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Inferences of biogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian binary MCMC analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies)

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• *Background and Aims* Subfamily Hyacinthoideae (Hyacinthaceae) comprises more than 400 species. Members are distributed in sub-Saharan Africa, Madagascar, India, eastern Asia, the Mediterranean region and Eurasia. Hyacinthoideae, like many other plant lineages, show disjunct distribution patterns. The aim of this study was to reconstruct the biogeographical history of Hyacinthoideae based on phylogenetic analyses, to find the possible ancestral range of Hyacinthoideae and to identify factors responsible for the current disjunct distribution pattern.
• *Methods* Parsimony and Bayesian approaches were applied to obtain phylogenetic trees, based on sequences of the *trnL-F* region. Biogeographical inferences were obtained by applying statistical dispersal-vicariance analysis (S-DIVA) and Bayesian binary MCMC (BBM) analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies).
• *Key Results* S-DIVA and BBM analyses suggest that the Hyacinthoideae clade seem to have originated in sub-Saharan Africa. Dispersal and vicariance played vital roles in creating the disjunct distribution pattern. Results also suggest an early dispersal to the Mediterranean region, and thus the northward route (from sub-Saharan Africa to Mediterranean) of dispersal is plausible for members of subfamily Hyacinthoideae.
• *Conclusions* Biogeographical analyses reveal that subfamily Hyacinthoideae has originated in sub-Saharan Africa. S-DIVA indicates an early dispersal event to the Mediterranean region followed by a vicariance event, which resulted in Hyacintheae and Massonieae tribes. By contrast, BBM analysis favours dispersal to the Mediterranean region, eastern Asia and Europe. Biogeographical analysis suggests that sub-Saharan Africa and the Mediterranean region have played vital roles as centres of diversification and radiation within subfamily Hyacinthoideae. In this bimodal distribution pattern, sub-Saharan Africa is the primary centre of diversity and the Mediterranean region is the secondary centre of diversity. Sub-Saharan Africa was the source area for radiation toward Madagascar, the Mediterranean region and India. Radiations occurred from the Mediterranean region to eastern Asia, Europe, western Asia and India.

Key words: Asparagaceae, biogeography, S-DIVA, Hyacinthoideae, Bayesian binary MCMC, RASP, Scilloideae.

INTRODUCTION

Phylogenetically based historical biogeographical reconstructions are now an important way to illuminate the evolutionary history of organisms in space and time. The enormous growth of biogeographical studies has resulted from the rapid accumulation of phylogenetic data during the last two decades. Recently, model- and event-based approaches have been used for biogeographical inferences. The Lagrange (likelihood analysis of geographical range evolution) implementing dispersal-extinction cladogenesis (DEC) model (Ree *et al.*, 2005; Ree and Smith, 2008) and the BIB (Bayesian island biogeography) method (Sanmartín *et al.*, 2008, 2010) were recently applied to biogeographical analysis, but the event-based method dispersal vicariance analysis (DIVA; Ronquist, 1997, 2001) has remained the most popular and widely used method for reasons of simplicity. In the DIVA method, ancestral distributions are inferred based on a three-dimensional cost matrix derived from a simple biogeographical model (Ronquist, 1997). Two problems, uncertainty in phylogeny and uncertainty in ancestral area optimization, are attached to it. Nylander *et al.* (2008) proposed a new method, Bayes-DIVA, to overcome the uncertainties in DIVA analysis. Similarly, Harris and Xiang (2009) proposed their approach, an alternative to Bayes-DIVA. Their method differs in its ability to handle uncertainty at some nodes.

The Statistical DIVA (S-DIVA; Yan *et al.*, 2010) method rectified the problems in DIVA analysis and the results are comparable with those obtained by Bayes-DIVA. RASP (Reconstruct Ancestral State in Phylogenies) (Yan *et al.*, 2011) is a useful tool to reconstruct evolutionary histories in phylogeny. Three different methods, S-DIVA, Bayesian binary MCMC (BBM) and maximum-parsimony (MP) analysis, are implemented in RASP to obtain ancestral ranges at each node. S-DIVA and BBM methods suggest possible ancestral ranges at each node and also calculate probabilities of each ancestral range at nodes.

The family Hyacinthaceae [= Asparagaceae subfamily Scilloideae *sensu* APG III (2009)and Chase *et al.* (2009)] consists of approx. 900 species and 70 genera (Speta, 1998*a*, *b*) in temperate and tropical regions (e.g. Mwafongo *et al.*, 2010). Members of this family can be found in different habitats, but most species are adapted to seasonal climates with pronounced dry and wet periods. Southern Africa has the highest diversity, followed by the Mediterranean region (Stedje, 1996). The Fynbos (Cape area) and succulent Karoo regions of southern Africa have the highest species diversity. The molecular analyses of Pfosser and Speta (1999) and Manning *et al.* (2004) resulted in the recognition of four monophyletic clades in the family Hyacinthaceae. These four clades are treated as subfamilies Oziroeoideae, Urgineoideae, Ornithogaloideae and Hyacinthoideae (Pfosser and Speta, 1999, 2001; Manning *et al.*, 2004). Alternatively, Hyacinthaceae is nested within Asparagaceae *sensu lato* and can be treated as subfamily Scilloideae. Hyacinthaceae are monophyletic within Asparagaceae and the subfamilies mentioned above are then treated as tribes Hyacintheae, Ornithogaleae, Oziroëeae and Urgineeae (e.g. APG III, 2009; Chase *et al.*, 2009). Here we use Hyacinthaceae at the family level. Subfamily Oziroeoideae is restricted to South America and consists of five species. The number of species in subfamily Urgineoideae is >100 (Manning *et al.*, 2004), and Ornithogaloideae consists of 200–300 species (Manning *et al.*, 2009; Martínez-Azorín *et al.*, 2011); the remaining 400+ species form Hyacinthoideae. As most of the sub-Saharan African representatives of Hyacinthaceae occupy early branching positions in the phylogenetic analyses (Pfosser and Speta, 1999, 2001; Pfosser *et al.*, 2003, 2006; Manning *et al.*, 2004; Martínez-Azorín *et al.*, 2011), it is commonly accepted that the whole family evolved from that region. Except for Oziroeoideae, all subfamilies exhibit a bimodal distribution pattern. A primary centre of diversity is located in sub-Saharan Africa, and a secondary centre of diversity occurs in the northern hemisphere around the Mediterranean, extending, at least for subfamily Hyacinthoideae, as far as East Asia. This split between northern and predominantly southern hemisphere taxa is most pronounced in subfamily Hyacinthoideae, which has been further subdivided into tribes Hyacintheae (northern hemisphere) and Massonieae (southern hemisphere, Madagascar, Arabia and India) (Pfosser and Speta, 1999; Pfosser *et al.*, 2003; Wetschnig and Pfosser, 2003).

Pseudoprospero Speta occupies an early branching position in the phylogenetic tree and is sister to Massonieae and Hyacintheae. If *Pseudoprospero* is excluded from Massonieae, then molecular data suggest that Massonieae and Hyacintheae evolved independently (Pfosser *et al.*, 2003). Tribe Massonieae is monophyletic upon the exclusion of genus *Pseudoprospero* (Pfosser *et al.*, 2003), and it has been suggested that *Pseudoprospero* should be placed into a tribe of its own (Wetschnig *et al.*, 2002; Pfosser *et al.*, 2003). This led to the creation of a third monotypic tribe Pseudoprospereae (Manning *et al.*, 2004).

Hyacinthaceae and its subfamilies (except Oziroeoideae), like many other plant lineages, show a disjunct distribution pattern. The Rand flora pattern is the best example of plant disjunction between the flora of the Mediterranean region/western Asia/north-west Africa and sub-Saharan Africa (Sanmartín *et al.*, 2010). Vicariance and dispersal hypotheses have been proposed to explain the origin of this disjunct distribution pattern. According to the first hypothesis, due to aridification (Sahara region), the widespread African flora underwent partial extinction and created the current pockets of distribution of extant species. The dispersal hypothesis suggests long-distance dispersal among these regions, followed by local diversification. Two dispersal routes, southward and northward, have been proposed. The source area of the southward route is either the Mediterranean region or western Asia and the dispersal is directed toward sub-Saharan Africa (Levyns, 1964), whereas the northward route is directed from sub-Saharan Africa to the Mediterranean region (Galley *et al.*, 2007).

The results of biogeographical histories of Hyacinthoideae obtained by S-DIVA and BBM analyses are presented here. The aims of this study are to find the possible ancestral range of Hyacinthoideae and to identify factors responsible for the current distribution pattern.

MATERIALS AND METHODS

Taxon sampling and outgroup selection

This analysis is based on material of subfamily Hyacinthoideae published in an earlier paper (Wetschnig *et al.*, 2007). The *trnL-F* region is composed of the *trnL* (UAA) intron and the intergenic spacer (IGS) between the *trnL* (UAA)-3′ exon and the *trnF* (GAA) gene. In many groups of plants, the intron region evolves more slowly than the spacer region (Gielly and Taberlet, 1994, 1996; Kita *et al.*, 1995; Gielly *et al.*, 1996). The different evolving rates of these regions are helpful in providing useful information; slowly evolving regions support the older divergences and quickly evolving regions provide resolution among closer relatives (McDade and Moody, 1999). In this study, we include 59 taxa (58 ingroups and one outgroup). Voucher information for all plant accessions, geographical origin and EMBL database accession numbers are provided in the Appendix. *Oziroe acaulis* is closely related to Hyacinthoideae and was selected as the outgroup in this analysis. Nomenclature follows that of the most recent nomenclatural synopsis available for a particular group of taxa. In particular, we adopted the nomenclature of Speta (1998*a*, *b*) for tribe Hyacintheae of subfamily Hyacinthoideae, and that of Manning *et al.* (2004) for all other taxa, with the exception of the genera *Ledebouria*, *Drimiopsis* and *Resnova*, which have later been treated as separate genera by Lebatha *et al.* (2006).

Phylogenetic analysis

Clustal X (Jeanmougin *et al.*, 1998) was used for DNA sequence alignment with a pairwise multiple alignment parameter. On average, <1 % of data matrix cells were scored as missing data and the region with ambiguous alignment was excluded in this analysis. Phylogenetic analysis using MP was performed in PAUP version 4·0b10 (Swofford, 2002). MP analyses were performed either without or with successive character weighting (rescaled consistency index) until tree

lengths remained the same in two successive rounds. Most-parsimonious trees were obtained by 1000 replicates of random sequence addition using tree bisection-reconnection (TBR) branch swapping under the Fitch criterion (Fitch, 1971). Confidence limits for the resulting tree topologies were assessed by 10 000 fast bootstrap replicates (Felsenstein, 1985) and the jackknife algorithm (50 % deletion). The Bayesian analysis was conducted in MrBayes 3·1 (Hülsenbeck and Ronquist, 2001; Ronquist and Hülsenbeck, 2003) under the GTR + I + G model. MrModeltest version 2·2 (Nylander, 2004) was used to select the best fit model under the Akaike information criterion. The Markov chain Monte Carlo chains were run simultaneously for 1000 000 generations. Trees were sampled every 100 generations. The first 1000 trees were eliminated (burn-in) and the remaining trees were used to construct a 50 % majority rule consensus tree with posterior probability (PP) distribution. One of the post-burn trees was drawn using Mesquite software (Maddison and Maddison, 2010), shown in Fig. 1A. The tree topologies obtained by Bayesian and parsimony analyses are similar (no significant differences). The Bayesian tree with Bayesian PP and parsimony bootstrap (BS) values is shown in Fig. 1A.

Dating the tree

The lack of a fossil record is a major constraint in the estimation of the ages of Hyacinthaceae and its subfamilies. However, we used the Bayesian analyses to date the tree with BEAST v1·6·1 (Drummond *et al.*, 2002; Drummond and Rambaut, 2007) under the hypothesis of the molecular clock based on the general substitution rates of the plastid sequence ($u = 1·0 \times 10^{-9}$ s s^{-1} year^{-1}; Zurawski *et al.*, 1984). Ten million generations of the MCMC chains were run, sampling every 1000 generations. Convergence of the stationary distribution was checked by visual inspection of plotted posterior estimates using the software Tracer v1·5 (Rambaut and Drummond, 2007). After discarding the first 1000 trees as burn-in, the samples were summarized in the maximum clade credibility tree using TreeAnnotator v1·6.1 (Drummond and Rambaut, 2007) with the PP limit set to 0 and summarizing mean node heights. The results were visualized using Figtree v1·3.1 (Rambaut, 2009).

Biogeographical analysis

The distribution range of Hyacinthoideae plus *Oziroe* was divided into eight areas, based on the presence of one or more endemic species, as shown in Fig. 1C. These areas are: A (India), B (South America), C (Madagascar), D (sub-Saharan Africa), E (Mediterranean region), F (Western Asia), G (Europe) and H (eastern Asia). In this analysis due to a lack of samples from eastern Africa, we treated sub-Saharan Africa as a single unit.

We used recently developed S-DIVA and BBM analyses implemented in RASP to reconstruct the possible ancestral ranges of subfamily Hyacinthoideae on the phylogenetic trees. In these methods, the frequencies of an ancestral range at a node in ancestral reconstructions are averaged over all trees (Yan *et al.*, 2010). To account for uncertainties in phylogeny, we used 9000 trees from MCMC output and ran S-DIVA on all of them. The number of maximum areas was kept as 2. The possible ancestral ranges at each node on a selected tree were obtained. BBM analysis was also conducted in a similar way. The MCMC chains were run simultaneously for 5000 000 generations. The state was sampled every 100 generations. Fixed JC + G (Jukes-Cantor + Gamma) were used for BBM analysis with null root distribution. The maximum number of areas for this analysis was kept as 6. The ancestral ranges obtained by BBM analysis are shown in Fig. 2.

RESULTS

The aligned matrix consisted of 1613 characters, 282 of which were excluded from this analysis. Of the remaining 1331 characters, 1070 were constant, 119 were autapomorphic and 142 were potentially parsimony-informative. The mean G + C content was 33·22 %. The pairwise divergence estimate was 0–6·18 %.

More than 1000 equally parsimonious trees had 418 steps, consistency index (CI) = 0·72, retention index (RI) = 0·86 and rescaled consistency index (RC) = 0·62. Without uninformative characters the parsimony trees had 290 steps, CI = 0·60, RI = 0·86 and RC = 0·51.

PP and BS support for the majority of clades were >0·95 and >90 %, respectively, thus showing high support for the tree topology. The tree topology also reflects the classification of subfamily Hyacinthoideae into a strictly northern hemisphere tribe Hyacintheae, a southern hemisphere tribe Massonieae and a monotypic sub-Saharan African tribe Pseudopropereae. Within the Hyacinthoideae clade, three sub-Saharan African, two Mediterranean, one southern hemisphere and one Eurasian subclade can be distinguished.

S-DIVA suggests a complex biogeographical history in which dispersal and vicariance have been vital in the shaping of the current distribution pattern in Hyacinthoideae. S-DIVA postulates 18 dispersals, the majority of which are located on the backbone of the tree. S-DIVA suggests two possible ancestral ranges, D (sub-Saharan Africa) and DE (sub-Saharan Africa + Mediterranean region), for node 116 and the occurrence of these ranges are 84·91 and 12·21 %, respectively (Fig. 1A). S-DIVA postulates that the ancestors of Hyacinthoideae originated in sub-Saharan Africa (optimal area reconstruction at basal node 116). This node suggests an early dispersal to the Mediterranean region, as shown in Fig. 3. The possible ancestral ranges at node 115 are DE and DH, the frequency of occurrence of these ranges being 82·81 and 17·17 %, respectively, with 68 % Bayesian support value. The most favoured ancestral range at node 115 is DE (sub-Saharan Africa + Mediterranean). A vicariance event is evident at this node, resulting in the Eurasian and sub-Saharan African lineages.

Node 83 represents members of tribe Hyacintheae, and the possible ancestor range at this node is EH (Mediterranean + eastern Asia) with 100 % marginal probability. This node suggests a vicariance event. One descendant remained in eastern Asia and another lineage underwent *in situ* diversification in the Mediterranean region. This *in situ* diversification resulted in two Mediterranean subclades. The ancestors of the node (82) of terminals 36–59 originated in the Mediterranean
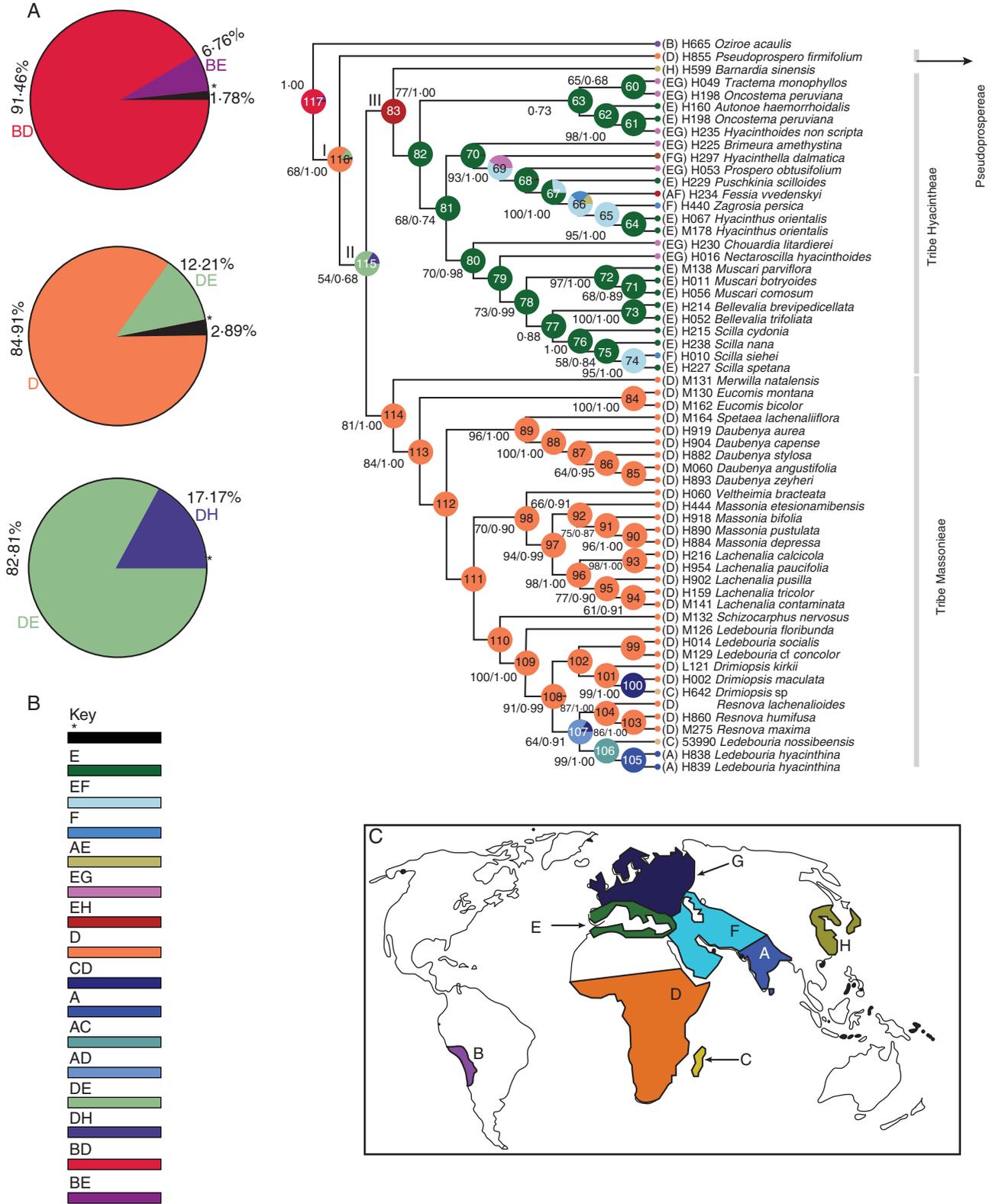
FIG. 1. Graphical output from S-DIVA (RASP). (A) Graphical results of ancestral distributions at each node of the phylogeny of subfamily Hyacinthoideae obtained by S-DIVA. Alternative ancestral ranges of nodes 117, 116 and 115 (with frequency of occurrence) are shown in pie chart form. Bootstrap support values (50 % and higher) and Bayesian credibility values (PP) are indicated above the pie chart on one of the post-burn Bayesian trees. (B) Colour key to possible ancestral ranges at different nodes; black with an asterisk represents other ancestral ranges. (C) Biogeographical regions: A, India; B, South America; C, Madagascar; D, sub-Saharan Africa; E, Mediterranean; F, Western Asia; G, Europe; H, Eastern Asia.
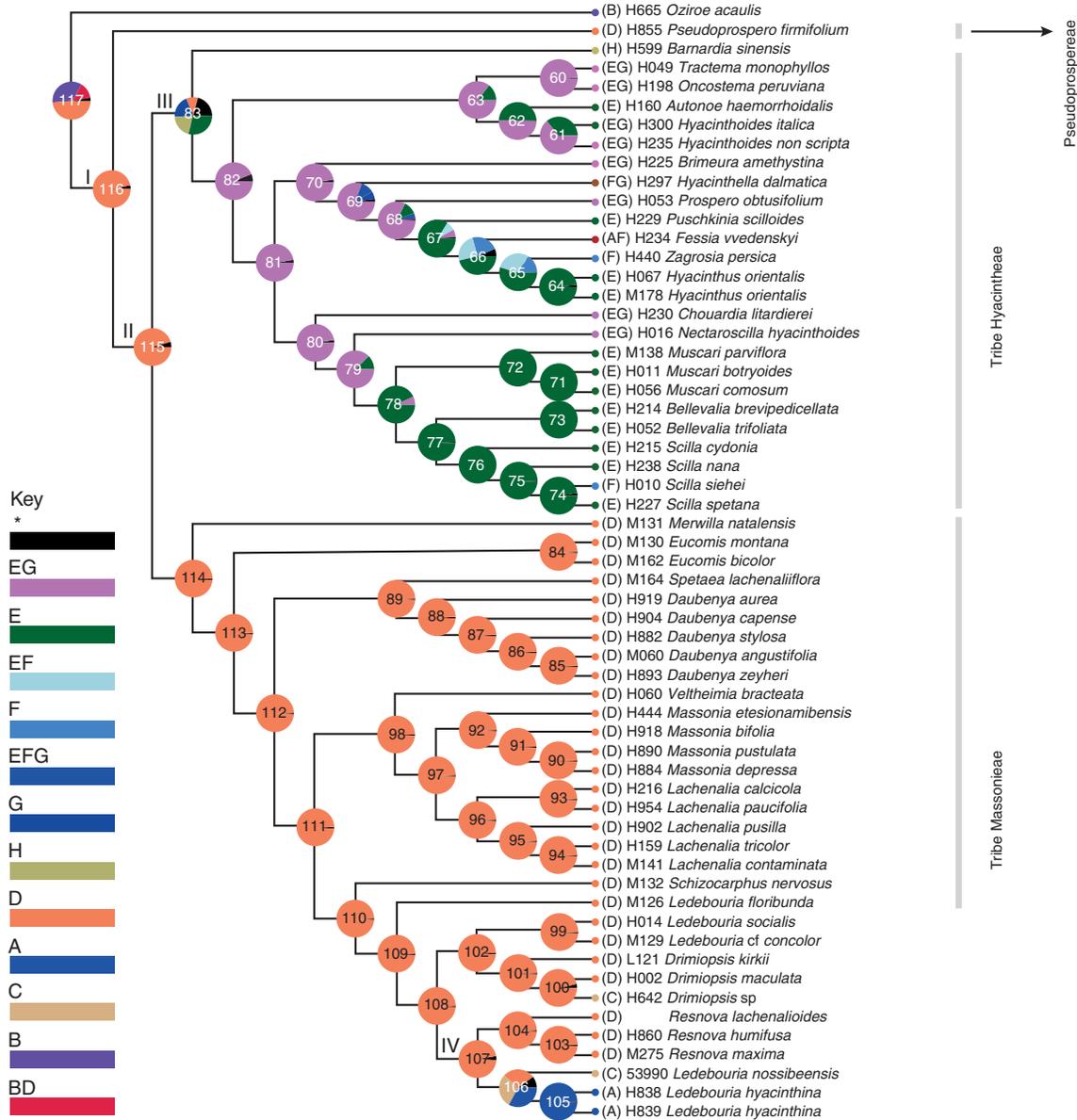
FIG. 2. Graphical output from BBM analysis (exported from RASP). Graphical results of ancestral distributions at each node of the phylogeny of subfamily Hyacinthoideae obtained by BBM analysis. Pie charts at each node show probabilities of alternative ancestral ranges. Colour key to possible ancestral ranges at different nodes; black with an asterisk represents other ancestral ranges.

region (E) with 100 % marginal probability (Fig. 1A); however, the Bayesian support value for this node is low (35 %). This is followed by a series of dispersals to Europe and western Asia. One dispersal event occurred from western Asia to India. These dispersals resulted in a Eurasian subclade.

Node 114 of terminals 3–14 includes the taxa of tribe Massonieae. The ancestors of the node of terminals 3–14 originated in sub-Saharan Africa (D) with 100 % marginal probability. The members of this tribe underwent local radiation in sub-Saharan Africa. The ancestral area reconstruction at node 100 suggests sub-Saharan Africa (D) + Madagascar (C) as ancestral areas with 100 % support value; the probability of this result is 100 %. This probably indicates a trans-oceanic

dispersal to Madagascar, with the source area of this dispersal being sub-Saharan Africa.

Node 107 of terminals 11–14 has two possible ancestral ranges, AD and CD. The most favoured ancestral range at this node is AD (India + sub-Saharan Africa) with 83·45 % marginal probability, indicating a second event of transoceanic dispersal (Fig. 3). The ancestral reconstruction at the next node is AC (India + Madagascar), suggesting another transoceanic dispersal. S-DIVA suggests that no dispersal occurred from sub-Saharan Africa to western Asia. Dispersals occurred to western Asia from the Mediterranean region. Similarly, no dispersal occurred from the Mediterranean region and western Asia to sub-Saharan Africa.
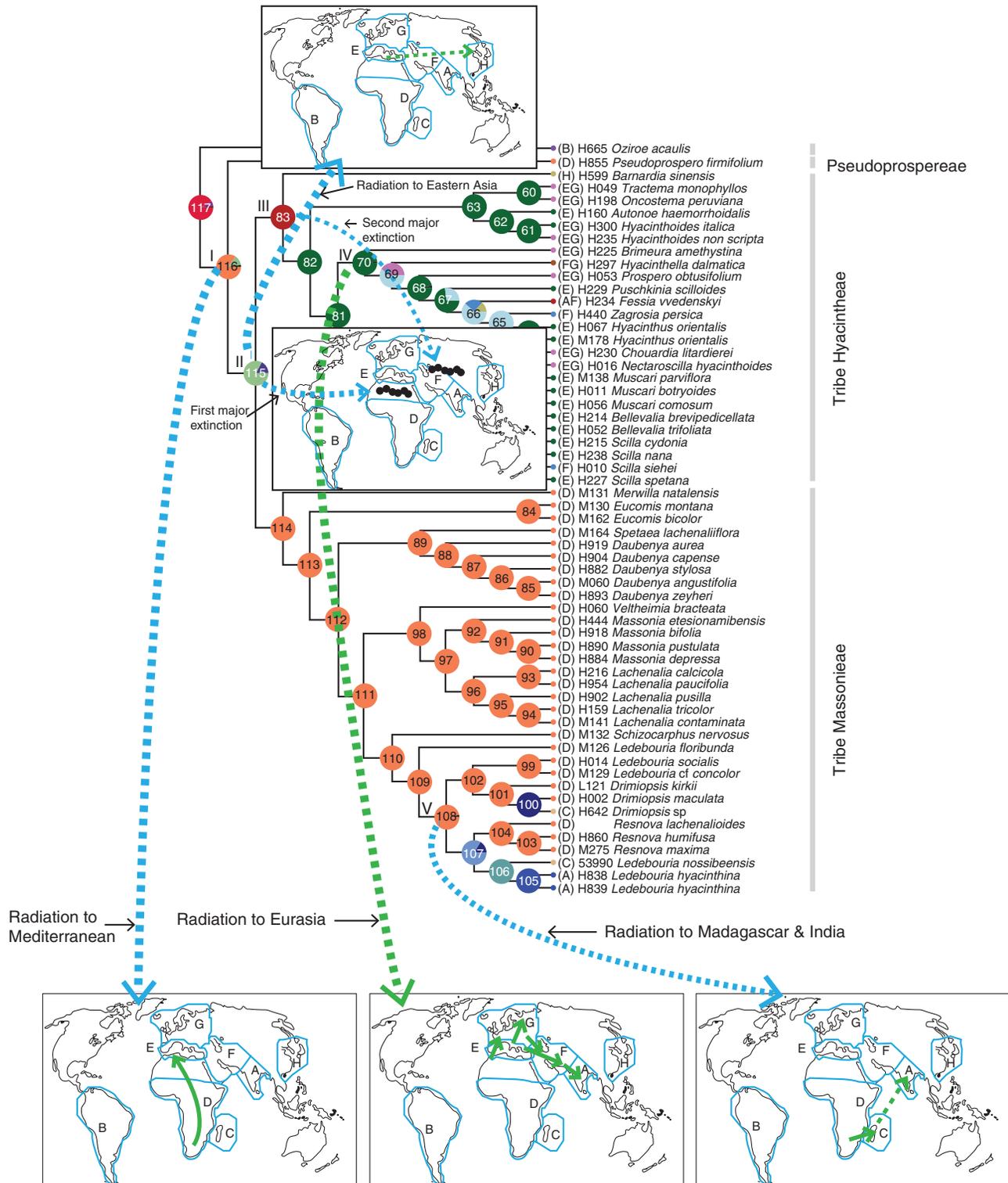
FIG. 3. Major radiation and extinction events in subfamily Hyacinthoideae. Major radiations from southern Africa and the Mediterranean region are shown on the blank world maps.

BBM analysis suggests slightly different ancestral ranges at basal nodes (Fig. 2). Node 116 represents all members of subfamily Hyacinthoideae. BBM analysis postulates that the ancestors of subfamily Hyacinthoideae originated in area D

(sub-Saharan Africa). The marginal probability for D is 96·63 %. Similarly, at node 115, the ancestral reconstruction indicates that sub-Saharan Africa is the ancestral area of terminals 14–35, with 95·06 % marginal probability. BBM

analysis suggests that the ancestors of Hyacinthoideae underwent *in situ* diversification in sub-Saharan Africa, as evident from ancestral ranges at basal nodes.

The ancestral reconstruction at node 83 is ambiguous and suggests four possible ancestral ranges, E, H, G and D. The occurrence of these ranges is: E, 29·00 %; H, 20·41 %; G, 19·67 %; and D, 10·07 %. BBM analysis suggests three dispersal events (internode leading to node 83) to eastern Asia, the Mediterranean region and Europe. Dispersal to the Mediterranean region occurred from sub-Saharan Africa. It is not clear whether dispersal to Europe and eastern Asia occurred from sub-Saharan Africa or the Mediterranean region. This node also suggests a vicariance event and one descendant underwent *in situ* diversification in EG (Mediterranean region + Europe), followed by dispersal to western Asia and India.

The ancestral reconstruction at node 106 is ambiguous, suggesting a number of possible ancestral ranges. Three ranges, A (India), C (Madagascar) and D (sub-Saharan Africa), had higher marginal probabilities of 32·43, 29·17 and 28·63 %, respectively. This suggests two transoceanic dispersals (Fig. 3).

DISCUSSION

Several biogeographical inferences can be made from this analysis. S-DIVA suggests that the ancestors of Hyacinthoideae originated in range D (sub-Saharan Africa, 84·91 %). This was followed by an early dispersal to the Mediterranean region indicated by the ancestral ranges at node 115. The favoured ancestral range at node 115 is DE (82·81 %), and the support value and this node also suggests a vicariance event between sub-Saharan Africa and the Mediterranean. Aridification of the Sahara region from the Miocene onward could explain this vicariance event, which resulted in the partial extinction of the widespread African flora and the creation of the current pockets of distribution of extant species. A similar vicariance pattern has been suggested within *Adenocarpus* (Fabaceae) (see Sanmartín *et al.*, 2010). The age of this event based on dated tree material in Fig. 4 is 17·92 Ma, consistent with the accelerated African aridification in the early Miocene due to the uplift of the continent and the formation of the East African Rift Valley (Axelrod, 1972; Baker *et al.*, 1972).

BBM analysis suggests that Hyacinthoideae originated in sub-Saharan Africa (D) with marginal probability >96 % (node 116). Dispersal to the Mediterranean region and eastern Asia took place early in the history of Hyacinthoideae (nodes 115 and 83). A similar pattern of sub-Saharan Africa origin and dispersal to the Mediterranean region has been suggested within *Androcymbium* (Colchicaceae) (del Hoyo *et al.*, 2007; Sanmartín *et al.*, 2010) and *Senecio flavus* (Coleman *et al.*, 2003). The arrival of members of Hyacinthaceae in the Mediterranean region would have been possible in the Oligocene/Miocene through north-west Africa (Pfosser and Speta, 2004). The dated tree in Fig. 4 suggests that Hyacinthoideae originated in the early Miocene (19 Ma) and arrived in the Mediterranean region between 19 and 18 Ma. Further northward movement was not possible due to the large Tethys Ocean (Rögl, 1998,

1999) barrier. The formation of the Gomphotherium land bridge (19 Ma) allowed the free exchange of floras and faunas between Africa and Eurasia (Rögl, 1998). The tree data in Fig. 4 suggests that dispersal to Eurasia took place about 15·3 Ma.

This single colonization from sub-Saharan Africa to the Mediterranean region, followed by rapid diversification and movements, resulted in the monophyletic Eurasian tribe Hyacintheae (Pfosser *et al.*, 2003; Wetschnig and Pfosser, 2003). *Pseudoprospero* occupies an early branching position in Hyacinthoideae in most of the *trnL-F* trees and is sister to the rest of the subfamily.

Barnardia sinensis occupies the earliest branching position in tribe Hyacintheae and suggests an early evolution of this genus. Its presence at an early diverging branch indicates that its ancestor was among the first members to colonize the Mediterranean region. Pfosser and Speta (1999) suggested that *Barnardia* was distributed from northern Africa to eastern Asia. *Barnardia sinensis* is found in Korea, Japan, China and Russia, whereas *Barnardia numidica* is distributed in northern Africa and the Balearic Islands. However, it is now believed that *B. numidica* is not related to *Barnardia* (data not shown) and should be transferred to a genus of its own.

S-DIVA suggests EH (Mediterranean + eastern Asia) as a possible ancestral range at node 83 with a 100 % marginal probability, and the most favoured ancestral range at node 115 is DE. Thus, S-DIVA suggests dispersal from the Mediterranean region to eastern Asia. BBM analysis suggests ambiguous ancestral ranges at node 83, but the Mediterranean is the most favoured ancestral range at this node. Node 83 also suggests a vicariance event between the Mediterranean and eastern Asia. Geological and climatic changes (aridification and the uplift of Tibet) were instrumental in the extinction of widespread flora and created the Mediterranean–eastern Asia disjunct distribution pattern. The aridification of central Asia and North China occurred between 22·0 and 6·2 Ma (Guo *et al.*, 2002). The dated tree in Fig. 4 suggests that this extinction event occurred about 13·74 Ma. A similar Mediterranean–eastern Asia disjunct distribution pattern has been suggested within *Helleborus* (Ranunculaceae) (Sun *et al.*, 2001).

According to S-DIVA, one descendant underwent local radiation in the Mediterranean region, followed by a series of dispersals to Europe (G), western Asia (F) and India (A). BBM analysis suggests an early dispersal to Europe. *In situ* diversification took place in these areas and then dispersals occurred to western Asia and India. Only one dispersal event was directed to India via western Asia. The Mediterranean region acted as a source area for these dispersals toward Europe and western Asia in tribe Hyacintheae.

The node (114) of terminals 3–14 represents all members of tribe Massonieae. Both S-DIVA and BBM analysis suggest D (sub-Saharan Africa) as the ancestral range at this node, with 100 and 99·05 % marginal probability, respectively. The sub-Saharan African ancestors underwent local radiation and *in situ* diversification within sub-Saharan Africa. Three transoceanic dispersals occurred in Massonieae to Madagascar and India. *Ledebouria nossibeensis* (Madagascar) shows strong affinity to *L. hyacinthina* from India and to members
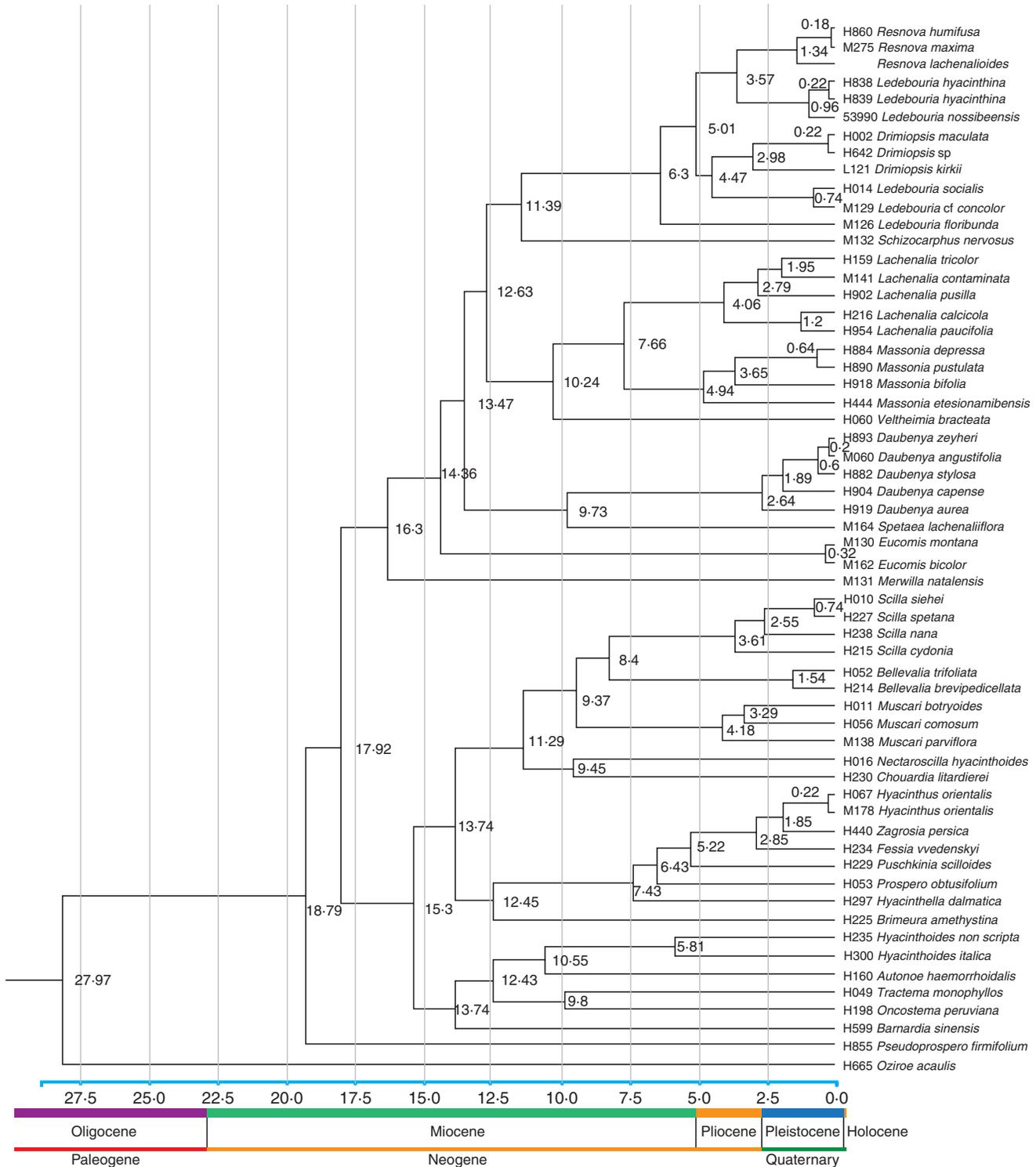
FIG. 4. Molecular clock based on the general substitution rates of the plastid sequence. Divergence age is shown below each node. Geological epoch is shown below the tree.

from the Arabian Peninsula, such as *L. grandifolia* from Socotra. The monophyly of *L. nossibeensis* from the north of Madagascar with *L. hyacinthina* from India is well supported (99/98 % bootstrap support and PP of 1·00).

BBM analysis suggests different ancestral ranges at nodes 107 and 106. The ancestral reconstruction at node 107 suggests sub-Saharan Africa as the sole ancestor area. The most recent common ancestors of nodes 106 and 104 are distributed in

sub-Saharan Africa. Discussing multiple overseas dispersals in amphibians, Vences *et al.* (2003) mentioned that the important part of the Malagasy fauna was the result of dispersal from Africa. This dispersal was probably facilitated by currently submerged islands via a stepping-stone mechanism (McCall, 1997). Important examples of long-distance dispersal between Africa, Madagascar and the Seychelles are provided by multiple lineages of frogs (Vences *et al.*, 2003, 2004), chameleons (Raxworthy *et al.*, 2002), snakes (Nagy *et al.*, 2003) and lemurs (Yoder and Yang, 2004). Slow climbing lorises distributed in Africa and Asia provide another striking example of dispersal between Africa and Asia (Masters *et al.*, 2005). A number of recent studies have attempted to explain the plant disjunction in Africa, Madagascar and Asia. The stepping-stone mechanism, birds capable of long-distance flight and monsoon trade winds coupled with oceanic currents are important tools of dispersal. Dispersal by a stepping-stone mechanism occurred through the Seychelles, Comoros and Chagos Archipelago between Africa and Asia. Diaspore dispersal and successful speciation occurred in both directions. Dispersal from Africa to Asia has been suggested in *Osbeckia* (Melastomataceae) (Renner and Meyer, 2001; Renner, 2004), *Gaertnera* (Rubaceae) (Malcomber, 2002), *Exacum* (Gentianaceae; from Madagascar to Asia) (Yuan *et al.*, 2005), *Cucumis* (Renner *et al.*, 2007) and tribe Sonerileae (Melastomataceae; from Africa to Madagascar and Asia) (Renner, 2004). Dispersal has been suggested from Asia to Africa in *Uvaria* (Annonaceae) (Richardson *et al.*, 2004), *Bridelia* (Phyllanthaceae) (Li *et al.*, 2009), and *Macaranga* and *Mallotus* (Euphorbiaceae) (Kulju *et al.*, 2007).

The second route of dispersal was opened after the collision of the African and Eurasian plates. In the Miocene, the collision of the African and Eurasian plates was followed by a series of palaeogeographical reorganizations in the circum-Mediterranean region (Rögl, 1998; Scotese *et al.*, 1988). Finally, a new dispersal route was established in the early Pliocene (5 Ma) between Africa and south-western Asia via the Arabian Peninsula and the Levant region (Thompson, 2000; Fernandes *et al.*, 2006) due to the closing of the Red Sea to the Mediterranean. Extensive faunal exchange occurred through this route between Africa and Eurasia (Vrba, 1993; Cox and Moore, 2005; Nylander *et al.*, 2008). The dispersal route between Africa and Eurasia via the Arabian Peninsula and the Levant region was used by the ancestors of current Campanulaceae (Roquet *et al.*, 2009) and other plants (Mummenhoff *et al.*, 2001; Oberprieler, 2005; Inda *et al.*, 2008; Mansion *et al.*, 2008).

Birds are considered to be important tools of dispersal. Birds that can cross the 400-km Mozambique Channel include the Madagascan squacco heron, the Madagascan cuckoo, the Madagascan pratincole, the broad-billed roller and the Mascarene martin (Moreau, 1966; Renner, 2004). The first three species spend winters in eastern Africa and the last one is a casual visitor (Moreau, 1966; Renner, 2004).

S-DIVA and BBM analyses suggest two independent transoceanic dispersals to Madagascar from the source area of sub-Saharan Africa. According to S-DIVA reconstruction, African ancestors colonized the Mediterranean region (115), Madagascar (nodes 106 and 100) and India (node 107).

BBM analysis suggests that African ancestors could have colonized Eurasia (node 83), Madagascar and India (node 106).

S-DIVA suggests early dispersal to the Mediterranean region in Hyacinthoideae, followed by a vicariance event (due to aridification of the Sahara region) between sub-Saharan Africa and the Mediterranean region, which resulted in sub-Saharan African and the Mediterranean lineages. A southward dispersal route is not plausible for members of Hyacinthoideae because the results of S-DIVA and BBM analyses suggest that sub-Saharan Africa is the ancestral area of the subfamily. Results also suggest an early dispersal to the Mediterranean region, and thus the northward route of dispersal is plausible for members of the subfamily. A similar dispersal route was shown for *Androcymbium* (Colchicaceae) (Sanmartín *et al.*, 2010). In the present study, no samples from eastern Africa were available; therefore, we treated the region south of Sahara as a single block (sub-Saharan Africa). The absence of taxa from eastern Africa will surely have had impact on the results because without representation from this area, a northward route of dispersal would not be clarified. Sanmartín *et al.* (2010) suggest that southern Africa has highest dispersal rates with eastern Africa, while eastern Africa has highest dispersal rates with north-west Africa (Macaronesia). Therefore, sub-Saharan Africa was treated as a single block in this study, to understand the northward dispersal scenario from southern Africa to the Mediterranean region in the absence of taxa from the important eastern African region. In their latest study on Ornithogaloideae, Martínez-Azorín *et al.* (2011) included taxa from eastern Africa, which nested within predominantly southern African taxa, probably suggesting dispersal from southern Africa to eastern Africa. The formation of the eastern African mountains was instrumental in the migration of some southern African lineages to eastern Africa via the Grand Rift and the Drakensberg mountains, which resulted in the eastern African endemic flora (Linder, 2005; Galley *et al.*, 2007; Sanmartín *et al.*, 2010). No nuclear gene data are available for this study; however, Martínez-Azorín *et al.* (2011) included the ITS region of nuclear ribosomal DNA in their study of Ornithogaloideae, a closely related group to Hyacinthoideae. They found the position of two clades were different in the phylogenetic tree based on the ITS region compared with the combined tree. Whether we include or exclude a nuclear region, sub-Saharan Africa is the ancestral area, but inclusion of this region will be helpful to explain some other dispersal and vicariance scenarios.

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APPENDIX

List of taxa investigated in this study, with vouchers, citation information and EMBL accession numbers

Species	Voucher	Locality	EMBL acc. no(s).
Autonoe haemorrhoidalis (Webb & Berthel) Speta	Klenner H160	Spain	AJ232518/AJ2326411
Barnardia scilloides Lindl.	Pfosser H599	Korea	AJ507998
Bellevalia brevipedicellata Turrill	Jahn H214	Greece	AJ232547/AJ2326701
Bellevalia trifoliata Kunth	Speta H052	Greece	AJ232548/AJ2326711
Brimeura amethystina (L.) Chouard	Pfosser H225	cult. ex B.G. Tallinn	AJ232510/AJ2326331
Chouardia litardierei (Breistr.) Speta	Pfosser H230	Croatia	AJ232541/AJ2326641
Daubenya angustifolia (L.f.) A.M.van der Merwe & J.C.Manning	Wetschnig 1101	South Africa	AJ507960
Daubenya aurea Lindl.	Wetschnig 1162	South Africa	AJ507956
Daubenya capensis (Schltr.) A.M.van der Merwe & J.C. Manning	Wetschnig 1129	South Africa	AJ507955
Daubenya stylosa (W.F.Barker) A.M.van der Merwe & J.C.Manning	Wetschnig 1160	South Africa	AJ507957
Daubenya zeyheri (Kunth) J.C.Manning & A.M.van der Merwe	Wetschnig 1153	South Africa	AJ507961
Drimiopsis maculata Lindl. & Paxton	Speta H002	cult. Linz	AJ232502/AJ2326251
Drimiopsis sp.	Pfosser H642	Madagascar	AJ507953
Drimiopsis kirkii Baker	Pfosser L121	cult. B.G. Vienna	AJ507952
Eucomis bicolor Baker	Schnabel M162	South Africa	AJ507933
Eucomis montana Compton	Schnabel M130	South Africa	AJ507932
Fessia vvedenskyi (Pazij) Speta	Speta H234	Uzbekistan	AJ232535/AJ2326581
Hyacinthella dalmatica (Baker) Chouard	Gutermann H297	Croatia	AJ232526/AJ2326491
Hyacinthoides italica (L.) Rothm.	Pfosser H300	France	AJ232519/AJ2326421
Hyacinthoides non-scripta (L.) Chouard ex W.Rothmaler	Pfosser H235	France	AJ232524/AJ2326471
Hyacinthus orientalis L.	Speta H067	Romania	AJ232539/AJ2326621
Hyacinthus orientalis L. var. *alba*	J. Plass M178	Syria	AJ508002
Lachenalia calcicola (U.Müll.-Doblies & D.Müll.-Doblies) J.C.Manning & Goldblatt	Müller-Doblies H216	South Africa	AJ232506/AJ2326291
Lachenalia contaminata (Soland.)	Pfosser M141	South Africa	AJ507985
Lachenalia paucifolia (W.F.Barker) J.C.Manning & Goldblatt	Wetschnig 1154	South Africa	AJ507990
Lachenalia pusilla Jacq.	Wetschnig 1115	South Africa	AJ507986
Lachenalia tricolor Jack. ex Murray	Pfosser H159	cult. B.G. Vienna	AJ232508/AJ2326311
Ledebouria cf. *concolor* (Baker) Jessop	Wetschnig 1412	South Africa	AJ507946
Ledebouria floribunda (Baker) Jessop	Wetschnig 1433	South Africa	AJ507937
Ledebouria hyacinthina Roth	Jha H838	India	AJ507944
Ledebouria hyacinthina Roth	Jha H839	India	AJ507945
Ledebouria nossibeensis (H.Perrier) J.C.Manning & Goldblatt	Andriantiana 53990	Madagascar	AN109129
Ledebouria socialis (Baker) Jessop	Pfosser H014	cult. B.G. Vienna	AJ232501/AJ2326241
Massonia bifolia (Jacq.) J.C.Manning & Goldblatt	Wetschnig 1130	South Africa	AJ507966
Massonia depressa Houtt.	Wetschnig 1142	South Africa	AJ507980
Massonia etesionamibensis (U.Müll.-Doblies & D.Müll.-Doblies) J.C.Manning & Goldblatt	Lavranos & Pehle-mann H444	Namibia	AJ232504/AJ2326271
Massonia pustulata Jacq.	Wetschnig 1148	South Africa	AJ507970
Merwilla natalensis (Planch.) Speta	Wetschnig 1534	South Africa	AJ507931
Muscari botryoides (L.) Mill.	Kleesadl H011	Austria	AJ232545/AJ2326681
Muscari comosum (L.) Mill.	Neuner H056	Italy	AJ232546/AJ2326691
Muscari parviflorum Desf.	J. Plass M131	Syria	AJ508003
Nectaroscilla hyacinthoides Parl.	Scheiblreiter H016	Portugal	AJ232542/AJ2326651
Oncostema peruviana (L.) Speta	Pfosser H198	Portugal	AJ232516/AJ2326391
Oziroe acaulis (Baker) Speta	Weigend s.n.	Peru	AJ507921
Prospero obtusifolium (Poir.) Speta	HC H053	Morocco	AJ232529/AJ2326521
Pseudoprospero firmifolium (Baker) Speta	Wetschnig 1322	South Africa	AJ507928
Puschkinia scilloides Adams var. *libanotica*	Pfosser H229	Lebanon	AJ508688
Resnova humifusa (Baker) U.Müll.-Doblies & D.Müll.-Doblies	Wetschnig 1524	South Africa	AJ507942
Resnova lachenalioides (Baker) Van der Merwe	Lebatha 019 (PUC)	South Africa	DQ313330
Resnova maxima Van der Merwe	Hankey M275	South Africa	AJ507943
Schizocarphus nervosus (Burch.) Van der Merwe	Saunders M132	South Africa	AJ507936

Scilla cydonia Speta	Jahn *et al.* H215	Greece	AJ232549/AJ2326721
Scilla nana (Schultes f.) Speta	Speta H238	Greece	AJ232552/AJ2326751
Scilla siehei (Stapf) Speta 'Pink Giant'	Speta H010	cult. Linz	AJ232551/AJ2326741
Scilla spetana Kereszty	Speta H227	Austria	AJ232556/AJ2326791
Spetaea lachenaliiflora Wetschnig & Pfosser	Saunders M164	South Africa	AJ507954
Tractema monophyllos (Link) Speta	Raus H049	Spain	AJ232513/AJ2326361
Veltheimia bracteata Harv. ex Baker	Speta H060	cult. Linz	AJ232503/AJ2326261
Zagrosia persica (Hausskn.) Speta	Leep H440	Turkey	AJ232537/AJ2326601