



Insights into Neogene Mediterranean biogeography based on phylogenetic relationships of mountain and lowland lineages of *Narcissus* (Amaryllidaceae)

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ABSTRACT

Aim Our aims were: (1) to reconstruct the phylogenetic relationships of daffodils (*Narcissus*), focusing on the lowland subgenus *Hermione* and the mountain section *Apodanthi*; (2) to estimate the temporal setting of diversification; (3) to reconstruct the migration patterns of the lineages; and (4) to examine the microevolutionary differentiation of the wide-ranging *Narcissus tazetta* group across the Mediterranean.

Location The Mediterranean Basin.

Methods Plastid (*trnT-L*, *trnL-F* and *ndhF*) sequences were obtained from 63 populations representing 23 species of *Narcissus* and combined with published data from 16 species. Phylogenetic relationships and dating were inferred by Bayesian analysis based on geological events and divergence estimates of closely related taxa. A dispersal–extinction–cladogenesis analysis was performed using maximum likelihood methods to infer ancestral geographical distributions, and phylogeographical reconstruction was performed using coalescence analysis.

Results Subgenus *Hermione* is not recognized as a monophyletic group because two of the nine species were found to have a close relationship with the subgenus *Narcissus*. The results on section *Apodanthi* confirmed previous findings of its monophyly and phylogenetic relationships within this mountain group. Molecular dating and ancestral range reconstructions suggest that the ancestor of *Narcissus* originated in the Iberian Peninsula during the Late Oligocene–Early Miocene. Eastward expansion of the lineage range proceeded from the western Mediterranean and involved colonization of mountain ranges in northern Africa. The phylogeography of the *N. tazetta* group revealed a widespread distribution of certain haplotypes, suggesting wide dispersal and a high level of colonization in the Mediterranean Basin.

Main conclusions Our study points to the role of three key historical events in *Narcissus* diversification: tectonic shifts of the Alboran domain in the western Mediterranean, the Messinian salinity crisis, and the onset of the Mediterranean climate followed by periods of repeated glaciation. Diversification of section *Apodanthi* probably resulted from allopatric speciation, while subgenus *Hermione* may have shown more sympatric speciation and high dispersal, despite the lack of apparent adaptations to long-distance dispersal. This is best exemplified by the presence of both ancestral and recent haplotypes of *N. tazetta* across the Mediterranean.

Keywords

Alboran domain, dispersal, Mediterranean Basin, Mediterranean palaeogeography, Messinian salinity crisis, *Narcissus*, phylogeography, Strait of Gibraltar, vicariance.

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INTRODUCTION

The Mediterranean Basin is characterized by a heterogeneous geomorphological and environmental mosaic and displays high plant species richness and endemism (Thompson, 2005). Proposed evolutionary forces driving speciation of the Mediterranean flora are the strong spatio-temporal variations in soils, a complex geomorphological history (mountains, straits, isthmuses, plate dynamics and island formation) and climate variation (e.g. Pleistocene glaciations) (Pignatti, 1978; Cowling *et al.*, 1997; Thompson, 2005).

There has been a great deal of interest in the patterns of diversification and speciation in Mediterranean plants, particularly of narrow-range endemic species. Traditionally, studies of these processes have been based on morphology and karyotype variation (Stebbins & Dawe, 1987; Dahlgren, 1991; Petit & Thompson, 1999; Mansion *et al.*, 2005). Moreover, the relative timing of the underlying evolutionary processes has been subjectively determined from the assumed triggering effects of palaeoenvironmental events, without proper tests. Typically, the underlying evolutionary processes can be inferred with a fair degree of accuracy when they are recent, i.e. linked to Pleistocene changes since the original onset of a Mediterranean-type climate, in which extinction has played a minor role, and complete clades can be examined (e.g. Vargas, 2003; Kropf *et al.*, 2006). For older taxa subject to more ancient processes, the reconstruction of a biogeographical history is more complicated. Although palaeobotanical and palaeoclimatological research has provided additional insights for taxa well represented in the fossil record (e.g. lauroid vegetation, widespread in the Late Tertiary in the Mediterranean; see Rodríguez-Sánchez & Arroyo, 2008, and references therein), for most taxa the necessary information is fragmentary or lacking.

Recently, the development of molecular phylogenies and dating, together with available knowledge of Mediterranean palaeogeography (Krijgsman *et al.*, 1999b; Rosenbaum *et al.*, 2002; Meulenkaamp & Sissingh, 2003; Garcia-Castellanos *et al.*, 2009), has begun to enable the estimation of clade divergence times on longer time-scales. However, the scarcity of studies still precludes any generalization about temporal patterns of plant diversification in the region (Cowling *et al.*, 2009). Most studies show that genera originated in the second half of the Tertiary, with radiation occurring at diverse times thereafter, in the Eocene–Miocene for some Araceae (Mansion *et al.*, 2008), in the Pleistocene for *Cistus* (Guzmán & Vargas, 2009) or even in the Late Pleistocene for *Nigella* (Bittkau & Comes, 2009). These differences may depend on the particular geographical distributions of the ancestral groups, as well as their life history (Kay *et al.*, 2006; Smith & Donoghue, 2008), vegetative or floral traits (Valente *et al.*, 2010), ecological requirements and hybridization (Paun *et al.*, 2005; Lo Presti & Oberprieler, 2009; Yesson *et al.*, 2009). All these factors must be understood within a common historical scenario framed by the palaeogeographical dynamics of the region. It should be noted that although hybridization is a common process among

plants, particularly in the Mediterranean (Thompson, 2005), it is possible to trace the biogeographical history of the lineages with the use of maternally inherited DNA, such as plastid and mitochondrial sequences (Rieseberg & Ellstrand, 1993). Plants expand their ranges through colonization, which requires previous dispersal of seeds or vegetative parts. Thus, the plastid genome, usually transmitted in angiosperms through seeds and vegetative propagules, but almost never through the pollen, is particularly appropriate for depicting the migration processes of lineages (Petit *et al.*, 2007). Hence, the vast majority of biogeographical and phylogeographical studies rely on cytoplasmic markers (Avice, 2000).

Given the limited number of studies, inferences about Mediterranean biogeography are strongly dependent on a careful selection of key taxa circumscribed to the Mediterranean Basin in order to reflect its historical processes (Yesson & Culham, 2006; Mansion *et al.*, 2008; Rodríguez-Sánchez *et al.*, 2009). A representative life-form in the Mediterranean Basin is the geophytic one (Dafni *et al.*, 1981; Shmida, 1981; Parsons & Hopper, 2003) with 1335 species (Procheş *et al.*, 2006). The geophyte life history provides for summer dormancy in a Mediterranean climate (Raunkjær, 1934; Thompson, 2005; Procheş *et al.*, 2006). Despite the high representation of geophytes in the current Mediterranean flora and their potential for illustrating its evolutionary history, there are few evolutionary studies with a proper temporal account of geophyte origin and diversification (Caujapé-Castells *et al.*, 2001; Yesson & Culham, 2006; Mansion *et al.*, 2008; del Hoyo *et al.*, 2009). An important factor that affects the reliability of molecular dating of geophytes is that the plant fossil records are poor, particularly for the Amaryllidaceae. However, calibration for the geophytes may rely on other temporal information such as geological events and divergence estimates of more internal nodes of the angiosperm phylogeny, which make time constraints similarly reliable (del Hoyo *et al.*, 2009).

Narcissus L. (daffodils; Amaryllidaceae) is a typical Mediterranean genus of geophyte, with 60–80 species (Fernandes, 1975; Dorda & Fernández Casas, 1989; Blanchard, 1990; Mathew, 2002), although some authors have raised this figure to c. 150 species (e.g. Haworth, 1831). The distribution of the genus is largely restricted to the Mediterranean region, and its centre of diversity occurs in the Iberian Peninsula and north-western Africa (Valdés *et al.*, 1987; Arroyo, 2002; Fernández Casas, 2002). *Narcissus* species cover a wide variety of habitats including different elevations, bioclimatic areas and substrates, and have diverse geographical ranges from narrow-range endemic species to widespread ones. These characteristics make *Narcissus* a good model for testing hypotheses about the role of the complex Mediterranean evolutionary history in the diversification of a rich and diverse group of species.

Here, we studied the phylogenetic relationships of *Narcissus* with special emphasis on the lowland subgenus *Hermione* and the mountain section *Apodanthi* which together show different ecogeographical characteristics. Our objectives were: (1) to infer the phylogenetic relationships of species in subgenus

Hermione, subgenus *Narcissus* (section *Apodanthi*), and representatives of the other taxonomic groups in the subgenus *Narcissus*; (2) to estimate the dates of lineage divergence based on geological events and divergence timing of closely related monocots; (3) to analyse whether range expansion–contraction events have been predominantly responsible for the current distribution area of the species; and (4) to reconstruct the phylogeography of one of the most widespread species groups of these daffodils, the *Narcissus tazetta* group. Finally, we discuss all these results in the light of the available palaeogeographical and climatic information for the Mediterranean floristic region.

MATERIALS AND METHODS

Study group

Numerous attempts at a *Narcissus* classification have been published, but the genus has never been properly monographed (but see Haworth, 1831 and Pugsley, 1933). Problems faced by taxonomists are mostly related to the biological features of *Narcissus*, such as continuous speciation via incomplete morphological differentiation across populations of some species, hybridization and polyploidy (Fernandes, 1968, 1975; Webb, 1980; Brandham & Kirton, 1987; Díaz Lifante & Andrés Camacho, 2007). Chromosome studies carried out by Fernandes (1951, 1968, 1975) have allowed a better understanding of the nature of hybrids, many of them being virtually sterile (e.g. Marques *et al.*, 2010). We have followed the taxonomic classification made by Fernandes (1975) with some modifications based on Valdés *et al.* (1987), Dorda & Fernández Casas (1989) and Fernández-Casas (2008).

Fernandes (1951, 1967, 1975) recognized two subgenera, *Narcissus* and *Hermione* (Haw.) Spach (*Hermione*), and 10 sections in *Narcissus*; recently one more section was accepted by Fernández-Casas (2008) (see Appendix S1 in Supporting Information). Four sections are circumscribed in *Hermione*: *Serotini* Parl., *Aurelia* (J. Gay) Bak., *Angustinii* (Fernández-Casas) and *Tazettae* DC. (= *Hermione* (Salisb.) Spreng.). Section *Tazettae* is subdivided into three subsections: the monospecific subsection *Angustifolii* A. Fernandes with *Narcissus elegans*, the subsection *Dubii* Fernández-Casas with *Narcissus dubius* and *Narcissus tortifolius*, and the subsection *Hermione*. The latter includes many species of dubious taxonomic value because of little morphological differentiation (Fernandes, 1975; Blanchard, 1990). For example, we considered *Narcissus panizzianus*, *Narcissus polyanthos* and *Narcissus barlae* together within *Narcissus papyraceus* Ker-Gawler based on morphological data from Valdés *et al.* (1987) and Pérez-Barrales (2005). Within the subgenus *Narcissus* seven sections are recognized: *Tapeinanthus* Traub (one species), *Apodanthi* Fernandes (eight species largely restricted to the mountains of Morocco, Spain and Portugal; see Fig. 1), *Jonquilla* (six species), *Bulbocodium* DC. (three species), *Ganymedes* (Haw.) Schult. F. (one species), *Pseudonarcissus* DC. (13 species) and *Narcissus* (one species).

Palaeogeography of the study area

Available evidence suggests that key palaeogeographical events in the western Mediterranean occurred as follows. The Betic (Iberian) and the Rif (Moroccan) cordilleras, denominated as the Alboran domain, formed a continuous Alpine orogenic belt together with the Kabylies (Algeria), Calabria, Corsica and Sardinia. From the Late Oligocene (*c.* 30 Ma) this plate started a south-eastward rotation with migration to the east, due to collision between the African plate and the Iberian microplate (Rosenbaum *et al.*, 2002; Meulenkamp & Sissingh, 2003). By 23 Ma, the component parts of the plate had become separated from each other, except for the Alboran domain that remained attached to the Iberian Peninsula until 12 Ma (see Fig. 2, Map 3), creating a corridor between Iberia and Africa (Rosenbaum *et al.*, 2002). The Messinian salinity crisis (5.96–5.33 Ma) initiated by the tectonic uplift of the Strait of Gibraltar, which cut the sea connection to the Atlantic (see Fig. 2, Map 5), together with a climatic aridification, involved a drying up of the basin (Krijgsman *et al.*, 1999a; Krijgsman, 2002). More recently, the onset of the Mediterranean climate in the Pliocene and the climatic oscillation during Pleistocene glaciations, with the consequent sea-level variations (Yokoyama *et al.*, 2000), have been the most important events.

Taxa sampling

Our sample aimed to represent the diversity of the two *Narcissus* subgenera and, in particular, to include all the species of *Narcissus* section *Apodanthi* and all the species of the sections of *Narcissus* subgenus *Hermione*. As a result, 9 of the 11 *Narcissus* sections were sampled (see Appendix S1).

We collected leaf material from species throughout the entire geographical range of subgenus *Hermione* and section *Apodanthi* (Portugal, Spain, France, Italy, Greece, Cyprus, Israel and Morocco; see Fig. 1). In total, 63 populations comprising 23 species of *Narcissus* were sampled (see Appendix S1). Most of the populations collected belonged to subgenus *Hermione*, sections *Serotini* (two populations), *Aurelia* (four populations), *Angustinii* (two populations) and *Tazettae* (36 populations) (see Fig. 3 for reference to population numbers). Additional samples represented the eight species of *Apodanthi* (11 populations) and one species from each of the sections *Jonquillae* (three populations), *Bulbocodium* (one population), *Tapeinanthus* (one population) and *Ganymedes* (two populations). Given that many *Narcissus* species are cultivated for ornamental purposes and may have escaped, we avoided sampling those populations in close proximity to apparent past or present human settlements. To maximize the robustness of our phylogenetic reconstruction, we also included sequences downloaded from GenBank for 20 species from different sections of the subgenus *Narcissus* which were not collected in our sampling design. *Sternbergia lutea* (L.) Kerl Gaw. ex Spreng., *Sternbergia colchiciflora* Waldst. & Kit., *Lapiedra martinezii* Lag. and *Pancratium canariense* Ker Gawl. were chosen as the outgroups for the Bayesian analysis

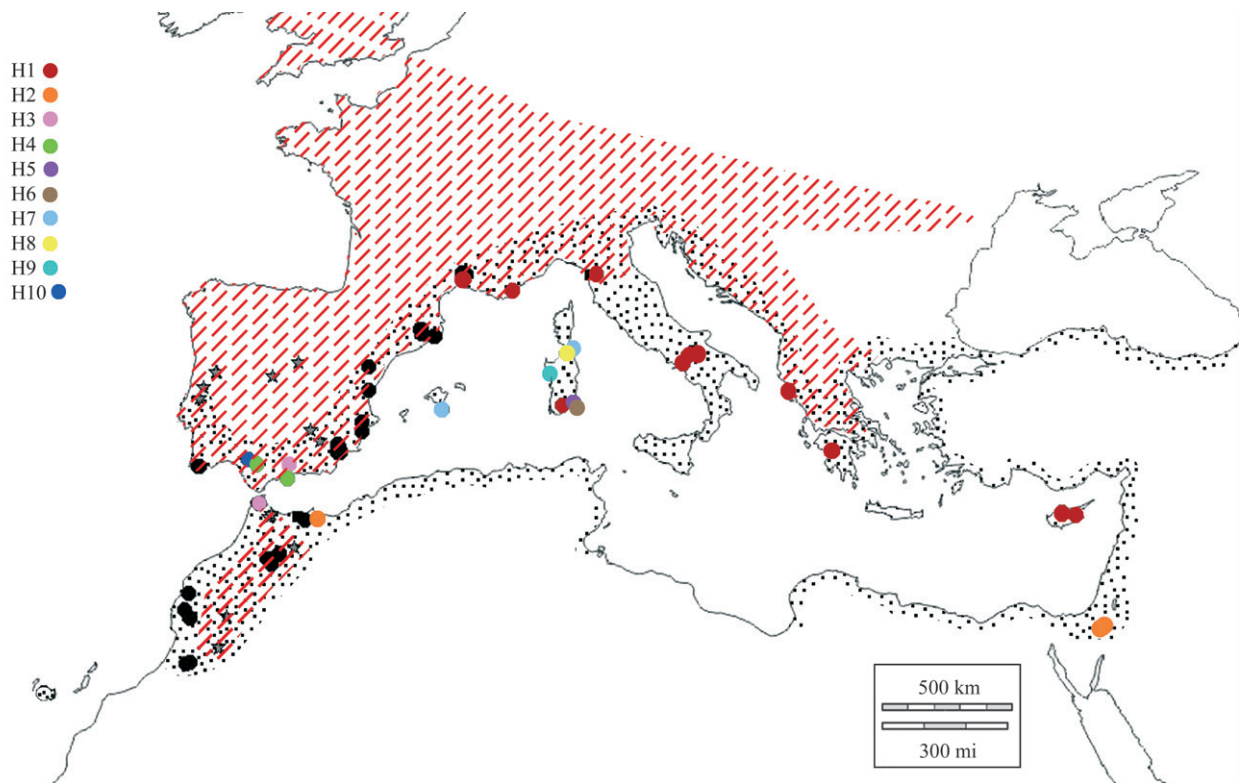


Figure 1 The distribution and location of sampled populations of *Narcissus*. Only two species (*N. poeticus* and *N. pseudonarcissus*) reach the north-easternmost part of the distribution range. The darker, dotted area corresponds to the range of subgenus *Hermione* and the red dashed area corresponds to subgenus *Narcissus*. Circles represent locations of samples used for the phylogenetic study (black) and common haplotypes (H1–H10) (see Fig. 6). Stars represent the locations of section *Apodanthi*.

based on previous phylogenetic analysis of the Amaryllidaceae (Meerow *et al.*, 2006). For the analysis of divergence time estimates, we also included 18 species belonging to the Amaryllidaceae to enable setting calibration points. See Appendix S1 for the geographical origin and GenBank accession numbers of the samples.

DNA amplification and sequencing

Genomic DNA was extracted from silica-dried leaves using the DNeasy Plant Mini Kit (Qiagen Inc., Chatsworth, CA, USA). The entire *trn* (*trnT*–L and *trnL*–F) and *ndhF* (3'-end) regions were amplified by polymerase chain reaction (PCR) with the primers designed by Taberlet *et al.* (1991) [a and b for *trnT*(UGU)–*trnL*(UAA), e for *trnL*(UAA)–*trnF*(GAA)] and those designed by Olmstead & Sweere (1994), 2110F for *ndhF*. Thermocycling conditions for the *trn* regions were 2 min at 95 °C, followed by 30 cycles at 95 °C for 1 min, 50 °C for 2 min, and 72 °C for 1 min with a final extension at 72 °C for 2 min. For *ndhF* the conditions were 2 min at 95 °C, followed by 30 cycles at 95 °C for 1 min, a gradient temperature with a range of 45 to 55 °C, and 72 °C for 1 min with a final extension at 72 °C for 2 min. Amplified products were cleaned using spin filter columns (PCR Clean-up kit; MoBio Laboratories, Solan Beach, CA, USA) following the protocols provided by the manufacturer. Sequencing was performed

using dye terminators (BigDye Terminator v2.0, Applied Biosystems, Little Chalfont, UK) and run into polyacrylamide electrophoresis gels (70%) using an Applied Biosystems Prism model 3700 automated sequencer.

Sequence data analysis

Three different matrices were reconstructed, corresponding to the non-coding *trnT*–L and *trnL*–F regions and the *ndhF* protein-coding region. We aligned each matrix using MAFFT v. 6.0 with FFT-NS-I (a slow iterative refinement method) (Kato *et al.*, 2009); misalignments were corrected manually and poorly aligned positions were eliminated using GBLOCKS v. 0.91b (Castresana, 2000). Single matrix analyses were performed to check for congruence followed by combined analysis. Having confirmed congruence, the three matrices were concatenated into a single one comprising 87 accessions and 1991 positions with CONCATENATOR v. 1.1.0 (Pina-Martins & Paulo, 2008). Insertions and/or deletions (indels) in the *trnT*–L and *trnL*–F regions were identified (42 in total) and this information was coded and added to the data matrix following the method of Müller (2005) using the SEQSTATE program and INDELCODER software (<http://www.nees.uni-bonn.de/downloads/SeqState/>). We selected a substitution model using jMODELTEST v. 0.1 (Posada, 2008). Bayesian inference was performed by analysing the 87-sequence matrix

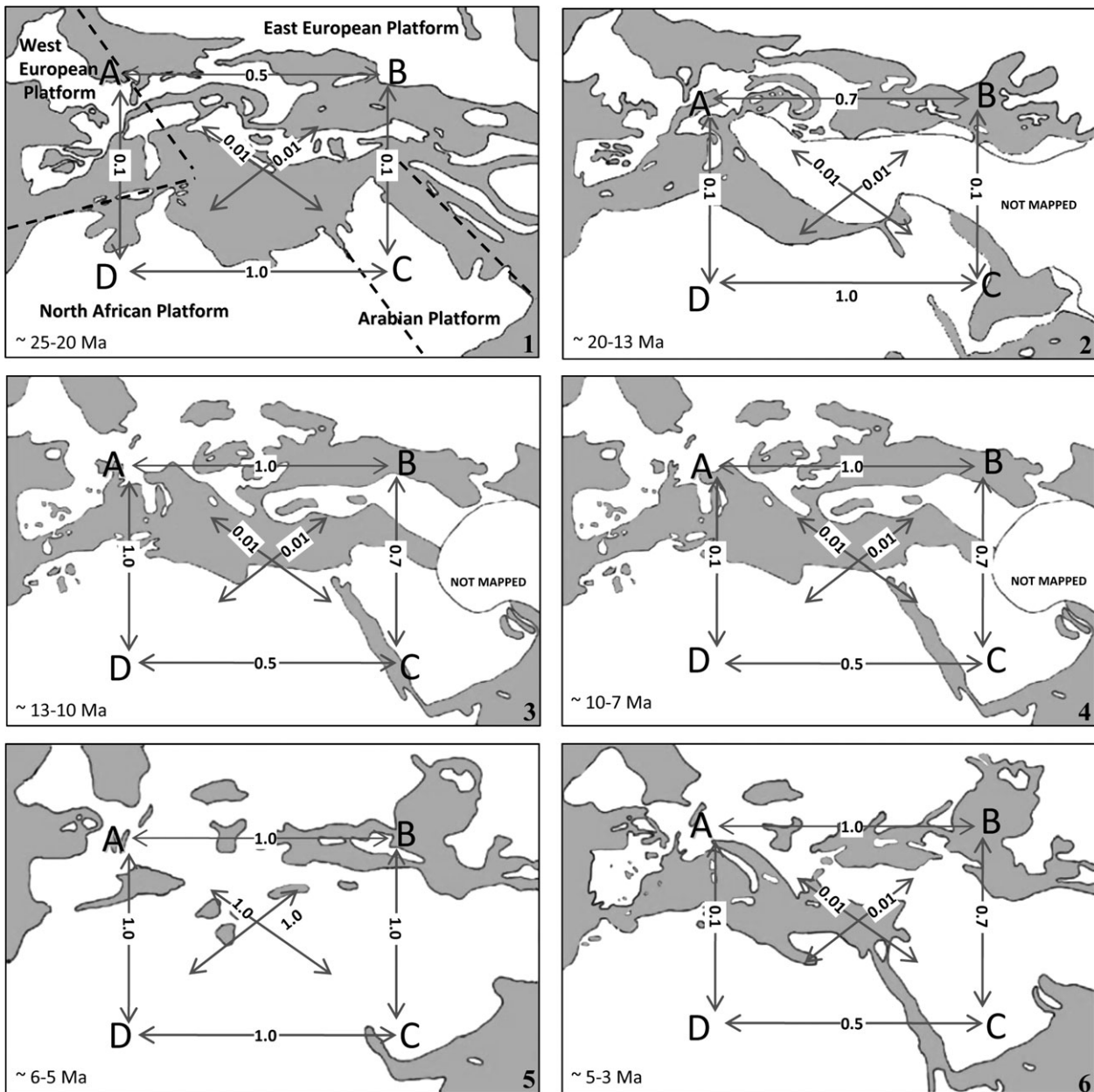


Figure 2 Palaeogeographical maps of the Mediterranean Basin representing a simplification of major tectonic events during Miocene based on Rosenbaum *et al.* (2002), Meulenkamp & Sissingh (2003) and Ree & Sanmartín (2009). Biogeographical areas used for ancestral reconstruction of *Narcissus* are: A, north-western Mediterranean (West European Platform); B, north-eastern Mediterranean (East European Platform); C, south-eastern Mediterranean (Arabian Platform); and D, south-western Mediterranean (African Platform). Arrows and numbers above indicate the dispersal rates between areas used for dispersal–extinction–cladogenesis (DEC) analysis (see Materials and Methods for details).

in MRBAYES v. 3.1.2 (Huelsenbeck & Ronquist, 2001) under a GTR+ Γ +I substitution model for DNA characters, whereas a F81-like model was implemented for indels setting the ascertainment bias to variable as suggested in the MRBAYES manual (Ronquist *et al.*, 2005). We ran two independent analyses with six chains each, for 5×10^6 generations sampling every 1000 generations. The first 25% of sampled trees were discarded to ensure convergence was reached and the remainder used to compute a consensus tree.

Divergence time estimates

Divergence time was analysed using a Bayesian approach as implemented in the program BEAST v. 1.5beta2 (Drummond & Rambaut, 2007). The analysis was performed on a 101-sequence matrix, of which 83 sequences belong to the genus *Narcissus* and 18 to the outgroup (see Appendix S1). We used GTR+ Γ +I as the evolutionary model. We allowed the program to estimate the mean substitution rate and divergence times.

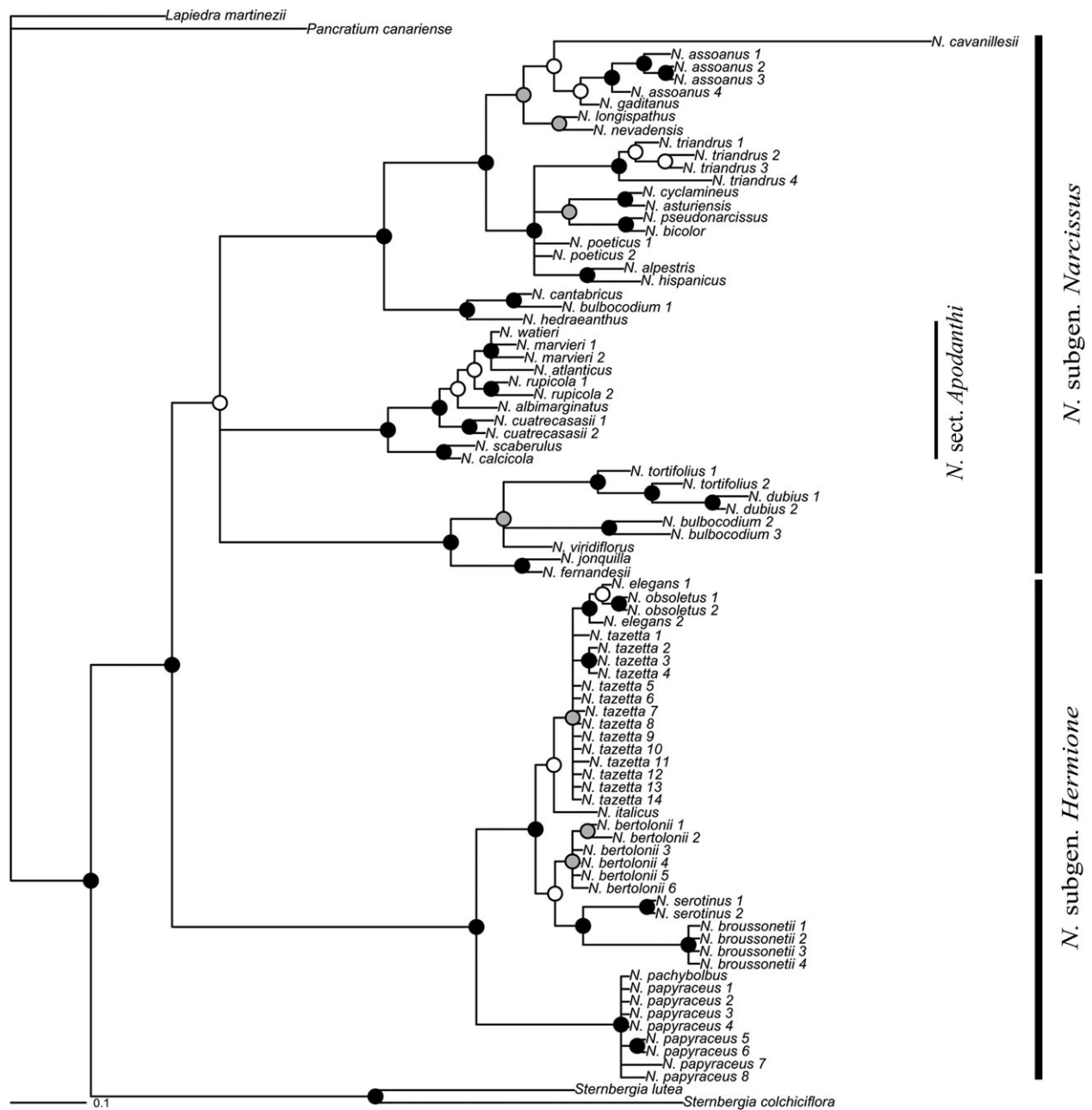


Figure 3 Bayesian phylogenetic tree of *Narcissus*, reconstructed using plastid (*trnL-F*, *trnT-L* and *ndhF*) sequences. Circles on the nodes show posterior probabilities (PP) from the Bayesian analysis under the GTR+ Γ +I model of DNA selected by jMODELTEST (Posada, 2008). Black circles: $95 \leq PP$; grey circles: $70 \leq PP < 90$; white circles: $PP < 70$. Population number follows those in Appendix S1. Scale bar indicates number of expected substitutions per site.

Model parameters to account for lineage-specific rate heterogeneity consisted of an uncorrelated relaxed lognormal clock, because preliminary analyses indicated that there was rate heterogeneity among lineages (ucl.d.stdev parameter > 1 ; Drummond & Rambaut, 2007). The analysis assumed a speciation Yule process, the most suitable tree prior for inferring relationships between species (Drummond & Rambaut, 2007). We used a consensus phylogeny from a Bayesian analysis (including the 101 sequences), instead of a random tree, as the starting tree in BEAST.

We used divergence estimates from an independent molecular dating study and one geological event. We applied two simultaneous calibration points to the outgroup (letters a and b in Fig. 4). First, the estimated age (33 ± 4.0 Ma) of the split between *Clivia* and *Hippeastrum* (Amaryllidaceae) as obtained by Wikström *et al.* (2001) (letter a in Fig. 4) and second, a 21 ± 1.0 Ma maximum age for the split between the endemic *Pancratium canariense* from the Canary Islands and two related species, *Pancratium zeylanicum* from the south of Asia and *Pancratium tenuifolium*, endemic to the western Sahara coast,

the closest continental territory to the Canary Islands (Maire, 1959) (letter b in Fig. 4). This corresponds to the age of the oldest island of the Canarian Archipelago (Fuerteventura; Balogh *et al.*, 1999), and thus the earliest available land mass for propagules dispersed from the African coast. However, recent investigations suggest a deeper time span of Canarian lineages could be possible via island-hopping from currently submerged islands (Fernández-Palacios *et al.*, 2011).

Bayesian posterior distributions for each parameter were obtained using Markov chain Monte Carlo (MCMC), running two independent analyses for 70 million generations, and sampling every 1000 generations. We altered the weight of the operators that work on the *treemodel* as recommended by Drummond *et al.* (2007). The performance of the analyses (convergence of the independent runs, effective sample sizes) were evaluated using TRACER v. 1.5 (Rambaut & Drummond, 2007a). The two independent BEAST runs were combined using LOGCOMBINER v. 1.5.3 (Rambaut & Drummond, 2007b) resampling each tree file at a frequency of 1:5000 trees. Mean and 95% highest posterior density (HPD) intervals of ages were then calculated from 25,200 post-burn-in trees using the software TREEANNOTATOR v. 1.5.4 (Rambaut & Drummond, 2007c). By default, TREEANNOTATOR only provides divergence dates for nodes with a posterior probability > 0.5 (Rambaut & Drummond, 2007c). Only a few nodes had a posterior probability lower than 0.5. Hence, to ensure all nodes in our tree were dated, we lowered the threshold value to 0.0.

Reconstruction of ancestral areas

The patterns of spatial and temporal distribution of *Narcissus* were inferred using dispersal–extinction–cladogenesis (DEC) likelihood analyses implemented in LAGRANGE v. 2.0.1 (Ree & Smith, 2008). This method does not assume a hierarchical organization of the biogeographical scenario, a property that is essential in regions with a complex palaeogeographical history as is the case in the Mediterranean Basin (Sanmartín, 2003; Oberprieler, 2005). Our selected method contrasts with event-based methods such as those implemented in dispersal–vicariance analysis (DIVA; Ronquist, 1996), which estimate ancestral area distribution minimizing the cost of vicariance over dispersal events. Despite its relevance in historical biogeography in the past, DIVA is limited by its dependence on parsimony (Ree & Sanmartín, 2009). In contrast, DEC is a continuous-time stochastic model for geographical range evolution in discrete areas, with maximum likelihood (ML) parameters estimated from dispersal rates between areas (range expansion) and local extinction within areas (range contraction), according to exponential rate parameters. The probability of dispersal and extinction events along any branch of the phylogeny is dependent on evolutionary time (Ree & Smith, 2008). Furthermore, the model allows dispersal rates between areas to be constrained based on palaeogeographical information and assumptions on the ability for long-distance dispersal of species.

We focused on Tertiary palaeogeography of the Mediterranean Basin to define the geographical areas in which *Narcissus* species originated, diversified and migrated. According to Meulenkamp & Sissingh (2003) four major plates reflect the main collision and splitting events during tectonics of the basin. As suggested by Ree & Sanmartín (2009), we selected four geographical areas (see Fig. 2): (1) north-western Mediterranean (West European Platform), (2) north-eastern Mediterranean (East European Platform), (3) south-eastern Mediterranean (Arabian Platform), and (4) south-western Mediterranean (African Platform). We selected only four areas because an oversplitting of geographical areas reduces phylogenetic signal of species ranges within a clade because fewer areas are likely to be shared by descent. Furthermore, dispersal events are likely to involve the same areas, reducing the power to detect general trends (Ree & Sanmartín, 2009). Finally, oversplitting of ranges reduces computational feasibility (Ree & Smith, 2008). We compared two DEC models (i.e. a null and an alternative) for the four areas (Fig. 2). The null model (unconstrained) assumes that the spatial arrangement of plates has no effect on biogeographical patterns of evolution, thus assuming that dispersal rates were equal between all geographical areas (i.e. setting all possible transitions to equal values). Implicitly, this model assumes high long-distance dispersal capability. The alternative model (constrained according to the palaeogeographical information, Fig. 2) assumes that geological evolution of the Mediterranean Basin influenced the connectivity of biogeographical areas and therefore patterns of dispersal/vicariance among ancestral areas. For this model, we modified the transition matrix setting lower values (0.1) for distant, unconnected areas, and maximal values (1) for adjacent or connected areas (Ree & Sanmartín, 2009). The implicit assumption here is that long-distance dispersal is limited. DEC analyses were performed on a cropped chronogram of the BEAST analysis, in which each taxon was represented by one population (except *Narcissus bulbocodium*) and *Sternbergia colchiciflora* and *S. lutea* were included as the outgroups. Maximum range sizes were restricted between non-adjacent areas (Western European Platform/Arabian Platform/East European Platform/North African Platform). Area optimizations were reported and considered significant only if the fraction of the global likelihood at each split exceeded 0.5. For all nodes with a relative probability value < 0.5, we selected the reconstructed ancestral area whose relative probability was three times the value of the other possible reconstructions.

Phylogeography of the *Narcissus tazetta* group

The statistical parsimony method (Templeton *et al.*, 1992) as implemented in the software rcs v. 1.21 (Clement *et al.*, 2000) was used to infer the haplotype relationships in the *N. tazetta* group. According to the phylogenetic results (see below), this group includes the following species: *N. tazetta*, *N. elegans*, *Narcissus obsoletus* and *Narcissus italicus*. Furthermore, we included sequences of the sister clade to the *N. tazetta* group (*Narcissus bertolonii*, *Narcissus serotinus* and *Narcissus*

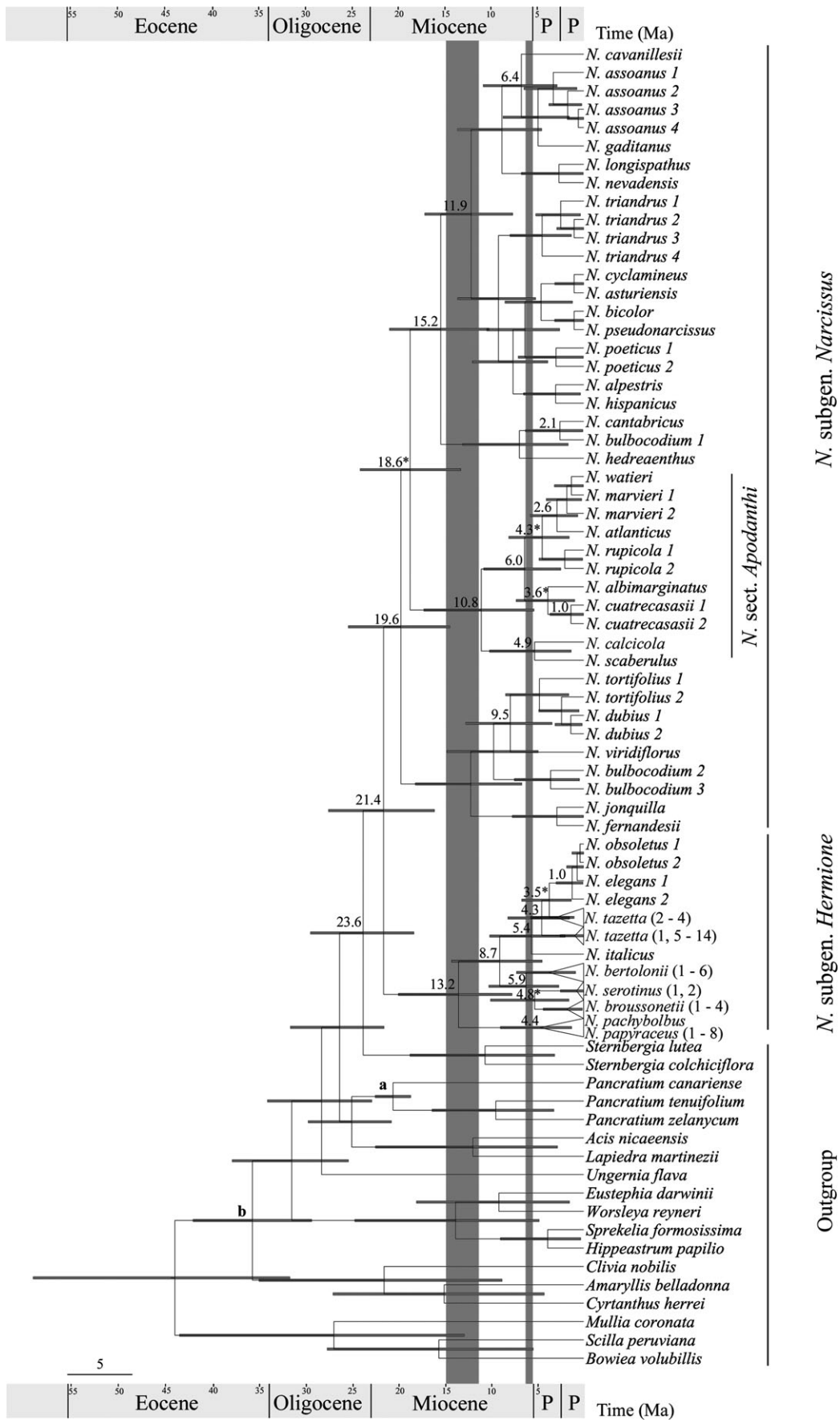


Figure 4 Bayesian molecular clock tree of 83 samples of *Narcissus* and outgroups using *trnL-F*, *trnT-L* and *ndhF* sequences. Nodes indicate mean age estimates (for simplicity we only show the node ages referred to in the paper) and 95% confidence intervals (grey bars). Asterisks show posterior probabilities lower than 0.5. Nodes a and b were used as calibration points for the estimates of divergence analysis (see Materials and Methods for details). Triangles represent the populations of a single taxon (except for the *N. papyraceus* populations and *N. pachybolbus*). P P, Pleistocene and Pliocene. Shaded bars indicate the two main palaeogeographical events: the Messinian salinity crisis (c. 6.0 Ma) and the tectonic events affecting the Alboran domain (c. 15–12 Ma). The scale bar represents million years ago (Ma).

broussonetii). We did not include *N. papyraceus* or *Narcissus pachybolbus* because the phylogenetic results clearly showed that these two species form a well-supported, separate clade. As a result, a final subset of 40 plastid sequences of seven species was analysed. We ran two independent analyses incorporating gaps as either a fifth character or non-informative. Haplotypes were mapped across the Mediterranean region and assigned to taxonomic identities.

RESULTS

Phylogenetic reconstruction

We obtained a total of 1991 bp divided into three plastid markers for most of the species in *Narcissus* as follows: 333 and 907 for *trnL-F* and *trnT-L*, respectively, and 751 for *ndhF*. Thirty-three per cent of the three DNA regions was found to be parsimony-informative. We obtained 42 indels that were coded as binary characters for the analysis. Finally, the combined 87-sequence matrix set included 2033 characters, except for the outgroup species for which we were able to obtain sequences from one or two regions only (*trnL-F* or *ndhF*). The analyses of the three chloroplast DNA (cpDNA) regions recovered congruent topologies under Bayesian criteria.

The trees derived from the 87-sequence matrix strongly supported [posterior probability (PP) = 100] the monophyly of the largest part of subgenus *Hermione*, except for *N. tortifolius* and *N. dubius* (Fig. 3). These two species formed a clade with species from subgenus *Narcissus* (section *Jonquilla* and section *Bulbocodium*, respectively). Thus, subgenus *Hermione* (hereafter *Hermione*) cannot be formally recognized as a monophyletic group. However, we considered that the clade of nine species (Fig. 3) should be assigned to this taxon because it retained the type species (*N. tazetta*) of the subgenus. *Hermione* formed a sister clade to the rest of *Narcissus*. A subclade of the widespread *N. papyraceus* and the North African narrow-range endemic *N. pachybolbus* branched off first. The populations of *N. papyraceus* and the sample from *N. pachybolbus* formed a polytomy because of high sequence identity (99.95%) even though they show evident morphological differences (R. Santos, unpublished data). The second subclade was more diverse and also subdivided into two subgroups. One (*N. tazetta* group) included all the samples of the widespread *N. tazetta* and *N. italicus* in a lineage sister to *N. elegans* and *N. obsoletus*. The other subclade was sister to the *N. tazetta* group and was formed by two subclades: one is represented by *N. bertolonii* from the islands of Sardinia (Italy) and Cabrera (Balearic Islands, Spain), and the second by

N. broussonetii (northern Africa) and *N. serotinus* (south-western Spain and north-western Morocco) (Fig. 3).

None of the sections sampled in subgenus *Narcissus* were monophyletic, except for section *Apodanthe*, which showed well-resolved relationships. Also, a highly supported clade (PP = 1.0) is formed by *N. bulbocodium* from Morocco, *Narcissus cantabricus* and *Narcissus hedraeanthus*, which form the section *Bulbocodium* previously described by Webb (1980), although two samples of *N. bulbocodium* (see Appendix S1 for origin of samples) were found within another clade (Fig. 2).

Estimating divergence times

Phylogenetic and divergence time analyses basically yielded identical topologies (no conflicting clades), except for the position of *Narcissus albimarginatus* (see Figs 3 & 4).

The western Mediterranean genus *Narcissus* apparently diverged from the Eurasian *Sternbergia* lineage in the Late Oligocene to Early Miocene, some time around 23.6 Ma (confidence interval: 29.3–18.1), and the basal split between subgenus *Narcissus* and *Hermione* occurred at about 21.4 Ma (27.4–16.1), when the North African and Arabian Platforms were connected (maps 1 and 2 in Fig. 2). The basal split within subgenus *Hermione* occurred during the Miocene 13.2 Ma (19.9–7.8), and a posterior diversification coincides with the Messinian period (5.9, 5.3 and 4.4. Ma for the three clades in this group) (maps 5 and 6 in Fig. 2) and continued at a rather constant rate during the Pliocene and Pleistocene (Fig. 4). The divergence estimate of the ancestor of *Apodanthe* remains unresolved (Fig. 3), although the first split within the section occurred during the middle Miocene (10.8 Ma) (Fig. 4), when the tectonics of the Alboran domain probably connected the African Platform with the Iberian Peninsula (maps 3 and 4 in Fig. 2) and most of the diversification took place after the Messinian salinity crisis had ended (5.33 Ma).

Reconstructing ancestral areas

Both constrained and unconstrained models yielded identical ancestral range reconstructions, although the global likelihood at the root under the unconstrained model provided a slightly better fit to the data (log-likelihood differences between models = 1.2; see Edwards, 1992). Basically, this result implies that the dispersal capacity of *Narcissus* may have been higher than previously assumed because the constrained model did not provide a better fit, nor did it influence ancestral area reconstruction results. The results (Fig. 5) suggest that the most recent ancestor of *Narcissus* was distributed in an area

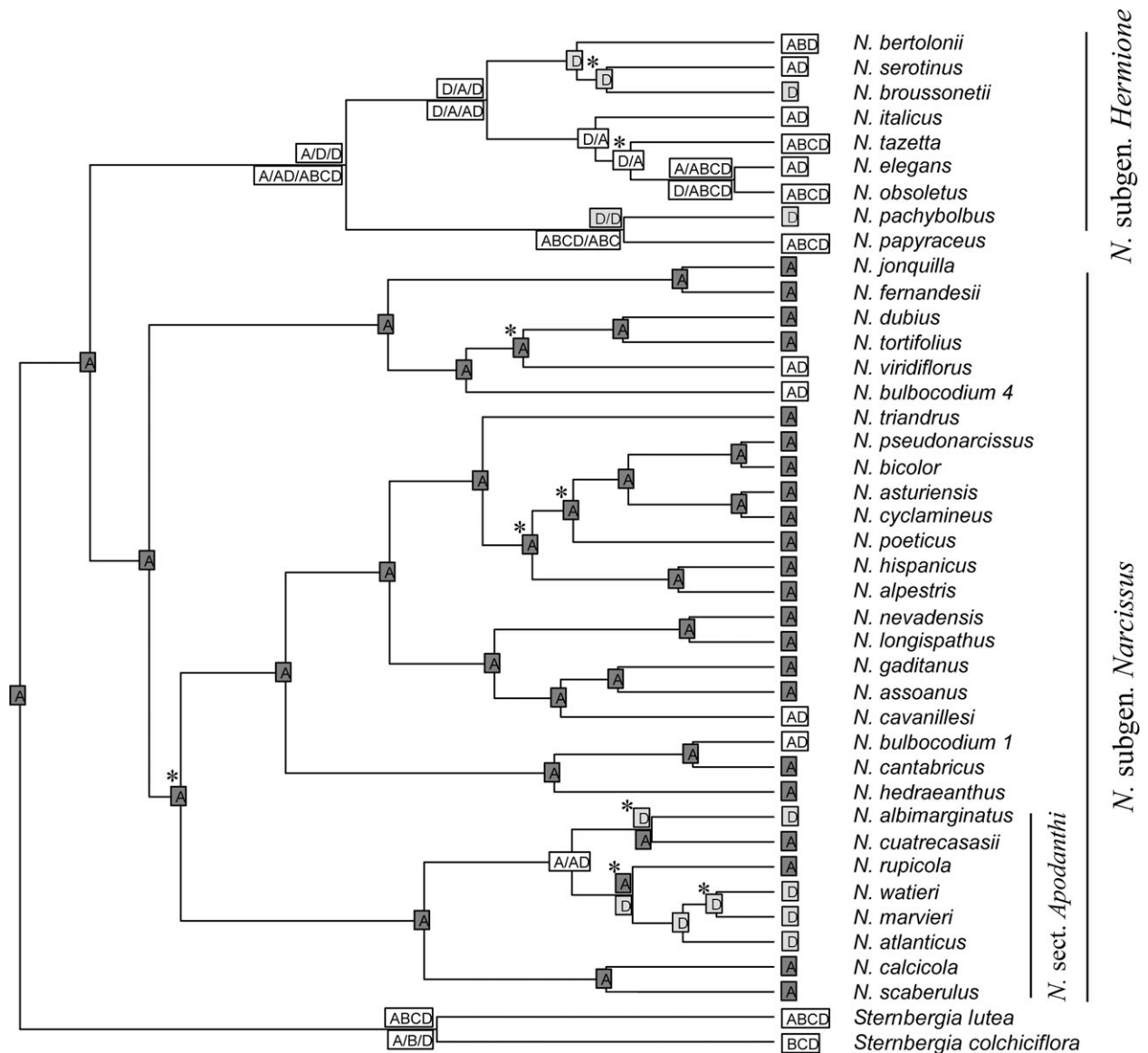


Figure 5 Maximum likelihood reconstruction of geographical range evolution of *Narcissus* under the unconstrained dispersal–extinction–cladogenesis analysis (DEC). Single-area ancestral ranges are shown at nodes, ranges inherited from widespread ancestors are shown above and below the node, where each represents the range inherited following cladogenesis, dark grey and light grey squares for ancestral areas A and D, respectively. When more than one likely alternative ancestral range was reconstructed, they are shown separated by a slash and within white boxes. Letters A, B, C and D indicate: north-western Mediterranean (West European Platform), north-eastern Mediterranean (East European Platform), south-eastern Mediterranean (Arabian Platform) and south-western Mediterranean (African Platform), respectively. Asterisks show nodes with posterior probabilities below 0.5.

corresponding to the current Iberian Peninsula, southern France and north-western Italy (A in Fig. 2). It is within this area that divergence of most sections of the subgenus *Narcissus* occurred and only a few taxa within sections *Jonquillae*, *Tapenanthus* and *Bullbocodium* (*Narcissus viridiflorus*, *Narcissus cavanillesii* and *N. bulbocodium*, respectively) dispersed to North Africa. For section *Apodanthi*, ancestral area reconstructions also include north-western Africa (D in Fig. 2). Our analyses indicated that the ancestral area for *Apodanthi* was western Europe. A secondary area included both the north-western Mediterranean and north-western Africa (area recon-

structions A/AD in Fig. 5). From those shared areas the ancestor diversified into four geographical lineages: *Narcissus cuatrecasasii*, currently found in the south Iberian Peninsula; *N. albimarginatus*, which is found on the northern tip of Africa; three species, namely *Narcissus marvieri*, *Narcissus watieri* and *Narcissus atlanticus*, which originated and colonized the current Moroccan mountains; and *Narcissus rupicola*, which occupied central Iberia.

For subgenus *Hermione*, the south-western Mediterranean and north-western Africa are the most likely ancestral areas (A and D in Fig. 5). The ancestor of the narrow-range endemic

N. pachybolbus probably differentiated within the northern tip of Africa while the likely ancestral area for *N. papyraceus* may have been distributed across the Mediterranean Basin (ABCD or ABC in Fig. 5). For the *N. tazetta* group, the north and south-western Mediterranean were the more plausible areas for ancestral diversification. The ancestor of the *N. bertolonii*–*N. broussonetii*–*N. serotinus* clade probably occupied the African Platform with further dispersal to the north-western and eastern Mediterranean, where *N. serotinus* and *N. bertolonii* are currently distributed. The north-western and south-western Mediterranean were also the most likely ancestral areas for the diversification of *N. tazetta* and closely related taxa.

Phylogeography of *N. tazetta* group

Ten haplotypes were obtained from the 40 plastid sequences and six species (*N. tazetta*, *N. elegans*, *N. obsoletus*, *N. italicus*, *N. serotinus* and *N. broussonetii*). The network analysis implemented by *tcs* depicted a clade of five haplotypes (H1–H5) for *N. tazetta*, *N. elegans*, *N. obsoletus* and *N. italicus* (Fig. 6). The other five haplotypes (H6–H10) are assigned to

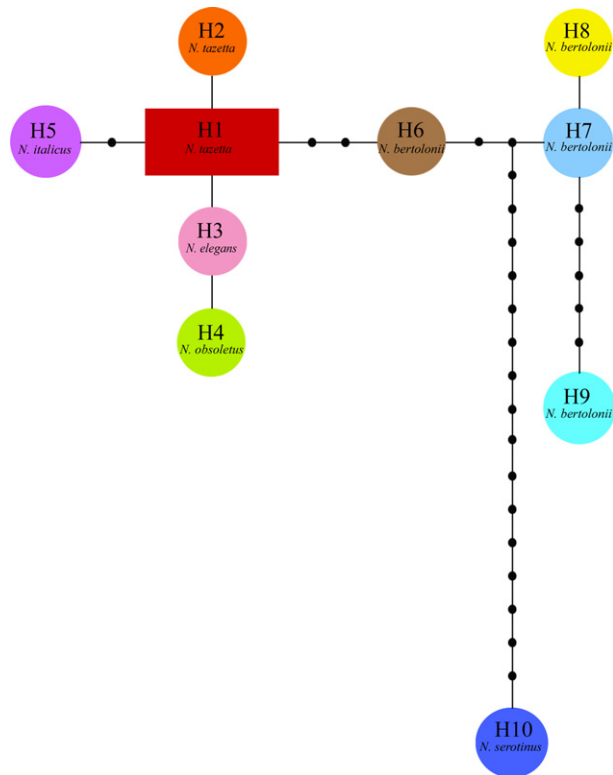


Figure 6 Network analysis of the *Narcissus tazetta* group and relatives using statistical parsimony. Haplotype numbers represent the following taxa: H1, *N. tazetta* from France, Italy, Greece and the islands of Corfu and Cyprus; H2, *N. tazetta* from Morocco and Israel; H3, *N. elegans* from Spain and Morocco; H4, *N. obsoletus* from Spain; H5, *N. italicus* from Sardinia; H6–H9, *N. bertolonii* from Sardinia and the Balearic Archipelago; H10, *N. serotinus* from Spain. The haplotypes numbers are the same as those in Fig. 1.

the other two species (*N. bertolonii* and *N. serotinus*). These two groups were separated by two missing haplotypes. Haplotype 1 was inferred as ancestral for the *N. tazetta* group and was found in *N. tazetta* from Italy, the south of France, Greece, and the islands of Corfu and Cyprus. One more *N. tazetta* haplotype (H2) differing in only one mutation step from H1 was detected in samples from north-western Africa and Israel. Also varying by a single step mutation were the haplotypes H3 and H4, found in two species (*N. elegans* and *N. obsoletus*) with a wide distribution range within the Mediterranean Basin. One more tip haplotype (H5) formed by *N. italicus* from Sardinia was separated from H1 by one absent (unsampled or extinct) haplotype. Haplotypes H6–H9 were found in *N. bertolonii* and distributed in the Balearic Islands and Sardinia. The most distant (18 mutation steps), albeit connected, haplotype H10 corresponds to *N. serotinus* from the south-western Iberian Peninsula. Finally, *N. broussonetii* was separated from the rest of the *N. tazetta* group because it exceeded the maximum number of mutational steps between pairs of sequences; hence it was not connected in the network (Fig. 6).

DISCUSSION

Phylogeny and molecular dating of *Narcissus* lineages

Our results support the basal evolutionary split of the genus *Narcissus* into two subgenera – *Hermione* and *Narcissus* (PP = 0.99) – as formerly recognized in traditional taxonomy, except for two species of subgenus *Hermione*, namely *N. dubius* and *N. tortifolius*, which are grouped together in the subgenus *Narcissus* (Fig. 3). These two species may have evolved by hybridization between ancestral species of the two subgenera (Fernandes, 1967) and deserve a comprehensive taxonomic study, which is beyond the scope of this work. Indeed, we found several elements of incongruence between morphology (particularly for flower traits, on which the traditional taxonomy has been based) and the phylogenetic relationships depicted by plastid sequences. These may be the result of convergent evolutionary processes, such as those claimed by Arroyo & Barrett (2000) and Pérez *et al.* (2004) for *Narcissus triandrus* and *N. albimarginatus*, or the result of a hybridization that cannot be resolved by plastid sequences (Marques *et al.*, 2010).

The other species of the subgenus *Hermione* were found to be monophyletic. At the section level, *Tazettae* should be considered non-monophyletic because its species are intermingled with those of the three monotypic sections *Angustinii*, *Serotinii* and *Aurelia*.

According to our calibrated molecular tree, the diversification of *Narcissus* dates back to the Late Oligocene–Early Miocene, around 23.6 Ma (29.3–18.1), with the subsequent split between subgenera *Narcissus* and *Hermione* at *c.* 21.4 Ma (27.4–16.1). Our dating is consistent with other molecular calibrations of geophytes in the Mediterranean Basin, all of which produced estimated origins around the Late Eocene to

Middle Miocene (Araceae, Mansion *et al.*, 2005; *Androcymbium*, del Hoyo *et al.*, 2009; *Cyclamen*, Yesson *et al.*, 2009). The diversification of *Narcissus* species contrasts with an increasing number of extensive radiations documented in the Mediterranean genera, because much of the geophyte differentiation appears to have pre-dated the Miocene. Dating is very variable when considering other life-forms or biogeographical groups, such as documented radiations of shrubby rockroses (*Cistus*, Guzmán & Vargas, 2009), herbaceous snapdragons (*Antirrhinum*, Vargas *et al.*, 2009) and European pinks (*Dianthus*, Valente *et al.*, 2010), all of which primarily diversified around the Late Miocene to Pleistocene. Therefore, our hypotheses for the biogeographical dynamics of *Narcissus* should be viewed with caution. Lack of an appropriate fossil record for the Amaryllidaceae precludes any further refinement of the dating. However, there is some concordance between molecular dating and certain critical palaeogeographical events. Irrespective of a fine-scale dating, our two main study groups (subgenus *Hermione* and section *Apodanthi*) showed similar temporal patterns of splitting. Both groups may have diverged during the Middle Miocene (10–13 Ma, see Fig. 3), with most of the subsequent diversification occurring after the Late Miocene. Therefore, the Alboran domain process (Middle to Late Miocene) followed by the Messinian salinity crisis (Miocene–Pliocene) and the onset of the Mediterranean climate (Pliocene) may have played important roles. These palaeoclimatic events may also have influenced the successful migration of the lowland subgenus *Hermione* and the speciation of the mountain section *Apodanthi*. The majority of the other clades within subgenus *Narcissus* include both mountain and lowland species. Differentiation of these species primarily occurred in the north-western Mediterranean, with a few dispersals to north-western Africa coinciding with the Messinian salinity crisis (*N. cavanillesii* c. 6.4 Ma) and the onset of the Pleistocene glaciations (*N. bulbocodium* c. 2.1 Ma). The aforementioned palaeogeographical events have been of key importance in the evolution of other Mediterranean groups (del Hoyo *et al.*, 2009; Vargas *et al.*, 2009; Yesson *et al.*, 2009). Although we have few studies for comparison, some common patterns are emerging as Mediterranean plant genera are studied in greater detail. The well-known genus *Cistus* also displays a mixed distribution pattern, in which lowland species have an extensive distribution across the Mediterranean (Guzmán & Vargas, 2005), while other species were inferred to be isolated by mountains (Fernández-Mazuecos & Vargas, 2010). Thus, our study suggests that there may be a general pattern of distinctive trends in lineage divergence in mountain and lowland areas of the Mediterranean. Further studies are needed to confirm this tendency.

Historical biogeography of *Narcissus*

Long-distance dispersal may explain part of the distribution patterns found in subgenus *Hermione*. At the time when diversification of this clade probably began (c. 13.2 Ma), the African and West European platforms were close together and

may have been a single area connected by the Betic and Rifian corridor (Rosenbaum *et al.*, 2002; Meulenkaamp & Sissingh, 2003). After the Messinian salinity crisis (5.96–5.33 Ma), separation of the two areas occurred, and north-western Africa and south-western Europe were reconstructed as the ancestral area of some subclades. In particular, our biogeographical analysis indicates that two subclades, *N. bertolonii*–*N. serotinus*–*N. broussonetii* and *N. pachybolbus*, diversified in north-western Africa (Fig. 5). In addition to the ancestral ranges inferred, multiple, long-distance dispersal events from either the south-western Mediterranean or north-western Africa to the rest of the Mediterranean Basin have been traditionally considered, because almost half of the extant species of the subgenus *Hermione* show a widespread distribution across the Mediterranean (Fig. 5). Despite the apparently limited existence of dispersal traits in this species (no anemochorous or zoochorous dispersal syndromes have been described), only three widespread ancestral ranges were reconstructed in agreement with the support of the constrained model mentioned before. It is possible that dispersal of vegetative parts (bulbs) played an additional role by natural means or by humans. Some *Narcissus* species have been cultivated as ornamentals (e.g. *N. italicus*; Blanchard, 1990), which could explain their wide distribution ranges. However, an explanation based purely on anthropogenic effects is unlikely. Some of the species of most recent origin, such as *N. elegans* and *N. obsoletus*, have never been cultivated and show a wide geographical range (Díaz Lifante *et al.*, 2009). Although *Narcissus* does not have considerable dispersal capacity, both its long-standing presence in the Mediterranean since the Miocene and its specialization to Mediterranean seasonal drought through dormant bulbs may account for the widespread distribution of some lineages. The habitats of many species of the subgenus *Hermione* are typical of the Mediterranean coastline, and their resistance to flooding (R.S.G. observations on *N. papyraceus* and *N. tazetta*) and brackish water (*N. obsoletus*, identified as *N. serotinus*; Fernandes, 1967) may additionally explain their spread by seasonal flooding and marine currents, as has been claimed for other coastal plants (Kadereit *et al.*, 2006). In geophytes, the diaspore is often the bulb or perennial unit, in addition to the fruits and seeds. It is interesting to note that in two other geophyte genera (del Hoyo *et al.*, 2009; Yesson *et al.*, 2009) there may have been similar dispersal mechanisms through water masses. In all these groups of plants, other long-distance dispersal mechanisms (anemochory and zoochory) are apparently lacking.

A contrasting pattern is described for section *Apodanthi*, where narrow-range endemism and non-overlapping ranges facilitated the reconstruction of ancestral areas and migration routes. The origin of the group is undoubtedly Iberian, where half of the species diversity (four of eight species) is currently found. One of the ancestral lineages remained in the Iberian Peninsula and diversified early (c. 4.9 Ma) into the current Portuguese endemics *Narcissus scaberulus* and *Narcissus calcicola*. The other ancestral area appeared to be

the Iberian Peninsula or a wider range encompassing Iberia and north-western Africa (A/AD, Fig. 5). The age of this node (*c.* 6.0 Ma) coincides with the Messinian salinity crisis, so that the connection across the Strait of Gibraltar appears to have played an important role. Descendants of this lineage are currently found in north-western Africa (*N. watieri*, *N. marvieri*, *N. atlanticus* and *N. albimarginatus*) and the Iberian Peninsula (*N. rupicola* and *N. cuatrecasii*). The divergence of these subclades is more recent and points towards its probable origin during the onset of the Mediterranean climate and Pleistocene glaciations (3.2 Ma and 2.5 Ma, respectively). This scenario stresses again that the colonization ability of these few species may have been facilitated by multiple palaeoclimatic changes (the Messinian period, the onset of the Mediterranean climate, and particularly Pleistocene glaciations for mountain species occurring at elevations up to 2500 m). Migrations from the Iberian Peninsula to northern Africa and coinciding with the Messinian salinity crisis and Pleistocene glaciations are also observed in two other sections within the subgenus *Narcissus*. *N. cavanillesii* (section *Tapeinanthus*) is dated as having diverged from its Iberian ancestral lineage at *c.* 6.4 Ma, while *N. bulbocodium* (section *Bulbocodium*) diverged at *c.* 2.09 Ma. For our studied species, colonization of mountains by bulbs appears to be unlikely; instead, seed dispersal is suggested to have permitted colonization across the Strait of Gibraltar and into the high mountains of the Moroccan Rif and Atlas. Indeed, palaeoclimatic and palaeogeographical events may have facilitated not only the north–south distribution of *Narcissus* species but may also have been critical in shaping the flora of both shores of the Strait of Gibraltar (Rodríguez-Sánchez *et al.*, 2007).

Phylogeography of the *N. tazetta* group

The ancestral haplotype (H1), which is widespread in the south of France, Italy, Greece (including Corfu) and Cyprus, indicates that the seeds or bulbs of *N. tazetta* have been successful in long-distance dispersal after the species diverged in the Messinian (Fig. 6). In contrast, populations of *N. tazetta* from Morocco and Israel shared the tip haplotype 2, which is in agreement with a recent long-distance dispersal. This may have occurred through human population movements and ornamental plantations. In any case, we cannot determine whether this long-distance dispersal occurred from east to west or vice versa, as ancestral and derived haplotypes show wide distribution across the Mediterranean Basin. This phylogeographical pattern may shed light on the poor resolution of DEC analysis for the ancestral range of the subgenus *Hermione*. Phylogeography of the *N. tazetta* group supports an origin in the western European–Mediterranean plate, rather than in north-western Africa, as also suggested by the DEC analysis (see Fig. 5). However, more extensive sampling from North Africa and Middle East populations and from other widespread *Hermione* species such as *N. papyraceus*, *N. elegans* or *N. obsoletus* is needed.

Concluding remarks

Our results provide a more detailed insight into the biogeographical histories of early divergent lineages of *Narcissus*, followed by contrasting evolutionary patterns. We have shown here that the genus *Narcissus* probably originated in the western Mediterranean during the Late Oligocene and gave rise to several new lineages in the Iberian Peninsula. In particular, section *Apodanthe* colonized Africa from the Iberian ancestral area, and then probably back-colonized Iberia. In contrast, many species of the lowland subgenus *Hermione* expanded throughout the Mediterranean Basin. The diversification of all these lineages coincided with three geological events: the orogeny and displacement of the Alboran domain, which formed a land bridge between Africa and Iberia 12 Ma (Rosenbaum *et al.*, 2002); the Messinian salinity crisis 5.96–5.33 Ma (Krijgsman *et al.*, 1999a; Krijgsman, 2002); and the climatic oscillations of the Pleistocene, coupled with sea-level fluctuations enabling connections between islands (Collina-Girard, 2001). Different *Narcissus* groups show very different ecogeographical patterns and speciation processes, which include hybridization and polyploidy in lowland coastal species (Díaz Lifante & Andrés Camacho, 2007; Díaz Lifante *et al.*, 2009) with largely overlapping ranges (subgenus *Hermione*), with some of them (e.g. *N. tazetta* and *N. obsoletus*) showing successful long-distance dispersal. Our study thus illustrates an unexpected possibility for long-distance dispersal of geophytes, despite their lack of evident morphological adaptations for such dispersal. In contrast, allopatric speciation without genetic exchange, mostly driven by specialization to abiotic (soil and climate) or biotic (pollinators) environments, especially in mountain habitats, represents the most likely evolutionary scenario for section *Apodanthe* (Pérez-Barrales *et al.*, 2006). These two different evolutionary histories illustrate the diversity of biogeographical processes acting on evolutionary diversification of plants from the same genus.

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REFERENCES

- Arroyo, J. (2002) *Narcissus*, la evolución de los polimorfismos florales y la conservación más allá de las 'listas rojas'. *Revista Chilena de Historia Natural*, **75**, 39–55.
- Arroyo, J. & Barrett, S.C.H. (2000) Discovery of distyly in *Narcissus* (Amaryllidaceae). *American Journal of Botany*, **87**, 748–751.
- Avise, J.C. (2000) *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, MA.
- Balogh, K., Ahijado, A., Casillas, R. & Fernández, C. (1999) Contributions to the chronology of the Basal Complex of Fuerteventura, Canary Islands. *Journal of Volcanology and Geothermal Research*, **90**, 81–101.
- Bittkau, C. & Comes, H.P. (2009) Molecular inference of a Late Pleistocene diversification shift in *Nigella* s. lat. (Ranunculaceae) resulting from increased speciation in the Aegean archipelago. *Journal of Biogeography*, **36**, 1346–1360.
- Blanchard, J. (1990) *Narcissus, a guide to wild daffodils*. Alpine Garden Society, Surrey, UK.
- Brandham, P. & Kirton, P. (1987) The chromosomes of species, hybrids and cultivars of *Narcissus* L. (Amaryllidaceae). *Kew Bulletin*, **42**, 65–102.
- Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, **17**, 540–552.
- Caujapé-Castells, J., Jansen, R., Membrives, N., Pedrola-Monfort, J., Montserrat, J. & Ardanuy, A. (2001) Historical biogeography of *Androcymbium* Willd. (Colchicaceae) in Africa: evidence from cpDNA RFLPs. *Botanical Journal of the Linnean Society*, **136**, 379–392.
- Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.
- Collina-Girard, J. (2001) Atlantis off the Gibraltar Strait? Myth and geology. *Comptes Rendus de l'Académie des Sciences, Series IIA, Earth and Planetary Science*, **333**, 233–240.
- Cowling, R.M., Rundel, P.W., Lamont, B.B., Arroyo, M.K. & Arianoutsou, M. (1997) Plant diversity in mediterranean-climate regions. *Trends in Ecology and Evolution*, **11**, 361–366.
- Cowling, R.M., Procheş, Ş. & Partridge, T.C. (2009) Explaining the uniqueness of the Cape flora: incorporating geomorphic evolution as a factor for explaining its diversification. *Molecular Phylogenetics and Evolution*, **51**, 64–74.
- Dafni, A., Shmida, A. & Avishai, M. (1981) Leafless autumnal-flowering geophytes in the Mediterranean region – phyto-geographical, ecological and evolutionary aspects. *Plant Systematics and Evolution*, **137**, 181–193.
- Dahlgren, G. (1991) Karyological investigations in *Ranunculus* subg. *Batrachium* (Ranunculaceae) on the Aegean islands. *Plant Systematics and Evolution*, **177**, 193–211.
- Díaz Lifante, Z. & Andrés Camacho, C. (2007) Morphological variation of *Narcissus serotinus* L. s.l. (Amaryllidaceae) in the Iberian Peninsula. *Botanical Journal of the Linnean Society*, **154**, 237–257.
- Díaz Lifante, Z., Andrés Camacho, C., Viruel, J. & Cabrera Camacho, A. (2009) The allopolyploid origin of *Narcissus obsoletus* (Alliaceae): identification of parental genomes by karyotype characterization and genomic *in situ* hybridization. *Botanical Journal of the Linnean Society*, **159**, 477–498.
- Dorda, E. & Fernández Casas, J. (1989) Estudios morfológicos del género *Narcissus* L. Anatomía de la hoja y el escapo III. *Fontqueria*, **27**, 103–162.
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Drummond, A.J., Ho, S.Y.W., Rawlence, N. & Rambaut, A. (2007) *A rough guide to BEAST 1.4*. Available at: http://beast.bio.ed.ac.uk/Main_Page.
- Edwards, A.W.F. (1992) *Likelihoods*. Johns Hopkins University Press, Baltimore, MD.
- Fernandes, A. (1951) Sur la phylogénie des espèces du genre *Narcissus* L. *Boletim da Sociedade Broteriana Series*, **2**, 113–190.
- Fernandes, A. (1967) Contribution à la connaissance de la biosystématique de quelques espèces du genre *Narcissus* L. *Portugaliae Acta Biologica serie B*, **9**, 1–44.
- Fernandes, A. (1968) Keys to the identification of native and naturalized taxa of the genus *Narcissus* L. *Daffodil and Tulip Yearbook*, **59**, 37–66.
- Fernandes, A. (1975) L'évolution chez le genre *Narcissus* L. *Anales del Instituto Botánico A.J. Cavanilles*, **32**, 843–872.
- Fernández Casas, J. (2002) *Narcissus. Catalogue des plantes vasculaires du nord du Maroc, incluant des clés d'identification* (ed. by B. Valdés, M. Rejdali, A. Achhal El Kadmiri, S.L. Jury and J.M. Montserrat), pp. 879–882. CSIC Biblioteca de Ciencias, Madrid.
- Fernández-Casas, F. (2008) *Narcissorum notulae*, X. *Fontqueria*, **55**, 547–558.
- Fernández-Mazuecos, M. & Vargas, P. (2010) Ecological rather than geographical isolation dominates Quaternary formation of Mediterranean *Cistus* species. *Molecular Ecology*, **19**, 1381–1395.
- Fernández-Palacios, J.M., de Nascimento, L., Otto, R., Delgado, J.D., García-del-Rey, E., Arévalo, J.R. & Whittaker, R.J. (2011) A reconstruction of Palaeo-Macaronesia, with particular reference to the long-term biogeography of the Atlantic island laurel forest. *Journal of Biogeography*, **38**, 226–246.
- García-Castellanos, D., Estrada, F., Jiménez-Munt, I., Gorin, C., Fernández, M., Vergés, J. & De Vicente, R. (2009) Catastrophic flood of the Mediterranean after the Messinian salinity crisis. *Nature*, **462**, 778–782.
- Guzmán, B. & Vargas, P. (2005) Systematics, character evolution, and biogeography of *Cistus* L. (Cistaceae) based on ITS, *trnL-trnF*, and *matK* sequences. *Molecular Phylogenetics and Evolution*, **37**, 644–660.

- Guzmán, B. & Vargas, P. (2009) Long-distance colonization of the Western Mediterranean by *Cistus ladanifer* (Cistaceae) despite the absence of special dispersal mechanisms. *Journal of Biogeography*, **36**, 954–968.
- Haworth, A.H. (1831) *A monograph on the subordo V of Amaryllidaceae, containing the Narcissineae*. Tilling, Chelsea.
- del Hoyo, A., García-Marín, J.L. & Pedrola-Monfort, J. (2009) Temporal and spatial diversification of the African disjunct genus *Androcymbium* (Colchicaceae). *Molecular Phylogenetics and Evolution*, **53**, 848–861.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, **17**, 754–755.
- Kadereit, G., Mucina, L. & Freitag, H. (2006) Phylogeny of Salicornioideae (Chenopodiaceae): diversification, biogeography, and evolutionary trends in leaf and flower morphology. *Taxon*, **55**, 617–642.
- Katoh, K., Asimenos, G. & Toh, H. (2009) Multiple alignment of DNA sequences with MAFFT. *Bioinformatics for DNA sequence analysis* (ed. by D. Posada), pp. 39–64. Humana Press, Hatfield, UK.
- Kay, K.M., Whittall, J.B. & Hodges, S. (2006) A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. *BMC Evolutionary Biology*, **6**, 36.
- Krijgsman, W. (2002) The Mediterranean: Mare Nostrum of earth sciences. *Earth and Planetary Science Letters*, **205**, 1–12.
- Krijgsman, W., Hilgen, F.J., Raffi, I., Sierro, F.J. & Wilson, D.S. (1999a) Chronology, causes and progression of the Messinian salinity crisis. *Nature*, **400**, 652–655.
- Krijgsman, W., Langereis, C.G., Zachariasse, W.J., Boccaletti, M., Moratti, G., Gelati, R., Iaccarino, S., Papani, G. & Villa, G. (1999b) Late Neogene evolution of the Taza–Guercif Basin (Rifian Corridor, Morocco) and implications for the Messinian salinity crisis. *Marine Geology*, **153**, 147–160.
- Kropf, M., Comes, H.P. & Kadereit, J.W. (2006) Long-distance dispersal vs vicariance: the origin and genetic diversity of alpine plants in the Spanish Sierra Nevada. *New Phytologist*, **172**, 169–184.
- Lo Presti, R.M. & Oberprieler, C. (2009) Evolutionary history, biogeography and eco-climatological differentiation of the genus *Anthemis* L. (Compositae, Anthemideae) in the circum-Mediterranean area. *Journal of Biogeography*, **36**, 1313–1332.
- Maire, R.D. (1959) *Flore de l'Afrique du nord*. Paul Lechevalier, Paris.
- Mansion, G., Zeltner, L. & Bretagnolle, F. (2005) Phylogenetic patterns and polyploid evolution within Mediterranean genus *Centaureum* (Gentianaceae - Chironieae). *Taxon*, **54**, 931–950.
- Mansion, G., Rosenbaum, G., Schoenenberger, N., Bacchetta, G., Rossello, J.A. & Conti, E. (2008) Phylogenetic analysis informed by geological history supports multiple, sequential invasions of the Mediterranean Basin by the angiosperm family Araceae. *Systematic Biology*, **57**, 269–285.
- Marques, I., Feliner Nieto, G., Draper Munt, D., Martins-Loução, M.A. & Fuertes Aguilar, J. (2010) Unraveling cryptic reticulate relationships and the origin of orphan hybrid disjunct populations in *Narcissus*. *Evolution*, **64**, 2353–2368.
- Mathew, B. (2002) Classification of the genus *Narcissus*. *Narcissus and daffodil* (ed. by G.R. Hanks), pp. 30–52. Taylor and Francis, London.
- Meerow, A.W., Francisco-Ortega, F., Kuhn, D.N. & Schnell, R.J. (2006) Phylogenetic relationships and biogeography within the Eurasian clade of Amaryllidaceae based on plastid *ndhF* and nrDNA ITS sequences: lineage sorting and reticulate area? *Systematic Botany*, **31**, 42–60.
- Meulenkamp, J.E. & Sissingh, W. (2003) Tertiary palaeogeography and tectonostratigraphic evolution of the Northern and Southern Peri-Tethys platforms and the intermediate domains in the African–Eurasian convergent plate boundary zone. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **196**, 209–228.
- Müller, K. (2005) SeqState: primer design and sequence statistics for phylogenetic DNA data sets. *Applied Bioinformatics*, **4**, 65–69.
- Oberprieler, C. (2005) Temporal and spatial diversification of Circum-Mediterranean Compositae-Anthemideae. *Taxon*, **54**, 951–966.
- Olmstead, R.G. & Sweere, J.A. (1994) Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology*, **43**, 467–481.
- Parsons, R.F. & Hopper, S.D. (2003) Monocotyledonous geophytes: comparison of south-western Australia with other areas of mediterranean climate. *Australian Journal of Botany*, **51**, 129–133.
- Paun, O., Lehnebach, C., Johansson, J., Lockhart, P. & Hörandl, E. (2005) Phylogenetic relationships and biogeography of *Ranunculus* and allied genera (Ranunculaceae) in the Mediterranean region and in the European Alpine System. *Taxon*, **54**, 911–932.
- Pérez, R., Vargas, P. & Arroyo, J. (2004) Convergent evolution of flower polymorphism in *Narcissus* (Amaryllidaceae). *New Phytologist*, **161**, 235–252.
- Pérez-Barrales, R. (2005) *La evolución de la heterostilia en Narcissus: análisis macro y microevolutivo*. PhD Thesis, University of Seville, Seville.
- Pérez-Barrales, R., Vargas, P. & Arroyo, J. (2006) New evidence for the Darwinian hypothesis of heterostyly: breeding systems and pollinators in *Narcissus* sect. *Apodanthi*. *New Phytologist*, **171**, 553–567.
- Petit, R.J. & Thompson, J. (1999) Species diversity and ecological range in relation to ploidy level in the flora of the Pyrenees. *Evolutionary Ecology*, **13**, 45–65.
- Petit, R.J., Pineau, E., Demesure, B., Bacilieri, R., Ducouso, A. & Kremer, A. (2007) Chloroplast DNA footprints of post-glacial recolonization by oaks. *Proceedings of the National Academy of Sciences USA*, **94**, 9996–10001.
- Pignatti, S. (1978) Evolutionary trends in Mediterranean flora and vegetation. *Vegetatio*, **37**, 175–185.

- Pina-Martins, F. & Paulo, O.S. (2008) Concatenator: sequence data matrices handling made easy. *Molecular Ecology Resources*, **8**, 1254–1255.
- Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.
- Procheş, Ş., Cowling, R.M., Goldblatt, P., Manning, J.C. & Snijman, D.A. (2006) An overview of the Cape geophytes. *Biological Journal of the Linnean Society*, **87**, 27–43.
- Pugsley, H.W. (1933) A monograph of *Narcissus*, subgenus *Ajax*. *Journal of the Royal Horticultural Society*, **58**, 17–93.
- Rambaut, A. & Drummond, A.J. (2007a) *Tracer*, version 1.5. Available at: <http://tree.bio.ed.ac.uk/software/tracer/>
- Rambaut, A. & Drummond, A.J. (2007b) *LogCombiner*, version 1.5.3. Available at: http://beast.bio.ed.ac.uk/Main_Page
- Rambaut, A. & Drummond, A.J. (2007c) *TreeAnnotator*, version 1.5.4. Available at: http://beast.bio.ed.ac.uk/Main_Page
- Raunkjær, C. (1934) *The life forms of plants and statistical plant geography*. Oxford University Press, Oxford.
- Ree, R. & Sanmartín, I. (2009) Prospects and challenges for parametric models in historical inference. *Journal of Biogeography*, **36**, 1211–1220.
- Ree, R. & Smith, S. (2008) Maximum likelihood inferences of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, **57**, 4–14.
- Rieseberg, L.H. & Ellstrand, N.C. (1993) What can molecular and morphological markers tell us about plant hybridization? *Critical Reviews in Plant Sciences*, **12**, 213–241.
- Rodríguez-Sánchez, F. & Arroyo, J. (2008) Reconstructing the demise of Tethyan plants: climate-driven range dynamics of *Laurus* since the Pliocene. *Global Ecology and Biogeography*, **17**, 685–695.
- Rodríguez-Sánchez, F., Pérez-Barrales, R., Ojeda, F., Vargas, P. & Arroyo, J. (2007) The Strait of Gibraltar as a melting pot for plant biodiversity. *Quaternary Science Reviews*, **27**, 2100–2117.
- Rodríguez-Sánchez, F., Guzmán, B., Valido, A., Vargas, P. & Arroyo, J. (2009) Late Neogene history of the laurel tree (*Laurus* L., Lauraceae) based on phylogeographical analyses of Mediterranean and Macaronesian populations. *Journal of Biogeography*, **36**, 1270–1281.
- Ronquist, F. (1996) *DIVA*, version 1.1. Uppsala University, Uppsala.
- Ronquist, F., Huelsenbeck, J.P. & Mark, P. (2005) *MrBayes*, version 3.1. Available at: <http://mrbayes.csit.fsu.edu/index.php>
- Rosenbaum, G., Lister, G.S. & Duboz, C. (2002) Reconstruction of the tectonic evolution of the western Mediterranean since the Oligocene. *Journal of the Virtual Explorer*, **8**, 107–130.
- Sanmartín, I. (2003) Dispersal vs. vicariance in the Mediterranean: historical biogeography of the Palearctic Pachydeminae (Coleoptera, Scarabaeoidea). *Journal of Biogeography*, **30**, 1883–1897.
- Shmida, A. (1981) Mediterranean vegetation in California and Israel: similarities and differences. *Israel Journal of Botany*, **30**, 105–123.
- Smith, S. & Donoghue, J. (2008) Rates of molecular evolution are linked to life history in flowering plants. *Science*, **322**, 86–89.
- Stebbins, G.L. & Dawe, J.C. (1987) Polyploidy and distribution of European flora: a reappraisal. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*, **108**, 343–354.
- Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, **17**, 1105–1109.
- Templeton, A.R., Crandall, K.A. & Sing, C.F. (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**, 619–633.
- Thompson, J. (2005) *Plant evolution in the Mediterranean*. Oxford University, Oxford.
- Valdés, B., Rejdali, M., Achhal El Kadmiri, A., Jury, S.L. & Montserrat, J.M. (1987) Flora vascular de Andalucía occidental *Narcissus*. *Flora vascular de Andalucía occidental* (ed. by B. Valdés, S. Talavera and E. Galiano), pp. 463–474. Ketres, Barcelona.
- Valente, L.M., Savolainen, V. & Vargas, P. (2010) Unparalleled rates of species diversification in Europe. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1489–1496.
- Vargas, P. (2003) Molecular evidence for multiple diversification pattern of alpine plants in Mediterranean Europe. *Taxon*, **52**, 463–476.
- Vargas, P., Carrió, E., Guzmán, B., Amat, E. & Güemes, J. (2009) A geographical pattern of *Antirrhinum* (Scrophulariaceae) speciation since the Pliocene based on plastid and nuclear DNA polymorphism. *Journal of Biogeography*, **36**, 1297–1312.
- Webb, D.A. (1980) *Narcissus*. *Flora Europaea* (ed. by T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters, D.A. Webb, A.O. Chater and I.B.K. Richardson), pp. 78–84. Cambridge University Press, Cambridge.
- Wikström, N., Savolainen, V. & Chase, M.W. (2001) Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society B: Biological Sciences*, **268**, 1–10.
- Yesson, C. & Culham, A. (2006) A phyloclimatic study of *Cyclamen*. *BMC Evolutionary Biology*, **6**, 72.
- Yesson, C., Toomey, N.H. & Culham, A. (2009) *Cyclamen*: time, sea and speciation biogeography using a temporally calibrated phylogeny. *Journal of Biogeography*, **36**, 1234–1252.
- Yokoyama, Y., Lambeck, K., De Deckker, P., Johnston, P. & Fifield, L.K. (2000) Timing of the last glacial maximum from observed sea-level minima. *Nature*, **406**, 713–716.

SUPPORTING INFORMATION

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Appendix S1 Species and populations of *Narcissus* sampled in this study.

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