



A tale of migrations from east to west: the Irano-Turanian floristic region as a source of Mediterranean xerophytes

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ABSTRACT

Aim The Irano-Turanian (IT) floristic region is characterized by high levels of endemism. Despite its potential role as a cradle of xerophytic taxa for neighbouring areas, its biogeographical history remains poorly studied. *Haplophyllum*, a diagnostic element of the IT region, was used as a model to discriminate between alternative biogeographical scenarios for the evolution of the region and, more specifically, to investigate whether it served as a source of xerophytes for the colonization of the Mediterranean Basin.

Location Irano-Turanian floristic region (Central Asia and West Asiatic areas) and Mediterranean floristic region (western and eastern parts of the Mediterranean Basin).

Methods Three chloroplast DNA regions were sequenced in 77 accessions of *Haplophyllum* and 37 accessions from other subfamilies of Rutaceae. To elucidate the temporal and spatial evolution of *Haplophyllum* in the IT and Mediterranean regions, we performed Bayesian molecular dating analyses with four fossil constraints and ancestral range reconstructions, respectively.

Results Our molecular dating and ancestral area reconstruction analyses suggest that *Haplophyllum* originated in the Central Asian part of the IT region during the early Eocene and started to diversify *in situ* during the early Oligocene, soon after the vanishing of the Tethys Ocean. Our results further imply that *Haplophyllum* later invaded the eastern Mediterranean Basin in the middle-to-late Miocene, concomitantly with the Paratethys Salinity Crisis and rapid palaeobiogeographical changes in the proto-Mediterranean. Finally, *Haplophyllum* diversified in the western Mediterranean in the early Pliocene at the end of the Messinian Salinity Crisis.

Main conclusions The IT floristic region can serve as a 'donor' of xerophytic taxa to 'recipient' neighbouring regions, including the Mediterranean floristic region. The climatic/geological processes during the Miocene–Pliocene, by increasing aridity and topographic heterogeneity, facilitated range shifts and allopatric speciation in the region.

Keywords

Climate, floristic region, geology, *Haplophyllum*, historical biogeography, Irano-Turanian, Mediterranean, migration, xerophytes.

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INTRODUCTION

The identification and comparison of different regional biotas, the elucidation of their connections via diverse biotic elements, and the clarification of how their composition

changes through time have represented key aspects of biogeographical research since the publication of von Humboldt's seminal work (1845). A comparative study of regional biotas requires a division of the world into natural floristic regions (Takhtajan, 1986). Specific floristic regions

have been defined as geographical areas with distinct floras characterized by endemic taxa at the rank of species or higher (see also Schouw, 1823; McLaughlin, 1994, and references therein). Floristic plant geography plays an important role in the understanding of biogeographical processes via its broad evaluation of modern patterns of plant distribution (McLaughlin, 1994). Therefore, rigorous studies on the connections between floristic regions, especially poorly known ones (e.g. the Irano-Turanian region), represent crucial new contributions to our knowledge of biogeography.

The Irano-Turanian floristic region (IT region) was first defined in relatively explicit terms by Edmond Boissier in *Flora Orientalis* (1867) and has subsequently been named and delimited in different ways by various authors (e.g. Zohary, 1973; Takhtajan, 1986, and references therein; White & Léonard, 1991; Davis *et al.*, 1994) (see Fig. 1). The IT region in Southwest Asia is one of the richest floristic areas of the Holarctic Kingdom, with most of its species diversity in the Iranian plateau, Anatolian plateau, and Central Asia (Zohary, 1973; Takhtajan, 1986; Davis *et al.*, 1994). Continentality, winter temperature and precipitation seasonality differentiate it from the surrounding regions, and its flora is characterized by a relatively high number of endemic genera and very high species endemism (Takhtajan, 1986). The region comprises two subregions: the Central Asian and the West Asiatic subre-

gions, the latter including the Iranian and the Anatolian plateaux (Takhtajan, 1986). The Irano-Anatolian area and the Central Asian mountains are two of the 34 global hotspots of biodiversity *sensu* Mittermeier *et al.* (2005). The IT region has long been regarded as the source of many taxa found in neighbouring regions, most notably the Mediterranean region (Akhani, 2007; Blondel *et al.*, 2010, and references therein). The evidence supporting such a claim, however, has often been speculative and is mainly based on floristic studies, rather than phylogenetic/biogeographical analyses.

The Mediterranean Basin is a crossroads for species from Europe, Asia and Africa, representing an area where biotic elements of the surrounding temperate, arid and tropical biogeographical regions converge (Quézel, 1985; Comes, 2004; Thompson, 2005; Blondel *et al.*, 2010; Migliore *et al.*, 2012). Mediterranean species either evolved *in situ*, or were filtered from the regional biota of neighbouring areas, or migrated from more distant regions (Mansion *et al.*, 2008; Ackerly, 2009). The onset of the current seasonal climate in the Mediterranean Basin, starting during the late Pliocene (Suc, 1984; Thompson, 2005), caused the demise of most of its tropical and subtropical taxa and the rise of many xerophytic species, including several IT taxa with centres of diversity in the semi-arid steppes of Central Asia. More recently, Quaternary glaciations eliminated additional meso-

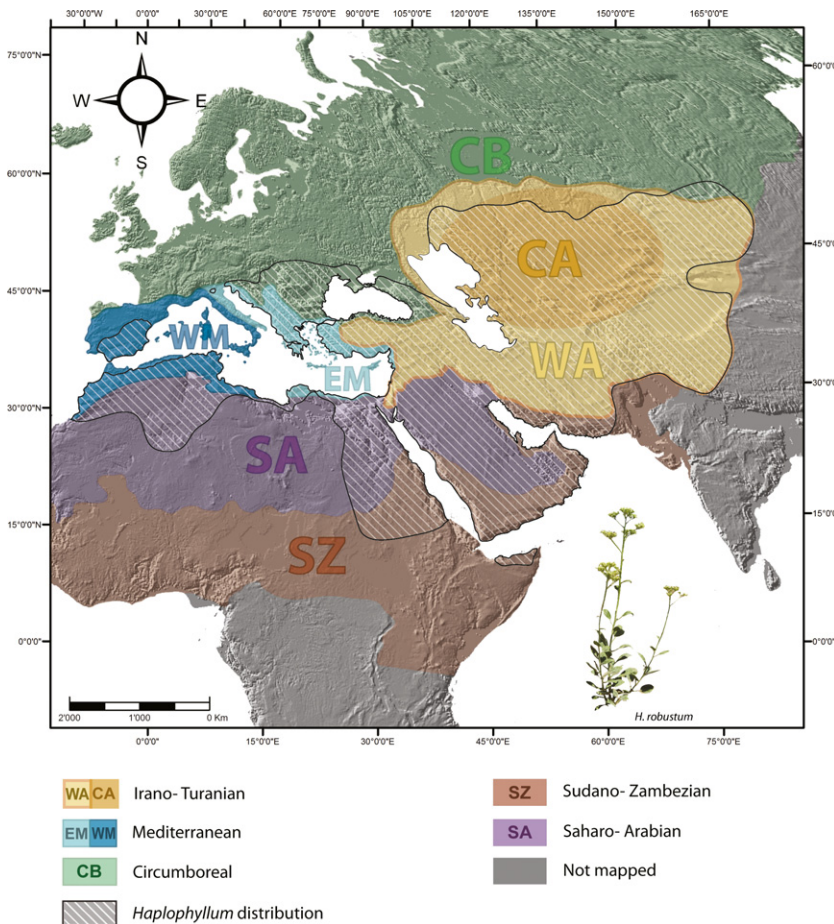


Figure 1 Distribution of *Haplophyllum* and relevant floristic regions. Hatched surface delimited by black continuous line shows the extant distribution of *Haplophyllum* (after Townsend, 1986) overlaid on the five major floristic regions (after Takhtajan, 1986) where its species occur. These floristic regions were used to define the seven areas for the reconstructions of ancestral areas: Irano-Turanian floristic region [consisting of the West Asiatic (WA) and the Central Asian (CA) areas]; Mediterranean floristic region [consisting of the East Mediterranean (EM) and the West Mediterranean (WM) areas]; Sudano-Zambezian floristic region (SZ); Saharo-Arabian floristic region (SA); Circumboreal floristic region (CB). Map modified from GDEM V1 black & white, courtesy of NASA/JPL-Caltech. Available at: <http://asterweb.jpl.nasa.gov/images/GDEM-10km-BW.png>.

thermophytic elements from the Mediterranean flora (Blondel *et al.*, 2010).

Despite its potential role as a source of xerophytic species for neighbouring areas, the IT region remains poorly studied, as demonstrated by searches on ISI Web of Knowledge (Fig. 2). Modern biogeographical analyses are scarce (Emadzade *et al.*, 2011; Jabbour & Renner, 2011) and the relationships between this region and adjacent ones, such as the Saharo-Arabian and Mediterranean regions, have not been investigated in detail (but see Migliore *et al.*, 2012). There is therefore a need to study key taxa of the IT flora in order to better understand the evolution of biodiversity and biogeographical links both within the IT region and between the IT and neighbouring floristic regions.

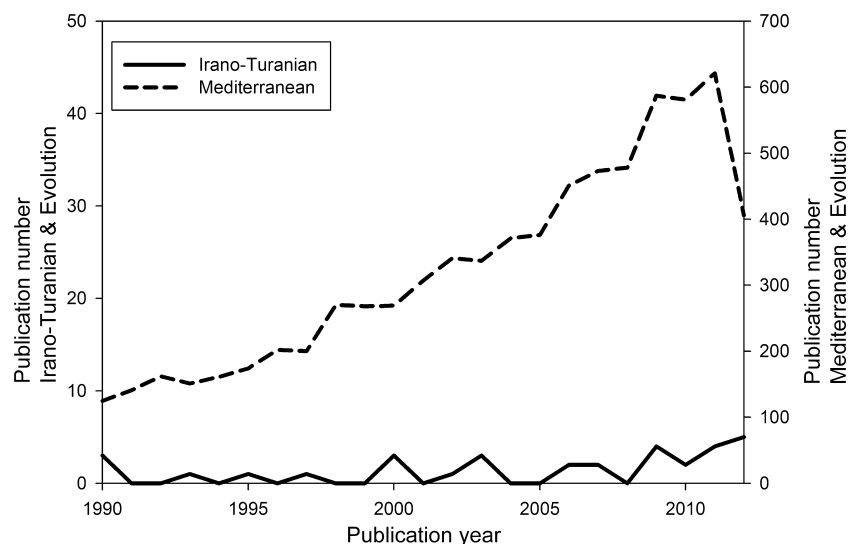
Haplophyllum A. Juss. (Rutaceae), comprising 68 species of perennial herbs and shrubs (Townsend, 1986; Navarro *et al.*, 2004; Soltani & Khosravi, 2005) and occurring in dry, stony hills and semi-deserts or steppes (Townsend, 1986), was chosen as a model to study the biogeographical history of the IT region, because it is a species-rich genus that reaches its maximum diversity in the area (60% of species), mostly in Turkey, Iran and Central Asia. It also includes species endemic to the Mediterranean region, thus allowing us to study the biogeographical connections between it and the IT region (13% of species; Salvo *et al.*, 2011). Moreover, both Zohary (1973) and Takhtajan (1986) used this genus to characterize the flora of the IT region. The fruits of *Haplophyllum* are often dehiscent and seed dispersal is probably autochorous, because the seeds lack any obvious adaptation to wind-dispersal (Navarro *et al.*, 2008; S.M., pers. obs.). A recent phylogenetic analysis of *Haplophyllum* and closely related genera ascertained its monophyly (Salvo *et al.*, 2011), but did not provide a temporal and spatial analysis of its evolution. Given the recognized importance of *Haplophyllum* as a key element of the IT flora, detailed knowledge of the evolution of its species diversity would yield novel insights into the biogeographical role of the IT region.

Different geological and climatic events are likely to have played a role in the origin and diversification of *Haplophyllum* in the IT and Mediterranean floristic regions. By using a combination of temporal and spatial phylogenetic evidence, it is now possible to evaluate alternative scenarios previously proposed for the evolution of the genus (Crisp *et al.*, 2011). With respect to time, two main scenarios have been suggested to account for the origin of *Haplophyllum*. In the first scenario, most of the xerophytic elements that form the steppe and arid flora of the IT region (e.g. in *Haplophyllum*) originated as the Tethys Ocean, which previously covered vast stretches of Southwest Asia, receded in the early Neogene (Zohary, 1973). Alternatively, the steppe and arid flora of the IT region may have originated much earlier, during the Late Cretaceous. Lithological data point to the existence of a broad arid zone extending from Spain, through North Africa, to West and Central Asia during this period (Takhtajan, 1969, quoting Strakhov, 1960; Zohary, 1973 quoting Engler, 1905).

With respect to space, two main scenarios have been proposed to account for the origin of *Haplophyllum* as an element of the IT flora. In the first case, Central Asia is considered to be the main source and centre of diversity of the present arid flora of Eurasia, the Mediterranean area, North Africa, and even South Africa (Bobrov, 1965; Pyankov *et al.*, 2002). In the second scenario, the West Asiatic subregion of the IT region, especially the Iranian plateau, represents the main centre of origin for the IT flora (Takhtajan, 1986).

A further geological event that is likely to have influenced the diversification of *Haplophyllum* within the IT region was the uplift of the Iranian plateau (Djamali *et al.*, 2012a, and references therein). Orogenic activities, especially mountain uplifts, have been shown to contribute crucially to processes of floristic diversification (Hughes & Eastwood, 2006; Antonelli *et al.*, 2009). The Arabia–Eurasia convergence is considered to be the main driver for the uplift of the Iranian plateau. The main stages of crustal thickening and uplift, which took

Figure 2 Two searches were performed on the ISI Web of Knowledge in October 2012, one using the combined terms 'Irano-Turanian' (IT) and 'Evolution' and a second one with the terms 'Mediterranean' and 'Evolution', the number of publications thus obtained was plotted on the y-axis. The plot demonstrates that the IT region is poorly studied from an evolutionary point of view.



place at discrete intervals, began around 25 Ma and accelerated from 15 to 12 Ma (e.g. Mouthereau *et al.*, 2012).

Beyond its main species diversity in the IT region, *Haplophyllum* also includes 16 species in the Mediterranean area, of which nine are endemic. Colonization of new areas by land plants can occur via long-distance dispersal (LDD) and/or land connections. In the latter case, two main geological/climatic processes might have affected the opportunities for colonizing the East Mediterranean from the neighbouring IT region. First, the movement of the Arabian Plate caused the closure of the eastern connection of the proto-Mediterranean Sea/Paratethys with the Indian Ocean during the middle Miocene, thus initiating drier climates in the Mediterranean Basin, Arabia, the Iranian plateau and northern Africa. The new climatic regime paved the way for waves of penetrations of IT elements (including *Haplophyllum*) into the Mediterranean region during the late Tertiary, as demonstrated by phylogenetic analyses and floristic similarities (Zohary, 1973; Quézel, 1985, 1995; Thompson, 2005; Mansion *et al.*, 2008; Jabbour & Renner, 2011; Salvo *et al.*, 2011). However, recent palaeobotanical evidence from the Thracian Plain of the present-day Balkans appears to support a second scenario, according to which the 'Oriental' elements of the Balkan flora reached the eastern Mediterranean Basin during the late-Quaternary glacial stages (Magyari *et al.*, 2008, and references therein). During the Quaternary interglacials, high-elevation mountains of the IT region might have served as refugia for xerophytic elements, while intensified continentality during glacial maxima might have favoured the expansion of cold-adapted IT species into surrounding regions (Djamali *et al.*, 2012a).

The following geological processes might have facilitated the colonization of the West Mediterranean area by *Haplophyllum*. A first scenario builds on the presence of land bridges that repeatedly separated and rejoined the Tethys and Paratethys seas during the Oligocene and Miocene (Quézel, 1985; Oosterbroek & Arntzen, 1992; Sanmartín, 2003, and references therein; Thompson, 2005). Such land bridges would have allowed dispersal across the entire Mediterranean region, and then promoted east-west disjunctions when rising sea levels disrupted land connections. A second scenario centres on the closure of the western part of the Mediterranean Sea with the Atlantic Ocean in the late Miocene (late Tortonian), which increased aridity in the Mediterranean Basin, especially in its western part. Moreover, during the Messinian Salinity Crisis, a drying trend enabled biotic dispersal between West/Central Asia and the Iberian Peninsula via North Africa (Zardoya & Doadrio, 1998; Sanmartín, 2003, and references therein; Navarro *et al.*, 2004). In general, the numerous examples of east-west vicariant species in the Mediterranean and their frequently observed taxonomic distinctness suggest that, at least in some cases, the initial divergence probably occurred before the Pleistocene (Davis & Hedge, 1971).

In order to discriminate between the alternative biogeographical scenarios outlined above, we generated a dated

phylogeny and used it to infer the spatio-temporal evolution of the genus, specifically addressing the following questions: (1) When and where did *Haplophyllum* originate? (2) When did the Mediterranean representatives of *Haplophyllum* originate, and along which route did they colonize the Mediterranean region? (3) Is the IT floristic region a potential source of xerophytic elements (e.g. in *Haplophyllum*) for the Mediterranean floristic region?

MATERIALS AND METHODS

Floristic regions

This study investigates the historical biotic interchanges among five floristic regions, focusing mainly on the IT and the Mediterranean regions and using *Haplophyllum* as a case study. We thus generated a distribution map of *Haplophyllum*, and divided it into floristic regions according to the designations of Takhtajan (1986). Takhtajan's classification is based on geographical patterns of endemism, particularly at the species and genus level. Its floristic regions, defined at the subcontinental scale, especially in the Holarctic Kingdom, are more suitable for biogeographical studies at large spatial scales than those defined at a more restricted scale (Zohary, 1973; White & Léonard, 1991). Therefore, Takhtajan's floristic regions have been used in several continental-level biogeographical studies (e.g. Cox, 2001; Fridley, 2008) and are appropriate for an investigation of the floristic connections between Asia, Europe and North Africa, using *Haplophyllum* as the focus group.

Sampling and phylogenetic analyses

We reconstructed phylogenetic relationships using DNA sequences from three loci of the chloroplast genome (cpDNA) that allowed us to combine our newly generated data with pre-existing sequences of Rutaceae (Salvo *et al.*, 2010, 2011): the *matK* gene, the *rpl16* intron and the *trnL-trnF* intergenic spacer. The final data set comprised a total of 3498 characters from 114 accessions: 77 from the ingroup (representing 37 of 68 *Haplophyllum* species, 54%) and 37 from the other subfamilies of Rutaceae (see Appendices S1 & S2 in Supporting Information). Accession numbers and sources of these sequences are listed in Appendix S3.

The choice of taxa was guided by principles that considered the taxonomic, geographical and palaeobotanical diversity of *Haplophyllum* and its relatives and by the requirements of molecular dating and ancestral area analyses. Details of taxon selection, especially in relation to fossil calibration for molecular dating and phylogenetic analyses, can be found in Appendix S1.

Molecular dating analyses

A likelihood-ratio test (LRT; Felsenstein, 1988) was performed to check for constancy of substitution rates among

the branches of the phylogeny. Because the LRT rejected rate constancy, molecular dating analyses were carried out within a Bayesian framework, which allows for rate variation between lineages, by employing an uncorrelated lognormal relaxed clock model in *BEAST* 1.6.2 (Drummond & Rambaut, 2007). The software uses Bayesian inference and a Markov chain Monte Carlo (MCMC) analysis to infer branch lengths, tree topology and nodal ages. All fossil calibration points were assigned a lognormal prior, with 95% of the weight falling within the geological interval that included each of the fossils used (Table 1). Details of fossil selection and assignment to specific nodes are given in Table S1 in Appendix S1.

After selecting a GTR substitution model for the *rpl16* region and a TVM substitution model for the *matK* and *trnL-trnF* regions, with four gamma categories, an uncorrelated relaxed-clock model and a Yule prior were applied to the specific Bayesian starting tree used as the tree prior. Four independent runs of 75×10^6 generations each were conducted, sampling every 1000th generation. Convergence between runs and the amount of burn-in were determined in *TRACER* 1.5 (Rambaut & Drummond, 2007) by using effective sample size (ESS) scores and checking for the consistency of the results between multiple runs. The four runs were combined, discarding the initial 10% as burn-in, using *LOGCOMBINER* 1.6.2 (Drummond & Rambaut, 2007). A maximum clade-credibility tree using a posterior probability limit of 0.5 was then calculated using *TREEANNOTATOR* 1.6.2 (Drummond & Rambaut, 2007) and visualized using *FIG-TREE* 1.3.1 (Rambaut, 2010). Further details of the molecular dating procedure are given in Appendix S1.

Ancestral area reconstruction analyses

The range of *Haplophyllum* was divided into seven areas, based on the extant distribution of the genus and the floristic regions it covers (Fig. 1). These areas are the Central Asian (CA) and the West Asiatic (WA) portions of the IT floristic region, the eastern (EM) and western (WM) portions of the Mediterranean floristic region, the Sudano-Zambezian floristic region (SZ), the Saharo-Arabian floristic region (SA) and the Circumboreal floristic region (CB).

We used the recently developed statistical dispersal–vicariance analysis (S–DIVA) and Bayesian binary MCMC analysis (BBM) implemented in *RASP* (Yu *et al.*, 2011) to reconstruct

the possible ancestral ranges of the genus on our phylogenetic trees. The latter method averages the frequencies of an ancestral range at every node over all input trees. BBM, unlike S–DIVA, offers a statistical procedure for inferring states at ancestral nodes using a full hierarchical Bayesian approach (Yu *et al.*, 2010, 2011).

Biogeographical analyses were performed on a Bayesian cladogram generated from the data set of 77 accessions of *Haplophyllum*. To account for topological uncertainties, we used 2000 randomly selected trees from the MCMC output of the Bayesian analysis of *Haplophyllum*, and ran S–DIVA on all of them. The possible ancestral ranges at each node on a selected tree were thus obtained. In the BBM analysis, the MCMC chains were run simultaneously for 5,000,000 generations and every 100th generation was sampled. An estimated F81+ Γ model was used for the BBM analysis, with null root distribution. Following Ronquist (1997), the number of areas inferred at internal nodes was restricted (e.g. Mansion *et al.*, 2008); the maximum number of ancestral areas was constrained to two, because most of the sampled species (with the exception of *H. tuberculatum*, six areas, and *H. buxbaumii*, three areas) currently occur in no more than two areas (Townsend, 1986). We used BBM analysis to generate a modified Gaussian distribution for a time-event algorithm modelled on dispersal and vicariance events at each node (Yu *et al.*, 2011). A list of selected taxa with their current distribution is shown in Table S2 in Appendix S1.

RESULTS

Divergence time estimation

The 50% majority rule consensus tree recovered by *MRBAYES* from the 114-taxon data set was topologically identical to the maximum clade-credibility tree inferred with *BEAST* on the same data set (see Fig. S1 in Appendix S1); the latter is displayed in Fig. 3 with 95% highest posterior density (HPD) intervals associated with nodal heights for the nodes of interest (nodes A–F; Fig. 3b; see also Table 2). The ESS and the trace of the parameters confirmed that the four runs had converged within 75 million generations.

The molecular dating analyses revealed a high level of substitution-rate variation across the sampled sequences, as indicated by the marginal posterior probability of the coefficient

Table 1 Settings of the lognormal prior distribution for fossil calibration and root height in the molecular dating analyses of the xerophytic genus *Haplophyllum*.

Prior distribution	<i>Clausena</i> Lognormal	<i>Toddalia</i> Lognormal	<i>Ptelea</i> Lognormal	<i>Skimmia</i> Lognormal	Rutaceae Lognormal
Offset	23	37.2	11.608	7.246	65
Lognormal mean	0.9933	0.4700	0.7798	0.7798	2.5
Lognormal SD	0.4219	0.4215	0.4214	0.4214	0.29
Median age (Ma)	25.7	38.8	13.789	9.427	77.75
Lower (5%) and upper (95%) boundary	28.4–23	40.4–37.2	15.97–11.608	11.608–7.246	90.5–65

SD, standard deviation.

(a)

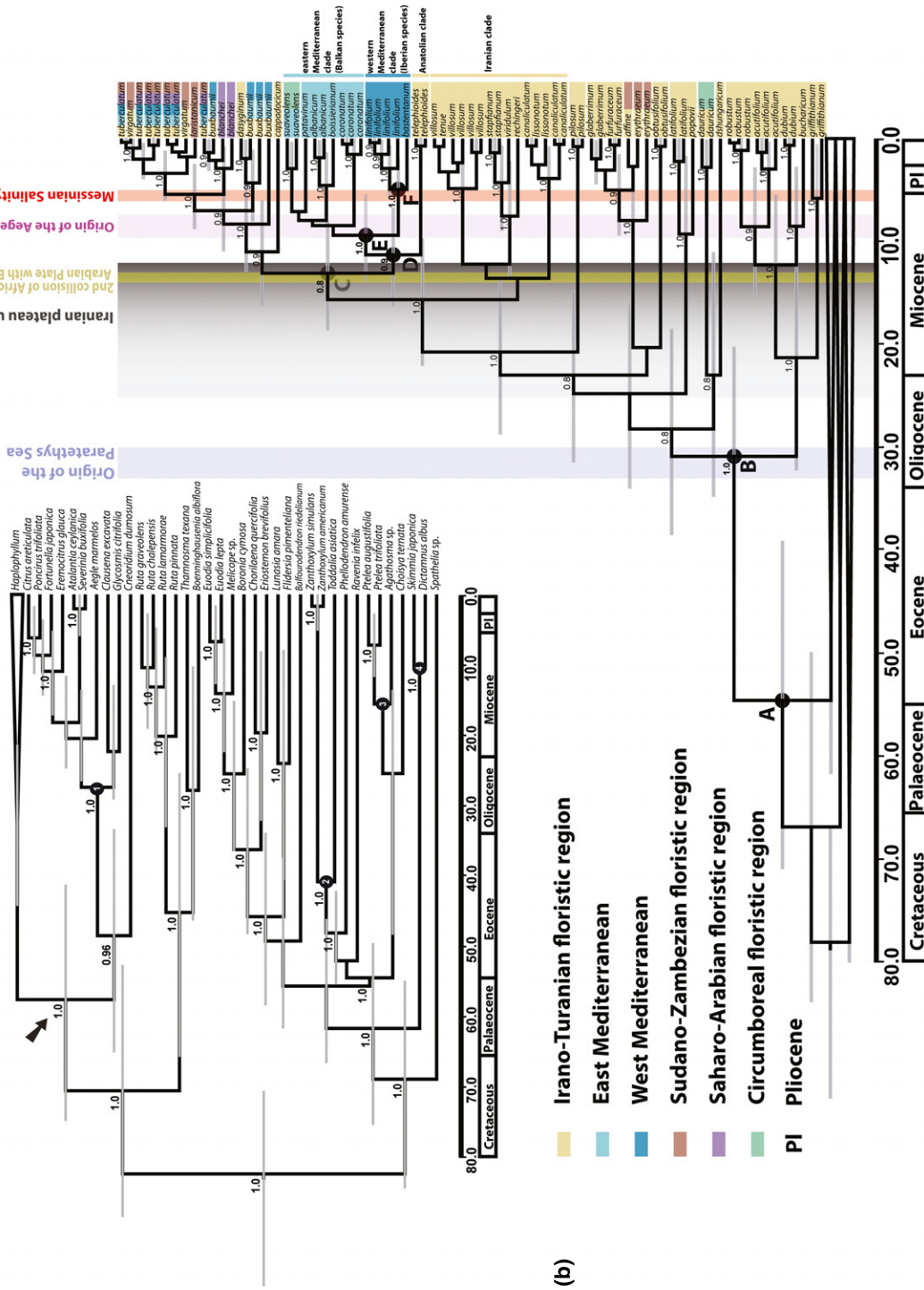


Figure 3 Chronogram inferred from Bayesian dating analysis (BEAST) of the 114-taxon data set including 77 accessions from *Haplophyllum* and 37 accessions from the other subfamilies of Rutaceae. Values above branches represent Bayesian posterior probabilities. Grey bars represent the 95% highest posterior density intervals around mean nodal ages. (a) Tree with the branches for all *Haplophyllum* species collapsed and the four fossil constraints applied to nodes in other clades of Rutaceae, as follows: 1, *Clausenia*; 2, *Toddalia*; 3, *Ptelea*; 4, *Skimmia*. (b) Tree with the branches for all genera except *Haplophyllum* collapsed and major geological/climatic events during the evolution of the genus represented by coloured vertical bars. Nodes of biogeographical interest: A, split of *Haplophyllum* from its sister; B, split between eastern and western Mediterranean clades; C, split of Anatolian and Mediterranean clades from the sister clade; D, split of the Mediterranean clade from the Anatolian clade; E, split between eastern and western Mediterranean clades; F, diversification of the western Mediterranean clade.

of variation of the rates [mean = 1.0051; 95% HPD interval = (0.7461, 1.228)]. Additionally, no evidence of rate autocorrelation between neighbouring branches was detected, as indicated by the marginal posterior probability of rate covariance [mean = 5.3744×10^{-2} , 95% HPD interval = (-0.0788, 0.1954)].

In summary, the molecular dating analyses suggest that: (1) *Haplophyllum* originated in the early Eocene, 54.56 Ma (95% HPD: 49.92–83.79 Ma; node A, Fig. 3b), and started to diversify in the early Oligocene, 30.86 Ma (39.12–70.86 Ma; node B, Fig. 3b); (2) *Haplophyllum* invaded the Mediterranean floristic region in the middle Miocene, between 13.08 Ma (8.13–18.58 Ma) and 11.28 Ma (6.73–16.25 Ma; nodes C & D, Fig. 3b), the split between the eastern and western Mediterranean clades occurred around 9.39 Ma (5.45–13.81 Ma; node E, Fig. 3b) and finally *Haplophyllum* diversified in the western Mediterranean in the early Pliocene, 4.94 Ma (1.86–8.46 Ma; node F, Fig. 3b; see also Table 2).

Ancestral area reconstruction

The results of the ancestral area reconstruction indicate that *Haplophyllum* required a total of either 111 dispersal and four vicariance events (inferred by BBM; Fig. 4b) or 80 dispersal and five vicariance events (inferred by S-DIVA) to reach its current distribution. Range reconstructions were identical for nodes F (colonization of the western Mediterranean, see Table 2 and Appendix S2), D (split of the Mediterranean clade from the Anatolian clade) and E (split between the eastern and western Mediterranean clades), although with small differences between BBM and S-DIVA in the probabilities of inferred areas at the two latter nodes (see Appendix S2). S-DIVA analyses inferred an ancestral area for node B (initial diversification of *Haplophyllum*) that also included the Circumboreal region, in addition to the West Asiatic and Central Asian portions. BBM inferred an ancestral area of EM in addition to WA for node C (split of the Mediterranean and the Anatolian clades from its sister) (Table 2). The time–event graph inferred by BBM analyses (Fig. 4b) illustrated that the number of dispersal events peaked about 4–5 times during the evolutionary history of *Haplophyllum*, and that vicariance events occurred at four different times (Fig. 4b; see discussion for details).

To summarize, the inferred ancestral areas at the nodes imply that: (1) the most recent common ancestor (MRCA) of the *Haplophyllum* clade (Fig. 4a, node B) most probably inhabited the Central Asian portion of the IT region, according to BBM analyses. BBM indicates a higher support value for this node (> 50% probability) than S-DIVA (40% probability); (2) the ancestor of the Mediterranean clade split from its Anatolian sister and invaded the eastern part of the basin via a vicariance–dispersal event, (Fig. 4a, node D); (3) the western Mediterranean species of *Haplophyllum* split from their eastern Mediterranean relatives via another vicariance–dispersal event (Fig. 4a, node E).

DISCUSSION

Geological events have profound effects on the biogeographical patterns of both terrestrial and marine biotas (Lomolino *et al.*, 2006). In particular, plate motions – with the often-associated opening and closing of seaways between oceans – and orogenic processes have changed both climatic conditions and the type and location of spatial barriers to biotic dispersal. Tectonic events have thus played a major role in shaping the floristic regions of the world, with profound effects on global plant evolution. For example, our study indicates that the closure of the Tethys Ocean in the late Eocene and the origin of the Paratethys Sea in the early Oligocene, events that were concomitant with the initial diversification of *Haplophyllum*, are likely to have influenced the biogeographical evolution of the genus, a key element of the xerophytic flora of the IT region. Furthermore, the closure of both the eastern and western proto-Mediterranean connections to the Indian Ocean (early Miocene) and the Atlantic Ocean (late Miocene), respectively, are associated with the diversification of *Haplophyllum* in the East and West Mediterranean, suggesting that such geological processes might have similarly influenced the evolution of other xerophytes in the basin.

Origin and diversification of *Haplophyllum* in the Irano-Turanian and Mediterranean regions

Molecular dating and ancestral area analyses suggest that *Haplophyllum* diverged from its sister group in the early

Table 2 Results of the analyses of molecular dating (BEAST) and ancestral range reconstruction (RASP) of the xerophytic genus *Haplophyllum*.

Nodes of interest	Description	BEAST analysis, mean nodal age (95% HPD)	RASP analyses (S-DIVA)	RASP analyses (BBM)
A	Split of <i>Haplophyllum</i> from its sister	54.556 Ma (49.916–83.7889)	—	—
B	Initial diversification of <i>Haplophyllum</i>	30.8647 Ma (39.1187–70.8615)	CA + IT + CB	CA + IT
C	Split of the Mediterranean and Anatolian clades from its sister	13.0803 Ma (8.1256–18.583)	IT	IT + EM
D	Split of the Mediterranean clade from the Anatolian clade	11.2877 Ma (6.7309–16.2492)	IT + EM	IT + EM
E	Split between eastern and western Mediterranean clades	9.3961 Ma (5.4497–13.8124)	EM + WM	EM + WM
F	Colonization of the western Mediterranean <i>Haplophyllum</i>	4.9459 Ma (1.8558–8.4604)	WM	WM

BBM, Bayesian binary MCMC analysis; HPD, highest posterior density; S-DIVA, statistical dispersal–vicariance analysis.

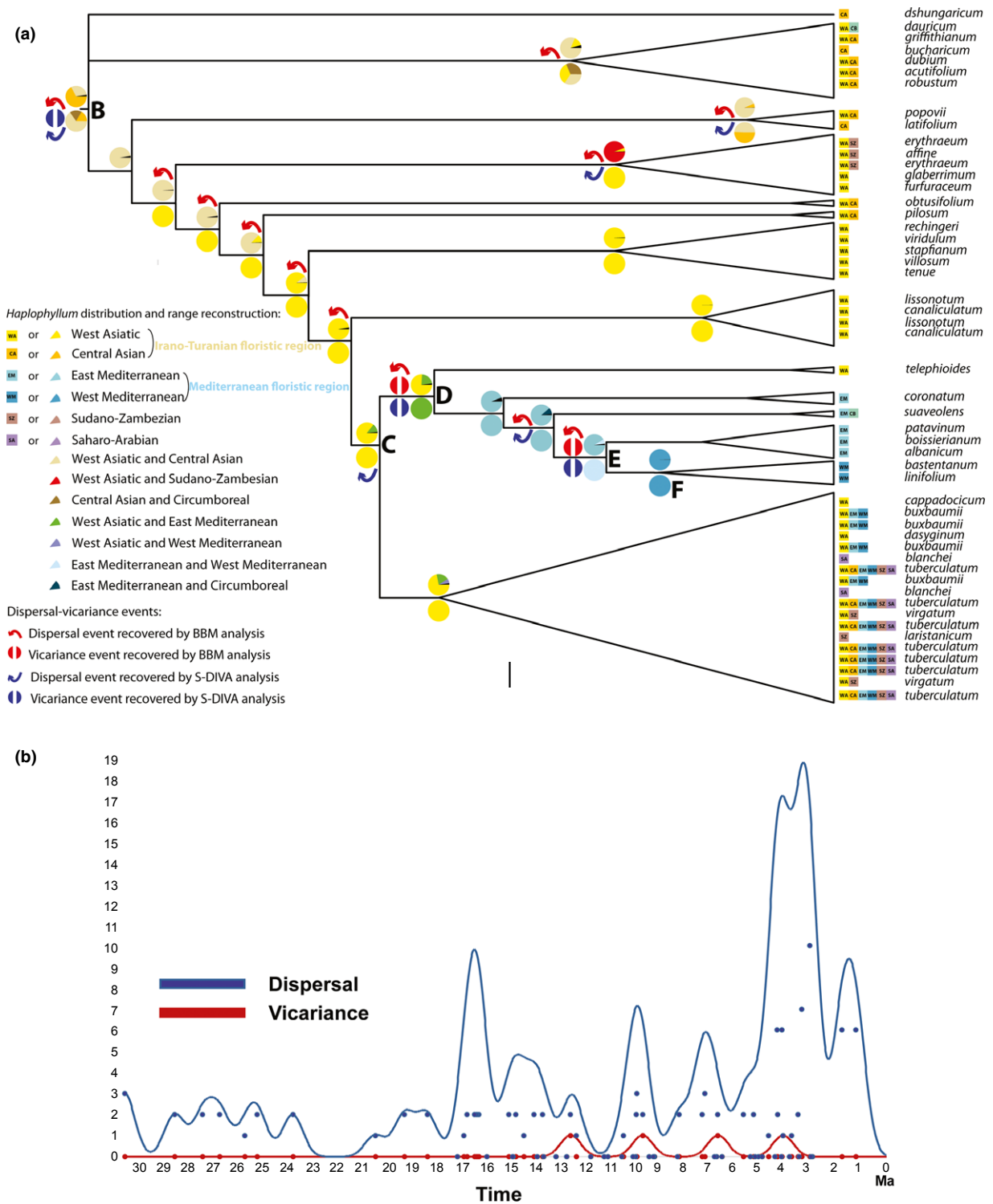


Figure 4 Biogeographical analyses. (a) Ancestral area reconstructions estimated with Bayesian binary MCMC analysis (BBM) and statistical dispersal–vicariance analysis (S–DIVA) analyses implemented in RASP. The ancestral areas inferred at the nodes are represented by pie charts of relative probabilities for each area above and below the branches for BBM and S–DIVA reconstructions, respectively. Symbols for *Haplophyllum* distribution and range reconstruction: the six square and triangular colour-codes in the upper half of the legend correspond to the areas defined in Fig. 1; the seven triangular colour-codes in the lower half of the legend designate combinations of areas inferred at the relevant nodes. Symbols for dispersal–vicariance events: arrows and split circles indicate dispersal and vicariance events, respectively (BBM inferences: red; S–DIVA inferences: blue). (b) Probability distributions of dispersal (blue) and vicariance (red) events at each node based on the time–event algorithm calculated by BBM analysis; x-axis, time range (0–31 Ma, right to left) corresponding to the time spanned by the tree of Fig. 4a; y-axis, number of events.

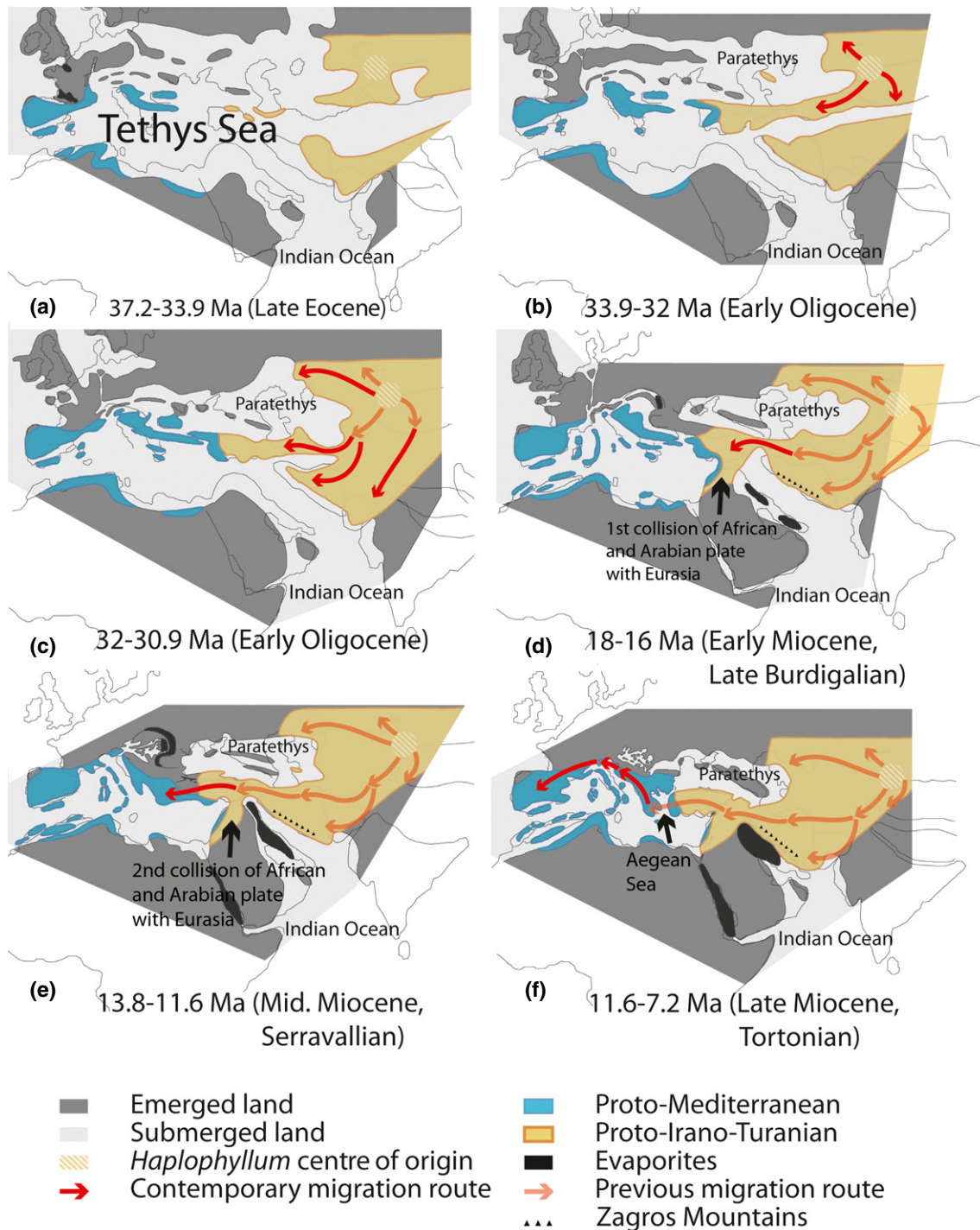


Figure 5 Historical biogeography of *Haplophyllum*. The palaeomaps show the position of plates and emerged land in different geological periods (based on Rögl, 1999). Yellow and blue areas display the hypothetical ranges of the proto-Irano-Turanian and proto-Mediterranean floristic regions, respectively, that are congruent with the time frames and ancestral areas inferred for the relevant nodes of Figs 3b & 4a. Red and orange arrows, respectively, denote the hypothesized *Haplophyllum* migration routes prior to and during each time frame.

Eocene (Fig. 3b, node A) and started to diversify sometime between the late Eocene and early Oligocene (Fig. 3b, node B), most probably in the Central Asian portion of the IT floristic region (Fig. 4a, node B; Table 2). In the early

Eocene, a zonal desert with a steppe vegetation and seasonally dry climate extended over part of the Central Asian region (Fig. 5a), while the rest of the IT region was covered by the Tethys Ocean (Zhang *et al.*, 2012).

Two key tectonic processes occurred between the late Eocene and early Oligocene that might have facilitated range expansion of the genus from Central Asia to the rest of the IT region. (1) By the end of the Eocene, the northward drift of India triggered the disappearance of the Tethys Ocean. Strong tectonic activities changed the Eurasian configuration, and the Tethys had completely vanished by the final collision of the Indian continent with Eurasia (Fig. 5b,c; Zohary, 1973; Rögl, 1999). (2) The Paratethys Sea, formed around the Eocene–Oligocene boundary (Báldi, 1980; Rusu, 1988; Rögl, 1999), eventually gave rise to the proto-Mediterranean Sea, which arose by separation from the Paratethys Sea via elongated and deep trenches stretching from the western Alps to the Trans-Caspian Basin (Fig. 5b,c; Rögl, 1999). The above-mentioned events are likely to have provided migration corridors for the initial expansion of *Haplophyllum* from its ancestral range in Central Asia (Fig. 5a) into the rest of the IT region by the early Oligocene (Fig. 5b,c; Fig. 3b, nodes A & B).

In summary, the results of our integrated molecular dating and ancestral range reconstruction analyses indicate that *Haplophyllum* originated *in situ* in the Central Asian part of the IT floristic region in the early Eocene, well before the complete vanishing of the Tethys Ocean. Our results thus reject Zohary's (1973) hypothesis that the steppe and arid flora of the IT region arose during the Neogene, after the drying out of the Tethys Ocean. They also fail to support a Cretaceous origin of the xerophytic IT elements (Takhtajan, 1969, quoting Strakhov, 1960; Zohary, 1973, quoting Engler, 1905) (Fig. 3b, node A). Similar analyses of additional xerophytes from the IT region would be needed to further test this hypothesis.

Our results indicate that the clade formed by the Mediterranean species of *Haplophyllum* and the Anatolian *H. telephioides* diverged from its geographically diverse sister clade in the middle Miocene (Fig. 3b, node C), probably in the IT region (Fig. 4a, node C). From there, it quickly invaded the eastern Mediterranean (Figs 3b & 4a; nodes C & D; Fig. 5d, e). During the middle Miocene (early Serravallian), strong tectonic activities caused the Paratethys salinity crisis and rapid palaeobiogeographical changes in the proto-Mediterranean Sea (Rögl, 1999). The second closure of the eastern connection of the proto-Mediterranean Sea/Paratethys with the Indian Ocean during the middle Miocene (Fig. 5e) also had significant climatic impacts, causing drier climates over the Mediterranean Basin, Arabia, the Iranian plateau and northern Africa. The aridification trend paved the way for waves of invasion of xerophytic IT elements into the Mediterranean (Fig. 5e). The above-mentioned geological and climatic events might have concomitantly caused the formation of a land corridor between the Iranian plateau and the East Mediterranean area via the Anatolian Plate (e.g. Meulenkaamp & Sissingh, 2003; Harzhauser & Piller, 2007; Fig. 5e).

Moreover, in the late Miocene (Tortonian), the formation of the Aegean Sea, increasing continentalization and the tectonic uplift of the Carpathians caused a new geological/cli-

matic configuration in the area (Rögl, 1999; Fig. 5f). These events might have allowed the vicariant divergence of the Mediterranean clade from its Anatolian sister species, *H. telephioides* (node D in Figs 3b & 4a; Fig. 4b; Table 2). Our results thus reject the hypothesis that the 'Oriental' elements of the continental steppes of the Balkan flora reached the eastern Mediterranean Basin during the late Quaternary glacial stages (e.g. *Celtis* and *Juniperus*; Magyari *et al.*, 2008, and references therein). However, our results support a scenario of older migrations of IT elements into the eastern Mediterranean during the late Tertiary, as previously proposed based on floristic similarities (Zohary, 1973; Quézel, 1985, 1995; Thompson, 2005) and phylogenetic analyses of a few other taxa (e.g. Araceae: Mansion *et al.*, 2008, *Ranunculus*: Emadzade *et al.*, 2011; Brassicaceae: Franzke *et al.*, 2011; Delphinidae: Jabbour & Renner, 2011; *Cousinia*: Djamali *et al.*, 2012a).

Colonization of the western Mediterranean

Our findings imply that the western Mediterranean clade diverged from the eastern Mediterranean clade towards the end of the Miocene (Tortonian) via vicariance–dispersal processes (node E in Figs 3b & 4a; Fig. 4b). Palaeogeographical reconstructions suggest that landmass connections existed across the Mediterranean at various times during the Miocene. Our results appear to confirm the crucial role of these connections for biotic dispersal across the region (e.g. Oosterbroek & Arntzen, 1992). Marine transgressions, by restoring connections between the Tethys and Paratethys, might have resulted in east–west vicariance of trans-Mediterranean lineages following dispersal via land corridors (Oosterbroek & Arntzen, 1992). Conversely, our results do not corroborate the scenario that *Haplophyllum* migrated to the Iberian Peninsula from North Africa via the Strait of Gibraltar (Zardoya & Doadrio, 1998; Sanmartín, 2003, and references therein; Navarro *et al.*, 2004).

The disjunct distributions of sister clades in *Haplophyllum* are congruent with the frequently observed patterns of disjunctions between western and eastern Mediterranean taxa, or between western Mediterranean and Central Asian taxa, termed 'Kiermack' disjunctions (Ribera & Blasco-Zumeta, 1998). It has been proposed that such disjunctions are likely to have originated via dispersal and vicariance cycles in response to the progressive fragmentation of tectonic belts, thus reflecting the complex geological history of the region (Oosterbroek & Arntzen, 1992; Palmer & Cambefort, 2000; Sanmartín, 2003; Oberprieler, 2005; Thompson, 2005; Mansion *et al.*, 2008).

Haplophyllum diversified in the western Mediterranean towards the end of the Messinian Salinity Crisis (node F in Fig. 3b; Table 2) (Rouchy & Caruso, 2006). At that time, increasing aridification triggered a cycle of partial or almost complete desiccation in the Mediterranean Basin (Suc, 1984; Thompson, 2005; van Dam, 2006), conditions that might have favoured the colonization of the western Mediterranean area by xerophytes such as *Haplophyllum*.

The Irano-Turanian floristic region

The main criteria used to define floristic regions rely on the geographical distributions of selected taxa and/or the association of several taxonomic groups within an area (Takhtajan, 1986). Additionally, climatic data are important factors in determining the floristic features and the nature of boundaries between floristic regions. A recent study by Djamali *et al.* (2012b) indicated that the IT region forms a distinct bioclimatic area and can be defined by a group of climatic parameters: continentality index, precipitation seasonality, cold winters and hot summers. Both the biogeographical and climatic delimitations of floristic regions underscore the importance of evolutionary studies on their characteristic elements, which can serve as proxies for the evolution of the region itself until a sufficient number of taxon-specific studies have accumulated to infer more general, comparative trends.

In addition to the climatic and biogeographical characteristics used to define floristic regions, geological events are also crucial to explaining their current biotic compositions. The IT region has a complex topography shaped by its complex tectonic history (e.g. Stoecklin, 1968; Djamali *et al.*, 2012a). The two main mountain ranges (Zagros and Alborz) of the Iranian plateau, for example, arose synchronously around the middle Miocene either within the plateau (Zagros Mountains) or between the plateau and Central Asia (Alborz Mountains; Djamali *et al.*, 2012a, and references therein). In particular, the Zagros Mountains of western Iran represent the largest mountain belt of the region and are part of the Alpine–Himalayan orogenic system. The onset of crustal thickening, caused by the first collision of the Arabian Plate with Eurasia, started about 25 Ma, but uplift, deformation and exhumation across the Zagros and throughout the Arabia–Eurasia collision zone took place later, between 15 and 5 Ma (e.g. Mouthereau, 2011; Fig. 5d–f). The Alborz Mountains of northern Iran are an intra-continental, east–west orientated orogen formed in response to crustal shortening and thickening induced by the ongoing Arabia–Eurasia convergence. The uplift of the Alborz and Zagros Mountains during the middle Miocene may have had a profound impact on the history of the IT floristic region. Variation in the amount of aridity during the mid-to-late Miocene might be chiefly due to the uplift of the Iranian plateau (Ballato *et al.*, 2012; Fig. 5d–f). The high rate and extent of mountain uplift in the Iranian plateau then promoted high levels of alpine endemism (Noroozi *et al.*, 2008, and references therein).

According to our molecular dating analyses, the large clade sister to *H. pilosum*, consisting of Iranian, Anatolian, Mediterranean and geographically diverse subclades, started to diversify in the IT region and Mediterranean Basin during the uplift of the Iranian plateau (Figs 3b & 4a). Geological heterogeneity in the IT region since the early Miocene, combined with a relatively stable continental climate, might be considered important factors limiting gene flow among populations and favouring allopatric speciation via both dispersal and vicariant processes (see Fig. 4a; Knapp, 1987; Linder,

2003). Nevertheless, a few speciation events in *Haplophyllum* may also have occurred in high-elevation refugia of the IT region during Quaternary glacial–interglacial cycles, probably via upward movement of cold-adapted xerophytic elements (Bennett & Provan, 2008; Djamali *et al.*, 2012a, and references therein). The climatic instability of the Quaternary might have influenced speciation processes in *Haplophyllum* and other IT elements (e.g. *Cousinia*: Djamali *et al.*, 2012a), although our current level of sampling in the genus does not allow us to draw specific conclusions on this possible scenario. However, our results allow us to conclude that the IT xerophytes (e.g. in *Haplophyllum*) survived the Quaternary glaciations and were probably more widespread during the previous eras than they are today, as also suggested for *Cousinia* (Djamali *et al.*, 2012a).

CONCLUSIONS

Our study shows that the IT floristic region is a source of xerophytic species for the neighbouring Mediterranean floristic region, and that in the late Tertiary, both geological and climatic changes influenced range expansion and speciation patterns of the xerophytic elements in the two regions (i.e. in *Haplophyllum*). In general, climatic conditions during the Miocene–Pliocene, influenced by the closure of the connections of the proto-Mediterranean Sea with neighbouring oceans and by the uplift of mountain ranges (e.g. Zagros Mountains), caused significant range shifts that promoted allopatric speciation in *Haplophyllum* and possibly other xerophytes of this area.

To further refine estimates of ancestral distributions, methods for the projection of ancestral ecological niches onto palaeogeographical configurations need to be improved and implemented (Crisp & Cook, 2012). Within *Haplophyllum*, the application of niche modelling tools will be useful to better explain the relative roles of geological versus climatic factors in speciation processes, and of niche conservatism versus niche evolution in shaping distributional patterns in the IT region. As a source of species for the neighbouring regions, the IT floristic region requires serious conservation strategies by the governments of those countries that host its major evolutionary and biodiversity centres.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Expanded materials/methods and results: Table S1 includes information on the four fossils of Rutaceae used as calibration points for the molecular dating analyses; Table S2 includes distributional ranges of *Haplophyllum* in the selected areas; and Fig. S1 shows the 50% majority-rule consensus tree of the Bayesian phylogenetic analysis.

Appendix S2 Detailed results of the RASP analyses.

Appendix S3 Sampled accessions of *Haplophyllum* and other Rutaceae taxa, including source, voucher information and GenBank accession numbers for three cpDNA regions studied.

BIOSKETCHES

Sara Manafzadeh is a PhD student at the University of Zurich. Her main interests are the evolutionary and ecological processes that have shaped plant distributions in the Irano-Turanian floristic region.

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Author contributions: S.M. and E.C. conceived the ideas; S.M. and G.S. collected the data; S.M. analysed the data; and S.M. and E.C. led the writing.

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