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The uneven phylogeny and biogeography of *Erodium* (Geraniaceae): radiations in the Mediterranean and recent recurrent intercontinental colonization

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• *Background and Aims* The genus *Erodium* is a common feature of Mediterranean-type climates throughout the world, but the Mediterranean Basin has significantly higher diversity than other areas. The aim here is to reveal the biogeographical history of the genus and the causes behind the evolution of the uneven distribution.
• *Methods* Seventy-eight new nrITS sequences were incorporated with existing plastid data to explore the phylogenetic relationships and biogeography of *Erodium* using several reconstruction methods. Divergence times for major clades were calculated and contrasted with other previously published information. Furthermore, topological and temporal diversification rate shift analyses were employed using these data.
• *Key Results* Phylogenetic relationships among species are widely congruent with previous plastid reconstructions, which refute the classical taxonomical classification. Biogeographical reconstructions point to Asia as the ancestral area of *Erodium*, arising approx. 18 MYA. Four incidences of intercontinental dispersal from the Mediterranean Basin to similar climates are demonstrated. Increases in diversification were present in two independent *Erodium* lineages concurrently. Two bursts of diversification (3 MYA and 0·69 MYA) were detected only in the Mediterranean flora.
• *Conclusions* Two lineages diverged early in the evolution of the genus *Erodium*: (1) subgenus *Erodium* plus subgenus *Barbata* subsection *Absinthioidea* and (2) the remainder of subgenus *Barbata*. Dispersal across major water bodies, although uncommon, has had a major influence on the distribution of this genus and is likely to have played as significant role as in other, more easily dispersed, genera. Establishment of Mediterranean climates has facilitated the spread of the genus and been crucial in its diversification. Two, independent, rapid radiations in response to the onset of drought and glacial climate change indicate putative adaptive radiations in the genus.

Key words: Biogeography, *Erodium*, divergence times, diversification rate, intercontinental dispersal, phylogenetics, radiation.

INTRODUCTION

Traditionally, disjunct distributions within taxonomic groups have been attributed to plate tectonic shifts or the historical presence of land bridges, such as the Beringian land bridge or the North Atlantic land bridge. These continental connections provide a clear explanation for the distribution of many groups, such as the Tertiary relict floras (Milne, 2006). However, the use of molecular techniques for dating divergence times is continually revealing younger ages than previously accepted for many taxa, uncovering patterns that are incompatible with tectonic shifts (Milne, 2006). An alternative hypothesis to explain distribution patterns is long-distance dispersal of organisms, a concept that is increasingly accepted as the last resort. In fact it has been pointed out that one single seed dispersal 'every few millions of years can have a large impact on biogeography' (Milne, 2006). Investigation of the role of dispersal in the evolutionary process has shed light on many aspects of evolutionary biology, such as selection pressures, dormancy, altruism and senescence (Ronce, 2007). The existence of many plant groups with disjunct distributions which are present in the Mediterranean Basin was thought to happen via arid corridors (Coleman *et al.*, 2003). However, this hypothesis has been refuted, and long-distance dispersal has been proposed as an alternative explanation for the colonization of these Mediterranean climate zones (Coleman *et al.*, 2003). The Mediterranean Basin region is a hotspot of global diversity, and evolutionary studies of its flora contribute to our understanding of Mediterranean ecosystems. Given its worldwide distribution and high diversity, especially in Mediterranean regions, *Erodium* provides an excellent model system to study these phenomena.

Under the current circumscription, Geraniaceae *sensu stricto* comprises five genera: *Geranium*, *Monsonia*, *Pelargonium*, *California* and *Erodium*. The genus *Erodium* has 74 species and is distributed on all continents, excluding Antarctica (Fig. 1; Fiz *et al.*, 2006). A major centre of diversity is observed in the Mediterranean Basin region (62 species), whereas, the other continents harbour only a few native species: one each in North and South America, five in Australia and four in

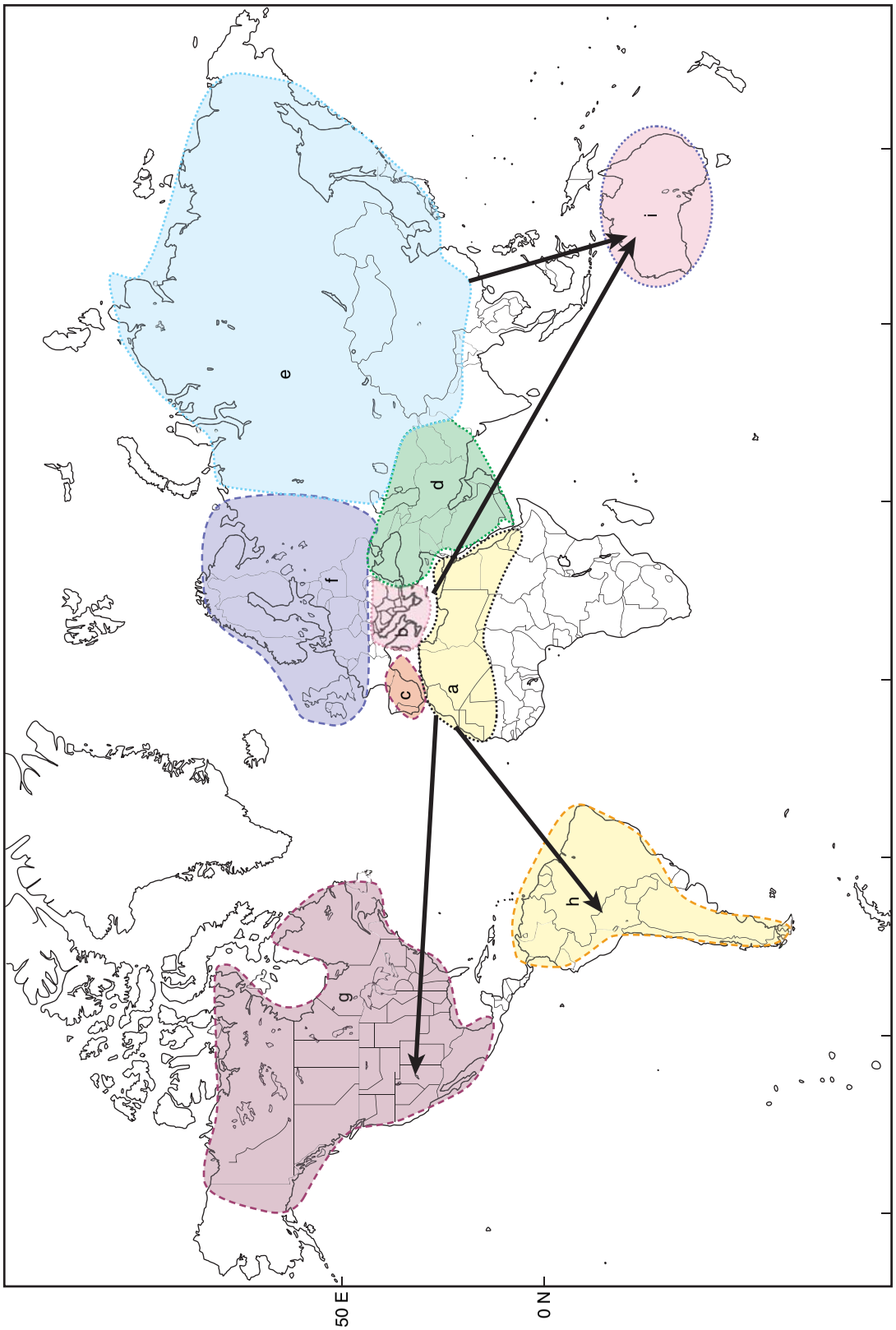

FIG. 1. Map detailing the distribution and areas of endemism for *Erodium* species (shaded). The arrows represent the four possible intercontinental dispersals (see main text). a = North Africa, b = Italy and Balkans, c = Iberian Peninsula, d = The Middle East, e = Asia, f = North Europe, g = North America, h = South America, i = Australia.

Asia. The dispersal and colonization ability of *Erodium* is reflected in this broad distribution. In fact, four species (*E. botrys*, *E. brachycarpum*, *E. cicutarium* and *E. moschatum*) each occupy Mediterranean floristic regions on all continents where these habitats exist. The monophyletic clade containing *Erodium*, *California* and *Geranium* has its origin in Asia, having migrated from southern Africa (Fiz *et al.*, 2008). The origin of *Erodium* is less clear as *California*, its monotypic sister genus, is endemic to California.

The Mediterranean Basin can be divided into two subregions of high species diversity for *Erodium*: subsection *Absinthioidea* (13 species) is centred in the eastern region, and the western region contains the core of section *Barbata*. In the western Mediterranean, 22 species are endemic to Spain and Morocco, eight of which are restricted to one or a few small populations. Rapid species radiations have been described for a number of genera with Mediterranean distribution (Valente *et al.*, 2010). This, together with the high rate of nucleotide substitution in *Erodium* (Fiz, 2005; Guisinger *et al.*, 2008), makes this genus an excellent candidate to explore putative rapid radiations.

It was not possible to resolve low-level relationships within *Erodium* by analysing the *trnL-F* gene region and morphological characters (Fiz *et al.*, 2006, 2008). However, these studies demonstrated the monophyly of *Erodium* and its division into two main clades, placed *California macrophylla* as its sister species, and highlighted an ancient diversification pattern. Finer-scale analyses of molecular regions with higher variability are needed to investigate more recent species formation and colonization patterns in *Erodium*. Sequences of nuclear ITS have been frequently used to study low-level phylogenetic relationships and to infer reticulation effects on phylogeny (Campbell *et al.*, 1997; Fuertes Aguilar and Nieto Feliner, 2003), to infer cases of allopolyploidy (Whittall *et al.*, 2000) and historical biogeographical patterns (Sang *et al.*, 1995; Campbell *et al.*, 1997), and as such this region is an appropriate candidate for further investigation of evolution and dispersal within *Erodium*.

The main objective of the study presented here is to examine the historical biogeography of *Erodium* in an attempt to explain its disjunct distribution over five continents. To this end, variation in newly sequenced nrITS sequences is used to search for fine-scale phylogenetic relationships and this is compared with phylogenetic information gathered using previously published plastid sequences (Fiz *et al.*, 2006, 2008). Greater phylogenetic resolution was necessary to reconstruct the evolutionary history of *Erodium*. A total evidence approach is adopted (combining nrITS and *trnL-F* matrices) and used to infer general biogeographical patterns in *Erodium*. Diversification rate shifts were also explored in an effort to elucidate putative, rapid radiations throughout the genus. These data along with divergence time estimates will lead to a better understanding of the evolutionary history of *Erodium*.

MATERIALS AND METHODS

Plant material and PCR

Seventy-eight nrITS sequences were generated, corresponding to 58 *Erodium* taxa and two individuals of *California macrophylla* (Appendix). DNA extracted from a previous study (Fiz *et al.*, 2006) was used, and DNA of several additional individuals was extracted from herbarium and field collections (Appendix) using DNeasy Plant Mini Kit (QIAGEN Laboratories, Germany).

The ITS region was amplified using the polymerase chain reaction (PCR) with following primer pairs ITS-4 and ITS-5 (White *et al.*, 1990) and 17SE and 26SE (Sun *et al.*, 1994. In addition, 0·5 μL of dimethyl sulfoxide was added to each reaction. PCR conditions for amplification were as described in Fiz *et al.* (2002). The PCR-Beads kit ('puRetaq Ready-To-Go'; Amersham Biosciences) was occasionally used for poorly preserved DNA from herbarium specimens. Amplified products were then purified using spin filter columns (PCR clean-up kit; MoBio Laboratories, CA, USA) following the protocols provided by the manufacturer. Forward and reverse sequences from cleaned products were then directly sequenced as in Fiz *et al.* (2002).

Alignment and phylogenetic analyses

Sequence data were edited using the program Seqed (Applied Biosystems). The limits of the ITS1-5·8S-ITS2 region were determined by comparison with secondary structure of the homologue in Asteraceae (Goertzen *et al.*, 2003). CLUSTAL W (Thompson *et al.*, 1994) was used as a first approach to the alignment of the sequences, followed by manual adjustment. IUPAC symbols were used to represent nucleotide ambiguities.

Three different matrices were constructed and analysed: the first included all 79 nrITS sequences (76 *Erodium*, two *California* and one *Geranium*); the second consisted of 60 nrITS sequences corresponding to one sequence per taxon (58 *Erodium*, one *California* and one *Geranium*); the third matrix combined nrITS and *trnL-F* sequences from 69 taxa (67 *Erodium*, one *California* and one *Geranium*) taken from a previous study (Fiz *et al.*, 2006). Sequences of the two molecular markers from the same individual (i.e. the same DNA extraction) were used for the combined matrix with the exception of 11 taxa (Appendix).

Matrices were analysed using maximum parsimony (MP) and Bayesian inference (BI) methods. *Geranium* and *California* were included as outgroups. Models were chosen based on the Akaike information criterion (AIC) as implemented in MrModeltest 1·1b (Nylander, 2002), which is a simplified version of Modeltest 3·06 (Posada and Crandall, 1998). The MP phylogenetic analyses were conducted using Fitch parsimony as implemented in PAUP* (Swofford, 1999), with unordered and equal weighting of all characters. All phylogenetic analyses were performed for the whole nrITS and for ITS1, 5, 8S and ITS2 independently. Heuristic searches were conducted using random taxon-addition sequences (100 replicates), tree bisection–reconnection branch swapping and with the options MULPARS and STEEPEST DESCENT in effect. Relative support for clades identified by parsimony analysis was assessed by both 'fast' bootstrapping (10 000 re-samplings using the heuristic search strategy as indicated above (Mort *et al.*, 2000) and 'full' bootstrapping (1000 re-samplings with simple taxon addition and SPR branch swapping but

TABLE 1. *Genetic distances (%) for nrITS at genus, species and intraspecific level*

Genus level: between different genera	35·23 % to 25·61 %: *Geranium biumcinatum/Erodium* 26·69 % to 25·81 %: *Geranium biumcinatum/California macrophylla* 25·32 % to 16·68 %: *California macrophylla/Erodium*
Species level: between *Erodium* species	20·96 %: *E. laciniatum* 1/*E. crassifolium* 0 %: *E. botrys/E. brachycarpun* 0 %: *E. glandulosum1/E. rupestre* 2/*E. lucidum/E. antariense* 2/*E. foetidum/E. foetidum cheilanthifolium/E. foetidum celtibericum* 0 %: *E. cossoni/E. laciniatum* 0 %: *E. touchyanum* 2/*E. moschatum* 0 %: *E. mouretti/E. moschatum* and *E. touchyanum*
Intraspecific level: between populations of the same species	6·18 %: *California macrophylla* 0·674 %: *E. gruinum*; 0·497 %: *E. touchyanum*; 0·162 %: *E. iconium*, *E. cygnorum*, *E. laciniatum and E. cazorlanum* 0 %: *E. arborescens*, *E. hoeftianum*, *E. alpinum*, *E. rupestre*, *E. antariense*, *E. reichardii*, *E. sanguis-christi*, *E. recoderii*, *E. rupicola*, *E. sebaceum*, *E. moschatum*, *E. tordylioides*

permitting only 10 trees per replicate to be held). Phylogenetic reconstructions using distance-based reconstructions were also explored in PAUP* using the neighbor-joining method. Genetic distances obtained from this analysis were used to study nrITS interspecific variability (Table 1).

Bayesian approaches were conducted using MrBayes 3·0b4 (Ronquist and Huelsenbeck, 2003), sampling for two million generations with four Markov Chain Monte Carlo (MCMC) chains (chain temperature 0·2; sample frequency 100). Combined analysis (nrITS and *trnL-F*) was partitioned, and different substitution models were applied and each partition had its own parameters. As such nrITS was coded with the $GTR + I + \Gamma$ model and *trnL-F* with the $GTR + \Gamma$ model. For all analyses burn-in was discarded. Posterior probabilities were examined to avoid sampling prior to convergence and mixing, and finally a majority-rule tree was reconstructed after discarding 1×10^5 generations.

For the biogeographical study, nine areas of endemism were defined, each having at least one endemic taxon present (Fig. 1). Lagrange v. 2·0·1 (Ree *et al.*, 2005; Ree and Smith, 2008) and DIVA (Ronquist, 1996) were used to estimate ancestral areas and dispersals along the evolution of *Erodium.* Both analyses were performed in one of the posterior probability trees from the Bayesian analysis of the combined matrix. The cosmopolitan outgroup taxon *Geranium* was removed from the analyses. The human-mediated recent colonizations of the New World and Australia by *E. botrys*, *E. brachycarpum*, *E. cicutarium* and *E. moschatum* were not taken into account in Lagrange analysis. Including them in the analysis resulted invariably in a 'convergence error'. All possible area combinations with a maximum of six simultaneous areas were permitted except for those containing non-adjacent areas and for intercontinental ranges of more than two areas. Four 'continents' or main regions weakly connected between them are considered here: North America, South America, Australia and Africa–Eurasia. The restrictions applied are justified given the distributions of extant taxa. The biogeographical model used was constant through time. Dispersals between all neighbouring areas were permitted bidirectionally, but they were given three different levels of probability (see Table S1 in Supplementary Data, available online).

Divergence times were reconstructed for nrITS (58 *Erodium* species plus *California*) using BEAST v. 1·4·7 (Drummond and Rambaut, 2007) under the uncorrelated lognormal dating method and the $GTR + I + \Gamma$ substitution model. Assignment of fossils to nodes is a significant problem in phylogenetic tree calibration, but the use of multiple fossils may reduce the effect of incorrect placements (Rutschmann *et al.*, 2007). The tree was constrained at the divergence between *Erodium* and *California* using a normal prior distribution [20·34 million years ago (MYA); after Fiz *et al.*, 2008] and a uniform prior distribution to constrain the dating to the only fossil available (8 MYA on Clade II; Fiz *et al.*, 2008). Ten million generations, with sampling every 1000th generation, were simulated and a tree was reconstructed after examination of all parameters, discarding the generations before MCMC-chain convergence. This analysis was repeated using extra outgroups [four from Geraniaceae and two from other families of Geraniales *sensu* APG III (2009)] to check for any distortion of the reconstructed divergence times. An additional calibration point was included with a normal prior distribution (42 MYA mean, four standard deviations) for the Geraniaceae crown node (Fiz *et al.*, 2008). Inclusion of more distantly related species was not possible as the high rate of mutation in Geraniales made homology assessment for the alignment unclear. All temporal reconstructions were compared with dates retrieved from the *rbcL* marker for all main nodes in Geraniaceae (Fiz *et al.*, 2008).

Three approaches were implemented for the diversification analysis. For the first, diversification rate shifts were examined, within the same tree as used in the analysis of biogeography, using a topological method. The likelihood-based diversification rate shift statistics, Δ_1 (Moore *et al.* 2004) and the Slowinski–Guyer statistic (Slowinski and Guyer, 1993) as implemented in SymmeTREE (Chan and Moore, 2005) were calculated. One hundred thousand trees equal in length to the study tree were generated by Monte Carlo simulation in order to estimate the null distribution of the test statistic, Δ_1. For the second, a temporal method (LASER; Rabosky, 2006) was used to test constancy of the overall rate of diversification. Since this model was rejected, to find out which rate-variable model fitted best to the data, between two (one rate change) and five rate parameters (four rate changes) were incorporated, enabling the detection of temporal increases in diversification rates. For the third, LASER was used to identify the position on the tree at which the diversification rate shift had the highest likelihood. All 74 species of *Erodium* were incorporated into the tree by assigning the number of species to each clade on the nrITS chronogram (with only *California* as an outgroup) (following Fiz *et al.*, 2006). When rate constancy was rejected, two values for the extinction rate (0 and 0·9) were taken into account.

RESULTS

Phylogenetic analysis

The length of the nrITS1 is 228 bp in *California macrophylla* and 208–231 bp in *Erodium.* The length of nrITS2 is 232–233 bp in *C. macrophylla* and 232–235 in *Erodium*. The numbers of variable and potentially parsimony informative sites in the nrITS matrix are 251/193 [687 steps; consistency index (CI) 0·51; retention index (RI) 0·86] and 209/276 (903 steps; CI 0·62; RI 0·86) in the combined matrix.

All phylogenetic analyses confirm the monophyly of Erodium. *California macrophylla* has lower genetic distance to *Erodium* (16·68 %) than to *Geranium* (25·81 %) (Table 1). BI and MP reconstructions using the nrITS region revealed two main clades (I and II; Figs 2 and 3). Clade I [73 % BS (bootstrap), 0·94 pp (posterior probability)] includes subgenus *Erodium* and subgenus *Barbata* subsection *Absinthioidea*. Clade II (59 % BS, 1·0 pp) comprises the remainder of subgenus *Barbata* and can be divided into a further two subclades: one comprising only Australian species (100 % BS, 1·00 pp) and the second containing the rest of the predominantly Mediterranean species of subgenus *Barbata* (50 % BS, 0·99 pp). Within the core subgenus *Barbata*, two cosmopolitan species (*E. brachycarpum* and *E. botrys*) are sister to the remainder; the rest of the core subgenus *Barbata* (81 % BS, 1·00 pp) is represented by several nested clades: section *Malacoidea* and subsection *Petraea* are successively sister to section *Cicutaria*. Topological resolution and support are congruent between MP and BI analyses of the nrITS sequences (Fig. 2). The incongruence length difference test shows significant incongruence between ITS1 vs. *trnL-F* ($P = 0{\cdot}01$) and ITS2 vs. *trnL-F* ($P = 0{\cdot}019$). In contrast, congruence between ITS1 and ITS2 is high ($P = 0{\cdot}92$).

In the combined tree (nrITS and *trnL-F*; Fig. 3) the species of subgenus *Erodium* are monophyletic, whereas in the nrITS reconstruction they are split between two clades (Fig. 2). Furthermore, in the nrITS reconstruction both clades form a polytomy with the clade containing *E. stephanianum* and subsection *Absinthioidea* (Fig. 2), whereas in the combined tree it appears as monophyletic with the *E. stephanianum* clade as sister group. There is a conflicting signal given by the two molecular datasets in clade 2 (Fig. 3). In the combined tree *E. hoeftianum* is highlighted as the first branching lineage, whereas in the nrITS reconstruction *E. ciconium* is placed as sister to other taxa in subsection Absinthioidea with poor statistical support. On the other hand, within subgenus *Barbata* (clade II) relationships among the *E. cygnorum* group, *E. botrys* group, subsection *Petraea*, section *Malacoidea* and section *Cicutaria* are completely congruent between nrITS and combined trees. Incongruences inside section *Malacoidea* include: (*a*) in the combined tree, *E. reichardii* and *E. boissieri* are sister to the core group, whereas in the nrITS tree they form a polytomy and (*b*) in the combined tree the *E. moschatum* clade is sister to the rest of section *Cicutaria* whereas in nrITS it is not. However, as both alternatives within section *Cicutaria* have low statistical support the phylogenetic relationship of these clades remains unclear.

The greatest nrITS sequence divergence between species was in the comparison of *E. laciniatum* 1 and *E. crassifolium* (20·96 %, GTR + Γ model). On the other hand, a total of 21 species pairs showed no divergence whatsoever. Intraspecific genetic distances range from 0·674 % in *E. gruinum* to 0 % within 12 other species (Table 1).

Biogeography and diversification analysis in Erodium

Both Lagrange and DIVA reconstruction methods broadly agree in assigning ancestral areas. As reconstructed by Lagrange, the estimated origin of *Erodium* is in Asia or North America; subsequent diversification extended the genus into the rest of Asia, the Mediterranean Basin and Africa. The DIVA analysis resulted in one optimal reconstruction requiring 79 dispersals and a combination of multiple areas is retrieved from DIVA (results not shown) for the origin of *Erodium*. Subgenus *Erodium* is assigned to North Africa while its sister subsection *Absinthioidea* is assigned to the eastern Mediterranean (plus Asia and Asia Minor with DIVA). The ancestral area of both clades is Asia (plus Asia Minor with DIVA). The ancestor of clade II is assigned to Asia or a combination of areas (Fig. 3) or to the Iberian Peninsula, Australia and South America when using DIVA. Within this clade, the ancestral area for sections *Malacoidea* and *Cicutaria* and subsection *Petraea* is the Iberian Peninsula. The ancestor of each individual clade stems from the Iberian Peninsula, but section *Cicutaria* is also assigned to six combined areas (Lagrange; Fig. 3) or to North Africa (DIVA). Biogeographical analysis suggests four long-distance dispersal events: two groups are endemic to Australia and two species are endemic to America (Fig. 3). The Australian group (clade 3) and a further Australian species nested within the body of sect *Malacoidea* (*E. aureum*) provide the ambiguity in the biogeographical origin of Clade II in the DIVA analysis. Moreover, the distant evolutionary relationship of *E. geoides* (South America) and *E. texanum* (North America) indicates two independent colonizations of the American continents.

The reconstructed divergence times of the major clades when using the nrITS marker are broadly congruent with those reported using the plastid *rbcL* (Fig. S1 in Supplementary Data, available online). Confidence intervals are broader when six outgroups are included (see Fig. S1 in Supplementary data). The use of *California* as a sole outgroup retrieved ages more congruent with those derived from *rbcL* for the crown age of the genus *Erodium* (18·34 MYA), the crown age of Clade II (14·81 MYA) and stem age of the core of Clade II (11·73 MYA) (chronogram in Supplementary Fig. S1). On the other hand, the age retrieved for clade I (13·68 MYA) was more similar when six outgroups were used, as was the crown age of section *Absinthioidea* (4·4 vs. 3 MYA). However, if *E. hoeftianum* is not taken into account, the age of this clade is similar among the three reconstructions (approx. 3 MYA). The use of six outgroups caused alignment ambiguities (an increase of 37 nucleotide positions), a result of the large genetic distance of *Erodium* from the majority of the outgroups included. This factor, coupled with the distortion of the crown and stem ages of *Erodium* from those expected from analyses of *rbcL* (Fig. S1 in Supplementary data), justify the rejection of reconstructed ages of the six-outgroup analysis.

Although no significant diversification rate shifts were detected with SymmeTREE, two marginally significant cases

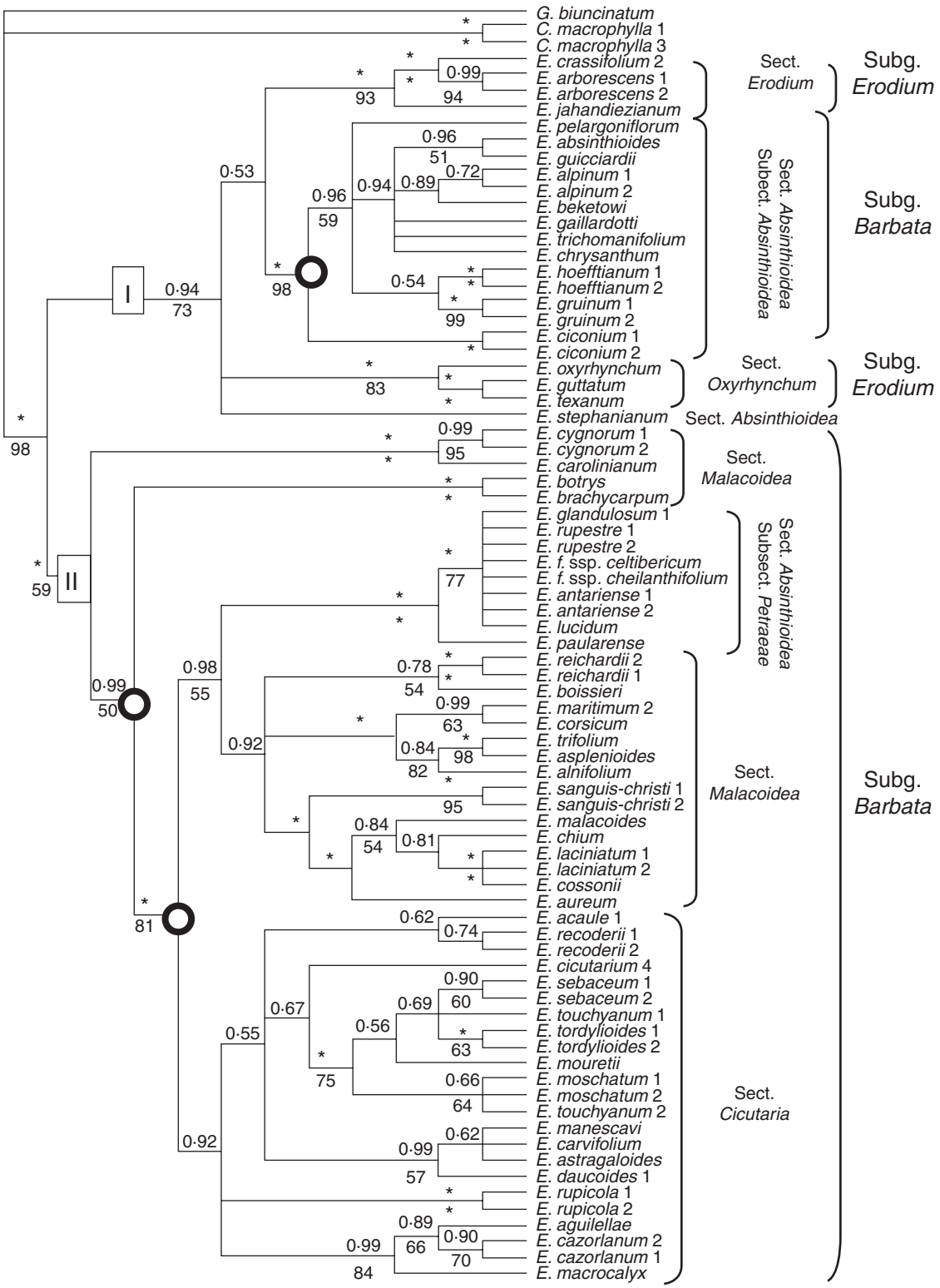

FIG. 2. Majority rule consensus tree obtained using Bayesian analysis of all ITS sequences available in this study. Posterior probabilities (pp) are given as values above branches. Bootstrap support (BS) values >50 % are below the branches. An asterisk denotes 1·0 pp or 100 BS. Diversification rate shifts (obtained from SymmeTREE and LASER) are indicated by circles. Clade names (I and II) according to Fiz *et al.* (2006) are highlighted in squares and taxonomic information according to Guittonneau (1990) is indicated on the right.

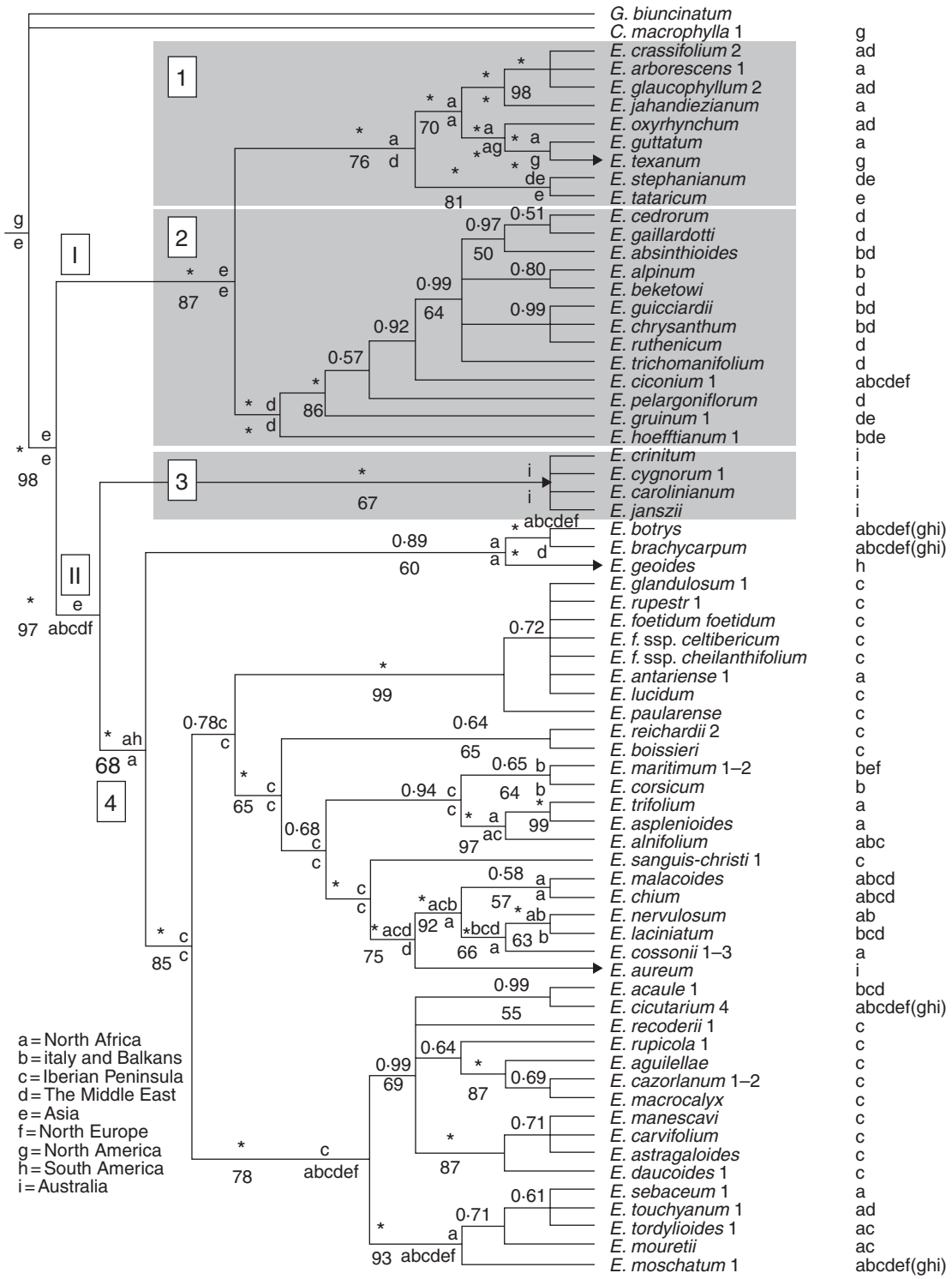

FIG. 3. Combined majority rule consensus tree (nrITS and *trnL-trnF* sequences) obtained from Bayesian analysis using one sequence per taxa. Values above branches are posterior probabilities (pp) and those below correspond to bootstrap (BS) supports >50 %. An asterisk denotes 1·0 pp or 100 % BS. Ancestral ranges recovered with the highest probability with Lagrange are shown at internal nodes (letters following those in Fig. 1). No letter indicates invariable estimated range for the remaining nodes of that lineage. Arrows highlight cases of intercontinental dispersals (see Fig. 1). Current ranges for each taxon are indicated on the right. For these reconstructions, it was assumed that cosmopolitan taxa have been recently introduced to the New World and Australia. Clade names (squares I and II, and 1–4) are also indicated.

were identified (Fig. 2). The first shift ($P = 0{\cdot}11$) occurs in subsection *Absinthioidea* after the divergence of *E. ciconium* and is significant when using and Slowinski–Guyer statistic ($P = 0{\cdot}04$). The second ($P = 0{\cdot}08$) occurs after the divergence of the group containing *E. botrys*, along the branch subtending the core of subgenus *Barbata* (Fig. 2). All other branches show balance in the diversification rate ($P = 1$).

The likelihood ratio test (LASER) for the 74 species tree, based on the nrITS chronogram (with *California* as the sole outgroup), also supported diversification rate heterogeneity when extinction was zero ($P = 0{\cdot}005$) but not when extinction was 0·9 ($P = 0{\cdot}16$). This analysis highlighted a diversification rate shift at the crown node of core subgenus *Barbata* in agreement with the second diversification rate shift detected using SymmeTREE (Fig. 2). Overall rate constancy was rejected (delta AICrc = 2·78); a three-rate variable model (Yule-3-rate) fitted the data most effectively. This analysis detected two inflection points at 0·069 and 3·09 MYA, indicating two increases in diversification rates (Fig. 4). An inflection point at 3·18 MYA (Yule-2-rate) was also detected from the six-outgroup chronogram (Fig. 4).

DISCUSSION

This study confirms that *Erodium* is a natural group as previously demonstrated using plastid DNA regions and morphology (Fiz *et al.*, 2006, 2008). A consistent structure is obtained across all phylogenetic analyses of *Erodium*, with two main clades (I and II) present in all analyses (*trnL-F* and *rbcL*; Fiz *et al.*, 2006, 2008). In a similar fashion, subclades 2, 3 and 4 are always recovered in phylogenetic analyses of nuclear and plastid markers. Subgenus *Erodium* (clade 1) is paraphyletic in the analyses of the nuclear marker (nrITS), but is a monophyletic group in the *trnL-F* (Fiz *et al.*, 2006) and combined analyses. This is probably an indication of reticulate evolution, potentially due to hybridization between members of section *Erodium* and subsection *Absinthioidea*. Section *Absinthioidea* (*sensu* Guittonneau) is split into three separate groups, and the paraphyly of section *Malacoidea* is also confirmed. Although there are different phylogenetic incongruences between markers at the species level (e.g. *E. cicutarium*), none of them is strongly supported.

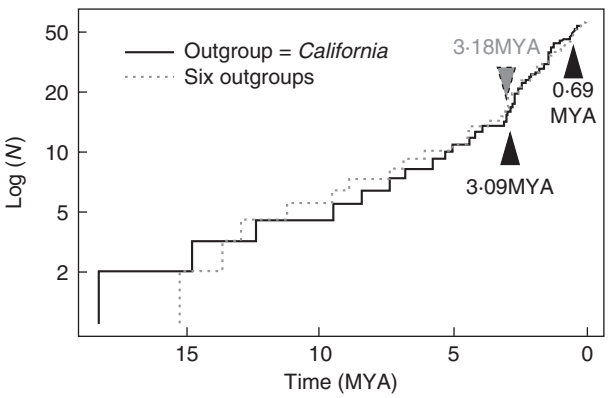

FIG. 4. Lineage-through-time plot for nrITS chronogram using *California* as the outgroup and using six outgroups, as indicated. The diversification rate shifts located by LASER are indicated as triangles.

In these cases incomplete lineage sorting and plastid capture may also explain these differences. Hence, the current and previous studies (*trnL-F* and *rbcL*; Fiz *et al.*, 2006, 2008) agree that a revision of the subgeneric and sectional circumscription of *Erodium* species is required.

Although special care must be taken with the nrITS marker when dealing with branch length in angiosperms (especially at low scales, Soria-Hernanz *et al.*, 2008; see also Bromham *et al.*, 2000), a signal was recovered that is broadly congruent with that gained from plastid regions in *Erodium* (Fiz *et al.*, 2008). The reconstruction of divergence times places the origin and diversification of *Erodium* in the early Miocene. The extension of steppes at the end of the Tertiary (Kers, 1968; Venter, 1983) has been linked to the evolution of *California*, *Erodium* and *Geranium* (Geraniaceae; Fiz *et al.*, 2008). As the American genus *California* is sister to *Erodium*, an origin in Asia spreading through to North Africa seems more probable [as for instance in Zygophyllaceae (Beier *et al.*, 2004) and Plantaginaceae (Meyers and Liston, 2008)]. One explanation for the disjunction between North America (*California*) and Asia (*Erodium*) is the breaking of the Beringian land bridge approx 20 MYA.

Multiple intercontinental colonizations

The phylogenetic evidence presented here highlights four incidences in which sister species/clades possess allopatric distributions on separate continents. Furthermore, biogeographical analyses using two reconstruction methods support the hypothesis that these four colonizations constitute long-range, intercontinental dispersal events. *Erodium texanum* stems from a predominantly Mediterranean lineage originating in Africa. Its presence in North America constitutes a convincing example of long-distance dispersal. The only endemic South American species, *E. geoides*, is sister to the clade formed by the cosmopolitan species *E. botrys* and *E. brachycarpum*. This clade also has its origins in Africa, supporting at least one long-distance dispersal between the continents. Two separate colonizations of Australia have occurred. The ancestor of *E. aureum* dispersed relatively recently from the Mediterranean Basin and Middle East (ACD), whereas the *E. cygnorum* clade represents a more ancient dispersal event, most likely from Asia.

Although *E. geoides* arrived in South America as long ago as 12 MYA (Fiz *et al.*, 2008 and the present study) when the Beringian land bridge and North Atlantic land bridge were in existence, a dispersal event across the Atlantic should not be ruled out as its origin appears to be in Africa. However, the alternative explanation that the ancestor of the clade was a more widespread species that evolved in Africa, spread to the New World via land bridges and diversified into the three modern species has some merit, as previously suggested for subfamily *Betoideae* [Chenopodiaceae (= Amaranthaceae *sensu* APG III, 2009); Hohmann *et al.*, 2006]. In contrast, the derived position of *E. texanum*, nested within a Mediterranean lineage, implies a recent (0·6 and 0·9 MYA from nrITS reconstructions) dispersal of *Erodium* making the North Atlantic land bridge migratory route impossible and strongly implicating a translocation across the Atlantic

from the Mediterranean Basin. Therefore, this is confirmed as another case of long-distance dispersal between Mediterranean–North Africa and western North America for taxa adapted to semi-arid habitats, as reported in other genera, e.g. *Senecio* (Asteraceae; Coleman *et al.*, 2003), *Oligomeris* (Resedaceae; Martin-Bravo *et al.*, 2009) and *Plantago* (Plantaginaceae; Meyers and Liston, 2008).

The most striking cases presented are the colonization of Australia by *E. aureum* and the *E. cygnorum* group. Human introduction of these species potentially explains these distributions, but the divergence of *E. aureum* towards the end of the Pliocene (from nrITS reconstructions) and that of *E. cygnorum* towards the end of the relatively warm Miocene (Fiz *et al.*, 2008, and the present study) pre-date human evolution, ruling out an anthropogenic introduction to Australia. This leaves long-distance dispersal as the most plausible explanation, especially as this type of colonization between the northern Hemisphere and Australia has been described previously [*Ceratocephala* (Garnock-Jones, 1984) and *Scleranthus* (Smissen 2003; Besnard *et al.*, 2009)]. *Erodium aureum* is closely related to a number of species with broad Mediterranean distributions indicating a propensity for dispersal, also seen in the relatives of *E. geoides.* Additionally, the occurrence of seeds of *E. cygnorum* in the stomach of an Australian babbler (Ridley, 1930) also suggests a potential for bird-mediated long-distance dispersal in this clade.

Although the Mediterranean Basin harbours the largest diversity of *Erodium* (Fiz *et al.*, 2006), many species are widespread, and four species have cosmopolitan distributions. Introductions due to human migration, such as that reported for *E. cicutarium* to California by Spanish missionaries in 1769 (Mensing and Byrne, 2003), may explain some of these broad distributions. Different features may also explain the vast distribution of the genus *Erodium*. In Israel, Zeide (1976) observed long-distance wind dispersal of mericarps of a desert species of subgenus *Erodium* (*E. crassifolium*). Most species in subgenus *Erodium* have plumose fruits (Fiz *et al.*, 2006), which may have facilitated the dispersal of this early lineage through anemochory. According to Stamp (1989) and Van Rheede and Van Rooyen (1999), *Erodium* species exhibit a combination of ballistic primary dispersal followed by hygroscopic secondary dispersal called trypanospermy. These authors suggested that the distances covered by this type of dispersal are short, and plant-colonization success depends on hygroscopic burial of mericarps. Also, Stamp (1989) stated that *Erodium* diaspores never dispersed >3 m in experimental conditions. It is difficult to reconcile this dispersal pace with a colonization of America or Australia from the Mediterranean Basin. Alternatively, Shmida and Ellner (1983) found a large number of *E. malacoides* diaspores attached to sheep wool in the Mediterranean chaparral of Israel. Thus phenomena such as epizoochory and endozoochory (*E. cygnorum*, see above) may operate in *Erodium* and potentially explain its present worldwide distribution.

Two periods of Mediterranean radiations in Erodium

The present study revealed two periods of significant increases in diversification rate within *Erodium*. As diversification in lineages occupying semi-arid regions outside the Mediterranean is minimal, the causes of these shifts in diversification are certainly linked to factors only affecting the lineages of the Mediterranean Basin. The most recent radiation was detected at approx. 0·69 MYA. This is coincident with the onset of the largest Mediterranean glaciation periods (0·65 MYA; Médail and Diadema, 2009), possibly indicating a role of isolation in glacial refugia as a major promoter of speciation and diversification in *Erodium*. Médail and Diadema (2009) identified 52 glacial refugia scattered throughout the Mediterranean Basin, 33 of which are located in montane or submontane regions. These data might explain the high level of species diversity and endemism for *Erodium* in the Mediterranean Basin. This is particularly evident in montane areas; different species of subgenus *Barbata* (e.g. *E. boissieri* and *E. trifolium*) are endemic to mountains from Iberian Peninsula and Morocco. The core of subgenus *Barbata* harbours over 70 % of the species in *Erodium* and it is mainly distributed in the western Mediterranean, the Iberian Peninsula and western North Africa, which were connected several times during the Quaternary (e.g. the Messinian Salinity Crisis, 5 MYA; Bocquet *et al.*, 1978).

The temporal analyses indicate that the second, more ancient shift occurs specifically in two lineages, the core of subgenus *Barbata* (section *Cicutaria*, section *Malacoidea* and subsection *Petraea*) and subsection *Absinthioidea*, in the last approx. 3 MYA. These groups diversified over the last 1–6 MYA (nrITS and *rbcL*), a period when seasonal drought was already present. Definitive stabilization of the summer drought in the Mediterranean Basin was at approx. 2·8 MYA (Suc, 1984). The data presented here could be interpreted as evidence of ancient divergence associated with adaptation to the onset of seasonal drought (see also Fiz *et al.*, 2006). The Mediterranean Basin contains the highest percentage of annual species (31–54 %) among the five Mediterranean climates (Fiz *et al.*, 2002), and this strategy is common in *Erodium*. Over 32 % of *Erodium* species are autogamic annuals (Fiz *et al.*, 2008). Long-lasting disturbance regimes are associated with annuality in Mediterranean floras (Shmida, 1981; Pons and Quezel, 1985; Herrera, 1991). *Erodium* has the greatest number of species growing in disturbed habitats in Geraniaceae (Fiz *et al.*, 2006). Selfing dominates in the 22 annual *Erodium* species, which are widely distributed, suggesting a possible correlation with the ability to colonize new areas (see Fiz *et al.*, 2006). The colonization and radiation of *Erodium* in the Mediterranean could also have been facilitated by acquisition of a new set of pollinators (Fiz *et al.*, 2008). These authors identified an association between a shift to generalist pollinators and higher reproductive flexibility with the colonization of disturbed habitats in the Mediterranean Basin (Fiz *et al.*, 2008). This evidence provides some insight into how this radiation may have been an adaptive response to regular drought in the Mediterranean Basin.

Subsection *Absinthioidea* arose from an Asian ancestor, and the earliest diverging species (*E. hoeftianum* and *E. ciconium*) inhabit disturbed sites from the east Mediterranean Basin to central Asia. In general, species of subsection *Absinthioidea* inhabit mountain crevices, shrublands and meadows from Turkey, Caucasus and Greece, with distributions matching the SW Asia Tertiary refugia (Tiffney and Manchester, 2001).

This link to Tertiary flora refugia is also supported by the divergence from its sister group at the end of the Miocene warm period. However, this radiation may have happened after diversification of the core group approx. 3 MYA (nrITS and *rbcL*) in agreement with diversification rate shift, and so it is unclear whether it is a tertiary lineage or it colonized these Tertiary refugia as a response to the start of Mediterranean climate. The two main features of this subsection are that dioecy is common (>70 % of the group) and that many species are endangered. It is common for clades of angiosperms to show low levels of dioecy compared with their sister taxa (Heilbuth, 2000; Vamosi and Vamosi, 2005). However, high proportions of dioecy have been found in rapid radiations of the flora of Hawaii (Sakai *et al.*, 1995) and of the dioecious group of *Momordica* (Cucurbitaceae; Schaefer and Renner, 2010). Furthermore, the dioecious group within *Gaertnera* (Rubiaceae) has been reported to have the highest rate of diversification and of nucleotide substitution (Malcomber, 2002). A striking feature of subsection *Absinthioidea* is that it has the highest rate of nucleotide substitution, not only within *Erodium* (Fiz, 2005; Guisinger *et al.*, 2008), but also within Geraniaceae (Guisinger *et al.*, 2008). This is particularly remarkable as Geraniaceae has one of the fastest substitution rates for organellar genomes within the angiosperms (Parkinson *et al.*, 2005; Bakker *et al.*, 2006; Guisinger *et al.*, 2008). A close relationship between rates of nucleotide substitution, rates of diversification and dioecy can be inferred for *Erodium* subsection *Absinthioidea*. Dioecy may provide an escape from the negative effects of inbreeding (Baker and Cox, 1984; Freeman *et al.*, 1997), a useful strategy in isolated refugia.

Conclusions

The evidence presented here clearly demonstrates four cases for which the current distribution of sister species or clades can only be explained by intercontinental dispersal events between regions with Mediterranean-type climates.

Although *Erodium* has fewer species than most other Geraniaceae (e.g. *Geranium* or *Pelargonium*), it can be seen that it has undergone significant shifts in diversification that are probably adaptive responses to radical changes in climate. Acquisition of some traits, such as selfing, annuality and fruit-dispersal structures, may have been crucial key innovations for the successful colonization of arid steppes and dry habitats, whereas dioecy may have allowed colonization of mountane crevices in subsection *Absinthioidea*. These adaptive responses may have allowed the survival and diversification of the genus during the onset of Mediterranean drought regimes and the Quaternary and Tertiary glaciation periods. Confirmation of correlations between the particular traits and the environments suggested here, and reconstructions of trait variation on the phylogenetic trees could potentially reveal that the rapidly radiated sections within *Erodium* have, in fact, undergone adaptive radiations.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following files. Table S1: Matrix of weights of dispersal events between areas used for Lagrange analyses. Fig. S1: Divergence times and 95 % HPD retrieved from BEAST on the ITS tree.

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APPENDIX

nrITS accessions provided for individuals used in the present work, including their locality, voucher and herbarium and GenBank accession numbers. trnL-F sequences are taken from a previous work (Fiz et al., *2006).*

Taxa	Geographical origin	Voucher sample	ITS Genbank accession no(s)	trnL-F Genbank accession no(s)
California				
macrophyllum 1	USA, California, Riverside Co., Murrieta Region: Skink Hollow, Santa Gertrudis Creek Drainage	J. Easton s.n. (MA)	EF185337	DQ072015
macrophyllum 3	USA, California, Riverside Co., Temescal Valley, 0·9 miles SE of Indian Truck Trail and 30 m south of De Palma Ra.	I. Gillespie 10 (MA)	EF185338	–
Erodium				
absinthioides	Turkey, Bursa, Uludag	G. Nieto Feliner 1580 (MA-393124)	EF185348	DQ072034
acaule 1	Italy, Sicily, Palermo, La Pizzuta, Portella della Paglia	C. Aedo & al. 5677 (MA-646287)	EF185392	DQ072089
aguilellae	Cultivated in MA, from seeds collected in Castellón, Onda, Sitjar	J. Aldasoro 2826 (MA)	EF185401	DQ072090
alnifolium	Tunisia, Nefta, 5 km to Segename	J. Aldasoro 2865 (MA)	EF185391	DQ072064
alpinum 1	Italy, Abruzzo, pendici del Mt Rosa Pinnola, Bisegna, L'Aquila	F. Conti 1656 (MA)	EF185352	DQ072029
alpinum 2	Italy	C. aedo CA8129 (MA)	EF185351	–
antariense 1	Morocco, Alto Atlas, Tizi-n-Aït-Hamed	J. Güemes 1549 (MA)	EF185373	DQ072078
antariense 2	Morocco	Staudinger 4800	EF185374	–
arborescens 1	Tunisia, Skhira	J. Aldasoro 3053 (MA)	EF185340	DQ072018
arborescens 2	Cultivated in MA, from seeds collected in Israel, Nahal Yarqon	J. Aldasoro 3488 (MA)	EF185341	–
asplenioides	Tunisia	A2935(1)	EF185390	DQ072065
astragaloides	Spain, Granada, Dilar, Trevenque, Los Alayos	C. Navarro & al. 2246 (MA-625117)	EF185400	DQ072091
aureum	Australia, Coolgardie, Eyre Higway, 59 km W of Madura	B. Archer 15 (MEL-2039223)	EF185385	DQ072066
beketowi	Ukraine, Biespars, Stavropol	Smababanova s.n. (LE)	EF185358	DQ072030
boissieri	Spain, Granada, La Zubia, Cortijo de la Cortichuela, Trevenque	M. Velayos & Navarro 9676 (MA-644606)	EF185378	DQ072054
botrys	USA, California, San Francisco, Mt Tamalpais	S. Castroviejo & al. 14575 (MA-590950)	EF185365	DQ072049
brachycarpum	Spain, Madrid, Rozas de Puerto Real	N. López 499 (MA)	EF185366	DQ072050
carolinianum	Australia, Olympic Dam Mine, Gairdner-Torrens	F.J. Badman 3597 (MA-592447)	EF185364	DQ072046
carvifolium 2	Spain, La Rioja, Montenegro de Cameros, N Puerto de Santa Inés	P. Vargas 230PV99 (MA)	EF185399	DQ072094
cazorlanum 1	Spain, Jaen, Sierra de Cazorla, Cortijo de la Cabrilla	C. Navarro & Benavente 3025 (MA-628379)	EF185404	DQ072097
cazorlanum 2	Spain, Jaen	MA580102	EF185403	–
cedrorum	Cultivated in MA from seeds collected in Bolkar Daglari, Nigde, Turkey	J. Aldasoro 3489 (MA)	–	DQ072031
chium	Spain, Cádiz, Monte Tavirana, Ronda	C. Navarro 3450 MA	EF185384	DQ072067
chrysanthum	Greece, Peloponeso, Killíni, N a NE-Siete des Gipfelmassiv	E. Hörandl & F. Hadacek 7612 (W)	EF185361	DQ072032
ciconium 1	Italy, Abruzzo, L'Aquila, pr. Santo Stéfano de Sessanio	C. Aedo & al. 8108 (MA)	EF185354	DQ072039
ciconium 2	Greece, Fthiotis, Othrys, Dhivri, 1991	Willing14594	EF185355	–
cicutarium 4	California	CA4439	EF185393	–
corsicum 3	Italy, Sardinia, Santa Teresa de Gallura, Capo Testa	C. Aedo & al. 9120 (MA-702081)	EF185380	DQ072061
cossonii 1	Morocco, Haut Atlas, Tiz-n- Test	J. Fernández Casas & al. 3277 (MA-252363)	–	DQ072073
cossonii 3	Morocco	Staudinger2852		EF185388
crassifolium 2	Tunisia, Coutinedes, cerca de Gabes	Aldasoro 3069 (MA)	EF185339	DQ072020
cygnorum 1	Cultivated in MA from seeds collected in Great Victoria Desert, Camp	Aldasoro 2842 (MA)	EF185362	DQ072044
cygnorum 2	Cultivated in MA	MEL1580207	EF185363	–
daucoides 1	Spain, Palencia, Velilla del río Carrión, Peña Cueto	C. Navarro & al. 1602 (MA-559982)	EF185402	DQ072096
foetidum subsp. *celtibericum*	Spain, Tarragona, ports de Beseit, ĹEngrillo	LL. Sáez s.n. (MA)	EF185375	DQ072081

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APPENDIX *Continued*

Taxa	Geographical origin	Voucher sample	ITS Genbank accession no(s)	trnL-F Genbank accession no(s)
foetidum subsp. *cheilanthifolium*	Spain, Granada, Sierra de Arana, Cueva del Agua	P. Vargas 100PV00 (MA)	EF185372	DQ072080
foetidum subsp. *foetidum*	Spain, Gerona, Cabo Norfeu, Rosas, Gerona	C. Aedo *et al.* 4920 (MA)	–	DQ072079
gaillardotti	Turkey, Malatya, Darende, 27 km de Gürün to Darende	F. Muñoz-Garmendia & al. 4567 (MA)	EF185353	DQ072035
geoides	Chile, Coquimbo, Choapa province, 1 km N of the border of Petarca province	Taylor 10620 (MO)	–	DQ072048
glandulosum 1	Spain, Leon, Puente de la Palanca	C. Aedo & Patallo 4451 (MA-621226)	EF185367	DQ072082
glaucophyllum 2	Spain, Barcelona, Montcau, St Llorenç de Munt	LL. Saéz 5001 (MA)	–	DQ072083
gruinum 1	Jordania, Gerassa (Jerash)	P. Vargas (MA)	EF185359	DQ072037
gruinum 2	Cultivated from Iran	Davis56574	EF185360	–
guicciardii	Cultivated in MA from seeds collected in Ohrid, Macedonia,	Aldasoro 2842	EF185356	DQ072036
guttatum 1	Tunisia, Feriana	J. Aldasoro 2973 (MA)	EF185344	DQ072024
hoefftianum 1	Turkey, Göreme, Ask Vadisi, dept. Nevsehir	F. Muñoz-Garmendia & al. 4626 (MA)	EF185349	DQ072033
hoefftianum 2	Iran	J. Aldasoro 10000 (MA)	EF185350	–
jahandiezianum	Morocco, Anti-Atlas, Igherm	F. Gómiz s.n. (BC).	EF185342	DQ072022
janszii	Australia, Far Western Plains, near Mt Robe, 35 km of Broken Hill	M.G. Corrick 7271 (MEL-592017)	–	DQ072047
laciniatum 1	Letur, Albacete	I. Alvarez 1239 (MA-591697)	EF185387	DQ072071
laciniatum 2	Palestine, Petra, Jordania	P.Vargas (MA)	EF185386	–
lucidum	Cultivated in MA, from seeds taken in Huesca, Aneto	J. Aldasoro 2821(MA)	EF185370	DQ072084
macrocalyx	Spain, Cuenca, Tragacete	C. Navarro 2469 (MA)	EF185405	DQ072087
malacoides 2	Australia, Volcanic plain, SE Organ Pipes, S side of Jacksons Creek	V. Stajsic 852 (MEL-2020988)	EF185383	DQ072070
manescavi	Cultivated in MA, from seeds taken in Valle de Ossau, France	J. Aldasoro 2829 (MA)	EF185398	DQ072098
maritimum 1	Cultivated in MA, from seeds taken in Devon, Great Britain	J. Aldasoro 905 (MA-614528)	–	DQ072057
maritimum 2	Spain, A Coruña, San Andres de Teixido	MAL129(2)	EF185379	–
moschatum 1	Australia, Southern Lofty, Angas River, Strathalbyn	N.M. Smith 2393 (MEL-1621233)	EF185409	DQ072086
moschatum 2	Morocco, Chefchaouen, Campsite	O. Fiz 152of00	EF185408	–
mouretii	Spain, Alange, Castillo	M.A. Moreno 9 (MA-643352)	EF185414	DQ072099
nervulosum	Morocco, Ifrane to Inmouzer	Mateos and Montserrat 6038 (BC-826634)	–	DQ072072
oxyrhynchum	Cultivated in MA from seeds collected in Egypt, Cairo-Suez Desert Road	J. Aldasoro 3487 (MA)	EF185343	DQ072023
paularense	Spain, Guadalajara, Cañamares, Atienza	C. Aedo 4097 (MA-588866)	EF185371	DQ072077
pelargoniflorum	Cultivated in MA from seeds collected in SE Turkey.	J. Aldasoro 2838 (MA)	EF185347	DQ072041
recoderii 1	Spain, Málaga, Monte Tavirana, Ronda	C. Navarro 3449 (MA-685241)	EF185394	DQ072104
recoderii 2	Spain, Cádiz, Pto Palomas	JR4, 4-5-2000	EF185395	–
reichardii 1	Spain, Baleares Islands, Palma de Mallorca, Lluc, collado de Massanella	R. Morales *et al.*1831 (MA-618180)	EF185377	–
reichardii 2	Cultivated in MA from seeds collected in Menorca, Cabo Favaritx	J. Martinez 173JM03	EF185376	DQ072063
rupestre 1	Spain, Lérida, Pallars Jussa, Trem, Serra de Gurp, Roques de Codó	C. Aedo & Pedrol 4782 (MA)	EF185368	DQ072085
rupestre 2	Barcelona, Montserrat	Ll. Saenz 17-III-2002	EF185369	–
rupicola 1	Spain, Granada, Guejar Sierra, Barranco del Guarón, 30SVG4106469	M. Ruiz & S. Vidal (GDA-41392)	EF185397	DQ072105
rupicola 2	Spain, Almeria	GDAC40231	EF185396	–
ruthenicum	Ukraine, Dniepopetrovskaia, Sabrilovka, SE Kiev	Deryiova s.n. (LE)	–	DQ072042
sanguis-christi 1	Spain, Murcia, La Azohia, castillo	C. Navarro & al. 1922 (MA-612356)	EF185381	DQ072055
sanguis-christi 2	Spain, Castellón, Peñíscola, Barranco de la Torre Nova	C. Fabregat & al. 51 (MA-580737)	EF185382	–
sebaceum 1	Morocco, middle Atlas, Ben Smine, Azrou, 67 S of Meknes	F. Damblon 82/36 (MA-596076)	EF185407	DQ072103
sebaceum 2	Morocco, Boumia, 8 km NW of Er-Rachidia	Podlech 43213 (MA-464889)	EF185406	–

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APPENDIX *Continued*

Taxa	Geographical origin	Voucher sample	ITS Genbank accession no(s)	trnL-F Genbank accession no(s)
stephanianum	China, Qinghai, Nangqên Xian, NW of Jangkar, E side of Za Qu (upper Mekong), on road between Jangkar and Yushu	Ho & al. 2892 (MO).	EF185346	DQ072027
tataricum	Russia, Jakasia, Payon, Ust-Bior	M. Voroniena s.n. (LE)	–	DQ072028
texanum	Cultivate in MA, from seeds taken in Yavapai Co., Arizona, USA	J. Aldasoro 3492 (MA)	EF185345	DQ072026
tordylioides 1	Spain, Huesca, Agüero, Los Mallos	C. Navarro 3485 MA	EF185412	DQ072101
tordylioides 3	Morocco	Saudinger 2856	EF185413	–
touchyanum 1	Morocco, Sk-el-Had-de-Reggada	J. Arrington & al (MA-654483)	EF185411	DQ072088
touchyanum 2	Iran	E91240	EF185410	–
trichomanifolium	Turkey, Palandoken Dag, Erzürüm	A. Herrero 1705 (MA)	EF185357	DQ072040
trifolium 1	Tunisia, Rohnia a Maktar, 30 km of Rohnia	J. Aldasoro 2936 (MA)	EF185389	DQ072075
Geranium biuncinatum	Jebel Burá, between Hilla and Attuba	J.R.I. Wood 3126 (MA648734) Yeo3	DQ525076	–
Monsonia speciosa			AF505648	–
Pelargonium zonale			DQ345326	
Geraniales: Melianthaceae				
Bersama lucens			DQ435401	–
Melianthus elongatus			DQ435418	–