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The invasion of *Senecio pterophorus* across continents: Multiple, independent introductions, admixture and hybridization

Roser Vilatersana[1] · María Sanz[2] · Almudena Galian[1] · Eva Castells[2,3,*]

[1]Botanic Institute of Barcelona (IBB-CSIC-ICUB), Passeig de Migdia s/n, 08038 Barcelona, Catalonia, Spain
[2]Departament de Farmacologia, Terapèutica i Toxicologia, Univ Autonoma de Barcelona, Cerdanyola del Vallès 08193, Spain
[3]CREAF, Cerdanyola del Vallès 08193, Spain

*Corresponding author: eva.castells@uab.cat, Phone: +34 93581 3833, Fax: +34 93581 2959

Abstract

Senecio pterophorus (Compositae) is a perennial shrub native to eastern South Africa that was introduced into the Western Cape in South Africa and Australia approximately 100 years ago and into Europe (Italy and Spain) more than 25-30 years ago. In this study, the aims were to unravel the putative sources of the introduced populations and identify the changes in genetic diversity after invasion using molecular markers and phylogeographic and population genetic analyses. We sampled the entire area of distribution for *S. pterophorus* extensively. Based on the results, three lineages were established along a latitudinal and climatic gradient in the native range (south, central, central/north) with high levels of admixture. Multiple, independent introductions occurred in the four invaded ranges. The central/northern lineage (humid climate) was the primary source for all of the invaded regions (with drier climates), although a secondary role was revealed for the southern lineage in the Western Cape and the central/northern lineage in Australia and Spain. The genetic diversity was slightly lower in the Spanish and Australian populations than that in the native populations. A variety of demographic and genetic processes affected the amount and structure of genetic diversity in the invaded areas, including multiple introductions and admixture (Western Cape, Australia and Spain) as well as pre-invasive hybridization (Italy). The patterns of dispersion support a hypothesis of rapid evolution of *S. pterophorus* after invasion in response to novel climatic conditions.

Keywords

Admixture; AFLP markers; genetic diversity; genetic structure; hybridization; invasion routes

Introduction

One of the primary characteristics of the Anthropocene, the new geological epoch defined by human activities (Steffen et al. 2007; Zalasiewicz et al. 2011), is the rearrangement of the current biogeographic barriers at global, regional and local scales (Sax and Gaines 2003; Simberloff 2013). Biological invasions are a well-documented component of human-caused global environmental change and one of the causes of biodiversity loss with the extinction of species and lineages and homogenization of ecosystems (Olden et al. 2004; Vitousek et al. 1997). Nevertheless, biological invasions are also ideal systems to study the evolutionary changes associated with recent colonization events. With an understanding of the ecological and genetic mechanisms that drive successful invasions, predictions of the effects of exotic species on newly invaded ecosystems and also the response of native species under a global change scenario will improve (Strayer et al. 2006, Hoffmann and Sgrò 2011).

According to classic population genetics theory, a loss of genetic diversity is predicted after invasion caused by the founder effect (Nei et al. 1975). This phenomenon is associated with failures of species to naturalize and spread into new habitats because exotic populations with low genetic variability are less capable of adapting to novel environmental conditions (Theoharides and Dukes 2007). However, based on recent studies, genetic variability is not always depleted after introduction, and different factors related to the idiosyncrasy of an invasion can mitigate or nullify a reduction in genetic variability, including a large founder population, multiple introduction events, admixture in the introduced lineages, and hybridization or introgression before or after colonization (Gaskin and Schaal 2002; Kolbe et al. 2004, 2008; Doorduin et al. 2010). Moreover, specific biological traits of some species help to maintain the genetic diversity even when the propagule pressure is low. For example, with an outcrossing mating system, a few immigrants may contain most of the genetic variation within a species (Novak and Mack 2005). Through the propagation of advantageous alleles, these factors all increase gene flow and counteract the negative effects of bottlenecks and founder events, promoting the spread of the species across the landscape into new ranges (Lee 2002; Lavergne and Molofsky 2007).

Whether an invasion is successful may also be determined by the environmental conditions both in the native and non-native ranges. A species distributed over a wide

range of climates in the native area or introduced into a new area with a climate similar to that of the source location is more likely to be preadapted to the new environmental conditions and become invasive (Theoharides and Dukes 2007, Dainese et al. 2014, Hamilton et al. 2015). However, during the last decade, the rapid evolution of exotic species in response to selective pressures (Vandepitte et al. 2014) or to epigenetic variations (Richards et al. 2012) has also been proposed as an important mechanism affecting invasiveness.

The identification of the population source(s) and the vector of invasion are key not only to understand the mechanisms of successful colonizations but also to develop management strategies to reduce the effects of future biological invasions. For example, management strategies can be improved with the characterization of novel habitats with similar ecological niches that may be susceptible to invasion, finding suitable agents for biological control, and improving quarantine measures. Additionally, with identification of the putative source populations for the invaded regions, an essential starting point is provided for comparative ecological and evolutionary studies (e.g., phenotypic traits, fitness and genetic diversity; Muirhead et al. 2008; Estoup and Guillemaud 2010; Colomer-Ventura et al. 2015). Molecular markers, both nuclear and plastid, are a good tool to reconstruct the routes of invasion (Estoup and Guillemaud 2010); however, sampling strategies must be well designed and include a large portion of the distribution range of the species to avoid erroneous assignments to the putative original populations (Muirhead et al. 2008; Fitzpatrick et al. 2012). To assist with sampling, herbarium records and literature reports provide valuable information on the distribution of species and the chronology of dispersion. Although historical information may be temporally and spatially biased because of irregular collection intensities (Delisle et al. 2003), when historical data are combined with the use of molecular markers, a better comprehension of the patterns of dispersion, the spatial variation of the genetic diversity and the population structure is achieved (Chun et al. 2010, Vandepitte et al. 2014).

Senecio pterophorus DC is a perennial shrub native to eastern South Africa (Eastern Cape and KwaZulu-Natal provinces) that expanded into western South Africa 100 years ago, where the species is invasive (Levyns 1950). The shrub was introduced cross-continentally to Australia approximately 70-100 years ago (Parsons and Cuthbertson 2001) and to Europe more than 25-30 years ago (Italy and Spain; Castells et al. 2013).

This species is a good model system to characterize the genetic events associated with a recent invasion because it has a restricted worldwide distribution in its native and introduced ranges and the chronology of the different colonization histories is well documented in literature and herbarium records (Castells et al. 2013). Recent studies show that *S. pterophorus* from the non-native populations have genetically determined plant traits, including reproductive capacity, biomass, leaf morphology and chemical composition, that are different compared with the native populations, which is consistent with rapid evolution postinvasion (Castells et al. 2014b; Colomer-Ventura et al. 2015).

We used amplified fragment length polymorphism (AFLP) markers to assess the relationships between the indigenous *S. pterophorus* and the introduced populations using methods based on phylogeography and population genetics. Plastid DNA (cpDNA) markers were also analyzed but were excluded from the genetic study because no intraspecific variation was evident in sequences across regions. Combining the genetic results with information from herbaria and literature, the history of the invasion events of *S. pterophorus* was reconstructed. Our goals were as follow: (1) to identify the dispersion routes of *S. pterophorus*, (2) to determine whether introduced populations were the result of single or multiple introductions from genetically distinct sources, (3) to compare the genetic diversity and population genetic structure among native and introduced populations, (4) to detect the bottlenecks in different areas, and (5) to evaluate the role of hybridization in the introduction events of *S. pterophorus*.

Materials and methods

Study species and invasion history

Senecio pterophorus is a perennial shrub native to South Africa (2*n* = 20; Lawrence 1985; Robinson et al. 1997) from the Eastern Cape and KwaZulu-Natal provinces in areas with annual rainfall of 500–1500 mm (Hilliard 1977; Parsons and Cuthbertson 2001). The species has a strong self-incompatibility system (Lawrence 1985; Caño et al. 2008) and is pollinated by insects. The seed production is high (more than 30,000 seeds per plant per year; Lawrence 1985; Parsons and Cuthbertson 2001), and the seeds have

an easily deciduous pappus adapted for wind and water dispersion (Parsons and Cuthbertson 2001). The high percentage of germination (around 80%) is favored by a mucilaginous coat segregated by the achenal hairs that increases the area of seed-water contact (Lawrence 1985). Similar to other species in the genus, *S. pterophorus* produces pyrrolizidine alkaloids, which are toxic compounds for defense against vertebrate and invertebrate herbivores (Kirk et al. 2004; Castells et al. 2014b).

Senecio pterophorus was introduced and naturalized in Western Cape Province in South Africa in approximately 1918 (Levyns 1950; Parsons and Cuthbertson 2001). The earliest records of *S. pterophorus* in Australia are 1908 and 1909 in Melbourne, although the lack of records during the following two decades suggests that this establishment was not successful (Walsh 1999). Later, in 1935, *S. pterophorus* was found in Port Lincoln, South Australia, most likely introduced via shipping ballast (Parsons and Cuthbertson 2001), from which the plant spread to Adelaide Hills (first record in 1942), southeast Victoria (1985) and Sydney and Newcastle in New South Wales (1987) (Levyns 1950; The Council of Heads of Australasian Herbaria 2013). *Senecio pterophorus* was introduced into Europe around woolen mills in Belgium during the late 19th century (Verloove 2006) and into the United Kingdom in the early 20th century (Preston et al. 2002). However, after no recordings in decades (in Belgium, the last citations were in 1908 and in the UK, in 1986; Preston et al. 2002; Verloove 2006), the species is currently considered extinct in both countries. In 1982, the species was first cited in Tarragona in NE Spain and then was rediscovered near Barcelona in 1995, widely distributed near the most important textile industrial area in Spain during the 20th century (Pino et al. 2000; Chamorro et al. 2006). Since 1990, the species is also reported in Liguria in NW Italy (Barberis et al. 1998; Verloove et al. 2007). In Europe, this xenophyte was apparently introduced associated with wool-processing areas (Verloove 2005; Castells et al. 2013). Individuals that are morphologically intermediate between *S. pterophorus* and other native *Senecio* species are found both in South Africa and Australia (Levyns 1950), which is consistent with the role of hybridization in the evolutionary history of both native and invasive species of the genus (Abbott 1992; Kirk et al. 2004; Calvo et al. 2013). In Europe, however, hybridization of other species with *S. pterophorus* has not been reported (Pino et al. 2000). The common habitats of *S. pterophorus* in the native areas in eastern South Africa are forest margins, grasslands and fynbos (Hilliard 1977; Castells et al. 2013). In the introduced ranges, however, *S.*

pterophorus forms dense populations in disturbed areas (e.g., railroads, road margins, abandoned fields and river beds) and occasionally settles into natural areas, causing significant loss of biodiversity (Heddle 1974; Parsons and Cuthbertson 2001; Chamorro et al. 2006; Castells et al. 2014a). In 1994, *S. pterophorus* was classified as a Declared Noxious Weed subject to eradication in Victoria (Australia; DEPI 2014) and in 2013, as an invasive species in Catalonia (Spain; Andreu et al. 2012).

Plant material

A total of 362 individuals from 55 populations were sampled, which covered the entire current distributional range of *S. pterophorus* (Fig. 1; Table S1), including the native area (eastern South Africa, 18 populations), the native range expansion (western South Africa, 5 populations) and the three centers of cross-continental introductions: Australia (13 populations), Italy (6 populations) and Spain (13 populations). Although there is no barrier between the two, populations in western South Africa were considered independently from those in the native area because the areas are geographically disconnected (Castells et al. 2013). Belgium and the UK were not included in analyses because the species is currently eradicated in these countries. Leaves were collected in the field between 2009 and 2011 during flowering to ensure plant identifications were correct. A detailed description of the sampling procedure is found in Castells et al. (2013). Leaf material from the South African populations S20-S23 was obtained from seeds germinated and grown in the greenhouse facility of the Botanical Garden of Barcelona (Spain). All leaf samples were immediately stored in silica gel. Voucher specimens are deposited in the herbarium at the University of Barcelona (BCN) (see codes in Table S1).

DNA methods

AFLP markers. Total genomic DNA was extracted from dried tissue (*ca.* 10 mg). The DNA extraction from plant material followed the CTAB method of Doyle and Dickson (1987) with the modification of Tel-Zur et al. (1999), including three steps with a sorbitol buffer wash. The quantity and quality of isolated DNA were determined with a NanoDrop 1000 v3.7.1 spectrophotometer (Thermo Fisher Scientific). Negative controls

were run in each step of the genotyping process as checks for exogenous contamination. After testing 42 selective primer pairs in four individuals from different geographical areas, three pairs were chosen for the analysis that were highly reproducible and easy to score (*Eco*RI-ACT/*Mse*I-CAA, *Eco*RI-AGG/*Mse*I-CTC, and *Eco*RI-AAG/ *Mse*I-CAA).

The AFLP procedure followed that of Vos et al. (1995) with a few modifications according to Vilatersana et al. (2007). A double digestion with *Eco*RI and *Mse*I was performed independently from the ligation. The restriction reaction was incubated at 37º C for 3 h and the ligation reaction at 16º C for 16 h. Selective amplifications were performed using the primer *Eco*RI labeled with the fluorescent dye 6-FAM. The fluorescence-labeled selective amplification products were separated on a polymer matrix with an internal size standard (GeneScan LIZ600; Applied Biosystems) on an automated sequencer (ABI 3730xl DNA Analyzer; Applied Biosystems) at the Interdisciplinary Center for Biotechnology Research (ICBR) facility at the University of Florida. Fluorescence signals of fragments between 50 and 600 bp were scored using GeneMarker v.1.85 (Softgenetics). Data were scored manually, and the results were exported as a presence/absence (1/0) matrix. The error rate (Bonin et al. 2004) was calculated as the ratio of mismatches (scoring of 0 *vs.* 1) to overmatches (1 *vs.* 1) in the AFLP profiles of replicated individuals (mean 10% of individuals). AFLP loci were excluded from the data set when ambiguous, nonreproducible or scored as present for fewer individuals than that indicated by the error rate.

Plastid markers. The variation of the chloroplast genome was tested using several noncoding cpDNA intergenic regions (*ndhC-trnV*^{UAC}, *rpl32-trnL*^{UAG}, *trnL-trnF* and *trnT-trnLb*) and introns (*rpl16*, *trnG*, 5'*trnK* and *trnL*; see Table S2) in a pilot study that included six individuals from each of South Africa, Australia and Europe (Spain). The PCR reactions were performed following Barres et al. (2013), and the amplifications were performed in PTC-200 (MJ Research) and Flex Cycler (Analytik Jena) thermal cyclers using the conditions described in Table S2. PCR products were purified with ExoSAP-IT (USB corporation), and the amplified DNA segments were sequenced using BigDye Terminator Cycle Sequencing v.3.1 (Applied Biosystems) and following the manufacturer's protocol on an ABI 3730xl capillary sequencer (Applied Biosystems) at the University of Florida ICBR Core Facility. Sequences were edited using BioEdit v.7.0.9.0 (Hall 1999) and aligned manually.

Data analyses

To construct a neighbor-joining (NJ) dendrogram, we used NTSYSpc v.2.02j (Rohlf 1998) applying the midpoint rooting mode and NEIGHBOR and CONSENSE of the package PHYLIP v.3.695 (Felsenstein 2005). The Nei's pairwise genetic distance among populations was calculated with AFLP-SURV v.1.0 (Vekemans 2002). The branch support of the NJ tree was assessed by a bootstrap analysis (500 replicates). The genetic relationships among populations were also represented graphically with a principal coordinate analysis (PCoA) based on Nei's genetic distance using GENALEX v.6.5 (Peakall and Smouse 2012).

To identify genetically homogeneous groups, we performed the Bayesian model-based clustering approach implemented in the software STRUCTURE v.2.3.4 (Pritchard et al. 2000; Falush et al. 2007). Because the colonization history of *S. pterophorus* is exceptionally well documented (Castells et al. 2013), we conducted various STRUCTURE analyses following the chronology of invasion: Western Cape in South Africa (over 100 years), Australia (over 70 years), Spain (over 30 years) and Italy (over 25 years). Thus, we first analyzed the structure of the South African native populations and then each of the non-native regions was added sequentially for a total of 5 regional combinations, i.e., native + Western Cape, native + Western Cape + Australia, etc. For each combination, we performed 10 independent runs per each K value (K = 1 to 10) with a 125,000 burn-in period and 10^6 Markov chain Monte Carlo iterations. These analyses were performed using a model with admixture, correlated allele frequencies and recessive alleles with no *a priori* information on the sample location of an individual.

To identify the most probable number of genetic groups, we used the ΔK approach (Evanno et al. 2005) with STRUCTURE HARVESTER v.0.6.93 (Earl and vonHoldt 2012). The results from different runs were summarized with CLUMPP v.1.1.2b (Jakobsson and Rosenberg 2007). For the native region, the average of each cluster for all individuals within a population was graphically represented by sector graphs and located on a map. When additional regions were included, structure results were represented using DISTRUCT v.1.1 (Rosenberg 2004) in which each individual corresponded to a vertical line formed by colored segments proportional to each of the inferred clusters. In some studies (Frantz et al. 2009; Jombart et al. 2010), clustering problems are detected under

isolation by distance (IBD); therefore, Mantel tests were performed within each region to determine the relationship between the genetic differentiation (F_{ST}; estimated through AFLP-SURV v.1.0) and the geographic distance per population pairs, using 1,000 permutations with the Isolation by Distance Web (IBDWS v.3.23; Jensen et al. 2005). For those regions showing IBD (in this study, only the South African native range), individuals were clustered using the Bayesian nonspatial model BAPS v.5.3 (Corander et al. 2004) without admixture, and the results were compared with those of STRUCTURE analyses.

To explore the origins of introduced populations, we performed assignment tests based on the multilocus genetic data using AFLPOP v.1.0 (Duchesne and Bernatchez 2002) that followed the chronology of invasion. The following settings were used: first, marker frequencies equal to zero were replaced by $1/n+1$, where n is the sample size; second, the minimal log likelihood difference to assign an individual to a certain region was tested using three levels of stringency (0, 1 and 2), which indicated that the probability for an assignment was 1-, 10- or 100-fold higher than that to another region, respectively; and third, the number of artificial (simulated) genotypes to compute *P*-values was set to 1,000. To better address our objectives, the populations for these analyses were arranged in a third, hierarchical subregional level that was defined by the lineages obtained by the STRUCTURE and BAPS analyses for the native area and under geographic criteria for the introduced regions. Thus, the Australian populations were grouped into five subregions: A-ADE (Adelaide area), A-BM (Barker-Mallee area), A-EYRE (Eyre Peninsula), A-MEL (Melbourne area) and A-SYD (Sydney area), and the Spanish populations were grouped into two subregions: CAT-S (Catalonia South), containing only population C01, and CAT-N (Catalonia North), containing the other Spanish populations (Fig. 1 and Table S1). Populations from the Western Cape and Italy were distributed homogeneously across the distributional areas and therefore were not divided into subregions.

An analysis of molecular variance (AMOVA) was used to examine the distribution of the variance components of genetic diversity within and among populations, based on the complete sample set and several nested analyses using ARLEQUIN v.3.5.1.2 (Excoffier and Lischer 2010) (see Table 2). The statistical significance of genetic differentiation (F_{ST} values) was tested nonparametrically after 1,000 permutations.

The genetic differentiation was calculated using the AFLP data matrix assuming that fragments of the identical size were homologous loci. Allele frequencies were estimated for each population, subregion and region using a Bayesian approach (Zhivotovsky 1999) based on a nonuniform prior distribution of null allele frequencies and Hardy–Weinberg genotypic proportions that was implemented in AFLP-SURV v.1.0. The proportions of polymorphic markers (*PLP*) and the estimates of gene diversity (*H*j) based on Nei's genetic distance were determined using the approach of Lynch and Milligan (1994). Because differences in sampling intensity between populations could bias the comparisons of genetic diversity, we computed the band richness (*B*r) standardized to population size $n = 5$ with a rarefaction method using AFLP-DIV v.1.0 (Coart et al. 2005). Statistical significance among regions for each genetic metric (*PLP*, *H*j and *B*r) was determined with one-way ANOVAs. We also used linear models to test differences in these genetic diversity metrics between the native and each non-native region. All statistical analyses were conducted in the R statistical software package (R Development Core Team 2012) with the DEDUCER package (Fellows 2012). Finally, to test for a recent bottleneck, we calculated the marker frequency distribution following Luikart et al. (1998).

Results

AFLP

Using three combinations of primers, we scored 327 unambiguous DNA fragments (ranging from 142 in population A13 to 243 in population S10; Table S1). To assess the reproducibility and reliability of the AFLP fragments, we replicated 10.23% of the samples (an average of 37 individuals per primer combination); the reproducibility ranged between 97.0% and 99.7% (Table S3) with an acceptable error rate of 2.02% (Bonin et al. 2004). All markers were polymorphic, and the individuals studied presented unique AFLP phenotypes. Eight private markers were found, one in Australia, one in Spain and six in Italy.

The NJ analysis revealed four distinct clusters (Fig. 2): cluster A, which contained four native South African populations and two populations from the Eyre Peninsula in

Australia (A-EYRE) with high bootstrap support; cluster B, which included all Italian populations and two native populations with low bootstrap support; cluster C, which included all of the northern Spanish populations (CAT-N), five native populations and two populations from the Western Cape of South Africa; and cluster D, which was divided into two separate subclusters: cluster D1 contained the Australian Melbourne populations (A-MEL) and a mix of native and Western Cape populations, and cluster D2 included the Australian populations from all subregions except Melbourne and the Spanish population C01 (CAT-S). The native South African populations S12-S14 were recovered as basal to the other populations when a midpoint rooting was applied. The PCoA analysis revealed three highly distinct groups (Fig. 3): the first group was formed by all Australian and South African populations, plus the Spanish population C01, the second group included all other Spanish populations and the third group included all Italian populations.

The STRUCTURE analysis for the native region showed three optimal genetics groups (K = 3; Fig. S1) with high levels of admixture (Fig. 4A). When individuals were averaged by population, these lineages were broadly correlated with geographical distributions within the native area, with one lineage located in the south of the distributional area (SA-1), one located central (SA-2) and one located central/north (SA-3), with few exceptions (Fig. 4A). A Mantel test showed a significant positive correlation between the pairwise genetic differentiation (F_{ST}) and the geographic distance within the native region in South Africa (r = 0.347; P < 0.001) but not for the other regions (Fig. S2). Therefore, we performed BAPS analysis only for the native region (Fig. S3). The STRUCTURE and BAPS results were concordant, and therefore, the lineages described above (i.e., SA-1, SA-2 and SA-3) were included as subregional levels for the native region in the genetic diversity and AFLPOP analyses.

The STRUCTURE analyses revealed K = 3 as the optimal number of groups when the native, Western Cape and Australian populations were included (Fig. S1). Most Australian populations presented high levels of admixture (Fig. 4B); however, populations from the Melbourne area (A-MEL) had low admixture and were classified in a group that was distinct from the other Australian subregions. When the Spanish populations were added to the analysis, we obtained K = 4, with results similar to those found for K = 3 for South Africa and Australia and a distinct group that included all of the Spanish populations located in the north (CAT-N) but not population C01 (CAT-S)

(Figs. 4C and S1). Finally, when all regions were included, we obtained only $K = 2$, with one class for the Italian populations and one class for all of the other regions (Figs. 4D and S1).

The summary of the assignment analyses by AFLPOP using an intermediate stringency condition is shown in Fig. 5. The average for individuals not assigned to a group (full region or subregion, depending on the classes established) was below 20%, with the exception of A-ADE, which exceeded 50%. The results for the three stringency conditions (0, 1 and 2) were consistent, and the individuals not assigned to a group increased in the highest stringency analyses as expected (Tables S4 and S5). Populations from the Western Cape in South Africa were predominantly associated with SA-1 and SA-3 lineages from the native range (Fig. 5). All subregions in Australia were primarily assigned to SA-3 and secondarily to SA-2. The Spanish subregion CAT-S, represented only by population C01, was assigned to SA-3 and to the Australian subregion A-ADE; whereas the subregion CAT-N was primarily (but not exclusively) associated with SA-3 and SA-1. Finally, more than 80% of the Italian individuals were assigned to SA-3 lineage.

A nonhierarchical AMOVA attributed most of the genetic variation to within populations (62% to 87%) rather than among populations or among regions, regardless of the type of analysis (Table 2). The F_{ST} values for the genetic differentiation among populations were higher in the Western Cape (0.168), Australia (0.217) and Spain (0.223) than those in the native region (0.134). By contrast, the F_{ST} value in Italy (0.127) was lower than that in the native region (Table 2).

The percentage of polymorphic bands (*PLP*) ranged from 43.4% (population A13) to 74.3% (population S10), gene diversity (*H*j) from 0.12 (population A13) to 0.28 (population S11), and band richness after rarefaction [*B*r(5)] from 1.23 (population A12) to 1.5 (population A10) (Table S1). The *PLP* of the Spanish populations, *H*j of the Australian populations and *B*r(5) of the Australian and Spanish populations were significantly lower than those of the native populations (Fig. 6; Table S6). No significant differences were found for any metric of genetic diversity between the Italian and the native populations, although the variability for this region was extremely low compared with that in all other regions, particularly for *H*j (Fig. 6). We did not detect a distortion of the band frequency at the regional level (Fig. S4); however, the populations in Melbourne (A-MEL), Sydney (A-SYD) and the southern Spanish (CAT-S)

subregions showed a loss of rare bands that was compatible with a recent bottleneck (Fig. S5).

Plastid DNA sequences

No genetic variability was found using chloroplast markers. The amplification products of all regions were good, but no intraspecific genetic variation was detected for *S. pterophorus* among regions (i.e., South Africa, Australia and Europe). Sequences were deposited in the GenBank database (see Table S2).

Discussion

Invasion history of *Senecio pterophorus*

Senecio pterophorus, a shrub native to eastern South Africa, has colonized Western Cape Province (South Africa), Australia, Spain and Italy through independent invasions. In the native range, we characterized three different lineages of *S. pterophorus* with high levels of admixture, approximately localized geographically (south: SA-1; central: SA-2; and central/north: SA-3), and with weak but significant genetic isolation by distance. One population, S16, was the exception to this geographical arrangement, which was located in the north but assigned to the southern lineage, possibly because of a subsequent secondary contact. According to our results, the central/northern lineage (SA-3) was the primary genetic source for all of the novel regions, although the southern (SA-1) and the central lineages (SA-2) also contributed to the colonization of the Western Cape in South Africa and Australia, respectively. The most plausible routes of colonization of *S. pterophorus* are shown in Fig. 7.

We also identified multiple introductions of *S. pterophorus* within each invasive range, except into Italy. Thus, two separate colonization events occurred in the Western Cape in South Africa: the southern native lineage SA-1 originated populations S01 and S05, and the northern lineage SA-3 originated populations S02, S03 and S04, with some admixture between these lineages at the contact zone.

Senecio pterophorus was also introduced multiple times into Australia. First, two independent introductions occurred at the Eyre Peninsula (A-EYRE; populations A03-A06): one from the central lineage SA-2 and one from the northern lineage SA-3. The historical records from herbaria show that the Eyre Peninsula was the entry point of *S. pterophorus* into South Australia in the 1930s from which the plant spread toward the southeast to Adelaide (1940s), the Limestone Coast (1960s), western Victoria (1970s) and Melbourne and Sydney (1980s) (The Council of Heads of Australasian Herbaria 2013). The genetic similarity between plants from the Eyre Peninsula with those from Adelaide (A-ADE; populations A07-A09), Barker-Mallee area (A-BM; populations A10–A11) and Sydney (A-SYD; populations A01-A02) was consistent with a wave of colonization from the Eyre Peninsula toward the southeast, with the northern native lineage SA-3 predominant in this dispersion. The populations of the Melbourne subregion (A-MEL; populations A12-A13) were also derived from lineage SA-3; however, the structure and NJ analyses revealed that those populations were statistically distinct from the other Australian populations. The first historical record in Melbourne was in 1908 in the city port, but *S. pterophorus* was not reported again until 1985 (The Council of Heads of Australasian Herbaria 2013). Whether this gap in the record was caused by a bias in the sampling effort during which the populations remained in a latent state for decades or by the actual disappearance of the species remains unclear. Because we detected only one introduction event into this region, two scenarios may explain the origin of the current Melbourne populations. First, populations derived from the introduction early in the century could have merged with plants from the dispersion wave from the Eyre Peninsula in the 1980s. Second, the original populations in Melbourne could have been extinguished, with the current populations derived exclusively from those introduced at the Eyre Peninsula. The detection of a recent bottleneck in the Melbourne populations, most likely caused by the comprehensive weed control program in the state of Victoria (DEPI 2014), could also be the cause of the genetic differentiation of the Melbourne plants from the other Australian populations. Finally, because of the considerable genetic similarity between the northern native lineage SA-3 and some populations of the Western Cape in South Africa (S02 to S04), we could not reject that these populations played a role in the Australian invasion. However, the chronological information based on herbaria did not support this hypothesis because when the colonization events in Australia were occurring (70-100

years ago), *S. pterophorus* was still incipient in the Western Cape (first record 100 years ago) (Levyns 1950; Walsh 1999; The Council of Heads of Australasian Herbaria 2013).

We found two distinct invasion centers in Spain. The first one, CAT-N, which contained most of the *S. pterophorus* populations, came from a single introduction from the central/north parts of the South African native range (lineage SA-3), with a significant admixture with the southern populations (lineage SA-1). The invasion of *S. pterophorus* into this area was most likely associated with the manufacture of wool (Verloove 2005; Castells et al. 2013); however, the geographical origin of these populations was uncertain because much wool was imported from South Africa and Australia during the second half of the 20th century (Dirección General de Aduanas de España 1951–1986; Fig. S6). Although both regions were potential sources, our results unequivocally point to a South African origin. Moreover, data were consistent with a lineage admixture of the native populations at the initial focus of introduction, suggesting that the imported wool, which carried the seeds, came from different areas within South Africa (Kowarik and von der Lippe 2007; Lachmuth et al. 2010). This hypothesis was also consistent with the AFLPOP assignment analyses performed at the population level within this subregion, which showed a strong admixture within populations and different origins or lineages between populations that were separated by only a few kilometers (data not shown).

The second Spanish center of introduction, CAT-S, currently located approximately 100 km south of CAT-N, was formed by a single, small-sized population (C01) in a very disturbed habitat without any known relationship with wool manufacture (Chamorro et al. 2006; Castells et al. 2013). The assignment analyses identified the northern native lineage SA-3 as the most likely origin of CAT-S (50% of individuals under an intermediate stringency value). However, we also found a significant Australian component in this subregion (20% of individuals assigned to A-ADE), which was maintained even when the stringency value was increased. Moreover, population C01 clustered with the Australian populations in the NJ analysis. Based on these results, a definitive conclusion about the origin of C01 was not possible, and different reasons could explain the ambiguous assignment of population C01 to South Africa and Australia. For example, small populations are more susceptible to genetic drift, which could cause a change in the genetic differentiation between the native and the invasive populations and hinder the identification of the source. By contrast, the

discordance between analyses could be caused by a shared ancestor of the invasive populations, a phenomena favored by the multiple introduction events, which could also explain the loss of resolution and support in some NJ branches (Sanz et al. 2013).

The third introduction of *S. pterophorus* into Europe, Italy, came from a single source from the central/north lineage of the native distribution (SA-3) that was independent of the other two European invasions in Spain. For Italy, the introduction vector remains unknown. However, dispersal along traffic routes and hydrographic networks, which favors the dispersal characteristics of the achene, was compatible with the localization of the Italian populations (Kowarik and von der Lippe 2007).

Genetic diversity and structure of *Senecio pterophorus* in South Africa, Australia and Spain

The levels of genetic variation in *S. pterophorus* in the native range were consistent with other species with similar life history traits (Nybom 2004). The loss of genetic diversity caused by a founder event is repeatedly demonstrated in biological invasions (Dlugosch and Parker 2008). In this study, the Australian and the Spanish populations had lower genetic diversity (estimated by *PLP*, *H*j and *B*r) than that of the native populations, although the differences were weak and not always significant. These results are similar to those for other taxonomically related invasive species such as *Jacobaea vulgaris* (Doorduin et al. 2010). The slight decrease in the genetic diversity after invasion was not accompanied by a loss of rare markers in the non-native regions, which normally indicates recent bottlenecks (Luikart et al. 1998). Only three subregions (A-MEL, A-SYD and CAT-S) showed evidence of recent bottlenecks, although these bottlenecks were most likely associated with events not linked to the species introduction; for example, the comprehensive weed control programs in the state of Victoria (DEPI 2014) for that of A-MEL and the low population sizes (Frankham 1996) for those of A-SYD and CAT-S. The self-incompatibility mating system of *S. pterophorus* and the circumstances of introduction, such as multiple introductions and admixture, could have attenuated the loss of genetic diversity after invasion. For the Western Cape, the relative proximity with the native populations and the absence of geographical barriers might have favored frequent entries of native genotypes, thereby maintaining genetic diversity.

Most of the genetic diversity of *S. pterophorus* was found within populations (75% averaged for all AMOVA analyses) rather than among populations, which was consistent with the type of mating system (Nybom 2004; Novak and Mack 2005). One important prediction of invasion theory is that population genetic structure will decline after an invasion when the alien populations are the result of a single introduction (Bossdorf et al. 2005; Novak and Mack 2005; Marrs et al. 2008). Thus, the substantial increase in the genetic differentiation statistics (F_{ST}) for the Western Cape, Australia and Spain was consistent with several genetically distinct introductions, as also indicated by the NJ, AFLPOP and STRUCTURE analyses. With the loss of IBD in the invasive populations, the premonitory indication was that sufficient time had not passed for the populations in the new range to be differentiated by genetic drift.

Role of hybridization in the invasion of *Senecio pterophorus* into Italy

In Italy, *Senecio pterophorus* had strong, distinctive genetic traits in comparison with the introductions in the Western Cape, Australia or even Spain, with the Spanish invasions geographically close and occurring at similar periods. First, the Italian populations contained an elevated number of private markers (6) and 27 alleles found in all other regions were absent. Second, the genetic diversity did not decrease after invasion as occurred in Australia and Spain, and the deviation in the genetic diversity metrics, particularly *H*j, was very low compared with that in all other regions. Third, regarding population structure, the genetic differentiation value F_{ST} was lower in Italy than that in the native range, which was opposite to those values of the other introductions. Given the nature of the molecular markers studied (neutral genetic markers), it is unlikely that all of these differences are due to evolutionary processes such as mutations and selection or with genetic drift caused by a founder effect because we did not find a decrease in gene diversity or changes in gene frequencies. Moreover, because of the extensive sampling and because the primary component of genetic diversity was within (not among) populations, the differences between Italy and the other invasive areas were not likely to be an artifact of insufficient sampling. Therefore, gene flow is the most likely hypothesis to explain the strong differentiation of Italian populations.

Gene flow could result from different and successive invasions, admixture, or hybridization or introgression. No trace of multiple introductions was detected in the Italian populations, and *S. pterophorus* in this region is classified separately compared with all other regions in the structure analyses; therefore, hybridization between *S. pterophorus* and other *Senecio* species was the most likely event. Hybridization success was likely favored by the self-incompatibility mating system in *S. pterophorus* (Lawrance 1985; Caño et al. 2008) and by the weak genetic barriers among the species of the genus (Calvo et al. 2013). Furthermore, several lines of evidence suggest that hybridization occurred in the native range before the introduction into Italy. First, the footprint of hybridization was found across all populations studied in this range. Second, based on the low deviation in genetic diversity metrics, all Italian populations were genetically uniform. Moreover, because of the short time since the establishment of the species (25 years), hybridization in the novel range was less plausible. Many studies detect post-invasion hybridization, typically between introduced and native species (e. g. Abbott 1992; Culley and Hardiman 2009) or between two invasive species (Ayres et al. 1999; Gaskin and Schaal 2002). However, hybridization rarely occurs as a preliminary event to an invasion and has been reported in only a few species of the genera *Centaurea* (Blair and Hufbauer 2010; Lai et al. 2012) and *Onopordum* (O'Hanlon et al. 1999) in Compositae. We did not identify the hybridized source populations in the native range that could have originated the Italian invasion; however, this result was consistent with our sampling strategy to avoid well-known hybrid areas (Castells et al. 2013).

Typically, hybridization events are linked to the generation of new genotypes, with an increase in the genetic variability of hybrid lineages because of the increase in heterozygosity (Reed and Frankham 2003). However, the hybridization in Italian populations was not accompanied by a phenotypic divergence compared with that of *S. pterophorus* populations in the other regions (E. Castells pers. comm., Verloove et al. 2007). The absence of such a divergence could occur when the interspecific genetic barriers remain incomplete (Calvo et al. 2013), causing a partial exchange of the genome that may not affect the morphology and ecology of the hybrid species (Kane et al. 2009).

Events explaining invasion success

The period between the introduction of a species and the invasive spread into a new habitat often involves a considerable lag-time, which can be caused by adaptive, demographic, ecological or genetic events, or some combination of these events (Sakai et al. 2001; Aikio et al. 2010). In Australia, *S. pterophorus* was introduced between the 1900s and the 1930s, but the large infestations in South Australia and Victoria did not occur until the 1980s (Parsons and Cuthbertson 2001). The admixture of SA-1 and SA-3 lineages by multiple introductions could have accelerated the geographic expansion by simple diffusion toward the southeastern parts of Australia (i.e., Adelaide, Melbourne and Sydney), as shown by the herbarium records (The Council of Heads of Australasian Herbaria 2013). In Spain, the high levels of admixture at the initial focus of invasion may also have contributed to the expansion of *S. pterophorus* in the last 15-20 years (Chamorro et al. 2006; Castells et al. 2013). Long lag-phases from the initial introduction event to a posterior geographical expansion are a common feature in biological invasions (Kolbe et al. 2004; Aikio et al. 2010). The resurgence of invasive populations after a long lag-phase, approximately 80 years, has been reported in other invasive *Senecio* species, such as *S. inaequidens* (Lachmuth et al. 2010) and *S. madagascariensis* (Dormontt et al. 2014).

The maintenance of the genetic diversity in the invasive areas caused by admixture and multiple introductions might have facilitated the adaptation of *S. pterophorus* to novel environmental conditions. In the native range, *S. pterophorus* is distributed along a climatic gradient of summer drought, with the southern populations subject to high water deficits (low values of precipitation to potential evapotranspiration, P/PET) and the northern populations subject to more humid conditions (high values of P/PET; Colomer-Ventura et al. 2015). However, the populations in the invasive ranges (i.e., Western Cape, Australia, Spain and Italy) only occurred in the Mediterranean areas with drier climatic conditions, equivalent to those of the southern native lineage (SA-1). Because of the convergence of plant traits between the native and introduced populations that shared similar climates (e.g., biomass, reproductive capacity, leaf morphology and chemical composition; Colomer-Ventura et al. 2015) and because the primary population source for the invaded areas was the central/north lineage (SA-3) from a more humid environment, the hypothesis

was strongly supported that the invasive populations of *S. pterophorus* rapidly evolved after invasion in adaptation to the drier climate (Colomer-Ventura et al. 2015).

In addition to the current areas of distribution in Europe, several unsuccessful colonization attempts by *S. pterophorus* occurred in the late 19th century in Belgium (Verloove 2006) and the British Islands (Preston et al. 2002) in locations near wool processing industries. One of the factors related to invasion failure is low propagule pressure, including propagule size or propagule number in the vehicle of introduction; with a higher propagule pressure the likelihood of establishment increases and that of a genetic bottleneck decreases (Simberloff 2009; Zenni and Nuñez 2013). However, some evidence indicated that propagule pressure alone was not the determining factor in the establishment of *S. pterophorus*. For example, the species was rediscovered in the British Isles near wool processing industries several times before the 1980s but establishment failed in all of these introductions (Preston et al. 2002). The failure of *S. pterophorus* to establish in two areas in a temperate bioclimatic region is consistent with the importance of climate as a factor in determining a successful invasion. The summer drought index in the UK and Belgium (average P/PET = 0.56) was within the range of those in the native range (P/PET ranged from 0.37 to 1.32) and similar to the average in Australia (P/PET = 0.51). Thus, other climatic variables might be more plausible to explain the failures to establish, such as lower annual temperatures ($T_{UK} = 9.2^0C$ and $T_{BELGIUM} = 10.1^0C$, compared with $T_{NATIVE} = 16.6^0C$, $T_{WESTERN\ CAPE} = 16.1^0C$, $T_{AUSTRALIA} = 15.1^0C$ and $T_{SPAIN} = 15.3^0C$), winter minimum temperatures or frost tolerance. The absence of a similar climatic niche between the native and introduced range for an exotic species is correlated with a lower risk of invasion (Petitpierre et al. 2012).

Conclusions

Senecio pterophorus has a relatively restricted but disjoint worldwide distribution, with a range expansion within South Africa and two cross-continental introductions into Australia and Europe. This distribution combined with the exhaustive sampling performed in this study provided an excellent opportunity to evaluate the phylogeographic history of a species over the entire area of distribution. Our results are consistent with a scenario of multiple and independent introductions of *S. pterophorus*

into the Western Cape, Australia, Spain and Italy, with the South African native SA-3 lineage as the primary source of colonization (central/northern lineage) and the SA-1 lineage a secondary source (southern lineage; Fig. 7). The amount and structure of genetic diversity in the different invasive areas were the result of variety of genetic events, including multiple introductions, admixtures and preinvasive hybridization. The patterns of dispersion described in this study are useful not only to identify the source populations and mechanisms involved in the process of invasion but also to understand the evolutionary changes in exotic species in novel habitats.

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Table 1. Summary of genetic diversity metrics estimated as the percentage of polymorphic loci (*PLP*), the average gene diversity (*Hj*) and band richness after rarefaction (*Br*5) of *Senecio pterophorus* within regions and at the subregional level for the South African native range, the South African native range expansion range (Western Cape), Australia, Spain and Italy (n = number of individuals; NL = number of AFLP loci). Population codes correspond to those in Figure 1.

Region/Subregion[1]	Number of Populations	Population codes	Origin[2]	n	NL	*PLP* (%)	*Hj* (Mean ± SE)	*Br*(5)
Regions								
South Africa (native)	23	S06-S23	N	117	210	64.2	0.209 ± 0.009	1.469
Western Cape	5	S01-S05	E	33	196	59.9	0.202 ± 0.009	1.404
Australia	13	A01-A13	I	91	211	64.5	0.195 ± 0.009	1.423
Spain	13	C01-C13	I	76	203	62.1	0.205 ± 0.009	1.435
Italy	6	I01-I06	I	45	219	67.0	0.233 ± 0.010	1.473
South Africa (native)								
SA-1	5	S06-S09, S16	N	44	209	65.9	0.210 ± 0.009	1.594
SA-2	4	S20-23	N	22	210	66.2	0.221 ± 0.009	1.561
SA-3	9	S10-S15, S17-S19	N	51	214	67.5	0.219 ± 0.009	1.643
Australia								
A-SYD	2	A01, A02	I	15	176	53.8	0.189 ± 0.010	1.451
A-EYRE	4	A03-A06	I	36	181	55.4	0.190 ± 0.010	1.527
A-ADE	3	A07-A09	I	17	181	55.4	0.193 ± 0.010	1.493
A-BM	2	A10, A11	I	12	253	77.4	0.236 ± 0.010	1.656
A-MEL	2	A12, A13	I	11	208	63.6	0.170 ± 0.009	1.425
Spain								
CAT-S	1	C01	I	8	174	53.2	0.177 ± 0.010	1.289
CAT-N	12	C02-C13	I	68	204	62.4	0.204 ± 0.010	1.428

[1] Abbreviation for subregional groups. In the South African native range: subgroups SA-1, SA-2 and SA-3 correspond to lineages following STRUCTURE analyses (see Fig. 5A). Populations were assigned to each subgroup based on the most frequent lineage. In Australia: A-SYD = Sydney area; A-EYRE = Eyre Peninsula; A-ADE = Adelaide area; A-BM = Barker-Mallee area; and A-MEL = Melbourne area. In Spain: CAT-S = South Catalonia and CAT-N = North Catalonia.

[2] Origin: N = native; E = native range expansion; and I = introduced.

Table 2. Analyses of molecular variance (AMOVA) for AFLP phenotypes of *Senecio pterophorus* from the South African native region, the South African native range expansion (Western Cape) and the invaded regions in Australia, Spain and Italy. *All *P*–values were < 0.001.

Sources of variation	d.f.	Sum of squares	Variance components	% Total variance*	F_{ST}*
Two hierarchical levels					
Among populations	54	5036.74	9.725	24.85	0.248
Within populations	307	9026.63	29.403	75.15	
Nested analyses (regions)					
Among regions	4	1549.32	4.512	11.25	0.267
Among populations within regions	50	3487.41.	6.20	15.47	
Within populations	307	9026.63	29.403	73.29	
Nested analyses (native and introduced regions)					
Among regions	1	275.83	1.020	2.57	0.258
Among populations within regions	53	4760.91	9.220	23.26	
Within populations	307	9026.63	29.403	74.17	
South African native region					
Among populations	17	1090.36	4.973	13.44	0.134
Within populations	99	3171.00	32.030	86.59	
Western Cape					
Among populations	4	253.60	5.534	16.87	0.168
Within populations	28	763.30	27.261	83.13	
Nested analyses (invasive regions)					
Among regions	2	1106.78	6.930	16.42	0.330
Among populations within regions	29	2143.45	6.991	16.56	
Within populations	180	5092.32	28.291	67.02	
Australia					
Among populations	12	926.96	7.334	21.74	0.217
Within populations	78	2058.75	26.394	78.26	
Spain					
Among populations	12	865.74	7.783	22.30	0.223
Within populations	63	1708.58	27.120	77.70	
Italy					
Among populations	5	350.74	4.939	12.69	0.127
Within populations	39	1324.99	33.974	87.31	
European regions (Spain and Italy)					
Among regions	1	731.52	11.500	23.99	0.379
Among populations within regions	17	1216.48	6.704	13.98	
Within populations	102	3033.57	29.741	62.03	

FIGURES

Fig. 1 Sample locations of *Senecio pterophorus* in the South African native region, the South African native range expansion (Western Cape), Australia and Europe (Spain and Italy). Gray lines and text indicate subregional divisions. Note the different scales in the figure. Abbreviations for subregional groups in Australia: A-EYRE = Eyre Peninsula; A-ADE = Adelaide area; A-BM = Barker-Mallee area; A-MEL = Melbourne area; and A-SYD = Sydney area; and in Spain: CAT-S = South Catalonia and CAT-N = North Catalonia. Population numbers correspond to the codes in Table S1.

Fig. 2 Neighbor–joining tree showing the relationships of *Senecio pterophorus* populations based on Nei's pairwise genetic distance. Populations are grouped into four clusters: A, B, C and D (with the D group divided into subclusters D1 and D2). Numbers above the branches indicate the bootstrap support. Gray text corresponds to the subregional divisions in the introduced areas (see Fig. 1) and to the genetic lineages in native populations based on STRUCTURE analyses (see Fig. 5). Abbreviations for subregional divisions in Australia: A-EYRE = Eyre Peninsula; A-ADE = Adelaide area; A-BM = Barker-Mallee area; A-MEL = Melbourne area; and A-SYD = Sydney area; and in Spain: CAT-S = South Catalonia and CAT-N = North Catalonia; and Italy = ITA. Abbreviations for the South African native lineages: SA–1 = southern lineage; SA–2 = central lineage; and SA–3 = central/northern lineage.

Fig. 3 Principal Coordinate Analysis (PCoA) based on Nei's genetic distance among native and introduced *Senecio pterophorus* populations. The percentage of variation explained by each axis is indicated in parentheses.

Fig. 4 Results of STRUCTURE analyses: **A**, the native South African samples (K = 3); **B**, all South African samples (native, S06–S23; Western Cape, S01–S05) and Australian populations (K = 3); **C**, the South African, Australian and Spanish samples (K = 4); and **D**, all regions (K = 2). Each color corresponds to a K-defined group. See material and methods for details. Abbreviations for groups: South African native area: SA-1, SA-2 and SA-3 lineages; Australia: A-SYD = Sydney populations; A-EYRE = Eyre Peninsula populations; A-ADE = Adelaide populations; A-BM = Barker-Mallee populations; and

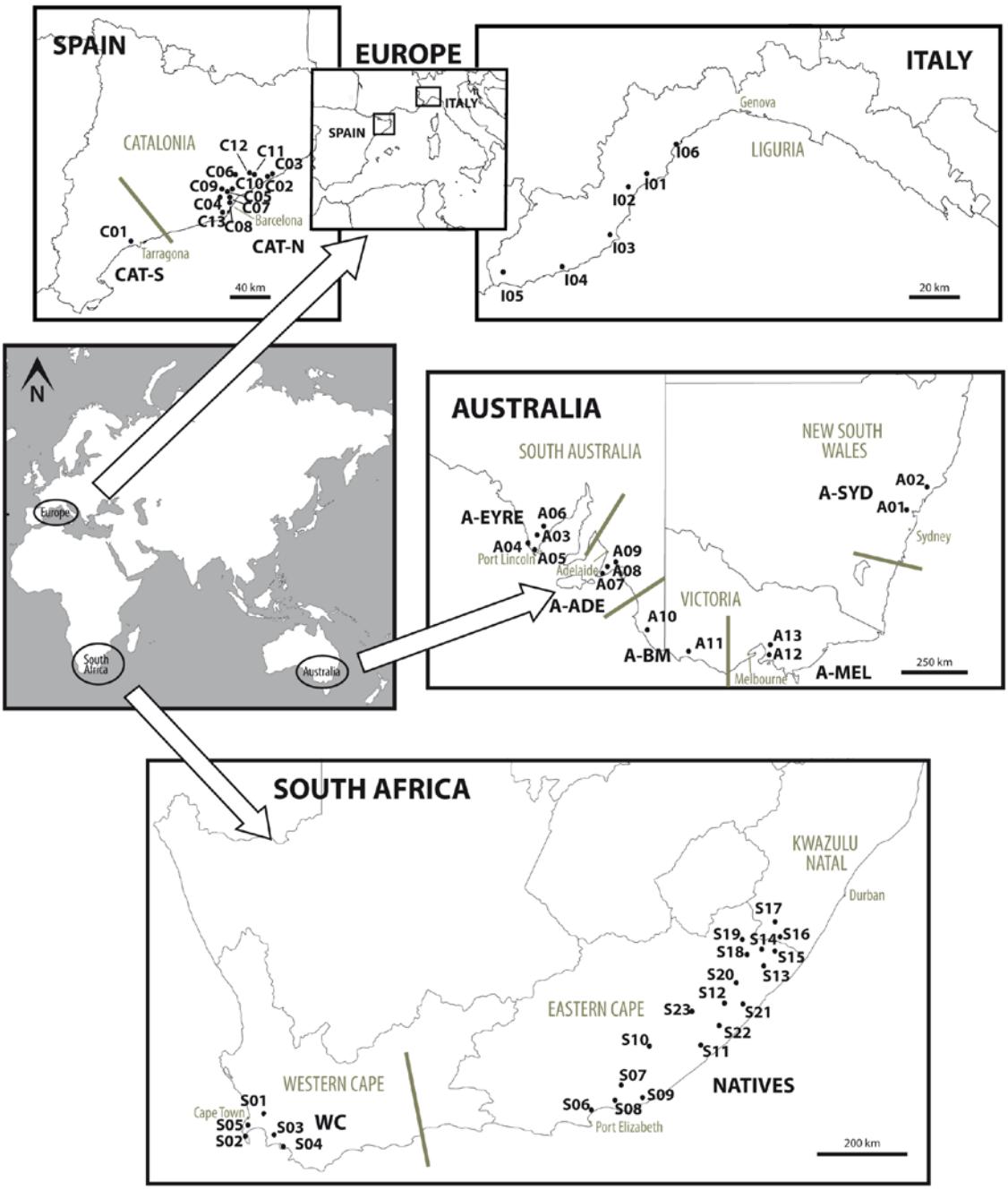
A-MEL = Melbourne populations; Europe: Italy = ITA populations and Spain: S-CAT = South Catalonian population and N-CAT = North Catalonian populations.

Fig. 5 Proportion of assignment test implanted in AFLPOP at minimal log likelihood difference 1 following the invasion chronology of *Senecio pterophorus*: **A**, between Western Cape populations and native South African lineages; **B**, between Australian subregions and native South African lineages; and **C**, between European regions and Australian subregions and native South African lineages. Abbreviation for groups: South African native area: SA-1, SA-2 and SA-3 lineages; Australia: A-EYRE = Eyre Peninsula populations; A-ADE = Adelaide populations; A-BM = Barker-Mallee populations; Europe: Italy = ITA populations and Spain: S-CAT = South Catalonian population and N-CAT = North Catalonian populations. None = no assignment success.

Fig. 6 Genetic diversity metrics (*PLP* = percentage of polymorphic loci; *H*j = average gene diversity; *B*r(5) = band richness after rarefaction to 5) of *Senecio pterophorus* from the native region (SA = South Africa), the South African native range expansion (W. CAPE = Western Cape) and the introduced regions (AUS = Australia, SPA = Spain and ITA = Italy). Results of ANOVAs that tested the differences in the genetic diversity metrics between regions are listed below the graphs. Open circles represent outlier values. An asterisk indicates significant differences based on linear models for the genetic diversity metrics that compared those of the native with each introduced region (see Table S6).

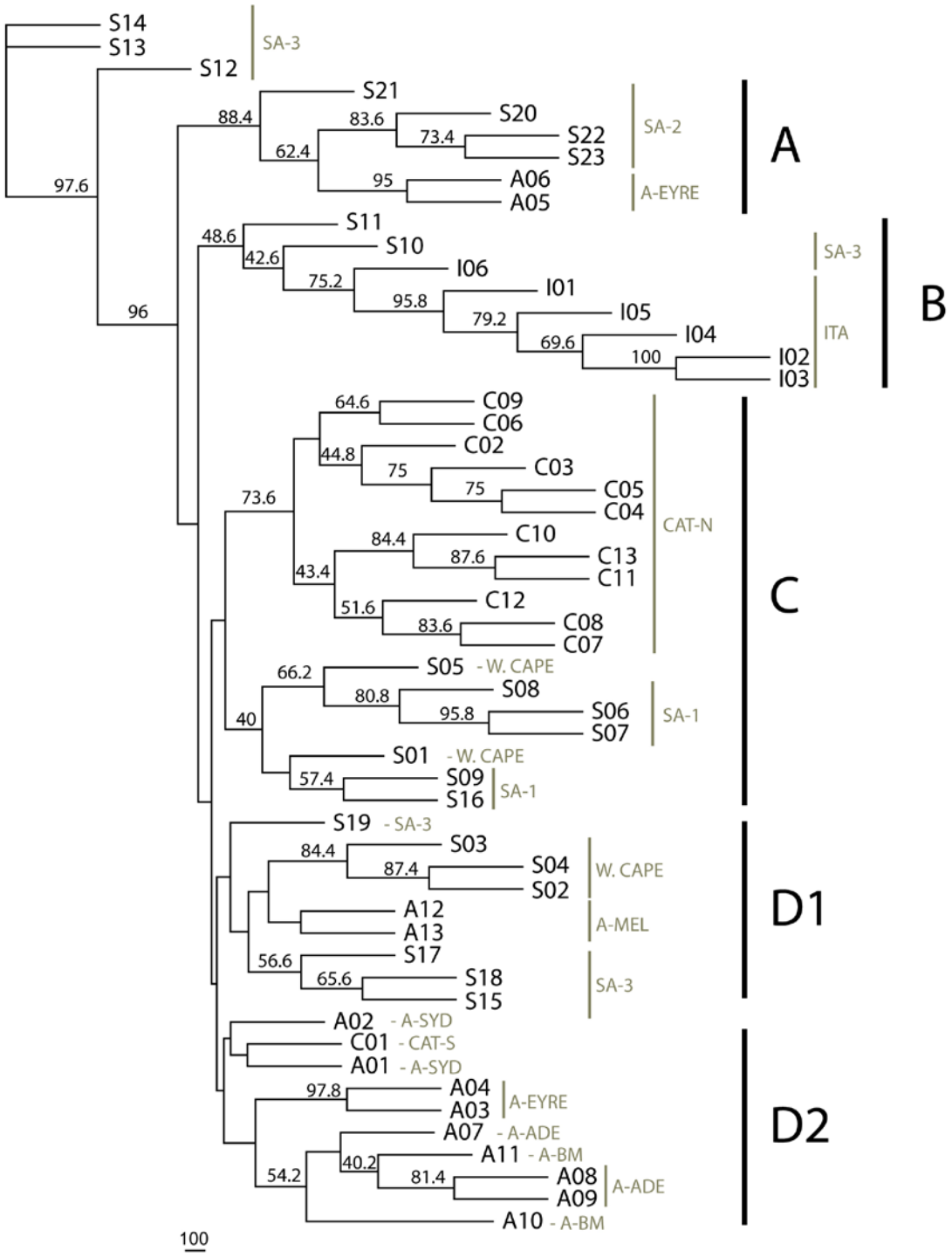
Fig. 7 Colonization routes of *Senecio pterophorus* within South Africa and across continents. The arrows indicate putative pathways: White arrow = SA-1 lineage; Gray arrow = SA-2 lineage; and Black arrow = SA-3 lineage. Question marks indicate uncertain introductions.

Figure 1



Vilatersana R, Sanz M, Galian A, Castells E (2016) Biological Invasions
DOI: 10.1007/s10530-016-1150-1

Figure 2



Vilatersana R, Sanz M, Galian A, Castells E (2016) Biological Invasions
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Figure 3

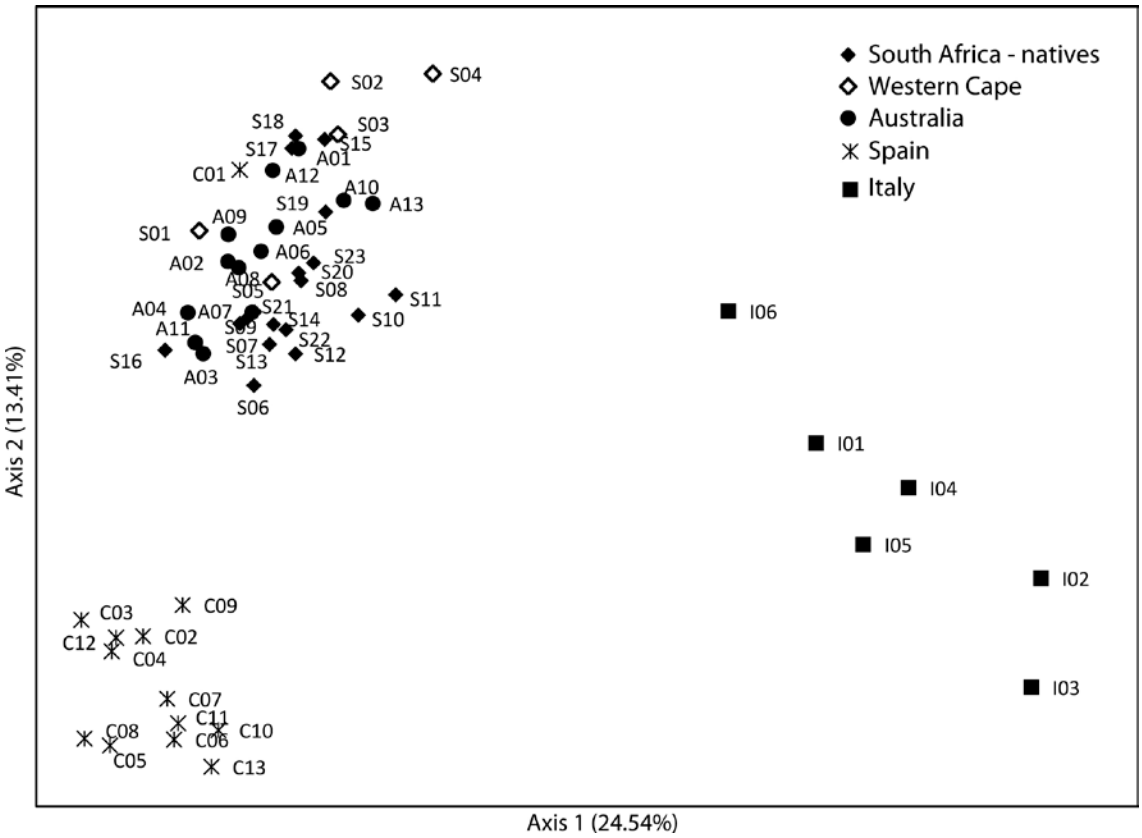
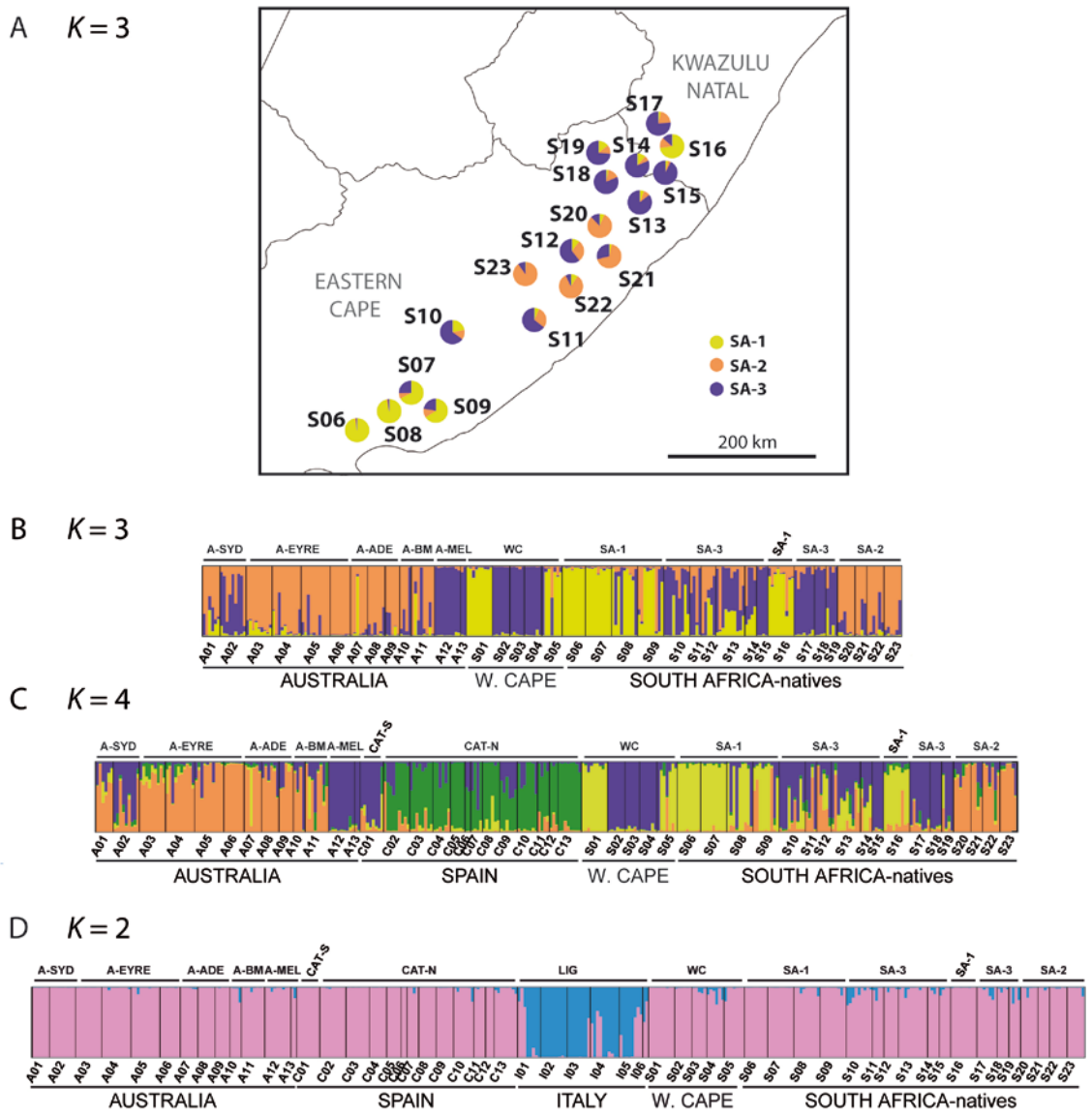

South Africa - natives
Western Cape
Australia
Spain
Italy
S02
S04
S18
S03
S17
S15
A01
C01
A12
A10
S19
A13
A09
S01
A05
A06
S23
A02
S20
S08
S11
A04
A07
S21
S14
S10
A11
S09
S07
S22
S16
S13
S12
A03
S06
I06
I01
I04
I05
I02
I03
C03
C09
C12
C02
C04
C07
C11
C08
C10
C06
C05
C13
Axis 2 (13.41%)
Axis 1 (24.54%)

Figure 4



Vilatersana R, Sanz M, Galian A, Castells E (2016) Biological Invasions
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Figure 5

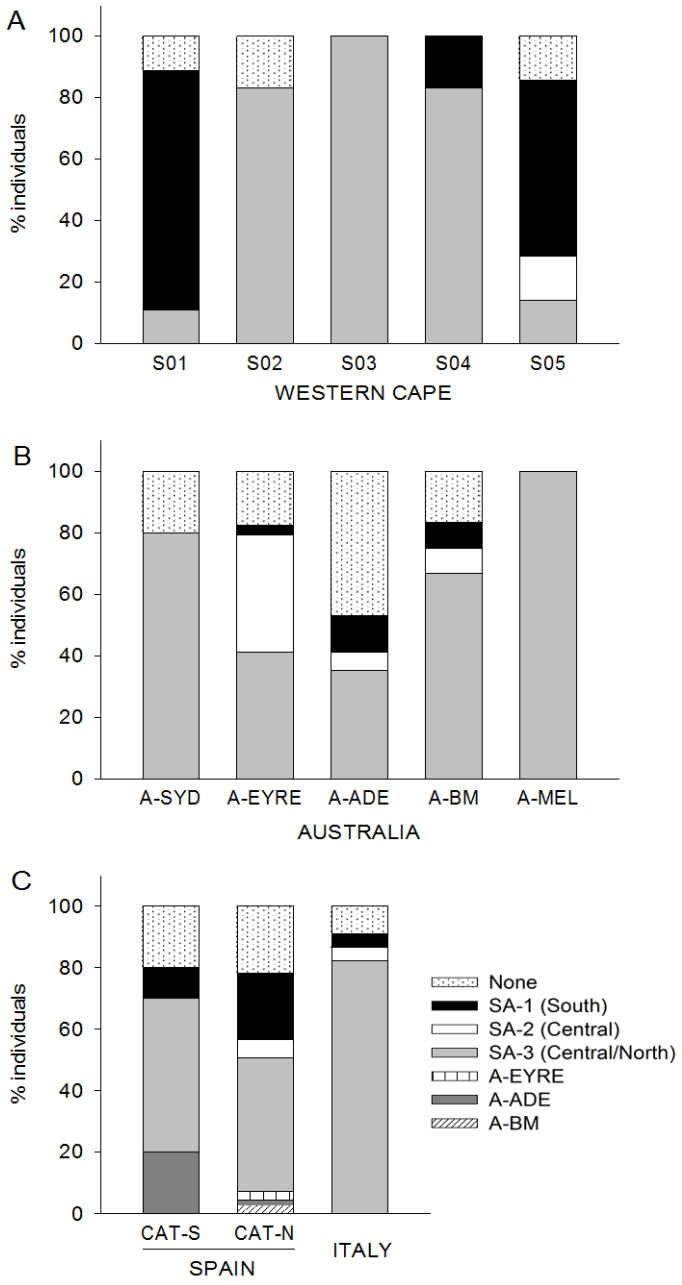

A
100
80
60
40
20
0
% individuals
S01
S02
S03
S04
S05
WESTERN CAPE
B
100
80
60
40
20
0
% individuals
A-SYD
A-EYRE
A-ADE
A-BM
A-MEL
AUSTRALIA
C
100
80
60
40
20
0
% individuals
CAT-S
CAT-N
SPAIN
ITALY
None
SA-1 (South)
SA-2 (Central)
SA-3 (Central/North)
A-EYRE
A-ADE
A-BM

Figure 6

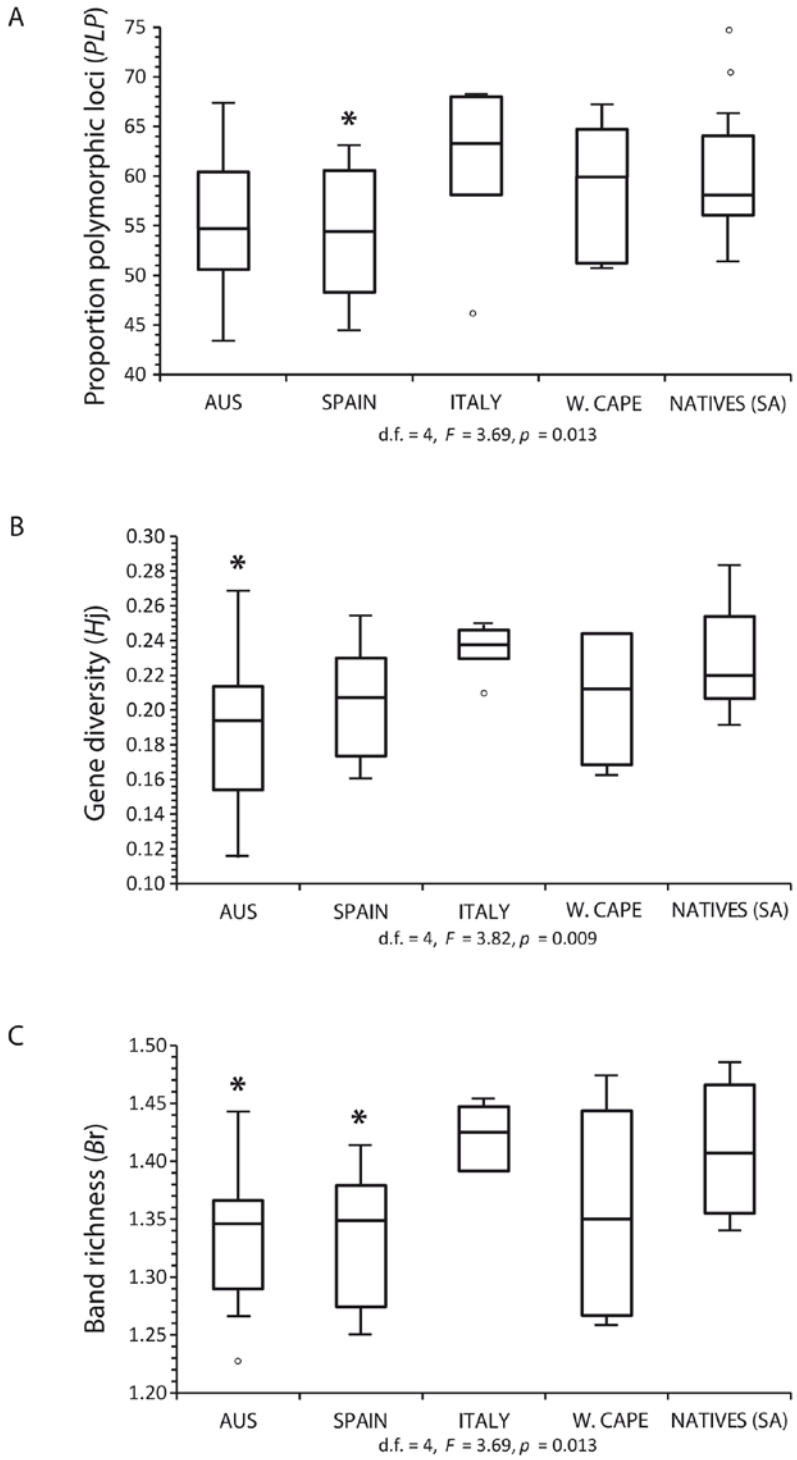
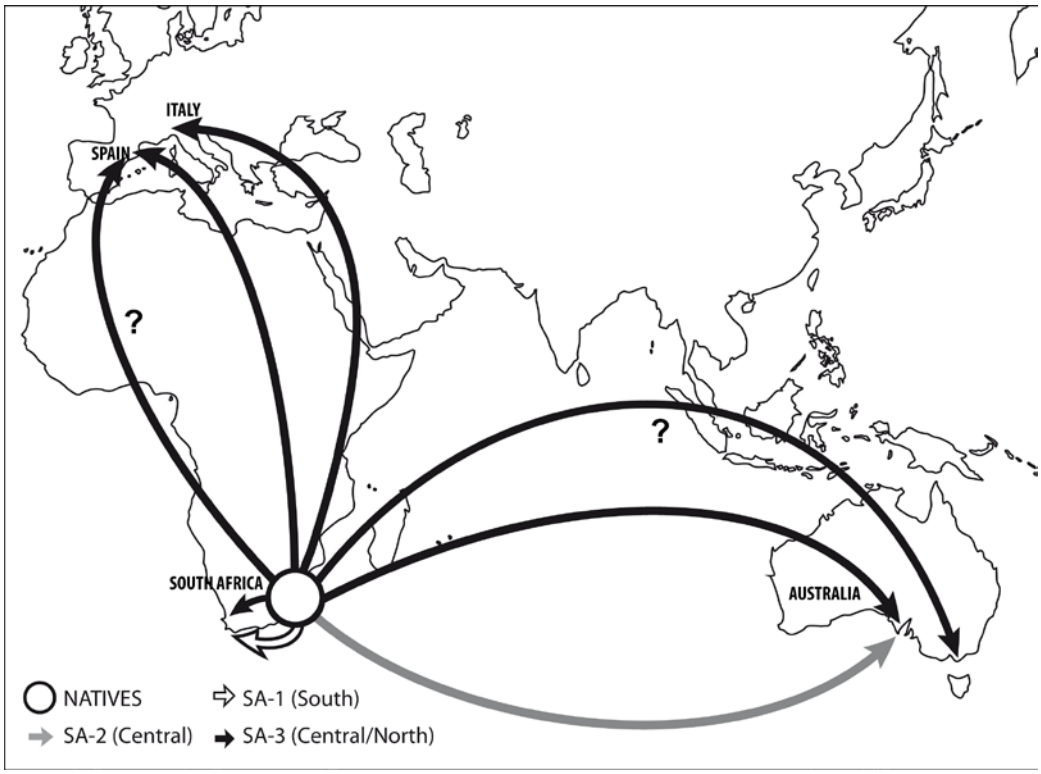

A
Proportion polymorphic loci (PLP)
75
70
65
60
55
50
45
40
AUS
SPAIN
ITALY
W. CAPE
NATIVES (SA)
d.f. = 4, F = 3.69, p = 0.013
B
Gene diversity (Hj)
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
AUS
SPAIN
ITALY
W. CAPE
NATIVES (SA)
d.f. = 4, F = 3.82, p = 0.009
C
Band richness (Br)
1.50
1.45
1.40
1.35
1.30
1.25
1.20
AUS
SPAIN
ITALY
W. CAPE
NATIVES (SA)
d.f. = 4, F = 3.69, p = 0.013

Figure 7


ITALY
SPAIN
?
?
SOUTH AFRICA
AUSTRALIA
NATIVES
SA-1 (South)
SA-2 (Central)
SA-3 (Central/North)