggen, S., K. Hoernle, P. van den Bogaard, L. Rupke, and J. P. Morgan. 2003. Deep roots of the Messinian Salinity Crisis. Nature 422:602–606.

Ellner, S., and A. Shmida. 1981. Why are adaptations for long-range seed dispersal rare in desert plants? Oecologia 51:133–144.

Favarger, C., and J. Contandriopolous. 1961. Essai sur l'endémisme. Bull. Soc. Bot. Suisse 77:383–408.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. J. Mol. Evol. 17:368–376.

Fochetti, R., V. Ketmaier, M. Oliverio, J. M. T. de Figueroa, and E. Sezzi. 2004. Biochemical systematics, biogeography and evolutionary rates in species of the Mediterranean genus *Tyrrhenoleuctra* (Plecoptera, Leuctridae). Insect Syst. Evol. 35:299–306.

French, J. C., M. G. Chung, and Y. K. Hur. 1995. Chloroplast DNA phylogeny of the Ariflorae. Pages 255–275 *in* Monocotyledons: Systematics and evolution (P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries, eds.). Royal Botanic Gardens, Kew.

Fridlender, A. 2000. Répartition, écologie et conservation de l'Arum mange-mouches (*Helicodiceros muscivorus* (L. fil) Engler) (Araceae) en Corse. Webbia 55:7–35.

Fridlender, A., and M. C. Boisselier-Dubayle. 2000. Comparison of the genetic diversity (RAPD) of *ex situ* collections and natural populations of *Naufraga balearica* Constance & Cannon. Comptes Rendus de l'Académie des Sciences, Série III. Sci. Vie/Life Sci. 323:399–406.

Friis, E. M., K. R. Pedersen, and P. R. Crane. 2004. Araceae from the Early Cretaceous of Portugal: Evidence on the emergence of monocotyledons. Proc. Natl. Acad. Sci. USA 101:16565–16570.

Fromhage, L., M. Vences, and M. Veith. 2004. Testing alternative vicariance scenarios in Western Mediterranean discoglossid frogs. Mol. Phylogenet. Evol. 31:308–322.

Galbany-Casals, M., N. Garcia-Jacas, A. Susanna, L. Saez, and C. Benedi. 2004. Phylogenetic relationships in the Mediterranean *Helichrysum* (Asteraceae, Gnaphalieae) based on nuclear rDNA ITS sequence data. Austral. Syst. Bot. 17:241–253.

Gamisan, J., and D. Jeanmonod. 1995. La flore de Corse: Bilan des connaissances, intérêt patrimonial et état de conservation. Ecol. Mediterranea 21:135–148.

Garfunkel, Z. 2004. Origin of the Eastern Mediterranean Basin: A reevaluation. Tectonophysics 391:11–34.

Gielly, L., M. Debussche, and J. D. Thompson. 2001. Geographic isolation and evolution of the Mediterranean endemic *Cyclamen*: Insights from chloroplast *trn*L (UAA) intron sequence variation. Plant Syst. Evol. 230:75–88.

Gillespie, R. G., and G. K. Roderick. 2002. Arthropods on islands: Colonization, speciation, and conservation. Annu. Rev. Entomol. 47:595–632.

Greuter, W. 1972. The relict element of the flora of Crete and its evolutionary significance. Pages 161–177 *in* Taxonomy, phytogeography and evolution. (D. H. Valentine, ed.). Academic Press, London.

Greuter, W. 1991. Botanical diversity, endemism, rarity, and extinction in the Mediterranean area: An analysis based on the published volumes of Med-Checklist. Bot. Chron. 10:63–79.

Gueguen, E., C. Doglioni, and M. Fernandez. 1998. On the post-25 Ma geodynamic evolution of the western Mediterranean. Tectonophysics 298:259–269.

Hruby, J. 1912. Le genre *Arum*: Aperçu systématique avec considérations spéciales sur les relations phylogénétiques des formes. Bull. Soc. Bot. Genève 4:113–160.

Hsü, K. 1972. When the Mediterranean dried up. Am. Sci. 227:27–37.

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Hugues, P. D., J. C. Woodward, and P. L. Gibbard. 2006. Quaternary glacial history of the Mediterranean mountains. Prog. Phys. Geogr. 30:334–364.

Jakob, S. S., A. Ihlow, and F. R. Blattner. 2007. Combined ecological niche modelling and molecular phylogeography revealed the evolutionary history of *Hordeum marinum* (Poaceae)—Niche differentiation, loss of genetic diversity, and speciation in Mediterranean Quaternary refugia. Mol. Ecol. 16:1713–1727.

Johnson, L. A., and D. E. Soltis. 1995. Phylogenetic inference in Saxifragaceae sensu-stricto and *Gilia* (Polemoniaceae) using *mat*K sequences. Ann. Miss. Bot. Gard. 82:149–175.

Jolivet, L., R. Augier, C. Robin, J. P. Suc, and J. M. Rouchy. 2006. Lithospheric-scale geodynamic context of the Messinian Salinity Crisis. Sed. Geol. 188:9–33.

Jonsson, K. A., and J. Fjeldsa. 2006. Determining biogeographical patterns of dispersal and diversification in oscine passerine birds in Australia, Southeast Asia and Africa. J. Biogeogr. 33:1155–1165.

Ketmaier, V., R. Argano, and A. Caccone. 2003. Phylogeography and molecular rates of subterranean aquatic Stenasellid Isopods with a peri-Tyrrhenian distribution. Mol. Ecol. 12:547–555.

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

Krijgsman, W. 2002. The Mediterranean: Mare Nostrum of Earth sciences. Earth Planet. Sci. Lett. 205:1–12.

Lepichon, X., and J. Angelier. 1979. Hellenic arc and trench system—Key to the Neotectonic evolution of the eastern Mediterranean area. Tectonophysics 60:1–42.

Lieberman, B. S. 2000. Paleobiogeography: Using fossils to study global change, plate tectonics, and evolution. Kluwer Academic Publishers, New York.

Lobin, W., and P. C. Boyce. 1991. *Eminium koenianum* (Araceae), a new species from N.E. Turkey and a key to the genus *Eminium*. Willdenowia 20:43–51.

Lomolino, M. V., B. R. Riddle, and J. H. Brown. 2006. Biogeography, 3rd edition. Sinauer Associates, Sunderland, Massachusetts.

Lorenz, C., J. Butterlin, C. Cavelier, J. Clermonte, M. Colchen, J. Dercourt, R. Guiraud, C. Montenat, A. Poisson, L. E. Ricou, and M. Sandulescu. 1993. Late Rupelian Palaeoenvironments (30–28 Ma). Pages 211–224 *in* Atlas Tethys palaeoenvironmental maps (J. Dercourt, L. E. Ricou, and B. Vrielynck, eds.). BEICIP-FRANLAB, Rueil-Malmaison.

Maire, R. 1957. Monocotyledonae. Pages 221–249 *in* Flore de l'Afrique du Nord (M. Guinochet, ed.). Paul Lechevalier, Paris.

Major, J. 1988. Endemism: A botanical perspective. Pages 117–146 *in* Analytical biogeography. An integrative approach to the study of animal and plant distribution (A. A. Myers, and P. S. Giller, eds.). Chapman & Hall, London.

Mariotti, M. 1990. Floristic connections between the Corso-Sardinian domination and the Ligurian area. Atti Convegni Lincei 85:429–448.

Mayo, S. L., J. Bogner, and P. C. Boyce. 1997. The genera of Araceae. Royal Botanic Gardens, Kew.

Médail, F., and P. Quézel. 1997. Hot-spot analysis for conservation in the Mediterranean Basin. Ann. Miss. Bot. Garden 84:112–127.

Meulenkamp, J. E., and W. Sissingh. 2003. Tertiary palaeogeography and tectonostratigraphic evolution of the Northern and Southern Peri-Tethys platforms and the intermediate domains of the African-Eurasian convergent plate boundary zone. Palaeogeogr. Palaeoclimatol. Palaeoecol. 196:209–228.

Meulenkamp, J. E., W. Sissingh, S. V. Popov, M. Kovac, E. Abbate, S. Abdel-Gewad, A. S. Andreyeva-Grigorovich, S. Bouaziz, B. Cahuzac, J. P. Calvo, R. Daams, G. Georgiev, F. Hirsch, L. Londeix, M. Maruntеanu, M. Monzer, J. P. Platel, J. Roger, A. Sadek, M. Sagri, I. G. Scherba, A. S. Stolyarov, B. Studencka, C. Yaich, and M. Sandulescu. 2000a. Early Burdigalian. Map 20 *in* Atlas Peri-Tethys, paleogeographical maps (J. Dercourt, M. Gaetani, B. Vrielynck, E. Barrier, B. Biju Duval, M. F. Brunet, J. P. Cadet, S. Crasquin, and M. Sandulescu, eds.). CCGM/CGMW, Paris.

Meulenkamp, J. E., W. Sissingh, S. V. Popov, M. Kovac, F. Bergerat, E. Abbate, S. Abdel-Gewad, A. S. Andreyeva-Grigorovich, S. Bouaziz, B. Cahuzac, J. P. Calvo, R. Daams, G. Georgiev, F. Hirsch, L. Londeix, M. Monzer, J. P. Platel, J. Roger, A. Sadek, M. Sagri, I. G. Scherba, A. S. Stolyarov, B. Studencka, C. Yaich, and M. Sandulescu. 2000b. Early Campanian. Map 19 *in* Atlas Peri-Tethys, paleogeographical maps (J. Dercourt, M. Gaetani, B. Vrielynck, E. Barrier, B. Biju Duval, M. F. Brunet, J. P. Cadet, S. Crasquin, and M. Sandulescu, eds.). CCGM/CGMW, Paris.

Moggi, G. 1959. La distribuzione dell'*Arisarum proboscideum* (L.) Savi ed il suo valore sistematico e fitogeografico. Webbia 15:95–110.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature 403:853–858.

Nie, Z. L., H. Sun, H. Li, and J. Wen. 2006. Intercontinental biogeography of subfamily Orontioideae (*Symplocarpus*, *Lysichiton*, and *Orontium*) of Araceae in eastern Asia and North America. Mol. Phylogenet. Evol. 40:155–165.

Nylander, J. 2004. MrModelTest 2.1. Uppsala: Evolutionary Biology Centre.

Oberprieler, C. 2005. Temporal and spatial diversification of Circum-Mediterranean Compositae-Anthemideae. Taxon 54:951–966.

Oliverio, M., M. A. Bologna, and P. Mariottini. 2000. Molecular biogeography of the Mediterranean lizards *Podarcis* Wagler, 1830 and Teira Gray, 1838 (Reptilia, Lacertidae). J. Biogeogr. 27:1403–1420.

Oosterbroek, P., and J. W. Arntzen. 1992. Area-cladograms of the Circum-Mediterranean taxa in relation to Mediterranean paleogeography. J. Biogeogr. 19:3–20.

Oxelman, B., M. Liden, and D. Berglund. 1997. Chloroplast *rps*16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). Plant Syst. Evol. 206:393–410.

Palamarev, E. 1987. Paleobotanical evidences of the Tertiary history and origin of the Mediterranean sclerophyll dendroflora. Plant Syst. Evol. 162:93–107.

Patriat, P., and J. Achacha. 1984. India-Eurasia collision chronology has implications for crustal shortening and driving mechanisms of plates. Nature 311:615–621.

Pérez-Mellado, V., G. Cortázar, M. López-Vicente, A. Perera, and N. Sillero. 2000. Interactions between the Balearic lizard *Podarcis lilfordi* and the plant *Dracunculus muscivorus*. Amphibia-Reptilia 21:223–226.

Philip, J., J. F. Babinot, G. Tronchetti, E. Fourcade, J. Azema, R. Guiraud, Y. Bellion, L. E. Ricou, B. Vrielynck, J. Boulin, J. J. Cornee, and J. P. Herbin. 1993. Late Cenomanian. Pages 153–178 *in* Atlas Tethys Palaeoenvironmental Maps (J. Dercourt, L. E. Ricou, and B. Vrielynck, eds.). BEICIP-FRANLAB, Rueil-Malmaison.

Philip, J., M. Floquet, J. P. Platel, F. Bergerat, M. Sandulescu, E. Baraboshkin, E. Amon, A. Poisson, R. Guiraud, D. Vaslet, Y. Le Nindre, M. Ziegler, S. Bouaziz, J. C. Guezou, and C. Lepvrier. 2000. Early Campanian. *in* Atlas Peri-Tethys, Paleogeographical maps (J. Dercourt, M. Gaetani, B. Vrielynck, E. Barrier, B. Biju Duval, M. F. Brunet, J. P. Cadet, S. Crasquin, and M. Sandulescu, eds.). CCGM/CGMW, Paris.

Polunin, O., and B. E. Smithies. 1973. Flowers of South-West Europe. Oxford University Press, London.

Popov, S. V., I. G. Shcherba, L. B. Ilyina, L. A. Nevesskaya, N. P. Paramonova, S. O. Khondkarian, and I. Magyar. 2006. Late Miocene to Pliocene palaeogeography of the Paratethys and its relation to the Mediterranean. Palaeogeogr. Palaeoclimatol. Palaeoecol. 238:91–106.

Quézel, P. 1978. Analysis of the flora of Mediterranean and Saharan Africa. Ann. Miss. Bot. Garden 65:479–534.

Quézel, P. 1985. Definition of the Mediterranean region and origin of its flora. Pages 9–24 *in* Plant conservation in the Mediterranean area (C. Gomez-Campo, ed.). Kluwer, Dordrecht.

Quilichini, A., A. Torre, and M. Gibernau. 2005. Preliminary data on the biology and reproduction of *Ambrosina bassii* L. (Araceae) in Corsica. Aroideana 28:37-42.

Rambaut, A. 1996. Se-Al: Sequence Alignment Editor. Available at http://evolve.zoo.ox.ac.uk/.

Raven, P. H. 1973. The evolution of the Mediterranean floras. Pages 213–324 *in* Mediterranean type ecosystems. Origin and structure. (F. di Castri and H. A. Mooney, eds.). Springer-Verlag, Berlin.

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

Renner, S. S., and L.-B. Zhang. 2004. Biogeography of the *Pistia* clade (Araceae): Based on chloroplast and mitochondrial DNA sequences and Bayesian divergence time inference. Syst. Biol. 53:422–432.

Riedl, H. 1980. Tentative keys for the identification of species in *Biarum* and *Eminium*, with notes on some taxa included in *Biarum*. Aroideana 3:24–31.

Robertson, A. H. F., P. D. Clift, P. J. Degnan, and G. Jones. 1991. Palaeogeographic and palaeotectonic evolution of the Eastern Mediterranean Neotethys. Palaeogeogr. Palaeoclimatol. Palaeoecol. 87: 289–343.

Roca, E. 2002. The Northwest-Mediterranean Basin (Valencia Trough, Gulf of Lions and Liguro-Provencal basins): Structure and geodynamic evolution. Pages 671–706 *in* Peri-Tethys memoir 6. PeriTethyan Rift/Wrench basins and passive margins (P. A. Ziegler, W. Cavazza, A. F. H. Robertson, and S. Crasquin-Soleau, eds.). Editions du Muséum, Paris.

Rögl, F. 1999. Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short overview). Geol. Carpath. 50:339–349.

Ronquist, F. 1996. DIVA version 1.1. Computer program and manual available by anonymous FTP from Uppsala University (ftp.uu.se or ftp.systbot.uu.se).

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

Rosenbaum, G., G. S. Lister, and C. Duboz. 2002a. Reconstruction of the tectonic evolution of the western Mediterranean since the Oligocene. J. Virtual Explorer 8:107–130.

Rosenbaum, G., G. S. Lister, and C. Duboz. 2002b. Relative motions of Africa, Iberia and Europe during Alpine orogeny. Tectonophysics 359:117–129.

Rosselló, J. A., and L. Saez. 1997. Notes on some Balearic Araceae. Acta Bot. Barcinonen. 44:169–174.

Ruddiman, W. F., and J. E. Kutzbach. 1991. Plateau uplift and climatic change. Sci. Am. 264:66–74.

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

Sanderson, M. J. 2003. r8s: Inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19:301–302.

Sang, T., D. J. Crawford, and T. F. Stuessy. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). Am. J. Bot. 84:1120–1136.

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

Schettino, A., and E. Turco. 2006. Plate kinematics of the Western Mediterranean region during the Oligocene and Early Miocene. Geophys. J. Int. 166:1398–1423.

Scotese, C. R. 2004. A continental drift flipbook. J. Geol. 112:729–741.

Smith, S. Y., and R. A. Stockey. 2003. Aroid seeds from the Middle Eocene Princeton chert (*Keratosperma allenbyense*, Araceae): Comparisons with extant Lasioideae. Int. J. Plant Sci. 164:239–250.

Speranza, F., I. M. Villa, L. Sagnotti, F. Florindo, D. Cosentino, P. Cipollari, and M. Mattei. 2002. Age of the Corsica-Sardinia rotation and Liguro-Provencal Basin spreading: New paleomagnetic and Ar/Ar evidence. Tectonophysics 347:231–251.

Stampfli, G., J. Marcoux, and A. Baud. 1991. Tethyan margins in space and time. Palaeogeogr. Palaeoclimatol. Palaeoecol. 87:373–409.

Stampfli, G. M. 2000. Tethyan oceans. Pages 1–23 *in* Tectonics and magmatism in Turkey and the surrounding area (E. Bozkurt, J. A. Winchester, and J. D. A. Piper, eds.). Geological Society, London, Special Publications.

Stampfli, G. M., G. D. Borel, R. Marchant, and J. Mosar. 2002. Western Alps geological constraints on western Tethyan reconstructions. J. Virtual Explorer 8:77–106.

Steininger, F. F., and F. Rögl. 1984. Paleogeography and palinspatic reconstruction of the Neogene of the Mediterranean and Paratethys. Pages 659–668 *in* The geological evolution of the eastern Mediterranean (J. E. Dixon, and A. H. F. Robertson, eds.). Blackwell Scientific Publications, Oxford.

Suc, J.-P. 1984. Origin and evolution of the Mediterranean vegetation and climate in Europe. Nature 307:429–438.

Swofford, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods) Version 4. Sinauer Associates, Sunderland, Massachusetts.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three noncoding regions of chloroplast DNA. Plant Mol. Biol. 17:1105–1109.

Talavera, S. 1976. Revision de las especies españolas del genero *Biarum* Schott. Lagascalia 6:275–296.

Tam, S.-M., P. C. Boyce, T. M. Upson, D. Barabé, A. Bruneau, F. Forest, and J. S. Parker. 2004. Intergeneric and infrafamilial phylogeny of subfamily Monsteroideae (Araceae) revealed by chloroplast *trn*L-F sequences. Am. J. Bot. 91:490–498.

Thompson, J. D. 2005. Plant evolution in the Mediterranean. Oxford University Press, Oxford.

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

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

Valido, A., and M. Nogales. 1994. Frugivory and seed dispersal by the Lizard *Gallotia-Galloti* (Lacertidae) in a xeric habitat of the Canary Islands. Oikos 70:403–411.

van Veller, M. G. P., and D. R. Brooks. 2001. When simplicity is not parsimonious: *A priori* and *a posteriori* methods in historical biogeography. J. Biogeogr. 28:1–11.

Wiens, J. J., and M. J. Donoghue. 2004. Historical biogeography, ecology and species richness. Trends Ecol. Evol. 19:639–644.

Wilde, V., Z. Kvacek, and J. Bogner. 2005. Fossil leaves of the Araceae from the European Eocene and notes on other aroid fossils. Int. J. Plant Sci. 166:157–183.

Winkworth, R. C., J. Grau, A. W. Robertson, and P. J. Lockhart. 2002. The origins and evolution of the genus *Myosotis* L. (Boraginaceae). Mol. Phylogenet. Evol. 24:180–193.

Wotton, D. M. 2002. Effectiveness of the common gecko (*Hoplodactylus maculatus*) as a seed disperser on Mana Island, New Zealand. N. Z. J. Bot. 40:639–647.

Yang, Z. H. 2000. Phylogenetic analysis by maximum likelihood (PAML). 3.0 ed. University College, London.

Yesson, C., and A. Culham. 2006. A phyloclimatic study of *Cyclamen*. BMC Evol. Biol. 6. Article 72.

Young, N. D., and E. Healy. 1987. Gapcoder automates the use of indel characters in phylogenetic analysis. BMC Bioinformatics 4. Article 6.

Zetter, R., M. Hesse, and A. Frosch-Radivo. 2001. Early Eocene zona-aperturate pollen grains of the *Proxapertites* type with affinity to Araceae. Rev. Palaeobot. Palynol. 117:267–279.

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