STUDIES IN TAXONOMY, BIOGEOGRAPHY AND EVOLUTION OF *SENECIO* SECT. *SENECIO* (COMPOSITAE)

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ABSTRACT

Senecio L. (Compositae) remains poorly circumscribed. The taxonomy, biogeography and evolution of sect. *Senecio* were explored using morphology, cytology, crossing experiments and molecular systematic methods. Phylogenies based on nuclear ribosomal DNA (ITS) sequences strongly support the section comprising widely disjunct species in southern Africa, southwest North America and the Mediterranean basin. A southern African origin is supported, with molecular clock estimates placing this in the Early Pliocene (~5 Mya). Two factors that appear to have influenced the evolution of the group are the development of Mediterranean climate during the Pliocene, and Pleistocene climatic fluctuations. Two dispersal events to the New World are supported. Firstly, during the Mid-Pliocene, and secondly, the dispersal of *S. mohavensis* from Southwest Asia sometime in the last 150,000 years. Long-distance dispersal represents the only plausible explanation, as no suitable land bridges have existed since the Mid-Tertiary. Whether the first dispersal occurred directly from southern Africa or from the Mediterranean basin is unresolved. *Senecio mohavensis* is newly recognized as a disjunct species based on a taxonomic reassessment of the allied *S. flavus*, itself disjunct between southern and northern Africa. Novel pappus characteristics are described from both species that are suggestive of dispersal by birds. Dispersal of the section within Africa may have utilized an East African arid corridor. Two population-level studies of Mediterranean taxa using randomly amplified polymorphic DNA (RAPD) markers indicate that long-distance seed dispersal is rare. Atypical populations of the Moroccan Atlantic coast endemic *S. leucanthemifolius* var. *casablancae* probably reflect divergence due to restricted gene flow. Western, central and eastern Mediterranean populations of the widespread *S. glaucus* subsp. *coronopifolius* are differentiated by morphology and RAPDs. RAPD markers indicate that subspecies *glaucus* and *S. hesperidium* have been independently derived from eastern and western Mediterranean populations respectively. Divergence from subspecies *coronopifolius* may reflect adaptation to maritime conditions in both cases.

DECLARATION

I, Max Coleman, hereby certify that this thesis, which is approximately 33,000 words in length, has been written by me, and that it is a record of work carried out by me, and that it has not been submitted in any previous application for a higher degree.

Max Coleman

September 2002

STATEMENT

I was admitted as a research student to the School of Biology, University of St Andrews, in October 1998 and as a candidate for the degree of PhD in October 1999; the higher study for which this is a record was carried out in the University of St Andrews between 1998 and 2002.

Max Coleman

September 2002

CERTIFICATE

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of PhD in the University of St Andrews and that the candidate is qualified to submit this in application for that degree.

Richard J. Abbott

September 2002

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Max Coleman

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NOTE ON FORMAT

The format adopted in this thesis is the production of a general introduction and conclusion at either end of a series of chapters written in the format of a scientific paper. For consistency the style conventions of the *American Journal of Botany* have been adopted here. Three of these chapters have been independantly published in separate journals. Titles and author details remain as they appear here, but minor text differences exist:

Chapter 2 – *Edinburgh Journal of Botany* 58: 389-403 (2001).

Chapter 3 – *American Journal of Botany* (In press).

Chapter 4 – *Molecular Ecology* 12: 423-434 (2003).

"The story, here told very briefly and imperfectly, is as worthy of an epic as the wanderings of Ulysses or the travels of Marco Polo; in fact, all the wanderings of primeval men are dwarfed into hurried events of recent and local interest in comparison with these journeys of the Groundsel in which about sixty million square miles were traversed in some forty million years."

Professor James Small, Queen's University, Belfast, 1921

Taken from a lecture – The Wanderings of the Groundsel – printed in the proceedings of the Belfast Natural History and Philosophical Society, 1920-1921.

CONTENTS

1.1 INTRODUCTION

1.1.1 Taxonomic background

1.1.1.1 Generic and sectional circumscription—*Senecio* L. (Compositae) is an almost cosmopolitan genus including ~1,250 species (Bremer, 1994; Mabberley, 1997). At this size, *Senecio* is among the largest angiosperm genera. The high species diversity and occurrence on all continents except Antarctica indicate that *Senecio* offers potential for evolutionary and biogeographic study. However, unclear generic and infrageneric classifications (Jeffrey et al., 1977; Nordenstam, 1977, 1978; Jeffrey, 1979; Vincent and Getliffe, 1992; Bremer, 1994; Pelser, Gravendeel and van der Meijden, 2002) have hindered such studies.

The circumscription of *Senecio* has been clarified in recent decades. During the nineteenth-century Bentham (1873a, b) and Hoffman (1892) established a broadly defined *Senecio*. Their classifications represented a convenient grouping of more or less related species that were appropriate to the needs of the time. By the late twentieth-century the genus contained an estimated 3,000 species and displayed a range of variation exceeding the combined ranges exhibited by several other genera (Jeffrey et al., 1977). Consequently, taxonomists have sought to reduce an unwieldy and undoubtedly artificial super-genus (Jeffrey et al., 1977; Nordenstam, 1977, 1978; Jeffrey, 1979; Jeffrey, 1992; Vincent and Getliffe, 1992; Bremer, 1994). This has been achieved by the recognition of a number of satellite genera either resurrected from synonymy or newly described (for commentaries and references see Barkley, 1985; Bremer, 1994; Barkley, Clark and Funston, 1996; Nordenstam, 1996; Barkley, 1999).

It has now become widely accepted that classifications should attempt to reflect evolutionary relationships (Backlund and Bremer, 1998; Judd et al., 1999). Ideally, taxa should represent monophyletic groups (groups containing all descendents of a common ancestor). Such groups are defined by synapomorphies (shared derived characters). The value of this approach is that classifications based on the principle of monophyly offer the greatest predictive ability, due to common descent. In addition, classifications based on phyletic relationships are of universal value for general biological research.

Despite having undergone a significant reduction in size via the removal of putative monophyletic satellite genera, *Senecio* is still probably not monophyletic (Bremer, 1994; Barkley, Clark and Funston, 1996; Kadereit and Jeffrey, 1996; Nordenstam,

1996). Bremer (1994) has suggested that similarities in floral details between the remaining *Senecio* species represent plesiomorphic (ancestral) character states, which are only capable of defining a paraphyletic group. Because *Senecio* accounts for approximately one third of the tribe Senecioneae, the generic classification of the tribe must be considered unresolved until *Senecio* itself has been more robustly defined.

A problem closely associated with the circumscription of *Senecio* is the highly artificial nature of the infrageneric classification. The division of *Senecio* into sections has generally been carried out for particular geographic regions in the absence of a global perspective. Jeffrey et al. (1977) considered the widely different concepts of infrageneric taxa in *Senecio* to have rendered them virtually meaningless. The majority of the ~150 sections are not distinguished from each other by unique characters or syndromes of characters (Pelser, Gravendeel and van der Meijden, 2002). Consequently, some taxonomists (Barkley, 1978; Barkley, Clark and Funston, 1996) have paid little attention to sections, opting instead to define "groups-of-convenience" with no presumption of evolutionary status.

The problem of sectional delimitation is exemplified by the case of sect. *Senecio* and sect. *Jacobaea* (Mill.) Dumort. in Europe. Pelser, Gravendeel and van der Meijden (2002) have recently reexamined the limits of sect. *Jacobaea* with a view to clarifying its evolution. Section *Jacobaea* is mainly perennial, and sect. *Senecio* is mainly annual. However, no clear morphological distinctions between them have been found, although each section does have a discernible facies that allows separation with some experience. Nevertheless, a few species have been inconsistently placed (for details see Pelser, Gravendeel and van der Meijden 2002). Using a molecular phylogenetic approach based on chloroplast (cp) DNA and nuclear ribosomal (nr) DNA sequences, these workers have shown that sect. *Jacobaea* is a well-supported monophyletic clade restricted to Eurasia and not closely related to sect. *Senecio*.

In a situation in which distantly related lineages may display little morphological distinction, the reliability of morphology as an indicator of relationships may be questioned. In fact, the majority of work clarifying the limits of *Senecio* has been based on morphology. Micromorphology, in particular, has provided some highly consistent characters for delimiting *Senecio* (Vincent and Getliffe, 1992) and subtribal groupings within the Senecioneae (see Bremer, 1994, for details).

An important series of papers aimed at clarifying generic and sectional limits in *Senecio* from a global perspective have been written by Jeffrey and co-workers (Jeffrey

et al., 1977; Jeffrey, 1979, 1987, 1992). As a global revision is impractical, the approach adopted in these papers was the scoring of a set of macro- and micromorphological characters in "modal" species chosen to represent larger species groups. The conclusion of the initial study (Jeffrey et al., 1977) was that *Senecio* should be restricted to a group identified as IX, but that further work was required to delimit sections. In the most recent paper (Jeffrey, 1992) a synthesis of the accumulated data was used to allocate modal species to genera and sections.

1.1.1.2 *Senecio* sect. *Senecio*—The focus of the four studies presented here is the predominantly annual sect. *Senecio*. This section represents the core of *Senecio* as it revolves around the type species for the genus *S. vulgaris* L. Synonyms of sect. *Senecio* include sects. *Obaejacae* DC., *Obaejacoideae* DC., *Annui* Harv. and *Annui* O. Hoffm. These names were established prior to the nomenclatural rule that sections containing the type species must bear the generic name.

Section *Senecio* is reasonably diverse in Europe with the main concentration of species in the Mediterranean basin. Europe and the Mediterranean are here termed the "core area" of sect. *Senecio*, because the type species *S. vulgaris* probably originated in this region (Kadereit, 1984a). Alexander (1979) enumerated 24 species from the Mediterranean and adjacent areas. Via a series of controlled crosses Alexander (1975) established that interfertility was generally high among members of this group. Indeed, some morphologically quite distinct species displayed high interfertility (Alexander, 1975).

A few species from the core area extend eastwards into Central Asia and Siberia (Alexander, 1979; Shishkin, 1995; Wiebe, 2000). Two species with distinctly eastern distributions not found in the core area are *S. subdentatus* Ledeb. and *S. kraschenninikovii* Schischk. The status of the former species is unclear as Alexander (1979) regarded it as conspecific with *S. glaucus* L. subsp. *coronopifolius* (Maire) Alexander, a widespread Mediterranean species. Beyond these areas, the distribution of the section must be viewed as provisionally defined.

According to Jeffrey's (1992) sectional placement of modal species, the global distribution of sect. *Senecio* includes the Mediterranean basin, Eurasia, southern Africa, montane tropical Africa, Madagascar, Australia, South America and North America. Some of these areas, such as South America and Australia appear to support a few taxonomically isolated species. The species of montane tropical Africa may be more

distantly related as they were not placed in the same subgroup as *S. vulgaris* by Jeffrey et al. (1977).

A connection to southern African species was made during the nineteenth century by Harvey (1865) who placed 27 annual species from the Cape area of South Africa in his sect. *Annui*, including *S. vulgaris*, which is introduced in southern Africa. Support for a link with many southern African species has also been provided by similarities in micromorphology (Vincent and Getliffe, 1992).

Southwestern North America is the other main area where species have been allocated to sect. *Senecio*. Greenman (1902) placed five American species and three introduced European species in sect. *Annui*, whereas Barkley (1978) defined an informal group "Annui" consisting of eight American and three introduced European species. Two of the native species were subsequently moved to the satellite genus *Packera*. Most recently, Jeffrey (1992) has placed *Senecio californicus* DC. in sect. *Senecio* as one of his modal species.

Assuming sectional placements are appropriate, it seems to be clear that the area of greatest diversity for sect. *Senecio* is southern Africa. In addition to Harvey's (1865) 27 annual species, Namibia supports an uncertain number of closely allied species (Merxmüller, 1967), whereas Jeffrey (1992) has placed Harvey's (1865) sect. *Sinuosi*, consisting of 21 species, into sect. *Senecio*. The occurrence of greatest diversity in southern Africa would be consistent with this area being a known centre of diversity for both *Senecio* and the tribe Senecioneae (Nordenstam, 1977; Bremer, 1994; Koekemoer, 1996). *Senecio* needs to be revised in southern Africa before any accurate assessment of species number for the genus or the section can be made.

1.1.1.3 Species delimitation and species concepts—Species delimitation in sect. *Senecio* has been complicated by a remarkable degree of morphological variation within species. Cultivation experiments (Alexander, 1975) using taxa from the core area have demonstrated considerable phenotypic plasticity. Other possible causes of this variation are introgression due to low barriers to hybridization and divergence resulting from restricted gene flow. Hybridization and introgression are discussed more fully below (section 1.1.3.1).

Species concepts have been debated at length, and the only clear conclusion seems to be that no one concept is universally applicable. The pros and cons of the different concepts are not discussed here, but for a recent review from a botanical perspective,

see Levin (2000). The species concept adopted here is outlined simply to provide additional background and is viewed merely as a conceptual tool. Taxonomists have generally viewed species as recognizable entities separated from other such entities by morphological discontinuities. This definition is accepted here but is brought up to date by accepting alongside it Mallet's (1995) genotypic cluster definition of species. In fact, these two definitions reflect the same basic conception of species as discreet clusters. Mallet's (1995) modification of the traditional taxonomists view simply reflects the tools of molecular genetics now frequently used in modern systematic research. This definition is practical rather than theory laden and suits the taxonomic focus of the studies presented here.

1.1.2 Biogeographic background

1.1.2.1 Ancestral areas and cladistic biogeography—The notion of a centre of origin of a particular group dominated historical biogeography until the 1970s. The search for centres of origin and associated dispersal explanations turned biogeography into a narrative approach. This resulted in the centre of origin concept being criticized (e.g., Croizat, Nelson and Rosen, 1974). In contrast, vicariance biogeography posits that distributions are explained by vicariance events rather than dispersal. Vicariance is the splitting of a formerly continuous taxon range, for example by continental drift or mountain building. A vicariance hypothesis can be falsified by a pattern of phyletic splitting that does not match the geological history. In other words, if vicariance alone explains the distribution then the order of splitting of lineages will reflect the order of vicariance events, and a particular clade will be restricted to a particular area. Long-distance dispersal, on the other hand, will be reflected in a breakdown of clade-area relationships.

The widespread application of techniques for phylogeny reconstruction has resulted in what has come to be called phylogenetic or cladistic biogeography (Nelson and Platnick, 1981; Humphries and Parenti, 1986; Brundin, 1988). Under this approach, the terminal taxa in a cladogram are replaced by their distribution to give an "area cladogram". Area cladograms are often only regarded as interesting to the extent that they reflect general patterns seen among unrelated groups, thereby highlighting "area relationships".

Aside from the search for area relationships, the historical biogeography of individual groups is of interest (Bremer, 1992). In situations for which a taxon may reasonably be assumed to have originally occupied a smaller area than its present distribution (e.g., cosmopolitan taxa) the search for a centre of origin is a reasonable goal. Cladistic methods have been used to provide a more rigorous means of identifying centres of origin. Bremer (1992) has devised a method for identifying what he prefers to call "ancestral areas". This method is based on the concept that positionally plesiomorphic areas are more likely to be the ancestral area than positionally apomorphic areas (Platnick, 1981). Another approach is to optimize "area" as a multistate character across a phylogeny using standard phylogenetic software (e.g., Hileman, Vasey and Parker, 2001).

1.1.2.2 Biogeography of sect. *Senecio*—In the case of *Senecio*, no recent attempt has been made to identify an ancestral area. Small (1919) believed that *Senecio* had originated in the Andes and that due to its vast distribution it must represent the ancestor of the entire family. This was based on Willis's (1918) age and area hypothesis, in which the largest group equates with the oldest. It is now known that *Senecio* occupies an apomorphic position in the Compositae (Bremer, 1994) and cannot therefore represent an ancestral form. In addition, the age and area hypothesis is now recognized to be a gross simplification.

An intriguing feature of the biogeography of sect. *Senecio* is that its extant distribution appears to display an association with Mediterranean-type climates. Greatest diversity appears to occur in southern Africa and the Mediterranean basin, with a lesser centre of diversity in southwestern North America. All of these areas share the characteristic "Mediterranean" summer-dry climate. Until the status of certain taxa outside of these areas has been assessed, the strength of this association is uncertain. Nevertheless, this observation does highlight the possibility that the history of the group is connected to the history of Mediterranean climates. This is discussed in more detail below (section 1.1.3.1).

An important question, assuming these disjunct lineages are closely related, is how disjunction occurred. Vicariance hypotheses involving Mediterranean taxa are problematic in that the five regions of Mediterranean climate are generally not thought to have been in direct contact (Raven, 1971; Axelrod and Raven, 1978; Quézel, 1978; Raven and Axelrod, 1978). Mediterranean climates are thought to have arisen in the last

five million years (Raven, 1971; Axelrod, 1975, 1977; Spect, 1979) and hence the five Mediterranean floras must be viewed largely in terms of contemporary geography. In general, each of the Mediterranean floras is highly distinctive and displays exceptional endemism (Raven, 1971). A generalist dry-adapted flora was proposed by Axelrod (1973, 1975) to have formed a nearly continuous belt from North America to Central Asia from the late Eocene to the end of the Oligocene (38-25 Mya). At this time a North Atlantic land bridge existed via Greenland, which would have supported tropical floras during the Eocene climatic optimum (Tiffney and Manchester, 2001). A vicariance hypothesis involving the North Atlantic land bridge would be supported by a sister group relationship exhibiting an estimated divergence time of at least 25 million years. Such an ancient split in Mediterranean lineages has been supported in Old World and New World Arbutoideae (Ericaceae) (Hileman, Vasey and Parker, 2001) and *Datisca* (Datiscaceae) (Liston, 1997). However, *Senecio* as a recognizable lineage may not have existed this early. The earliest fossils reliably attributed to the Compositae are pollen grains from Oligocene deposits (Graham, 1996). Bremer (1994) considers that the Compositae probably split from its sister group during the Early Tertiary.

Another possible corridor of arid habitat that may have enabled movement of Mediterranean taxa within the Old World has been suggested through the East African mountains (Balinsky, 1962; Verdcourt, 1969). This corridor is thought to have attained greatest development during the Pleistocene glaciations (Verdcourt, 1969; van Zinderen Bakker, 1975; Goldblatt, 1978). Evidence in support of such a corridor is seen in some southwest-northeast African species disjunctions and rather more clearly in disjunct genera (Verdcourt, 1969; de Winter, 1971; Thulin, 1994).

Long-distance dispersal must also be considered possible in the case of *Senecio*. *Senecio* may be adapted to wind dispersal as most species have small achenes (fruits) with a well-developed pappus that acts like a parachute. Experimental work on *S. vulgaris* (Small, 1919) has indicated that under conditions of optimal humidity and wind speed there may be no limit to the distance an achene could travel. Contrary to this conclusion, extremely few *Senecio* species are disjunct between continents; a phenomenon noted by Bentham (1873b) for the entire Compositae. Species that are widely disjunct mainly represent cosmopolitan weeds spread by man. Nevertheless, extremely rare long-distance dispersal events have founded new lineages as the floras of isolated oceanic islands attest (Carlquist, 1974).

Evidence of presumably recent long-distance dispersal in sect. *Senecio* is provided by two extreme disjunctions at or about the species level. *Senecio flavus* (Decne.) Sch. Bip. occurs as two distinct subspecies: (i) the type subspecies in the Canary Islands, North Africa, and disjunctly in southern Africa, and (ii) *S. flavus* subsp. *breviflorus* Kadereit in similar environments in Southwest Asia. The two subspecies have a small zone of overlap in Sinai. The second disjunction involves *S. flavus* subsp. *breviflorus* and *S. mohavensis* A. Gray in southwest North America. Although treated as different species, the Southwest Asian and southwest North American taxa are morphologically very similar. They also display higher isozyme and cpDNA similarity than do the two subspecies of *S. flavus* (Liston, Rieseberg and Elias, 1989; Liston and Kadereit, 1995). Liston Rieseberg and Elias (1989) inferred a southern African origin of *S. flavus* based on the relative ages of the desert environments. Under their biogeographic hypothesis, northward migration during the Pleistocene via East Africa was followed by subspecific differentiation in Southwest Asia and long-distance dispersal to the New World. It was suggested that dispersal from the Old World to the New World occurred via achenes attached to birds (Liston, Rieseberg and Elias, 1989).

1.1.3 Evolutionary background

1.1.3.1 Evolution of *Senecio* sect. *Senecio*—A number of studies have examined the evolution of sect. *Senecio* in the Mediterranean basin (Comes and Abbott, 1998, 1999a, b, 2000, 2001) and the British Isles (reviewed in Abbott and Lowe, 1996) and found evidence for evolution involving introgression, allopolyploid speciation and divergence. The morphological intergradation of infraspecific taxa commonly encountered in the section suggests that introgression and/or divergence are ongoing processes.

The timing of appearance and diversification of the section is of interest because of the apparent association with Mediterranean regions. This suggests a link to the development of the characteristic summer-dry climate of these regions, which began during the Pliocene (5.2 to 1.6 Mya) (Raven, 1971; Axelrod, 1975, 1977; Spect, 1979). Aridity has been suggested as a stimulus to evolution due to higher levels of population genetic subdivision (Stebbins, 1952; Axelrod, 1972). However, this hypothesis has been rejected in sect. *Senecio* by a comparison of genetic structure in closely related species that differ in their ecogeographic regimes (Comes and Abbott, 1999b). Nevertheless, the emergence of new habitats/ecological conditions has been identified as a cause of recent

evolutionary radiations [e.g., *Espeletia* (Compositae): Monasterio and Sarmiento, 1991; *Dendrosenecio* (Compositae): Knox and Palmer, 1995].

Climatic fluctuations during the Pleistocene may also have promoted diversification. Repeated fragmentation of species ranges may restrict gene flow, thereby favouring divergence (Wright, 1978; Slatkin, 1985). Further, small isolated populations would be more prone to genetic drift. Support for the influence of Pleistocene climate fluctuations would be provided by evidence of radiation within the last 1.6 million years. A nrDNA phylogeny of Mediterranean *Senecio* has supported diversification between 0.44 and 0.88 Mya in two subclades of the section (Comes and Abbott, 2001). However, it is unknown whether similar recent diversification has occurred in other regions where the section is putatively found. Studies of other Mediterranean plant groups (Bell and Patterson, 2000; Richardson et al., 2001) have established recent speciation, although in these cases diversification extended back into the Pliocene.

Based on the ecology and reproductive biology of the section additional factors that may have influenced the evolution of sect. *Senecio* are outlined below.

Hybridization is a common and creative evolutionary process in some plant groups (Abbott, 1992; Arnold, 1997; Rieseberg, 1997). The potential importance of hybridization in sect. *Senecio* is well established (Abbott and Lowe, 1996; Abbott et al., 2000, 2002; Comes and Abbott, 1999a, 2001). As already noted, Alexander (1975) found generally high levels of interfertility between Mediterranean members of the section. The unspecialized pollination syndrome of *Senecio* would make intertaxon pollination likely in mixed natural populations.

Achene dispersal by wind may be highly effective in *Senecio* (Small, 1919) and this may maintain gene flow among distant populations. However, rare extremely long-distance dispersal events could establish isolated populations beyond the range of significant gene flow. Such populations would then begin to diverge. This is most likely in self-compatible species (Baker, 1967), of which a number exist in sect. *Senecio*. Extremely long-distance dispersal could also bring congeners into contact that may subsequently hybridize. A possible example of this is seen in *S. flavus* subsp. *breviflorus*, which occurs in Southwest Asia. Chloroplast and nrDNA indicates that this taxon has a Mediterranean progenitor (Liston and Kadereit, 1995; Comes and Abbott, 2001), whereas morphology suggests that *S. flavus* subsp. *flavus* is the other parent. *Senecio flavus* subsp. *flavus* is thought to be of southern African origin (Liston, Rieseberg and Elias, 1989).

A rich diversity of habitats will also increase the possibilities for speciation. Superficially, sect. *Senecio* appears to be uniform in its ecological requirements for open and rather disturbed habitat. However, many species appear to have specific habitat requirements. A number of species are strictly coastal, whereas inland species may be associated with a particular range of altitude or geology. The three varieties of *S. leucanthemifolius* Poiret in Morocco provide a good illustration of this. *Senecio leucanthemifolius* var. *casablancae* Alexander is restricted to the Atlantic coast, whilst var. *fradinii* (Pomel) Batt. and var. *major* Ball are inland varieties, the former of calcareous soils and the latter of calcareous rocky hillsides. Similarly, *S. ertterae* T. M. Barkley is restricted to an unusual volcanic soil in Malheur County, Oregon (Barkley, 1978).

1.1.3.2 Molecular data—The examination of evolutionary relationships has been greatly aided by the application of molecular markers to a wide range of biological questions (Avise, 1994). Examination of phylogenetic relationships has benefited from the development and acceptance of explicit phylogenetic approaches and the use of DNA sequences as a data source. Molecular markers have also been widely applied to population-level evolutionary questions such as levels of gene flow, mating patterns and natural selection. The earliest work of this type utilized isozymes, and such markers are still used (e.g., Comes and Abbott, 1998, 1999b). In recent times, the use of polymerase chain reaction (PCR) amplified DNA regions as a data source has become widespread (Arnold and Emms, 1998).

The study of evolutionary relationships using DNA data needs to take account of hybridization. A second process that must also be considered is the retention of ancestral polymorphisms through speciation events (Avise, 1994). This process is called "incomplete lineage sorting" and has received less attention than reticulate evolution (Comes and Abbott, 2001). Both processes can influence the interpretation of the mainly uniparentally inherited plastid genome as this passes through subsequent generations and speciation events (Wendel and Doyle, 1998). A possible example of incomplete lineage sorting of chloroplast polymorphisms has been documented in *S. gallicus* Vill. from the Iberian Peninsula (Comes and Abbott, 2001).

To distinguish between introgression and divergence it is necessary to adopt a phylogeographic sampling strategy (e.g., Comes and Abbott, 1998, 1999a, b, 2001). Under such an approach a large number of loci are sampled in multiple populations

spread across the range of a taxon. Combining phylogeographic and phylogenetic approaches enables exploration of both microevolutionary processes and phylogenetic relationships. This is particularly valuable as it can provide a temporal dimension through which to view evolutionary processes (Comes and Abbott, 2001).

1.1.3.3 Phylogenetics and sect. *Senecio*—Because sect. *Senecio* has so many potential members it is not possible to achieve the ideal of complete taxon sampling. To estimate a phylogeny from a random sample of taxa would risk inferring incorrect relationships. DNA sequence data frequently displays variation in evolutionary rate across a phylogenetic tree. Rapidly evolving lineages tend to be connected, whether or not they are related, because with only four alternative states (A, G, C, T) for each position in a sequence, random changes in parallel (homoplasy) can outnumber characters indicating common ancestry. This situation is called "long-branch attraction" (Felsenstein, 1978) and is mainly solved by adequate sampling. Therefore, the only reliable way to construct a phylogeny of sect. *Senecio* is to work outwards from the centre establishing progressively comprehensive sister-groupings.

The close relationship of many of the Mediterranean taxa is well-established (Alexander, 1975; Comes and Abbott, 1999a, 2001). Consequently, sampling effort in the present study has focussed on southern Africa and southwest North America, as these areas appear to support the next most closely related species groups. Consequently, the main aim of the phylogenetic work was to explore the historical biogeography of the section. Phylogenies were estimated from sequences of the internal transcribed spacers (ITS1 and ITS2) of nrDNA. ITS sequences have demonstrated great potential for resolving phylogenetic relationships at lower taxonomic levels (Baldwin et al., 1995) and have been widely used in Senecioneae (Bain and Golden, 2000; Comes and Abbott, 2001; Pelser, Gravendeel and van der Meijden, 2002). The ribosomal genes and spacer regions are highly repetitive in the nuclear genome, and hybridization can produce genomic polymorphism. In cases where this is suspected the cloning of ITS sequences is necessary to establish the polymorphism with certainty (Hershkovitz, Zimmer and Hahn, 1999). Such polymorphism may be lost through time via the process of concerted evolution (Hillis and Dixon; Hillis et al., 1991) in which unequal crossover at meiosis can homogenize gene copies in the direction of one parent. Such a process has been inferred in *S. flavus* subsp. *breviflorus* (Comes and Abbott, 1999a, 2001), which contains an ITS sequence closely similar to *S. glaucus*.

1.1.3.4 Population-level study of sect. *Senecio*—In the present study two DNA-based marker systems have been used to assess population-level genetic variation: (i) restriction fragment length polymorphism (RFLP) of PCR-amplified fragments of cpDNA, and (ii) randomly amplified polymorphic DNA (RAPD) (Williams et al., 1990).

Organelle markers, due to their uniparental inheritance, are of particular value for identifying cases of hybridization when used in conjunction with nuclear markers (Ennos et al., 1999). Theoretically, the chloroplast genome will display reduced mutation rates because of its haploid condition and lack of recombination (Ennos et al., 1999). This makes the chloroplast genome a good source of species-specific markers. However, extensive intraspecific cpDNA variation has enabled phylogeographic study (e.g., Soltis et al., 1997; Comes and Abbott, 1998, 1999a, b, 2001). For PCR-based assays the non-coding regions of the genome located outside the inverted repeats generally provide useful levels of variation (Taberlet et al., 1991; Demesure, Sodzi and Petit, 1995).

RAPD fragments represent a random sample of the entire genome, but primarily reflect the nuclear genome due to the small size of the organelle genomes (Lorenz, Weihe and Börner, 1994). The main advantage of RAPDs is the high level of polymorphism exhibited due to the large number of anonymous and, supposedly, neutral loci screened (Williams et al., 1990). Consequently, RAPDs generally provide greater resolution of genetic variation than isozymes (e.g., Wolff and Morgan-Richards, 1999; Comes and Abbott, 2000). For this reason, they have been widely used to study population structure, hybridization and introgression (Wolff and Morgan-Richards, 1999).

An important limitation of RAPDs with respect to studying population structure is that they represent mainly biallelic dominant markers; the alleles being fragment presence (+) or absence (-). Consequently, heterozygotes (+-) and homozygotes (++) are indistinguishable, and direct estimation of allele frequency is not possible. Indirect estimates can be made by assuming Hardy-Weinberg equilibrium, but in many plant populations the random mating assumption is likely to be violated leading to biased results. In fact, in three species of sect. *Senecio* so far examined with co-dominant isozymes Hardy-Weinberg equilibrium has been rejected in 36% of populations of *S. gallicus* and *S. vernalis* Waldst. and Kit. and 64% of populations of *S. glaucus*, despite all of them being obligate outcrossers (Comes and Abbott, 1998, 1999b). However, with

the use of appropriate analytical techniques RAPDs may be used to assess population genetic structure (e.g., Chalmers et al. 1992; Yeh, Chong and Yang, 1995).

1.1.4 Outlines of the studies

Four separate studies were conducted using a combination of traditional taxonomic, phylogenetic and phylogeographic approaches. The background to each is briefly outlined along with the methods used.

Chapter 2 reports a taxonomic reassessment of *S. flavus* and the apparently closely allied *S. mohavensis*. *Senecio flavus* was split into two distinctive subspecies by Kadereit (1984b). The type subspecies occurs in desert environments in the Canary Islands, North Africa, and southern Africa, whilst *S. flavus* subsp. *breviflorus* occurs in similar environments in Southwest Asia. *Senecio mohavensis* occurs in southwest North American. Examination of isozymes (Liston, Rieseberg and Elias, 1989) and cpDNA (Liston and Kadereit, 1995) has shown that *S. flavus* subsp. *breviflorus* displays greater similarity to *S. mohavensis* than to *S. flavus* subsp. *flavus*. *Senecio mohavensis* is tetraploid ($2n = 40$) (Ornduff et al., 1963), whilst *S. flavus* has been recorded as diploid ($2n = 20$) (Alexander, 1979) and tetraploid (Diaz-Lifante, Luque and Santa Barbara, 1992). Whether this difference in ploidy follows the subspecific split is unclear. Relationships between all three taxa were reassessed by examination of herbarium material, conducting chromosome counts and carrying out controlled crossess to investigate interfertility.

Chapter 3 reports a phylogenetic study, based on ITS sequences, of primarily southern African, southwest North American and Mediterranean taxa, including the disjunct species studies in chapter 2. Relationships between these groups of species have not previously been examined phylogentically. The primary aim was to explore the historical biogeography of sectional and species disjunctions. The establishment of a molecular clock has enabled estimation of the timing of disjunctions and diversification in the section.

Chapter 4 reports a population-level study into the possible causes of morphological variation in *S. leucanthemifolius* var. *casablancae* along the Moroccan Atlantic coast. Three atypical populations south of the known range may reflect introgression from *S. glaucus* subsp. *coronopifolius* or divergence due to low levels of gene flow. RAPD markers and PCR-RFLP of cpDNA were used to test these competing hypotheses.

RAPD markers indicative of different taxa/morphologies were identified, and RAPD phenotypes were clustered using multivariate analysis. Population genetic structure was assessed based on the RAPD data.

Chapter 5 reports a population-level study of morphological and DNA-based variation in *S. glaucus* subsp. *coronopifolius* across the Mediterranean basin. The extent of variation was quantified by a morphometric analysis of material grown under standardized conditions. RAPDs were used to assess DNA variation. Both morphological and DNA data were subjected to clustering using multivariate analyses. The relationship of the widespread subspecies to two localized coastal taxa was also assessed. It has previously been suggested that *S. glaucus* subsp. *glaucus* in the east Mediterranean and *S. hesperidium* Jahandiez, Maire and Weiller in the west Mediterranean have been indepedently derived from *S. glaucus* subsp. *coronopifolius* (Alexander, 1975).

A NEW SUBSPECIES OF *SENECIO MOHAVENSIS* (COMPOSITAE) REVEALS OLD–NEW WORLD SPECIES DISJUNCTION

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ABSTRACT

Examination of morphology, ploidy and interfertility in the two subspecies of the Old World *Senecio flavus* (Decne.) Sch. Bip. (Compositae) and the closely related New World *S. mohavensis* A. Gray does not support the subspecific taxonomy of *S. flavus*. On the basis of our results *S. flavus* subsp. *breviflorus* Kadereit is transferred to *S. mohavensis* as a new subspecies: *S. mohavensis* subsp. *breviflorus* (Kadereit) M. Coleman *comb. nov.* The new subspecies has a distribution that includes Arabia, the Middle East, Sinai, Iran, Afghanistan, Djibouti, and the Thar Desert of Pakistan. The type subspecies of *S. mohavensis* occurs in the Mojave and Sonoran deserts of North America, providing an unusual disjunct distribution at the species level. Separation from *S. flavus* is based upon differences in morphology and chromosome number. *Senecio flavus* is diploid ($2n = 20$), while both subspecies of *S. mohavensis* are tetraploid ($2n = 40$). Further support for the new taxonomic treatment is provided by the results of controlled crosses. No artificial hybrids have been generated from crosses made between the previously recognized subspecies of *S. flavus*, while crosses between the newly recognized subspecies of *S. mohavensis* have produced fertile hybrids. The fertility of the hybrids is significantly lower than the parental taxa ($P < 0.001$), indicating partial genetic divergence since isolation. Previous studies of isozyme and chloroplast DNA variation in all three taxa also support the new treatment. The similarity of the *S. mohavensis* subspecies suggests a relatively recent separation, although the amount of genetic divergence does not support a post-Colombian introduction. Given that land bridges to North America via Beringia and the North Atlantic last existed in the Oligocene, long-distance dispersal seems the most likely explanation. Natural dispersal to rather than from the New World is supported, but whether this took place in an easterly or westerly direction is unclear. The evolution of *S. mohavensis* remains equivocal.

Key words: Asteraceae; long-distance dispersal; polyploidy; *Senecio flavus*.

2.1 INTRODUCTION

Senecio flavus (Decne.) Sch. Bip. is described in the most recent revision of *Senecio* L. sect. *Senecio* (Alexander, 1979) as a radiate or non-radiate short-lived annual of deserts and rocky habitats, with a distribution including the Canary Islands, northern Africa, Arabia and Israel. Records of *S. flavus* have also been made from Namibia (Merxmüller, 1967), Chad and Mauritania (SONNERAT database of P), Iran, Afghanistan and Pakistan (Nordenstam, 1989), and southeastern Spain (Kadereit, 1984b). We have seen herbarium material from throughout the range.

The present location of type material of *S. flavus*, collected from Sinai by Nicolas Bové in 1831, is unknown. The main recipients of Bové's collection were Brussels (BR), Cambridge (CGE), Florence (FI), Geneva (G), Leiden (L), Paris (P) and Vienna (W), all of which have been checked without success. The species was originally described in the genus *Crassocephalum* Moench by Decaisne (1834) and was reported to be non-radiate. However, it has previously been noted that *S. flavus* also occurs with small ray florets at the eastern end of its range (Feinbrun-Dothan, 1978; Alexander, 1979). Our examination of herbarium material of *S. flavus* has revealed that the radiate plants occur extensively in Southwest Asia, with the western limit in Sinai and the eastern limit in Pakistan. A single specimen was also identified from Djibouti, extending the known distribution to the south. Sinai represents the only area where radiate and non-radiate plants are sympatric.

Kadereit (1984b) described the radiate plants as *S. flavus* subsp. *breviflorus*. The basis for this was the virtually allopatric distribution of the floral polymorphism and the discovery that ray floret achenes are epappose, whilst the marginal florets in non-radiate plants produce normally pappose achenes. In all other respects Kadereit (1984b) regarded the radiate and non-radiate plants to be morphologically indistinguishable.

Radiate and non-radiate individuals also exist in *S. mohavensis* A. Gray. This species is morphologically similar to *S. flavus* subsp. *breviflorus* but occurs in the Mojave and Sonoran deserts of North America. Unlike *S. flavus* subsp. *breviflorus*, radiate individuals of *S. mohavensis* generally have rays of irregular formation composed of two or three teeth of varying length. Gray (1884) mentioned these rays as deformed in his original description of the species, although he described it as typically non-radiate.

The morphological similarity between *S. flavus* subsp. *breviflorus* and *S. mohavensis* is supported by the existing molecular data (isozymes and chloroplast DNA), which

have revealed higher similarity between these two taxa than between either of them and *S. flavus* subsp. *flavus* (Liston, Rieseberg and Elias, 1989; Liston and Kadereit, 1995).

Chromosome counts have previously been carried out for these taxa, although uncertainty about identifications limits the usefulness of some of these. Alexander (1979) reported a count of $2n = 20$ for *S. flavus*, which probably relates to subspecies *flavus* (Alexander, personal communication, 1999). It should be pointed out that Alexander's (1979) work predates the division of *S. flavus* into two subspecies. It has subsequently been assumed that both subspecies of *S. flavus* are diploid. The validity of this assumption has been brought into question by a chromosome count of $2n = 40$ for *S. flavus* (Diaz-Lifante, Luque and Santa Barbara, 1992). Unfortunately, the material used by Diaz-Lifante, Luque and Santa Barbara (1992) was not identified to subspecies, and we have not seen voucher material. However, based on distribution, its origin in Israel would indicate *S. flavus* subsp. *breviflorus*. *Senecio mohavensis* has only ever been recorded as a tetraploid (Ornduff et al., 1963).

The aim of this study has been to reassess the taxonomic relationships between all three taxa. The existing molecular data (Liston, Rieseberg and Elias, 1989; Liston and Kadereit, 1995) do not provide support for the current taxonomy, and chromosome numbers in the subspecies of *S. flavus* remain uncertain. Consequently, this study has re-examined morphology and chromosome number. Relationships have also been addressed through crosses between the two subspecies of *S. flavus* and with *S. mohavensis*.

2.2 MATERIALS AND METHODS

Chromosome counts were carried out from root squash preparations of cultivated plants from ten geographically separated populations representing all three taxa (Table 1). Voucher specimens were deposited at the Royal Botanic Garden, Edinburgh (E).

The same cultivated material was also used to investigate interfertility. All three taxa exhibit high levels of self-compatibility so it was necessary to emasculate the maternal plants. Gibbs (1971), working with short rayed and non-radiate *Senecio*, found that removal of the apical 1 mm of the capitulum at the correct stage of development removed most anther tissue. This method was less successful in *S. mohavensis* and *S. flavus*, and careful washing away of self-pollen was required. The emasculation method finally adopted was the use of water-based typewriter correction fluid to cover all but

the ray florets as these lack anthers. The same technique was also used to emasculate non-radiate plants successfully in the case of *S. flavus* subsp. *flavus* as the marginal florets are predominantly female.

Herbarium material of all three taxa was examined for diagnostic morphological characters.

TABLE 1. Location of *Senecio* used in cytological investigations and crossing experiments.

Country	Location	Voucher[a]
S. mohavensis		
North America	Arizona, Painted Rock State Park	Coleman 27/00
	California, Zzyzyx	Coleman 29/00
	Nevada, Eldorado Canyon	Coleman 28/00
S. flavus		
subsp. *breviflorus*		
Israel	Dead Sea, Khirbet Mezin	Coleman 23/00
	Arava Valley, S. of Hazeva	Coleman 05/00
	Paran, Be' er Menuha	Coleman 22/00
	Paran, Ha Meshar	Coleman 21/00
Sinai	Gebel Abbas	Coleman 08/00
subsp. *flavus*		
Morocco	Asni	Coleman 31/00
Sinai	Sharn-el-Sheik to Dahab	Coleman 10/00

[a] All voucher specimens at the herbarium of the Royal Botanic Garden Edinburgh (E).

2.3 RESULTS

Chromosome counts showed that all three individuals of *S. mohavensis* and all five individuals of *S. flavus* subsp. *breviflorus* were tetraploid ($2n = 40$), whereas both individuals of *S. flavus* subsp. *flavus* were diploid ($2n = 20$).

Despite repeated reciprocal crosses between *S. flavus* subsp. *breviflorus* and *S. flavus* subsp. *flavus*, no hybrids were generated. A small number of plants corresponding to the maternal species were raised from the crosses (due to self-pollen not being totally removed), but generally poor achenes (defined as unfilled and pale) were set.

Crosses between *S. mohavensis* and *S. flavus* subsp. *breviflorus* were successful (material from Israel and North America, see Table 1) and yielded fertile F_1 hybrids that displayed marked hybrid vigour and were generally of intermediate morphology. However, hybrids that had a *S. mohavensis* parent that was radiate produced ray florets that were larger than either parent and normally developed, rather than of the deformed type seen in *S. mohavensis*. This may represent an example of transgressive segregation (Rieseberg, Archer and Wayne, 1999). No difficulty was experienced in repeating this

cross using either taxon as the maternal parent. Herbarium specimens of the hybrid plants produced have been deposited at the Royal Botanic Garden, Edinburgh (E).

Although the F_1 hybrids generated were fertile and F_2 plants were raised, casual observation of the percentage of normally developed achenes in capitula of the F_1 hybrids indicated a marked reduction in fertility. This was tested by calculating the percentage of normally developed achenes in ten capitula from each of three individuals of the F_1 hybrids and parental taxa. Due to the highly autogamous nature of the parental taxa the achenes produced probably resulted from self-pollination, although a low proportion of crosses may have occurred as the capitula were not bagged. In this case pollen is not a limiting factor to seed set so its source is not important; what is being measured is female fertility. Mean percentage of normal achenes with standard errors for the total of 30 capitula per taxon were as follows: *S. mohavensis* 71.23 ± 4.02, *S. flavus* subsp. *breviflorus* 74.38 ± 3.20 and F_1 hybrids 24.44 ± 2.00. The percentage values were arcsin transformed and subjected to a two-level nested analysis of variance, in which variance between taxa and variance between plants within taxa was tested (Table 2). The analysis showed the reduction of fertility in the hybrids to be highly significant ($P < 0.001$), whereas variance between plants within taxa was not significant ($P = 0.401$) (Table 2).

TABLE 2. Results of a nested analysis of variance (ANOVA) of fertility (arcsin transformed percentage of normal seed) in *S. mohavensis*, *S. flavus* subsp. *breviflorus* and their F_1 hybrid. Ten capitula from each of three plants in each taxon were sampled.

Source	d.f.[a]	SS[b]	MS[c]	*F* ratio	*P* value
Between taxa	2	19193.6	9596.8	69.98	< 0.001
Plants within taxa	6	862.1	143.7	1.05	0.401
Error	81	11108.2	137.1		
Total	89	31163.8			

[a] Degrees of freedom; [b] sum of squares; [c] mean squares.

Our findings, in conjunction with the existing molecular data (Liston, Rieseberg and Elias, 1989; Liston and Kadereit, 1995), lead us to conclude that it is necessary to transfer *S. flavus* subsp. *breviflorus* to *S. mohavensis* as a subspecies.

2.3.1 *Senecio mohavensis* A. Gray subsp. *breviflorus* (Kadereit) M. Coleman, *comb. nov.*—Illustrations: Nordenstam, Fl. Iranica 164: Tab. 63 (1989); Feinbrun-Dothan, Fl. Palaestina 3: Tab. 597 (1977). Basionym: *S. flavus* subsp. *breviflorus* Kadereit, Bot. Jahrb. Syst. 104: 510 (1984). Type: Jordan, Azraq ed Druz, *Gillet* 15569 (holo. G–n.v.).

2.3.1.1 Description—Erect, glabrous, glaucous annual. 5–20(–40) cm tall. *Stems* terete, finely ridged. *Leaves* 1–4 × 0.5–2 cm, simple or shallowly lobed, margin entire to dentate with minute wart-like projections, frequently purple below. Lower cauline leaves ovate, sessile or attenuate into petiole of 0.5–2 cm, middle and upper cauline leaves auriculate and amplexicaul. *Capitula* cylindrical, arranged in lax corymbs. *Calyculus bracts* 1–5. *Phyllaries* c.13, 6–8 mm. *Ray floret limbs* c.13, 1.5–3.5 mm, pale yellow, sometimes with reddish veins on the abaxial surface. *Achenes* 2–3 mm, subcylindrical, strigose. *Pappus setae* c.4 mm, absent from achenes of the ray florets. Flowering 3–5. Desert and rocky places, often in gullies and below north facing cliffs. Distribution: Egypt (Sinai), Djibouti, Israel, Jordan, Syria, Saudi Arabia, Oman, United Arab Emirates, Kuwait, Iran, Afghanistan and Pakistan (Thar Desert) (Fig. 1).

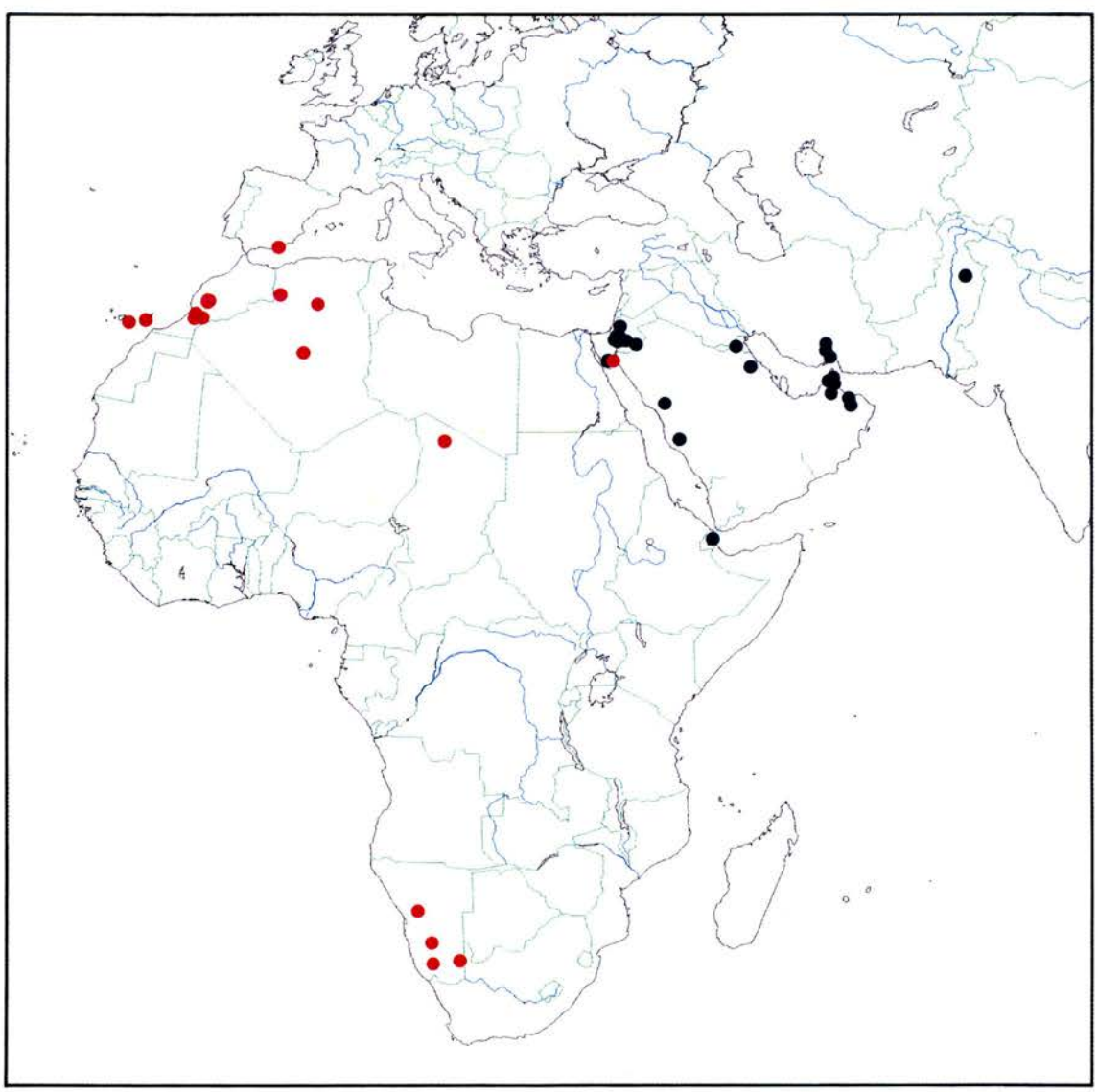

Fig. 1. Distribution of *Senecio flavus* (red) and *S. mohavensis* subsp. *breviflorus* (blue). All records from herbarium material.

2.3.1.2 Type material of *S. mohavensis* subsp. *mohavensis*—The material cited by Gray (1884) in his original description was collected by *Lemmon* from near the Colorado River and by *Pringle* from Sonora. No holotype was designated by Gray (1884), and formal lectotypification has not been carried out. However, Greenman (1915) incorrectly cited *Lemmon* 3129 (GH) as the holotype in *Annals of the Missouri Botanical Garden* 2: 580 (1915), and this constitutes a valid, albeit inadvertent, lectotypification. It is worth noting that the handwriting on this specimen indicates that Greenman added the number 3129, and the specimen should be cited as follows: Mojave desert, 11 V 1884, *Lemmon* s.n.

2.3.1.3 Nomenclatural notes—Although we do not consider the new subspecies sufficiently distinct to justify specific rank, the description of *S. decaisnei* DC. (one of three synonyms of *S. flavus* listed by Alexander, 1979) agrees well with *S. mohavensis* subsp. *breviflorus*, the only disagreement being the lack of ray florets. The three specimens of *S. decaisnei* in the De Candolle herbarium (microfiche E) come from Jordan, Saudi Arabia and Egypt. Duplicates of the specimens from Jordan and Saudi Arabia (E) (Arabiae Petraeae, Wadi Hebran, *Schimper* 1835: 344; Arabiae Felicis, Monte Gesser, *Schimper* 1837: 994) correspond to *S. mohavensis* subsp. *breviflorus*, whereas the Egyptian specimen (in deserto convallis, Meghegbe, *Acerbi* s.n.) corresponds to *S. flavus*. This may explain the confusing combination of characters described for *S. decaisnei*. The name *S. decaisnei* is also illegitimate because De Candolle cited *Crassocephalum flavum* Decne. in synonymy, thereby transferring a species with a legitimate name (*Crassocephalum flavum*) to a different genus without retention of the specific epithet (see International Code of Botanical Nomenclature, Greuter et al., 2000, Art. 11.4). This action means that, although two of the three specimens of *S. decaisnei* correspond to *S. mohavensis* subsp. *breviflorus*, the type of *C. flavum* must also typify *S. decaisnei*.

2.3.2 Specimens examined

S. mohavensis subsp. *mohavensis*

UNITED STATES OF AMERICA. CALIFORNIA, Death Valley National Monument, *DeBuhr and Wallace* 863 (E); Mojave Desert, *Lemmon* s.n. (GH, lectotype); Zzyzyx, *Liston* 645-3 (RSA); Death Valley, *Munz* 16468 (HUH); Death Valley National

Monument, *Steward* 7367 (HUH); ARIZONA, Painted Rock State Park, *Elias* 10190 (RSA); NEVADA, Eldorado Canyon, *Niles* 5375 (UNLV).

S. mohavensis subsp. *breviflorus*

EGYPT. Dchebel Ataka, *Bornmüller* 10703 (E); Mount Sinai, *Schimper* 1835: s.n. (E).

ISRAEL. Nahal Paran, *Danin and Knees* 1219 (RNG); 8 km southeast of Mizpe Ramon, *Danin and Knees* 1147, 1167 (RNG); Nahal Zin, *Liston* 474/3 (RNG).

JORDAN. El Inab, *Hunting Aero Survey* 73b (E); Arabiae Petraeae, Wadi Hebran, *Schimper* 1835: 344 (E, authentic material of *S. decaisnei*).

SYRIA. *Burton* s.n. (E); Wadi Jewerah, *Lowne* s.n. (E).

SAUDI ARABIA. Uema Figra 60 km west of Madinah, *Collenette* 7077 (E); Al-Figra, *Fayed* 1364 (E); Taif escarpment southeast of Mecca, *Lavranos and Collenette* 18508 (E); Jabal Dawmat al-Awdah, *Mandaville* 8861 (E); Jabal, *Naylor* 279 (E); Arabiae Felicis, Monte Gesser, *Schimper* 1837: 994 (E, authentic material of *S. decaisnei*).

OMAN. Wadi Bani Kharus, *Edmondson* 3225 (E); Wadi Falah, *Edmondson* 3258 (E); Al Fay, *FitzGerald* 39 (RNG); Muttrah, *Maconochie* 3288 (E); 51 km southeast of Nizwa-Sur, *Maconochie* 3386 (E); Bausher, *Rubens* 6a (E); Ar Rustaq, *Rubens* 100 (E).

UNITED ARAB EMIRATES. Sharjah, Jebel Mileiha, *FitzGerald* 9 (RNG); Wadi Shawka, *FitzGerald* 63 (RNG); Jebel Hafit Nr. Al Ain, *Western* 574 (E); 10 km north Masafi, *Western* 1153 (E); 10 km north Masafi, *Western* 1218 (E).

KUWAIT. *Macintyre* 19 (E).

IRAN. Bandar Abbas to Sirjan, *Léonard* 5937 (E); Kuh-e Genou, *Wendelbo and Foroughi* 15523 (E); 22 km north of Qotbabad, *Wendelbo and Foroughi* 15822 (E).

DJIBOUTI. Egerealeita, Gauda Mts., *Lavranos* 10492 (E).

PAKISTAN. Kirana Hills, Sargodha, *Stewart* 10930 (K); *Stewart* s.n. (E).

S. flavus

CANARY ISLANDS. Gran Canaria, Galdaz, *Murray* s.n. (K); Fuerteventura, Puerto de la Peña, *Nydegger* 26145 (RNG); Gran Canaria, *Pitard* 210 (L).

MOROCCO. 21 km from Asni, *Ait Lafkih* et al. 751 (RNG); Marrakech to Tizi n'Test, *Blanché* et al. 9459 (RNG); 88 km from Tiznit to Tafraoute, *Davis* 48741 (E); Ammeln valley, *Davis* 53853 (E); Marrakech, Ouirgane, *Castroviejo* et al. 4664 (RNG);

30 km southeast of Aït-Baha, Nr. Tioulit, *Jury* et al. 14388 (RNG); 27 km west of Tata, *Jury* et al. 14454 (RNG).

ALGERIA. Naama, Aïn Hadjhadj, *Bernedi* et al. 2368 (RNG); Ghardaïa, *Chevallier* s.n. (E); Metlili, *Cosson* s.n. (K).

CHAD. Tarso Tousside, Tibesti, *Grove and Johnson* 22 (K); Trouan Natron, Tibesti, *Hinchingbrookt* 28 (K).

EGYPT. Deserto convallis, Meghegbe, *Acerbi* s.n. (G–DC. microfiche E, authentic material of *S. decaisnei*); Sukari, *Sheded* 6205 (E).

NAMIBIA. Aroab, Keetmanshoop, *de Winter* 3365 (K); Maltahöhe, *Merxmüller and Giess* 28206 (M); Lüderitz-Süd, *Merxmüller and Giess* 32284 (M); Helmeringhausen, *Oliver* et al. 6491 (K); Haikamchab, *Searoch* 7668 (K); Chuosberge, *Seydel* 2016 (L); Kuisel, *Stuey* 2636 (K); Karibib, *Tölken and Hardy* s.n. (K); 22 km west of Rosh Pinah, *van Wyk* 8868 (M).

SPAIN. Sierra Alhamilla, Almeria, *Churchill* s.n. (K); Sierra Alhamilla, Almeria, *Ellman and Sandwith* 932 (K); Almeria, *Ripley* 72 (K).

2.3.3 Key to *Senecio flavus* and the two subspecies of *S. mohavensis*

1a. Lower cauline leaves triangular to cordate; petiole 2–5 cm_____***S. flavus***

1b. Lower cauline leaves ovate; petiole less than 2 cm or absent_____2

2a. Ray florets always present and normally developed; ray floret achenes epappose_____***S. mohavensis* subsp. *breviflorus***

2b. Ray florets present or absent but where present variable in length and often divided into two or three irregular teeth; marginal/ray floret achenes with an obvious pappus_____***S. mohavensis* subsp. *mohavensis***

2.4 DISCUSSION

The floral and pappus characters previously described (Kadereit, 1984b) allow easy separation of *S. mohavensis* subsp. *breviflorus* from *S. flavus*. Our examination of herbarium material has revealed further differences in the morphology that allow the determination of immature plants and those lacking flowers and fruit. The new morphological characters are leaf shape and nature of the margin. The lower cauline leaves of *S. mohavensis* subsp. *breviflorus* are ovate and sessile or have a petiole of up

to 2 cm into which the leaf blade is attenuate. The middle and upper cauline leaves are amplexicaul. The margins may be entire or dentate and frequently have minute wart-like projections. In contrast, the lower and middle cauline leaves of *S. flavus* have a triangular to cordate outline with angular teeth and a petiole of up to 5 cm. The upper cauline leaves are amplexicaul and strongly dentate. The important distinction between the species is that although both subspecies of *S. mohavensis* may have petiolate lower cauline leaves, these are never cordate in outline (Fig. 2). Morphological distinction of the two subspecies of *S. mohavensis* is less apparent from leaf morphology. The type subspecies usually exhibits more coarsely toothed leaf margins, although material of *S. mohavensis* subsp. *breviflorus* can exhibit the same characteristics (Fig. 2).

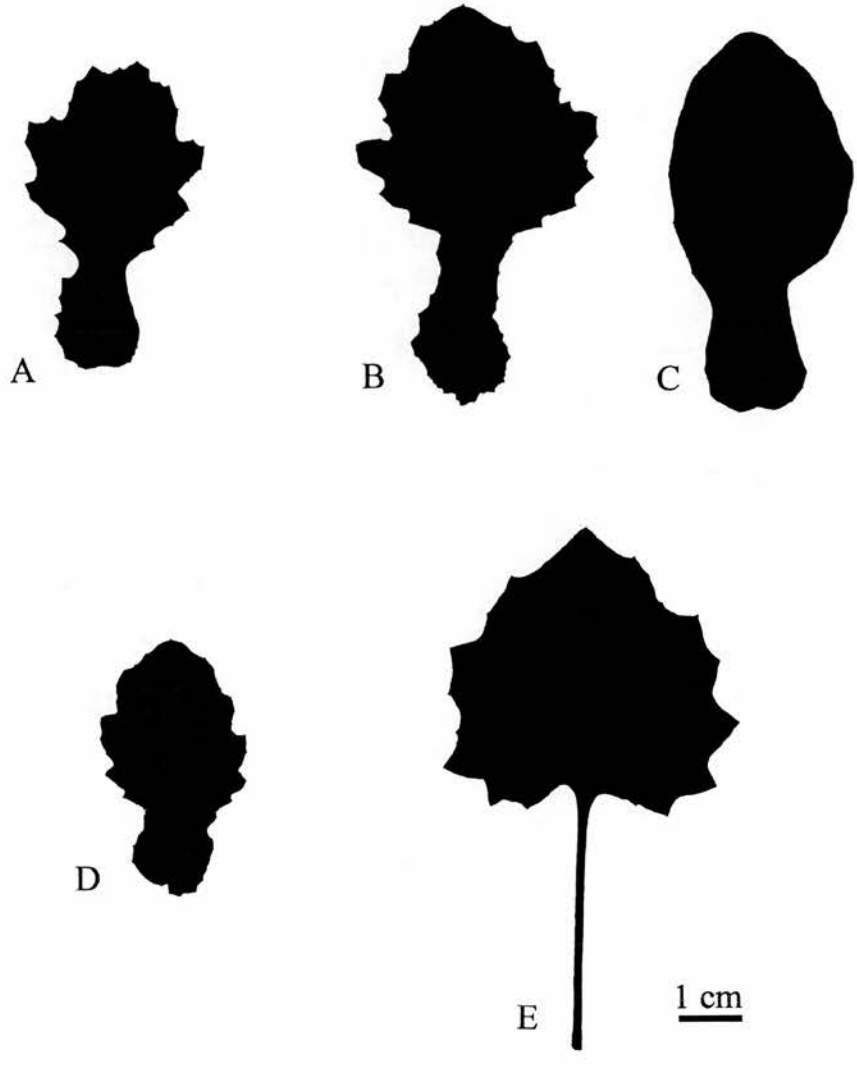

Fig. 2. A–E, mid-cauline leaf silhouettes. A, *S. mohavensis* subsp. *mohavensis*; B–D (B and C, Israel; D, Sinai), *S. mohavensis* subsp. *breviflorus*; E, *S. flavus*.

Marginal floret and ray floret morphology associated with pappus variation does provide taxonomically useful characters both for specific and subspecific distinction. In

S. flavus the capitula are disciform because the marginal florets differ from the inner florets in having four teeth rather than five and are female rather than hermaphrodite (rarely small anthers are present). When the four synonyms of *S. flavus* are considered, it is apparent that the disciform condition has not been treated uniformly. In two cases (*S. flavus* and *S. brevilimbus* S. Moore) very short (c.1 mm) ray florets have been described, whilst in the remaining three (*Crassocephalum flavum*, *S. decaisnei* and *S. claviseta* Pomel) non-radiate capitula are described. It is worth noting that the description of *S. flavus* by Schultz in *Phytographia Canariensis* (Barker-Webb and Berthelot, 1845) includes a detailed illustration (Tab. 107) that matches the non-radiate material we have seen and yet does not depict the short rays mentioned in Schultz's description. This apparent inconsistency, and the disagreement between the various descriptions, seems to result from the extremely small size of the three corolla teeth that could be interpreted as a ray. The significant differences in leaf morphology between *S. flavus* and *S. mohavensis* that have already been mentioned prevent this confusion from blurring the distinction between the species. In non-radiate plants of *S. mohavensis* subsp. *mohavensis* all florets are alike, and hence the capitula are discoid. Pappus morphology is useful in terms of distinguishing the subspecies of *S. mohavensis*. The achenes derived from the ray florets or marginal florets of the type subspecies have a normally developed pappus, whilst in *S. mohavensis* subsp. *breviflorus* a pappus is absent. Furthermore, the ray florets of the type subspecies are often divided into two or three irregular teeth. In contrast, *S. mohavensis* subsp. *breviflorus* has normally developed ray florets. Some apparently non-radiate plants of *S. mohavensis* subsp. *mohavensis* have highly reduced ray florets that cannot be detected without dissection of the capitulum.

The slight morphological distinction of the tetraploid taxa, shared ploidy and their reproductive compatibility all demonstrate close similarity between these taxa despite their large disjunction. However, the significant reduction in fertility of the F_1 hybrids generated between these taxa indicates that partial genetic divergence has occurred since isolation. Based upon this, it is most appropriate to treat the tetraploid taxa as two subspecies of a widely disjunct species. In contrast, the diploid *S. flavus* is morphologically and cytologically distinct, as emphasised by its reproductive isolation. Treatment of *S. flavus* as conspecific with either of the tetraploid taxa would not reflect the taxonomic or evolutionary relationships. The apparent reproductive isolation of *S. mohavensis* subsp. *breviflorus* and *S. flavus* does, however, need to be viewed in the

light of the small number of seed accessions used in the crosses. Nevertheless, in Sinai these taxa are sympatric and none of the herbarium specimens examined from this area was of intermediate morphology.

The results of existing molecular investigations (Liston, Rieseberg and Elias, 1989; Liston and Kadereit, 1995) also support the new classification. In a brief review of these results given below the taxa are primarily defined geographically as a return to the original classification (used in the papers cited) would be unnecessarily confusing.

High genetic similarity between the Southwest Asian and North American taxa was reported first by Liston, Rieseberg and Elias (1989) in an isozyme study of 13 enzyme systems. It was concluded that a relatively recent disjunction seemed most likely based upon the high degree of similarity. Chloroplast (cp) DNA restriction site variation has further demonstrated a close genetic relationship between these taxa (Liston and Kadereit, 1995). In addition, close cpDNA similarity was demonstrated between the tetraploid taxa and *S. squalidus* L., the outgroup used in the analysis. *Senecio squalidus* is a diploid species morphologically distinct from both the North American and Southwest Asian taxa. The explanation given for this unexpected similarity was that a Mediterranean species related to *S. squalidus* had been involved in the evolution of these taxa through interspecific hybridization and capture of the plastid genome (Liston and Kadereit, 1995).

The most recent molecular study (Comes and Abbott, 1999a) did not include the North American taxon but does shed further light on the evolution of the Southwest Asian taxon. This study used nuclear data sets (ITS sequences and RAPDs) in conjunction with cpDNA restriction site variation. The cpDNA results agree with the earlier study of Liston and Kadereit (1995) in that the previously recognized subspecies of *S. flavus* were well differentiated from each other, while the Southwest Asian taxon was closely allied to a group of four widespread Mediterranean diploids. The discovery that sympatric *S. glaucus* L. subsp. *coronopifolius* (Maire) Alexander possessed an identical cpDNA haplotype and very similar internal transcribed spacer (ITS) sequence was taken as evidence for the capture of these molecules via introgressive hybridization (Comes and Abbott, 1999a). Comes and Abbott (1999a) concluded that *S. glaucus* subsp. *coronopifolius* acted as the maternal parent as it is known that the plastid genome shows maternal inheritance in *Senecio* (Harris and Ingram, 1992). In the case of ITS capture the assumption is that homogenization of ITS repeats (Hillis and Dixon, 1991; Hillis et al., 1991) has occurred in the direction of the maternal parent.

The main nuclear data set examined by Comes and Abbott (1999a) was generated using randomly amplified polymorphic DNA (RAPD) primers. These markers are randomly scattered across all genomes but are generally assumed to reflect the nuclear genome due to the small size of the plastid genomes. The RAPD data indicated that the capture of ITS and cpDNA from *S. glaucus* subsp. *coronopifolius* had taken place in the absence of extensive nuclear introgression as the two former subspecies of *S. flavus* were grouped together (Comes and Abbott, 1999a).

The discovery that the newly recognized *S. mohavensis* subsp. *breviflorus* is tetraploid, combined with the earlier evidence of chloroplast and ITS capture from *S. glaucus* subsp. *coronopifolius* (Comes and Abbott, 1999a), means that future investigations of its origin need to consider polyploid modes of evolution. Either a combination of autopolyploidy and introgression or an allopolyploid hybrid of *S. flavus* and *S. glaucus* subsp. *coronopifolius* can be invoked. The far closer morphological resemblance of *S. mohavensis* subsp. *breviflorus* to *S. flavus* than *S. glaucus* subsp. *coronopifolius*, combined with the apparent absence of nuclear genetic markers of the latter (Comes and Abbott, 1999a), does not fit well with an allopolyploid origin. In allopolyploid species an additive profile of molecular markers would be predicted. In *Senecio* there is a documented case of diploid genetic material having introgressed into the tetraploid *S. vulgaris* L. (Lowe and Abbott, 2000), and this may represent a parallel to the present situation.

However, Liston, Rieseberg and Elias (1989) found that two isozyme loci in *S. mohavensis* subsp. *breviflorus* were duplicated relative to *S. flavus*, whereas in *S. mohavensis* subsp. *mohavensis* four loci were duplicated relative to *S. mohavensis* subsp. *breviflorus*. Examination of selfed progeny revealed fixed heterozygosity at these loci with no segregation, supporting duplication of differentiated genes. In light of the fact that *S. mohavensis* is tetraploid, this fixed heterozygosity could be interpreted as evidence for an allopolyploid origin. In addition, although having said that an additive profile of molecular markers would be expected in an allopolyploid, investigation of artificial allopolyploids has shown rapid rates of genome evolution (for a review see Wendel, 2000). It is, therefore, important to note that *S. mohavensis* subsp. *breviflorus* has a widespread distribution in Southwest Asia, indicating that the taxon may have arisen long enough ago for significant genome evolution and the loss of an additive pattern. Allopolyploidy also has the advantage of providing a simple explanation for the existence of a single plastid type as a single hybridization event could be responsible,

although many allopolyploids examined have shown evidence of multiple origin (Soltis and Soltis, 2000). It would be expected that an autopolyploid taxon that had undergone subsequent introgression would contain both parental plastid genomes, due to the uniparental inheritance of the plastid genome and no *a priori* reason to suppose that both parental species had not contributed to the maternal line. To date a *S. flavus* cpDNA haplotype has not been recovered from *S. mohavensis* subsp. *breviflorus*, although only small and geographically restricted samples have been examined. In conclusion, the molecular data remain equivocal regarding auto- as opposed to allopolyploidy, and further work is required.

Finally, the origin of the disjunct distributions exhibited by both *S. mohavensis* and *S. flavus* deserves comment. The disjunction of *S. flavus* between the arid zones of northern and southern Africa fits an established pattern of disjunction. A total of 41 species are listed as displaying this disjunction by de Winter (1971). In contrast, disjunction between the arid regions of the Old World and North America are poorly represented at the species level (Shmida, 1985). In the few cases that exist, most are assumed to be post-Colombian introductions to North America (Raven and Axelrod, 1978). However, at generic level this disjunction is not unusual. Stebbins and Day (1967) and Thulin (1994) list genera that display this disjunction. Stebbins and Day (1967) thought this pattern of vicarious species had arisen from migration between the Old and New Worlds by means of the Bering Strait land bridge during the climatically favourable Oligocene to Early Miocene. An alternative interpretation has been given by Thorne (1972), who viewed long-distance dispersal as a general explanation for floristic relationships between tropical Africa and tropical America.

The disjunction of *S. mohavensis* subsp. *breviflorus* between the Horn of Africa (Djibouti), Arabia and the adjacent countries of Southwest Asia, provides another example of an established disjunction at the species level (Thulin, 1994). This disjunction can be regarded as an extension of a larger disjunction between the Horn of Africa and southwest Africa, although here evidence at the species level is rather poor as recent studies have favoured the treatment of southwest and northeast African populations as distinct species (Thulin, 1990). The best remaining example of this disjunction at the species level is *Tribulocarpus dimorphanthus* (Pax) S. Moore (Verdcourt, 1957, 1969; Thulin, 1994). It is notable that this study continues this pattern by recognizing the Southwest Asian populations formerly ascribed to *S. flavus* as a separate species. Many genera show a disjunction between the arid regions of southwest

and northeast Africa, and an arid corridor during the Pleistocene has frequently been given as an explanation (Goldblatt, 1978; Werger, 1978).

Based upon the ages of the respective deserts, Liston, Rieseberg and Elias (1989) postulated a southwest African origin for the group, followed by migration to northeast Africa along an arid corridor during the Pleistocene, evolution of a distinct taxon in Southwest Asia, and finally dispersal from there across the Atlantic Ocean to North America. Liston, Rieseberg and Elias (1989) supported this scenario with evidence based upon the absence of close relatives in North America, the absence of seed-eating fruit flies (Tephritidae) found in all native American Compositae and the existence of a centre of diversity for the genus in southern Africa. Liston, Rieseberg and Elias (1989) argued for the native status of the North American taxon based upon its limited distribution and the remote and undisturbed nature of its habitats. This is less convincing as cases of introduced plant species invading remote and predominantly natural communities are well known, such as *Epipactis helleborine* (L.) Crantz in North America. The explanation of natural dispersal from Southwest Asia to North America was given as the attachment of seeds to migrating birds by the mucilage they exude upon wetting (Liston, Rieseberg and Elias, 1989).

As all three taxa are highly autogamous, long-distance dispersal events of single seeds could potentially establish new populations. The suggestion that migrating birds could have carried seed across the Atlantic Ocean cannot be ruled out, even though no birds now show this migration pattern (Liston, Rieseberg and Elias 1989). It does, however, seem rather surprising that *S. mohavensis* reached North America without spreading throughout the apparently suitable habitat of northern Africa. Likewise, the absence of *S. flavus* from the Horn of Africa and Arabia is difficult to explain if migration to northeast Africa took place as suggested along an eastern arid corridor (Liston, Rieseberg and Elias, 1989). These anomalies do point to subtle ecological differences between *S. flavus* and *S. mohavensis* that largely prevent sympatry.

An alternative route to North America through Central Asia has not previously been suggested, even though the distance is no greater. Despite the existence of *S. mohavensis* subsp. *breviflorus* in the Thar Desert of Pakistan, this still leaves Central Asia and the Pacific Ocean as barriers to dispersal, and there seems to be little to favour an eastward over a westward dispersal. Pushing the disjunction back to the Early Oligocene, at which time land bridges existed across the Bering Strait and the North Atlantic (Tiffney, 1985), does not seem reasonable given the close morphological and

molecular similarity, and the fact that the earliest Compositae fossils also date from this epoch (Graham, 1996). Consequently, long-distance dispersal does seem to be the most likely explanation of the disjunction.

Thulin (1994), in a review of disjunct distributions that include the arid parts of the Horn of Africa, did not favour either a Beringian migration or long-distance dispersal to North America. He considered both unlikely in the classic example of the genus *Thamnosma* Torr. and Frém (Thulin, 1994). In defence of long-distance dispersal in the case of *S. mohavensis*, we would point out that morphological and genetic differentiation is sufficient to doubt a post-Colombian introduction. It is also worth reiterating that the production of a heavy mucilage by the seeds provides a plausible means of long-distance dispersal via birds.

At the species level *S. mohavensis* is the only good example of a Southwest Asian–southwest North American disjunction. Two closely related species of *Plantago* L. with this disjunction have been studied by Stebbins and Day (1967), who demonstrated partial sterility of hybrids and minor morphological and cytological differences. Interestingly, these species also have seeds that produce a mucilage on wetting (Stebbins and Day, 1967). The *Plantago* example has been interpreted differently by Bassett and Baum (1969), who regard the pair as conspecific and of human introduction to North America. Raven (1971) has cited a number of genera with very closely related species that need re-evaluation, so further examples at the species level may come to light. In terms of future work, Liston (1997) has stressed the potential of molecular data, in particular the 'molecular clock' (Zuckerkandl and Pauling, 1965), to distinguish between vicariant (ancient) and dispersalist (recent) hypotheses. It would appear that a combination of cladistic biogeography and detailed study of potential long-distance dispersal mechanisms offers great potential to enhance our understanding of this unusual disjunct distribution.

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REPEAT INTERCONTINENTAL DISPERSAL AND PLEISTOCENE SPECIATION IN DISJUNCT MEDITERRANEAN AND DESERT *SENECIO* (COMPOSITAE)

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ABSTRACT

To explore the biogeographic history of Mediterranean/arid plant disjunctions phylogenetic analysis of Old and New World *Senecio* sect. *Senecio* was performed using nrDNA sequences (ITS). Strong support for a clade corresponding to sect. *Senecio* was found. Area optimization indicated this to be of southern African origin. The Mediterranean and southern Africa were not distinguishable as sources of the main New World lineage, estimated to have become established during the Mid-Pliocene. Another previously suspected recent dispersal to the New World from the Mediterranean was confirmed for the recently recognized disjunction in *S. mohavensis*. The loss of suitable land connections by the Miocene, means that both New World lineages must represent long-distance dispersal, providing the first evidence of repeat intercontinental dispersal in a Mediterranean group. In contrast, migration within Africa may have utilized an East African arid corridor. Recent dispersal to northern Africa is supported for *S. flavus*, which formed part of a distinct southern African lineage. Novel pappus modifications in both disjunct species may have enabled dispersal by birds. An estimated Early Pliocene origin of sect. *Senecio* coincides with the appearance of summer-dry climate. However, diversification from 1.6 Mya highlights the importance of Pleistocene climate fluctuations for speciation.

Key words: Asteraceae; biogeography; ITS; Mediterranean flora; molecular clock; phylogeny; plant disjunction; *Senecio*.

3.1 INTRODUCTION

The five Mediterranean floras (central Chile, southwest North America, southwest Australia, southern Africa and the Mediterranean basin) are associated with a characteristic summer-dry climate and exhibit high species diversity and endemism (Raven, 1971). Southern Africa displays exceptional diversity, with endemics comprising ~80% of the species and 29% of the genera (Goldblatt, 1978). Across all five floras it has been estimated that at least 40% of species and perhaps 10% of genera are endemic (Raven, 1971). These floras are thought to have evolved largely in isolation of each other from a combination of tropical and temperate ancestors in response to a drying trend throughout the Tertiary (Raven, 1971; Axelrod and Raven, 1978; Quézel, 1978; Raven and Axelrod, 1978). It is thought that the development of summer-dry conditions, largely since the beginning of the Pliocene (5.2 Mya), has been of central importance (Raven, 1971, Axelrod, 1975, 1977; Spect, 1979). Summer-dry conditions combined with climate fluctuations during the Pleistocene probably eliminated Tertiary mesophytic taxa, allowing the emergence of modern Mediterranean floras. Here we define "Mediterranean" broadly to include adjacent arid areas.

The biogeographic history of plant taxa disjunct between Mediterranean regions is complex and incompletely understood (Thulin, 1994; Fritsch, 1996, 2001; Liston, 1997; Caujapé-Castells et al., 2001; Hileman, Vasey and Parker, 2001). The possibility of arid corridors connecting Mediterranean regions has been rejected by some (Raven, 1971, 1973) because global ocean and air circulation patterns restrict Mediterranean climates to the tropical/temperate boundary between 30° to 40° north and south of the equator at the western margin of continents. In addition, the post-Tertiary origin of these floras means that they considerably post-date ecologically suitable land bridges between the Old and New World (Tiffney and Manchester, 2001). Consequently, long-distance dispersal has frequently been used to explain Mediterranean disjuncts (Raven, 1971, 1973; Thorne, 1972).

Nevertheless, continuous habitat links have been suggested between southwest and northeast Africa, through the mountains of East Africa, based on animal and plant disjunctions (Balinsky, 1962; Verdcourt, 1969). Balinsky (1962) called this the "arid track", and it is thought to have reached its greatest development during glacial periods of the Pleistocene (Verdcourt, 1969; van Zinderen Bakker, 1975; Goldblatt, 1978). Support for this comes from plant species and genera disjunct between southern and

northern Africa (Verdcourt, 1969; de Winter, 1971; Thulin, 1994; Jurgens, 1997), some of which occur within the arid track [e.g., *Androcymbium* (Colchicaceae), *Cephalaria* (Dipsacaceae), *Erica* (Ericaceae), *Gladiolus* (Iridaceae), *Lotonotis* (Fabaceae), *Olea* (Oleaceae), and *Scabiosa* sect. *Scabiosa* (Dipsacaceae)]. In addition, a three-way disjunction involving the New World is also seen in a few taxa (Thulin, 1994) [e.g., *Fagonia* (Zygophyllaceae), *Oligomeris* (Resedaceae), *Parkinsonia* (Fabaceae), *Senecio* sect. *Senecio* (Compositae), and *Thamnosma* (Rutaceae)].

Stebbins and Day (1967) have postulated a link between the Mediterranean basin and southwest North America via arid pockets in the Bering Strait land bridge (between eastern Asia and western North America) during the Early Miocene (~20 Mya). This is controversial in relation to Mediterranean taxa because it considerably predates the emergence of summer-dry conditions (Raven, 1971; Axelrod, 1975; Spect, 1979; Suc, 1984). In addition, it is thought that by the later Tertiary this land bridge was restricted to cool-tolerant and deciduous taxa and ultimately boreal taxa (Tiffney and Manchester, 2001). Axelrod (1973, 1975) has proposed that a generalist dry-adapted flora – the "Madrean–Tethyan" flora – formed a nearly continuous belt of sclerophyllous vegetation from North America to Central Asia from the Late Eocene to the end of the Oligocene (38-25 Mya). Subsequent climatic cooling and continued continental drift fragmented this flora, which may have represented an important source of xerophytic taxa that later adapted to summer-dry conditions in the Northern Hemisphere. However, the Madrean–Tethyan hypothesis is not universally accepted, and convergence from mesophytic ancestors has also been suggested to explain the observed distribution patterns (Wolfe, 1975).

To further explore the biogeographic history of Mediterranean disjunctions we have adopted a molecular phylogenetic approach focussed on *Senecio* sect. *Senecio*. The section contains a group of predominantly annual plants associated with Mediterranean climate in southern Africa, the Mediterranean basin and southwest North America. *Senecio* is also represented in South America and Australia, but few taxa appear on morphological grounds to be likely members of sect. *Senecio*. This group provides a useful comparison with other studies that have examined Mediterranean disjunction (Fritsch, 1996, 2001; Caujapé-Castells et al., 2001; Hileman, Vasey and Parker, 2001) because, unlike the taxa already examined, *Senecio* sect. *Senecio* appears to be adapted to long-distance dispersal via wind-blown pappose fruits (Small, 1919).

Sampling sect. *Senecio* is complicated by considerable taxonomic uncertainty. At ~1,250 species, *Senecio* is among the largest angiosperm genera. Generic and sectional limits are poorly circumscribed (Jeffrey et al., 1977; Jeffrey, 1979; Vincent and Getliffe, 1992) and monophyly of the genus has not generally been supported in cladistic analyses (Kadereit and Jeffrey, 1996; Pelser, Gravendeel and van der Meijden, 2002).

We have chosen to focus on two particularly striking disjunctions at the species level. *Senecio flavus* is disjunct between Namibia and the Mediterranean basin/northern Africa, whereas *S. mohavensis* is disjunct between southwest North America and Southwest Asia (Fig. 1). The disjunction of *S. mohavensis*, although only recently accepted at the species level (see Coleman, Forbes and Abbott, 2001), has previously been hypothesized to be of recent origin based on high isozyme similarity of the disjunct taxa (Liston, Rieseberg and Elias, 1989). In addition, evidence from nrDNA and cpDNA (Liston and Kadereit, 1995; Comes and Abbott, 2001) has indicated that *S. mohavensis* is a hybrid derivative of *S. flavus* and a second species, possibly *S. glaucus* subsp. *coronopifolius*, which is a member of a widespread Mediterranean diploid species complex. In *S. mohavensis* two subspecies are now recognized: the type subspecies in southwest North America and the new combination *S. mohavensis* subsp. *breviflorus* in Southwest Asia (Coleman, Forbes and Abbott, 2001). Although some Old World–New World disjunctions appear to result from accidental human introduction

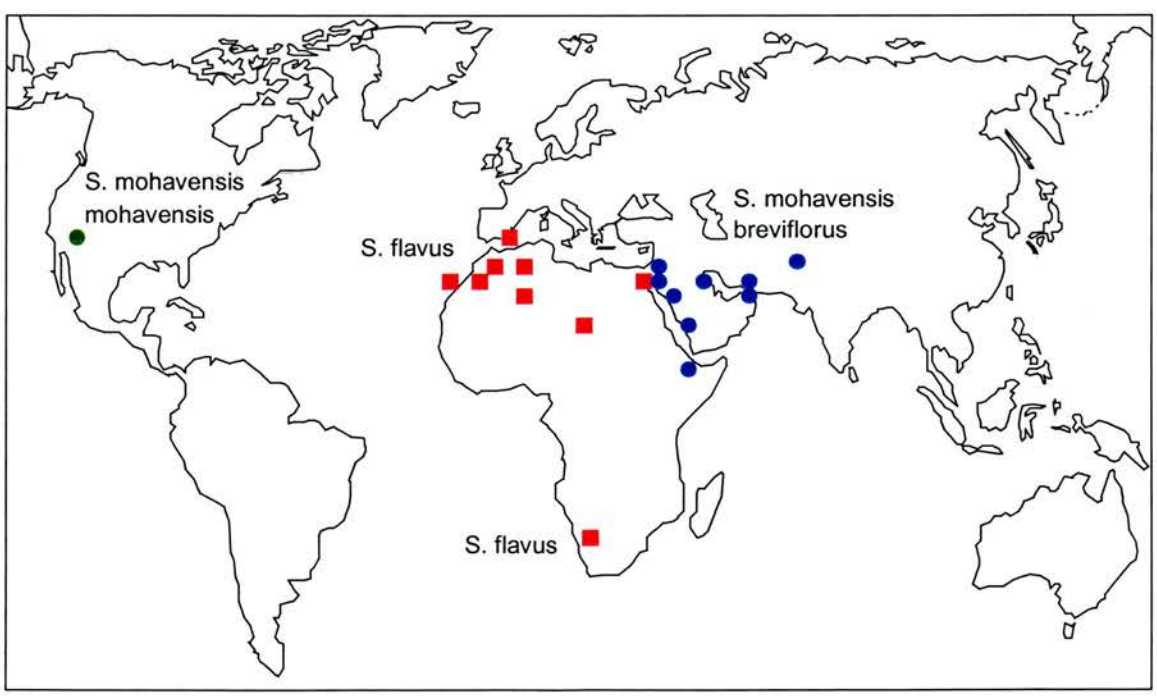

Fig. 1 Species disjunction in *Senecio flavus* and *S. mohavensis*.

(Bassett and Baum, 1969; Raven, 1971; Raven and Axelrod, 1978), morphological differences between the subspecies and reduced fertility in F_1 hybrids indicate that this is not the case in *S. mohavensis* (Coleman, Forbes and Abbott, 2001).

Following the most recent global taxonomic assessment of *Senecio* (Jeffrey, 1992) three subgroups may be distinguishable within sect. *Senecio*: a "basal austral subgroup", a "boreal subgroup" and an "australasian subgroup". As the australasian subgroup is peripheral to establishing the cause of the two species disjunctions in sect. *Senecio* we have not sampled this group. Instead, we have focussed on the other two subgroups, sampling primarily from southern Africa, southwest North America and the Mediterranean basin.

Phylogenies of Mediterranean taxa also have potential for examining the causes of the high species diversity so characteristic of Mediterranean floras. Enhanced rates of speciation have frequently been linked to the intensification of summer-dry conditions since the Pliocene (Axelrod, 1977; Axelrod and Raven, 1978; Raven and Axelrod, 1978; Goldblatt, 1978; Suc, 1984). Other suggested causes of diversification include Pleistocene climatic fluctuations, varied and generally poor soils, increasing topographic complexity due to mountain formation (Axelrod and Raven, 1978; Goldblatt, 1978; Quézel, 1978; Raven and Axelrod, 1978), pollinator shifts (Johnson, 1995) and alterations to mating system (Thompson, 1999). The few studies of Mediterranean plant groups that have dated diversifications have provided varied results. Pliocene and Pleistocene diversification has been supported in Californian Polemoniaceae (Bell and Patterson, 2000) and southern African *Phylica* (Rhamnacae) (Richardson et al., 2001). In contrast, diversification spread across much of the Tertiary has been supported in northern and southern African *Androcymbium* (Colchicaceae) (Caujapé-Castells et al., 2001), whereas Pleistocene speciation has been indicated in *Senecio* sect. *Senecio* from the Mediterranean basin (Comes and Abbott, 2001).

The aim of this study was to estimate a phylogeny from a representative sample of *Senecio* sect. *Senecio* using sequence data from the internal transcribed spacers (ITS) of nrDNA. The resultant phylogenetic trees were then used to: (i) explore the direction and timing of sectional and species disjunction, and (ii) the timing of diversification of the group. Where possible pappus morphology was examined to ascertain the distribution of characters such as barbs and hooks, which may be associated with animal dispersal of *Senecio* achenes (fruits).

3.2 MATERIALS AND METHODS

3.2.1 Plant material—Leaf material for ITS sequence analysis was obtained from cultivated material and herbarium specimens (Table 1). In addition, seven pre-existing sequences were obtained from GenBank (accession nos. AJ400803, AJ400807, AJ400810, AJ400813, AF459943, AF459965 and AF459968). Sampling was focussed on southern Africa, the Mediterranean basin and North America, reflecting the "basal austral" and "boreal" subgroups of Jeffrey (1992). Sampling was necessarily incomplete due to the large size and unclear limits of the section. Single representatives of three other genera in the tribe Senecioneae were included in the ingroup. *Erechtites* and *Crassocephalum* were included as allied genera from the New and Old World respectively, whereas the monotypic South African genus *Stilpnogyne* was included because it shares unusual morphological characteristics (petiolate leaves and disciform capitula) with *S. flavus*. The predominantly southern African genus *Euryops* was selected as outgroup based upon a cladistic analysis of morphological characters in the Senecioneae (Bremer, 1994).

TABLE 1. List of material sequenced of *Senecio* and allied genera providing location, collection and voucher references. Herbarium abbreviations: DAV, University of California, Davis (USA); DES, Desert Botanical Garden (USA); E, Royal Botanic Garden Edinburgh (UK); L, Leiden University (Netherlands); M, Botanische Staatssammlung München (Germany); OSC, Oregon State University (USA); RNG, University of Reading (UK); RSA, Rancho Santa Ana Botanic Garden (USA); S, Swedish Museum of Natural History (Sweden); STA, University of St Andrews (UK); UNLV, University of Nevada (USA). Specimens lacking a GenBank accession number have identical sequences to the preceding GenBank accession.

Taxon	Voucher	Location	GenBank accession
***Senecio* L.**			
S. abruptus Thunb.	van Rooyen et al. 95 (E)	S. Africa, Nieuwoudtville	AF457428
S. alliariifolius O. Hoffm.	Müller and Tilson 907 (M)	Namibia, Lemoenputs	AF457413
S. arenarius Thunb.	Giess and Müller 14431 (M)	Namibia, LU 70	AF457421
S. aff. *arenarius*	Merxmüller and Giess 28393 (M)	Namibia, Klinghardtberge	AF457422
S. aphanactis Greene	Preston 1097 (DAV)	USA, CA, Alameda Co.	AF457430
S. apiifolius (DC.) O. Hoffm.	Giess 15074 (M)	Namibia, Okaukuejo	AF457412
S. brasiliensis Less.	Davis et al. 2931 (E)	Brazil, Campos do Jordão	AF457434
S. cakilefolius DC.	Schlechter 8268 (E)	S. Africa, Karee Bergen	AF457423
S. californicus DC.	Chambers 10 (OSC)	USA, CA, Monterey Co.	AF457431
S. consanguineus DC.	Ross 1316 (E)	S. Africa, Ficksburg	AF457419
S. consanguineus	Giess 13464 (M)	Namibia, WIN 361	AF457420
S. cryphiactis O. Hoffm.	Giess 10519 (M)	Namibia, Porto Vehlo	AF457429
S. eenii (S. Moore) Merxm.	Jarman s.n. (E)	Namibia, Kwang Pan	AF457424
S. eenii	Van Wyk 8829 (M)	Namibia, Rosh Pinah	AF457425
S. englerianus O. Hoffm.	Müller and Loutit 1164 (M)	Namibia, River Huab	AF457417

TABLE 1. Continued

S. ertterae T. M. Barkley	Chambers 5888 (OSC)	USA, OR, Malheur Co.	AF457433
S. flavus (Decne.) Sch. Bip.-1	Nydegger 26145 (RNG)	Spain, Canary Islands	AF457415
S. flavus-2	Merxmüller and Giess 28206 (M)	Namibia, MAL 5	AF457416
S. flavus-3	Ait Lafkih et al. 751 (RNG)	Morocco, Asni	AF457414
S. flavus-4	Forbes cult. 70-4 (STA)	Morocco, Tafraoute	—
S. flavus-5	Jury et al. 14454 (RNG)	Morocco, Tata	—
S. flavus-6	Coleman cult. 10/00 (E)	Egypt, Sinai, Dahab	—
S. giessii Merxm.	Merxmüller and Giess 32233 (M)	Namibia, Aurusberge	AF457418
S. glaucus L. subsp. *glau.*	Forbes cult. 25-7 (STA)	Israel, Nof Yam	AF457440
subsp. *coro.* (Maire) Alexander-1	Forbes cult. 10-2 (STA)	Israel, Mizpe Ramon	AF457438
subsp. *coro.*-2	Forbes cult. 71-1 (STA)	Morocco, Tizi Mlil	AF457439
S. glutinosus Thunb.	Phillipson 1512 (E)	S. Africa, Port Alfred	AF457427
S. inaequidens DC.	Pelser cult. 9 (L)	Germany, Dullmen	AF459943
S. kraschenninnikovii Schischk.	McBeath 2272 (E)	India, Himachal Pradesh	AF457437
S. lemmonii A. Gray	Martin and Nilsson 44 (E)	USA, AZ, Pima Co.	AF457432
S. malacitanus Huter	Coleman cult. 01/01 (STA)	Morocco	AJ400813
S. mohavensis A. Gray subsp. *moha.*-1	Elias 10190 (RSA)	USA, AZ, Maricopa Co.	AF457436
subsp. *moha.*-2	Niles 5375 (UNLV)	USA, NV, Clark Co.	—
subsp. *moha.*-3	Engard and Hodgson 2121 (DES)	USA, AZ, La Paz Co.	—
subsp. *moha.*-4	Liston 645-3 (RSA)	USA, CA, San Bernadino Co.	—
subsp. *brev.* (Kadereit) M. Coleman-1	Coleman cult. 23/00 (E)	Israel, Khirbet Mezin	AF457435
subsp. *brev.*-2	Collenette 7077 (E)	Saudi Arabia, Uema Figra	—
subsp. *brev.*-3	Coleman cult. 05/00 (E)	Israel, Hazeva	—
subsp. *brev.*-4	Danin and Knees 1147 (RNG)	Israel, Mizpe Ramon	—
subsp. *brev.*-5	Western 1153 (E)	UAE, Masafi	—
S. squalidus L.	Ashton cult. s.n. (STA)	UK, Ainsdale	AJ400803
S. vernalis Waldst. and Kit.	Forbes cult. 18-2 (STA)	Israel, Zomet El Rom	AJ400807
S. vulgaris L.	no voucher	Israel, Bet Shean	AJ400810
S. windhoekensis Merxm.	Giess 15404 (M)	Namibia, WIN 134	AF457426
***Crassocephalum* Moench**			
C. crepidioides (Steen) R. O. Belcher	Pelser cult. 354 (L)	Nepal	AF459968
***Erechtites* Raf.**			
E. hieraciifolius (L.) DC.	Pelser cult. 352 (L)	Bolivia, La Paz	AF459965
***Euryops* (Cass.) Cass.**			
Eu. acraeus M. D. Hend.	Hilliard and Burtt 8855 (E)	S. Africa, Sani Pass	AF457410
***Stilpnogyne* DC.**			
St. bellidioides DC.	Goldblatt and Snijman 6972 (S)	S. Africa, Moordkuil	AF457411

Abbreviations of subspecies names: *glau.*, *glaucus*; *coro.*, *coronopifolius*; *moha.*, *mohavensis*; *brev.*, *breviflorus*.

3.2.2 DNA extraction and sequencing—Total genomic DNA was isolated using a modified hexadecyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1990). The entire ITS1–5.8S–ITS2 region was PCR amplified using primers ITS4 and ITS5 (White et al., 1990). Reactions were carried out in 25 μL volumes containing 1

unit Biotaq™ polymerase (Bioline, London, UK), 10% volume 10× Biotaq buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl, 0.1% Tween-20), 2 mM $MgCl_2$, 0.1 mM dNTPs, 0.2 mM of each primer and approximately 5 ng of template DNA. Denaturation at 94°C for 3 min was followed by a thermal cycle of: 1 min denaturation, 94°C; 1 min annealing, 55°C; 1.5 min extension, 72°C; 35 cycles. A final extension of 4 min at 72°C was carried out. PCR products were cleaned using Wizard™ PCR Preps (Promega UK, Southampton, UK) with the specified protocol. Both ITS strands were cycle sequenced using the amplification primers and BigDye™ (Applied Biosystems, Warrington, UK) with the specified protocol. Denaturation at 94°C for 30 s was followed by a thermal cycle of: 10 s denaturation, 96°C; 5 s annealing, 50°C; 4 min extension, 60°C; 30 cycles. Unincorporated dye terminators were removed using DyeEx™ Spin Kits (QIAGEN Ltd., Crawley, UK) with the specified protocol.

Due to the putative hybrid origin of the tetraploid ($2n = 40$) *S. mohavensis* involving progenitors with widely divergent ITS sequences (Comes and Abbott, 2001) cloning of ITS was carried out for this species. ITS was cloned using the pGEM®-T Easy Vector (Promega UK, Southampton, UK) with the specified protocol, followed by DNA extraction and purification using Plasmid Midi Kits (QIAGEN Ltd., Crawley, UK) with the specified protocol.

Sequences were obtained on an ABI PRISM 377 automated sequencer (Perkin-Elmer, Foster City, California, USA). Forward and reverse sequences were manually assembled using the computer program Chromas version 2.12 (Technelysium Pty. Ltd., Helensvale, Australia). Alignment was carried out manually using the computer program GeneDoc (Nicholas, Nicholas and Deerfield, 1997). Boundaries of ITS regions were determined by comparison with published *Senecio* sequences (Bain and Golden, 2000). Aligned sequences are available on request from the corresponding author.

3.2.3 Phylogenetic analysis—Phylogenetic analyses were conducted on the combined ITS1 and ITS2 regions (excluding the 5.8S gene) using PAUP* Version 4.0b8 (Swofford, 1998). Maximum parsimony (MP) analysis was performed with TBR, MULTREES, and COLLAPSE (max) options in effect. MP trees were generated by Fitch parsimony with a heuristic search that used 500 replicates of random sequence addition. Gaps (indels) were treated as missing data. Confidence in tree topologies was assessed using bootstrap analysis (Felsenstein, 1985) of 1000 replicates with the same settings as used in the searches, but with closest taxon addition. Maximum likelihood

(ML) analysis was performed using parameter estimates for ML obtained by a hierarchical likelihood ratio testing approach using the program MODELTEST Version 3.06 (Posada and Crandall, 1998). The substitution model selected, both for the full data set and a subset, was TrN + Γ. This uses a general time-reversible model and gamma-distributed (Γ) among-site rate variation. Heuristic searches were carried out with TBR, MULTREES, and COLLAPSE options in effect.

The hypothesis of clock-like evolution was tested using the likelihood ratio test (Goldman, 1993). Support for alternative hypothesized topologies was assessed by nonparametric Templeton tests (Templeton, 1983).

3.3 RESULTS

3.3.1 Sequence analysis—The total length of aligned ITS1 and ITS2 sequences was 494 bp (ITS1, 263 bp; ITS2, 231 bp), which comprised 263 (53.2%) constant characters, and 134 (27.1%) variable and parsimony informative characters. Sequence divergence (measured as uncorrected *p* distance) ranged from 0.0% (e.g., *S. mohavensis* subsp. *mohavensis–S. mohavensis* subsp. *breviflorus*) to 18.8% (*Crassocephalum crepidioides–S. californicus*) between ingroup taxa and from 14.8% (*S. flavus–Euryops acraeus*) to 20.6% (*Crassocephalum crepidioides–Euryops acraeus*) between ingroup and outgroup taxa. The TrN + Γ model of substitution under ML gave a transition/transversion ratio (Ts/Tv) of 1.86 across the whole matrix.

3.3.2 Phylogenetic analysis of ITS sequences—Fitch parsimony resulted in eight minimum-length trees of 427 steps, with a consistency index (CI) of 0.58 (excluding uninformative characters) and a retention index (RI) of 0.80 (Fig. 2). The single ML tree (-ln = 2929.69, not shown) was identical in topology to the MP tree in Figure 2. Four distinct lineages were resolved. Bootstrap (BS) support was maximal (100%) for three of these and lacking (<50%) for the fourth. Most species thought to represent sect. *Senecio* were placed in the "groundsel clade" (BS 100%) (Fig. 2). This clade was divided into five subclades: I (BS 82%) North American taxa except *S. brasiliensis* (South American), II (BS 92%) southern African taxa except *S. malacitanus* (Mediterranean), III (BS 78%) Mediterranean taxa except *S. mohavensis* subsp. *mohavensis* (North American), and IV and V (BS 98% and 58%) southern African taxa. Subclade V was sister to the remainder of the groundsel clade. Relationships among the

five subclades were poorly supported (<60%). *Senecio flavus*, previously considered part of sect. *Senecio* (Alexander, 1979), was isolated from the groundsel clade by a well-supported clade (BS 100%) composed of the Old World *Crassocephalum* and the New World *Erechtites*. A strongly supported (BS 100%) clade called the "petiolate clade" (Fig. 2) was composed of two petiolate-leaved species, *S. flavus* and *S. englerianus*. *Stilpnogyne* also has petiolate leaves, but no link to the petiolate clade was found. This leaf characteristic was not represented in the groundsel clade. Finally, sister to the rest of the ingroup was an unsupported clade composed of *S. apiifolius*, *S. alliariifolius* and *Stilpnogyne bellidioides* called the "African clade" (Fig. 2).

The failure of *Senecio* to form a monophyletic group was supported by a significant increase in tree length of 16 steps when monophyly was enforced ($Z = -2.51$, $P < 0.05$, Templeton-test). In contrast, a non-significant increase in tree length of four steps was found on enforcing the petiolate clade as sister to the groundsel clade ($Z = -0.90$, $P = 0.37$, Templeton-test), indicating that a sister relationship to the groundsel clade cannot be excluded. Enforcing the monophyly of the southern African members of the groundsel clade (subclades II, IV and V, Fig. 2) produced a non-significant increase of seven steps ($Z = -1.36$, $P = 0.17$, Templeton-test).

3.3.4 Rate constant evolution—The number of taxa in the full data set meant that clock-constrained ML searches did not run to completion. Consequently, a clock-constrained ML score was estimated from the unconstrained ML tree using the lscores option in PAUP*. Using the likelihood ratio test, the assumption of a molecular clock was strongly rejected for the complete data set [$-\ln L = 2(2969.12 - 2929.69) = 78.86$, d.f. = 34, $P < 0.001$]. The groundsel clade was also tested for clock-like evolution using *S. flavus* as outgroup. The smaller size of this data set meant that completion of clock-constrained ML heuristic searches was possible. The likelihood ratio test failed to reject a molecular clock in this reduced data set [$-\ln L = 2(1859.51 - 1842.22) = 34.58$, d.f. = 26, $P > 0.05$]. Using MP with the reduced data set generated two minimum-length trees of 204 steps, a consistency index of 0.67 and a retention index of 0.83 (not shown).

In the absence of a reliable fossil record or dated geological events, the molecular clock in the groundsel clade was calibrated using two ITS divergence rates from other Compositae (Sang et al., 1994, 1995). The relatively fast rate of 7.83×10^{-9} substitutions per site per year, estimated for *Robinsonia* from the Juan Fernández Islands

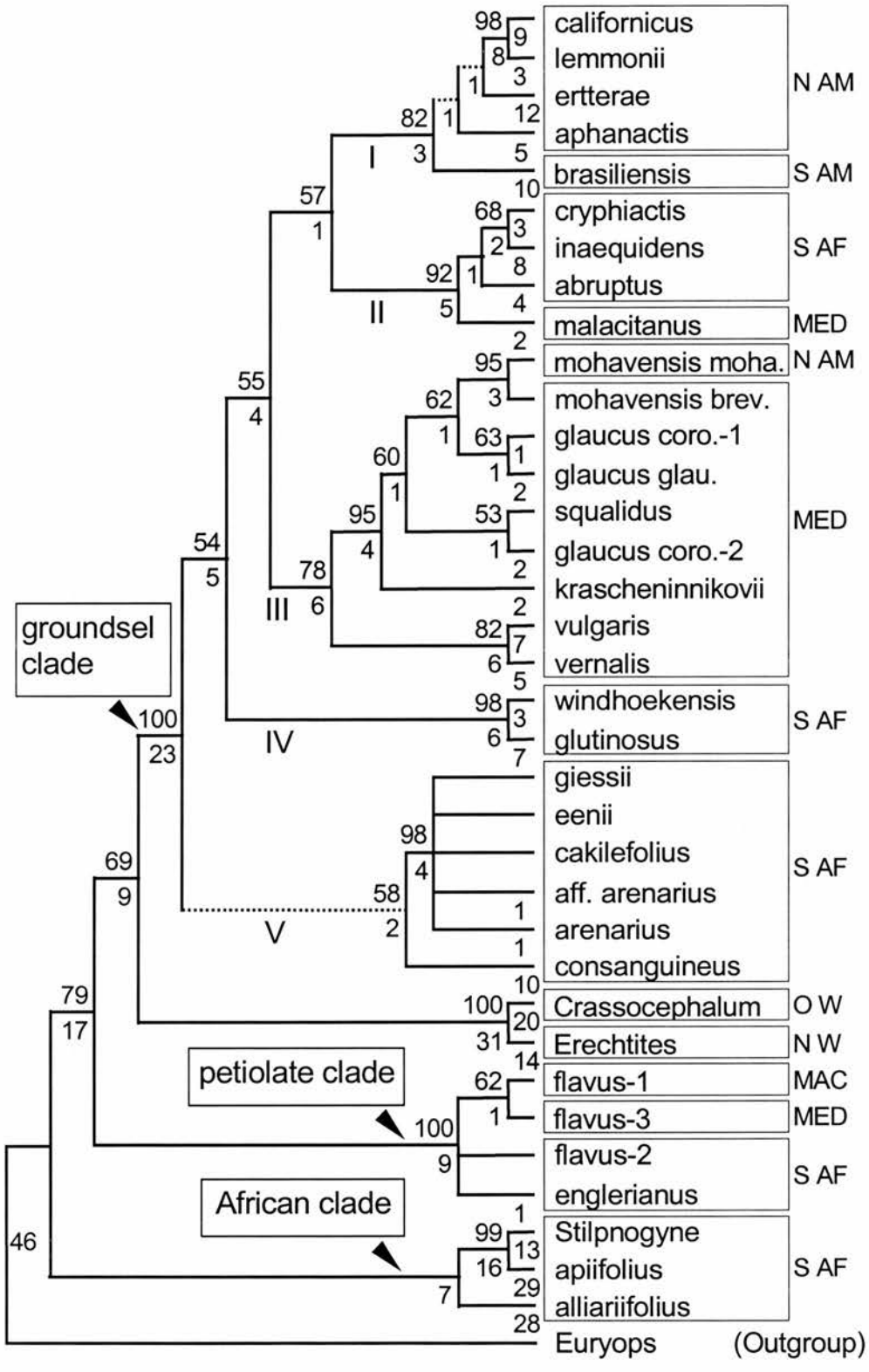

Fig. 2. One of eight maximum parsimony (MP) ITS trees found for *Senecio* sect. *Senecio* (427 steps; CI = 0.58; RI = 0.80). Tree branch values are number of nucleotide substitutions (below) and bootstrap values from 1000 replicates (above). Dotted lines indicate the three branches that collapse in the strict consensus tree. Roman numerals identify subclades within the groundsel clade. Area abbreviations: N AM, North America; S AM, South America; S AF, southern Africa; MED, Mediterranean basin; MAC, Macaronesia; O W, Old World; N W, New World. See Table 1 for taxon abbreviations. Maximum likelihood (ML) analyses produced a single tree of identical topology.

(Sang et al., 1995), was used to give a "fast clock". A "slow clock" was calibrated using a rate of 6.06×10^{-9} substitutions per site per year estimated for *Dendroseris* (Sang et al., 1994), also from the Juan Fernández Islands. *Robinsonia*, like *Senecio*, is in the tribe Senecioneae, while *Dendroseris* is in Lactuceae. Consequently, the *Robinsonia* rate may be more appropriate for calibrating a clock in *Senecio*. In addition to using both rates separately (Table 2), an average of the two was used to locate the boundaries of the Pliocene on the clock-constrained tree (Fig. 3). Because nucleotide substitution is not normally distributed, standard deviations (SDs) rather than confidence intervals are reported following the approach of Renner and Meyer (2001) (Table 2). All dates quoted in the discussion are based on the average rate (Table 2).

TABLE 2. Estimated age and standard deviation (SD) of nodes identified in Fig. 3 based on fast, slow and average rates of ITS divergence (see text).

Node	TrN + Γ Distance to tip ± SD	Fast clock Mya ± SD	Slow clock Mya ± SD	Average clock Mya ± SD
A	0.03633 ± 0.0084	4.64 ± 1.07	6.00 ± 1.39	5.23 ± 1.21
B	0.02987 ± 0.0077	3.81 ± 0.98	4.93 ± 1.27	4.30 ± 1.11
C	0.02259 ± 0.0067	2.89 ± 0.86	3.73 ± 1.11	3.25 ± 0.96
D	0.01160 ± 0.0048	1.48 ± 0.61	1.91 ± 0.79	1.67 ± 0.69
E	0.00712 ± 0.0038	0.91 ± 0.49	1.17 ± 0.63	1.02 ± 0.55
F	0.00165 ± 0.0018	0.21 ± 0.23	0.27 ± 0.30	0.24 ± 0.26
G	0.00106 ± 0.0015	0.14 ± 0.19	0.17 ± 0.25	0.15 ± 0.22

3.4 DISCUSSION

3.4.1 Ancestral area of the groundsel clade—Diverse criteria have been proposed for inferring the geographical origin of a taxon. Among the more useful is the location of the most ancestral forms (Platnick, 1981). A well-supported and appropriately polarized phylogeny provides an objective means of identifying ancestral and derived taxa. Both MP and ML phylogenies suggest a southern African origin of the groundsel clade. Within this clade both basal subclades (IV and V, Fig. 2) are exclusively southern African. However, a New World origin should also be considered because the North American *Erechtites hieraciifolius* is part of the sister group to the groundsel clade (Fig. 2). New World biogeographic links in the Senecioneae have also been indicated for the Macaronesian *Pericallis* (Kadereit and Jeffrey, 1996; Panero et al., 1999) and the Eurasian *Senecio* sect. *Jacobaea* (Pelser, Gravendeel and van der Meijden, 2002).

Manual area optimization under forward and reverse Camin-Sokal parsimony (Bremer, 1992), using the topology of Figure 2, also identified southern Africa as the

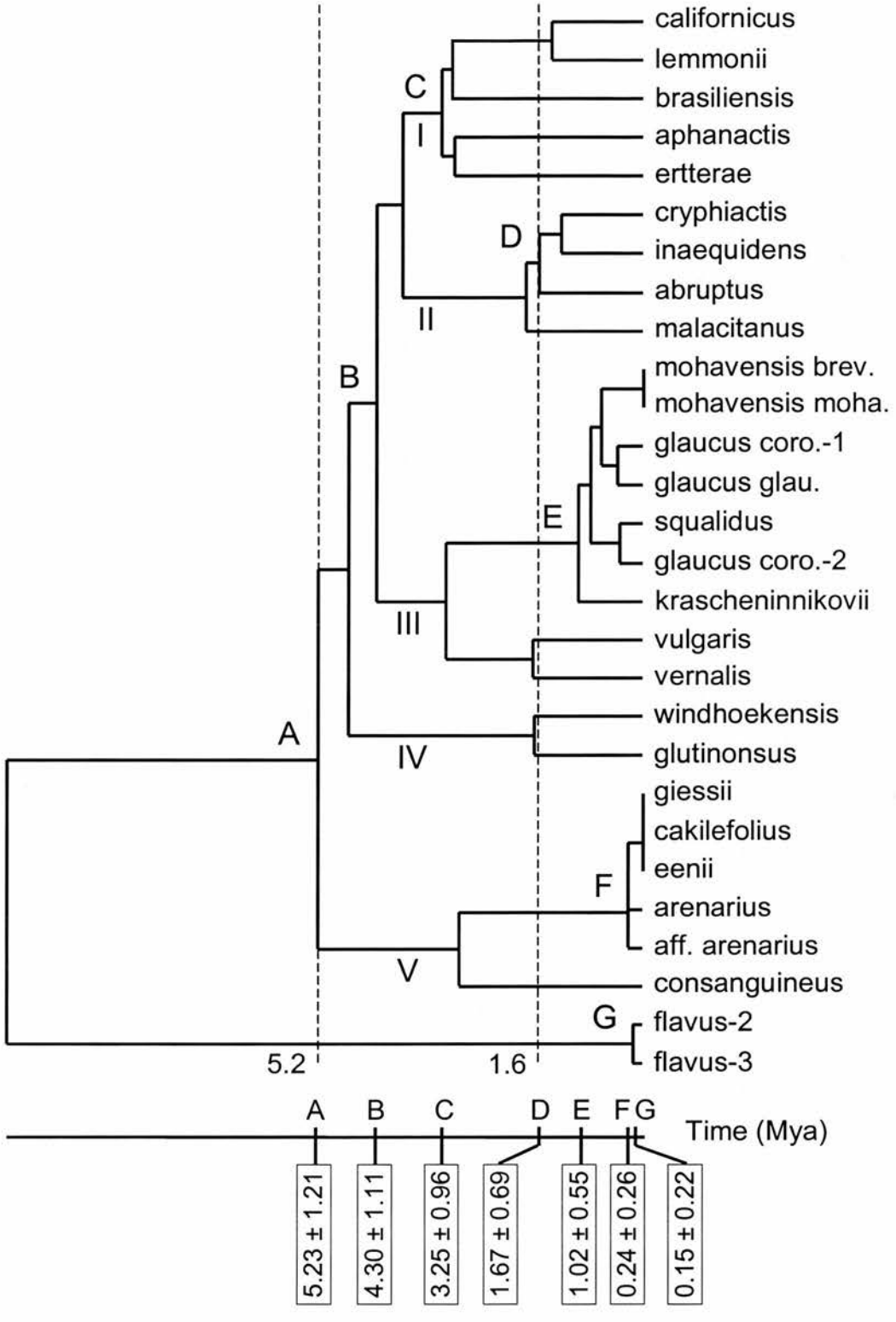

Fig. 3. Maximum likelihood (ML) clock-constrained ITS tree found for *Senecio* sect. *Senecio* under the TrN + Γ model of substitution (-lnL = 1859.51). Dashed lines mark the limits of the Pliocene as defined by calibration using an average of two published rates of ITS divergence in other Compositae (see text). Letters identify dated nodes (see Table 2) and roman numerals identify subclades within the groundsel clade. See Table 1 for taxon abbreviations.

ancestral area of the groundsel clade. This result was further supported by area optimization under PAUP* using both ACCTRAN and DELTRAN options. In addition, a New World origin seems unlikely based upon the known distribution of sect. *Senecio*. The section is not diverse in North America and may only be represented in South America by *S. brasiliensis*. In contrast, southern Africa is a known center of diversity for *Senecio* (Nordenstam, 1977; Bremer, 1994) and is probably the area of greatest diversity for sect. *Senecio*. Consequently, a southern African ancestral area appears to be most likely, although high diversity by itself does not provide evidence for an ancestral area due to the confounding effects of differential extinction/speciation rates.

3.4.2 Species disjunction—Vicariance involves a taxon of former wide distribution becoming split by a barrier to migration (Nelson and Platnick, 1981). This needs to be distinguished from long-distance dispersal if the causes of disjunction are to be understood. Phylogentic trees can be used to evaluate dispersalist and vicariant hypotheses. Vicariance may be inferred by large phylogenetic gaps associated with stable biogeographic barriers. This pattern has been termed "Deep history" (Riddle, 1996). Here isolation over long periods of time results in geographically distinct clades separated by long branches. In contrast, single or repeated long-distance dispersal events across a barrier will disrupt this pattern, leading to a situation where monophyletic groups contain members on either side of a geographical barrier.

Senecio mohavensis subsp. *mohavensis* is the only North American member of the otherwise Mediterranean subclade III. The lineages leading to the North American subclade I and Mediterranean subclade III separated 4.30 ± 1.11 Mya (Fig. 3, Table 2). The large phylogenetic gap between *S. mohavensis* subsp. *mohavensis* and the other North American species, and its derived position within subclade III, indicate dispersal from Southwest Asia to North America. The absence of variation in ITS sequence between the disjunct subspecies of *S. mohavensis* provides an estimated divergence 0.15 Mya. This confirms the hypothesized recent origin and direction of this disjunction (Liston, Rieseberg and Elias, 1989; Liston and Kadereit, 1995), although it does not limit the disjunction to the Holocene as previously suggested. As no case exists for an arid corridor linking Eurasia with North America during the Quaternary, this disjunction must be the result of long-distance dispersal.

Senecio flavus is disjunct between the northern Africa/Mediterranean basin and southern Africa. A southern African origin may be inferred from the restriction of *S.*

englerianus, the only other member of the petiolate clade (Fig. 2), to Namibia. The single nucleotide difference between Mediterranean and southern African *S. flavus* provides an estimated divergence time of 0.15 ± 0.22 Mya (Fig. 3, Table 2). This is consistent with Pleistocene migration along the arid track as previously suggested (Liston, Rieseberg and Elias, 1989; Liston and Kadereit, 1995). Consideration also needs to be given to the possibility of long-distance dispersal as our examination of pappus morphology has revealed novel characteristics suggestive of epizoochory (adhesive animal dispersal).

As an aside, these results are consistent with the putative hybrid origin of *S. mohavensis* in the Mediterranean basin (Liston and Kadereit, 1995; Comes and Abbott, 2001). The dispersal of *S. flavus* from southern Africa to the Mediterranean predates the dispersal of *S. mohavensis* to the New World, thereby providing time for the hypothesized hybridization to occur. Failure to recover a *S. flavus*-like ITS sequence from cloned *S. mohavensis* sequences indicates that interlocus concerted evolution has homogenized ITS repeats in the direction of the Mediterranean parent (see Wendel, Schnabel and Seelanan, 1995).

3.4.3 Sectional disjunction—Although the limits of sect. *Senecio* are unclear, the groundsel clade represents a part of the section because it includes the type species *S. vulgaris*. Establishing the biogeographic history of disjunction between the five subclades of the groundsel clade is complicated by poor resolution within subclades I and II. The position of *Senecio brasiliensis* (South America) and *S. malacitanus* (Mediterranean basin), within subclades I and II, respectively, is uncertain because both subclades exhibit little internal support (Fig. 2). Consequently, it is not possible to determine whether these taxa are ancestral or derived within their subclades. With regard to *S. malacitanus*, two possibilities exist. Assuming an ancestral position the southern African sister taxa represent a return to the south (subclade II, Fig. 2). This would also mean that an earlier dispersal to the New World, which established subclade I (Fig. 2), occurred from the Mediterranean basin. Alternatively, a derived position for *S. malacitanus* would mean that dispersal to North America occurred direct from southern Africa, and that the Mediterranean basin was reached twice by distinct southern African lineages. Similarly, an ancestral position for *S. brasiliensis* would mean colonization of North America via an intermediary step to South America, while a

derived position would suggest southward expansion from North America (subclade I, Fig. 2). Further sampling is required to clarify these questions.

Despite these uncertainties the timing of dispersal events does allow conclusions to be drawn on the likely causes of sectional disjunction. The establishment of each of the five lineages occurred before the Mid-Pliocene (Fig. 3, Table 2). Dispersal from the Old World to the New World occurred before 3.25 ± 0.96 Mya (node C, Fig. 3, Table 2). This is not coincident with putative arid pockets on the Bering Strait land bridge during the Early Miocene (Stebbins and Day, 1967). During the Late Tertiary the Bering Strait land bridge was restricted to cool-temperate taxa and ultimately boreal taxa (Tiffney and Manchester, 2001), and would therefore represent an unlikely migration route for Mediterranean species. Another land bridge between the Old World and New World existed across the North Atlantic. Geological evidence indicates the North Atlantic land bridge was broken in the Early Eocene (Tiffney and Manchester, 2001), at which time it would have been suitable for warm-temperate/tropical taxa. However, some estimates of divergence based on molecular clock studies imply contact through to the Middle or even Late Miocene (reviewed in Milne and Abbott, 2002). Regardless of the potentially wide timeframe for migration, it is unlikely that the North Atlantic land bridge enabled members of the groundsel clade to reach the New World. The groundsel clade emerged at the base of the Pliocene and good post-Miocene evidence for a North Atlantic land bridge does not exist. Consequently, the only plausible explanation is long-distance dispersal. Assuming a southern African origin of the section, the presence of *Senecio* in Australasia represents another example of long-distance dispersal in the genus, but this requires further examination of evolutionary relationships.

Repeat intercontinental long-distance dispersal appears to be a rare occurrence and we are unaware of any other examples from Mediterranean taxa, although two intercontinental colonizations of the New World have been supported in the predominantly warm temperate and tropical genus *Gossypium* (Malvaceae) (Wendel and Albert, 1992). In studies of southwest North American–Mediterranean disjunctions a vicariant relationship has generally been supported (Fritsch, 1996, 2001; Liston, 1997; Hileman, Vasey and Parker, 2001; Davis et al., 2002a). A monophyletic disjunct Mediterranean group within *Styrax* (Styracaceae), dated to a Late Miocene divergence, could possibly represent long-distance dispersal, as this is rather late for a North Atlantic land bridge, but its seeds appear to be maladapted for this (Fritsch, 1996, 2001). Although not Mediterranean, a vicariant pattern, probably involving the North Atlantic

land bridge, has also been supported in tropical dalbergioid legumes (Fabaceae) (Lavin et al., 2000) and Malpighiaceae (Davis et al., 2002b). Taken together these studies indicate that intercontinental long-distance dispersal across ocean barriers is rare in Mediterranean and tropical taxa. However, this is not a general pattern as studies of amphi-Atlantic arctic plant species have repeatedly indicated recent trans-Atlantic dispersal (Haraldsen, Ødegaard and Nordal, 1991; Haraldsen and Wesenberg, 1993; Hagen, Giese and Brochmann, 2001). Based on this evidence it is not possible to determine the direction of the two colonizations of the New World by *Senecio*.

Dispersal north in Africa either occurred once before 4.30 ± 1.11 Mya (node B, Fig. 3, Table 2), corresponding to ACCTRAN area optimization under PAUP*, or twice in the Mid-Pliocene and Pleistocene (subclades III and II respectively, Fig. 3), corresponding to DELTRAN optimization. In the single dispersal scenario a return to southern Africa occurred within subclade II before 1.67 ± 0.69 Mya (node D, Fig. 3, Table 2). Although the arid track is generally thought to have reached its greatest development in the Pleistocene, aridification during the Miocene and Pliocene may have provided an earlier corridor for arid adapted species. Molecular clock analysis in the disjunct genus *Androcymbium* (Colchicaceae) (Caujapé-Castells et al., 2001) has indicated that the arid track may have existed by the Late Miocene. Therefore, regardless of which of the two scenarios is accepted, it is possible that a northward expansion along the arid track occurred. However, because our results clearly demonstrate the potential for repeated long-distance dispersal in this group, the arid track may be of limited significance. For now, the means by which sect. *Senecio* migrated north in Africa remains uncertain. Some East African *Senecio* species have been assigned to sect. *Senecio* (Jeffrey, 1986) and inclusion of these in future work will be necessary to address this question further.

3.4.4 Pappus morphology and bird dispersal—Variation in pappus morphology provides some insight into how *S. flavus*, and possibly *S. mohavensis*, came to be widely disjunct. In most of the species examined variation was found to be limited and in agreement with previous findings (Drury and Watson, 1966). Typically almost all pappus hairs are covered with forward-pointing spines and the pappus is largely shed at achenial maturity. Frequently, a small number of pappus hairs (< 15 per achene) are of a distinctly different form. Such hairs are flexuous, firmly attached, and largely unornamented. These hairs have been called "fluked hairs" (Drury and Watson, 1966)

due to the frequent presence of backward-pointing projections at the swollen tip. *Senecio flavus* departed from this general pattern by having ~100 fluked hairs per achene, accounting for one third of the pappus. These fluked hairs are fused to each other near the junction with the achene (Fig. 4A). Above this the hairs separate and have a flexuous form, beyond which the shaft is straight. The hairs, which are of variable length, are each terminated with a regularly three-pronged grapple-like tip (Fig. 4B).

We believe this entire structure is novel and have called it "connate fluked pappus". A notable feature of connate fluked pappus is its very firm attachment to the achene, suggesting a dispersal function. Connate fluked pappus was observed in Mediterranean and southern African material of *S. flavus*, but was found to be absent in two accessions, one from Sinai and one from the Canary Islands. Connate fluked pappus was also observed in both subspecies of *S. mohavensis*, however, here the number of hairs is reduced by about 50% and the connate fluked pappus is less firmly attached. A further difference with *S. flavus* is that the grapple-like tips are variable in the number of prongs (not illustrated).

Barbs and hooks are typical of seeds adapted for epizoochory (Sorensen, 1986). The structure of the connate fluked pappus in *S. flavus*, and the strength of its attachment, strongly suggests epizoochory. Bird migration from southern Africa to northern Africa, and beyond, is an annual event in more than 300 species (Moreau, 1972; Curry-Lindahl, 1981; Walther, 2002). We believe that ground feeding migrants found in open habitats, such as Wagtails (*Motacilla* spp.), and Shrikes (*Lanius* spp.), represent possible vectors for dispersal of *S. flavus* from southern Africa. In the case of *S. mohavensis*, bird migrations between Southwest Asia and southwest North America are unknown (Alerstam, 1990). However, vagrant birds (migrants widely deviating from their normal route) are a common phenomenon throughout the world and well-documented examples of vagrants involve distances large enough to explain an Old World–New World disjunction (e.g., Thorup, 1998). Consequently, epizoochory may also explain the disjunction of *S. mohavensis*. Both *S. flavus* and *S. mohavensis* are self-fertile (autogamous) short-lived annuals. Autogamy is of great value in long-distance dispersal due to single colonist establishment, reduced dependence on pollinators, and reduced inbreeding depression. Baker's Rule (Baker, 1967) highlights the link between autogamy and long-distance dispersal. A good example is seen in the more than 130 Mediterranean species disjunct between Chile and California (Raven, 1963); all of which are herbs, and almost all autogamous. Circumstantial evidence for a dispersal role

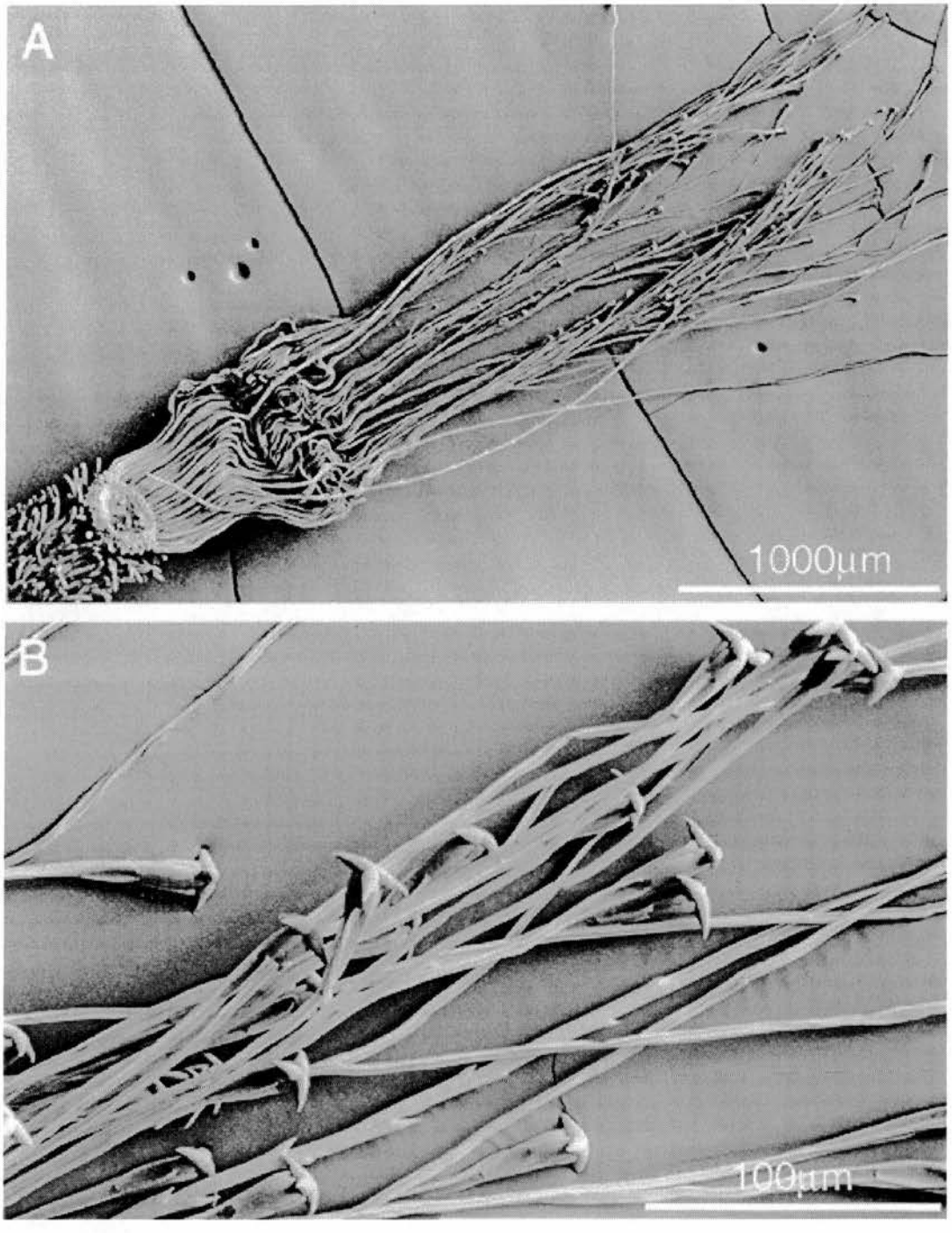

Fig. 4. Connate fluked pappus. (A) Entire structure in *S. flavus*, achene apex in bottom left corner; (B) detail of grapple tips in *S. flavus*.

comes from the fact that *S. englerianus*, a close relative of *S. flavus*, was found to lack any form of fluked pappus and is restricted to Namibia.

Another suggested mechanism for bird dispersal in these species is the mucilage produced by *Senecio* achenes after wetting (Liston, Rieseberg and Elias, 1989; Liston and Kadereit, 1995). This cannot be discounted, although it should be noted that temporary mucilage is generally thought to be associated with germination rather than dispersal (Sorensen, 1986).

It seems unlikely that wind dispersal caused these species disjunctions as in both cases the typical pappus is shed with extreme ease and the connate fluked pappus is ineffective as a parachute.

Connate fluked pappus was found to be absent in all other members of the groundsel clade that were examined. Consequently, its presence in *S. mohavensis* does provide additional support for the involvement of *S. flavus* in the evolution of this species.

3.4.5 Timing of diversification—The considerable species diversity of Mediterranean floras makes the factors that have driven speciation under these conditions of particular interest. The relative importance of the shift to summer-dry conditions and climatic fluctuations during the Pleistocene remains unclear. The few studies of Mediterranean plant groups that have estimated the timing of diversification from molecular clocks have presented varied results. Diversification in *Androcymbium* (Caujapé-Castells et al., 2001) was found to span the Oligocene and Miocene, while in *Linanthus* (Polemoniaceae) (Bell and Patterson, 2000) and *Phylica* (Rhamnaceae) (Richardson et al., 2001) considerable diversification since the Pliocene was supported. An earlier study of *Senecio* sect. *Senecio* restricted to taxa from the Mediterranean basin (Comes and Abbott, 2001) has indicated that, even using the most conservative rate calibration for ITS (Sang et al., 1994), rapid speciation occurred in the Pleistocene.

The position of the common ancestor of the groundsel clade at the base of the Pliocene (Fig. 3), combined with the establishment of the five subclades before the Mid-Pliocene is suggestive of a link with the spread of summer-dry climate. However, diversification is spread through the Pliocene and into the Pleistocene. All five lineages show Pleistocene diversification, although the extent of this is variable. In subclades I, II and IV diversification occurs at the beginning of the Pleistocene, whereas subclades III and V show rapid diversification in the Middle and Late Pleistocene, respectively (Fig. 3). The apparent lack of diversification in the *S. consanguineus* lineage of subclade V and the relatively low level of diversification in subclades II and IV may be an artefact of incomplete sampling. Because the North American and Mediterranean floras are relatively well known, we can be confident that the limited Pleistocene diversification seen in subclade I and the *S. vulgaris/S. vernalis* lineage of subclade III (Fig. 3) are not sampling artefacts.

Pleistocene climatic fluctuations were of global impact, and conclusions about their effects on diversification in other habitats and groups of organisms are needed to put our

results into context. Most work has been carried out on animals, and a growing number of studies (Hewitt, 1996; Klicka and Zink, 1997; Avise, Walker and Johns, 1998) have supported a protracted history of speciation spanning the Pliocene and Pleistocene. However, rapid recent speciation has also been recorded (Orr and Smith, 1998). Clearly rates of evolution are variable. Nevertheless, the importance of the dynamic nature of the Pleistocene for speciation is becoming generally appreciated (Hewitt, 2000). Our data, and the results of others (Bell and Patterson, 2000; Comes and Abbott, 2001; Richardson et al., 2001), lend weight to this view with regard to Mediterranean floras.

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POSSIBLE CAUSES OF MORPHOLOGICAL VARIATION IN AN ENDEMIC MOROCCAN GROUNDSEL (*SENECIO LEUCANTHEMIFOLIUS* VAR. *CASABLANCAE*): EVIDENCE FROM CHLOROPLAST DNA AND RANDOM AMPLIFIED POLYMORPHIC DNA

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ABSTRACT

Genetic variation was assessed in *Senecio leucanthemifolius* var. *casablancae* (Compositae), a Moroccan Atlantic coast endemic, in order to examine possible causes of atypical leaf morphology in three populations south of the known range. Evidence for introgression from *S. glaucus* subsp. *coronopifolius* and/or divergence was investigated with molecular markers. Both random amplified polymorphic DNA (RAPD) and chloroplast (cp) DNA restriction fragment length polymorphism (RFLP) differentiated the species well. Some evidence that hybridization may have occurred between the two species was provided by cp DNA markers. However, biparentally inherited RAPD markers failed to provide any support for the hypothesis that intermediate leaf morphologies in atypical populations arose through hybridization. Consequently, they are most likely to have arisen via divergence caused by drift and/or selection. Genetic distances among populations of *S. leucanthemifolius* were significant in all but one case. Isolation by distance was indicated by a significant positive correlation between genetic and geographic distances ($r = 0.68$, $P = 0.01$, Mantel test). These results suggest that long-distance achene dispersal is rare, despite the presence of a well-developed pappus. The observed loss of pappus at achene maturity may explain this unexpected result. Due to the morphological distinction of var. *casablancae* from other varieties of *S. leucanthemifolius*, we suggest elevation to species rank and treatment of atypical material at infraspecific rank.

Key words: Asteraceae; chloroplast DNA; divergence; hybridization; isolation by distance; randomly amplified polymorphic DNA.

4.1 INTRODUCTION

Geographic variation in species may reflect population divergence due to insufficient gene flow to counteract the forces of drift and selection (Wright, 1978; Slatkin, 1985). Such divergence leads to the formation of geographical races, and this has frequently been taken to represent the initial stages of speciation (Stebbins, 1950; Clausen, 1951; Grant, 1981; but see Levin, 2000). However, essentially similar patterns of variation can result from hybridization and introgression. Hybridization involving rare taxa has generally been viewed as a potential threat to survival (Avise, 1994; but see Arnold, 1997). Conservation concerns arise when introgression threatens to undermine the genetic integrity of a taxon (Brochmann, 1984; Rieseberg et al., 1989; Rieseberg, 1991; Rieseberg and Gerber, 1995; Levin, Francisco-Ortega and Jansen, 1996). On the other hand, introgressive hybridization can be a creative force, resulting in the evolution of new hybrid taxa on occasion (Abbott, 1992; Arnold, 1997; Rieseberg, 1997).

We have recently noted a potentially interesting pattern of leaf-shape variation in the endemic Moroccan groundsel *Senecio leucanthemifolius* Poiret var. *casablancae* Alexander (Compositae). This pattern could either be the product of population divergence or the introgression of genes from the related *S. glaucus* L. subsp. *coronopifolius* (Maire) Alexander. We have employed molecular markers in an attempt to distinguish between these two possible causes of the morphological variation observed.

Senecio leucanthemifolius var. *casablancae* is a diploid ($2n = 20$) annual restricted to dune habitats along the Moroccan Atlantic coast. Bagged capitula in this taxon set virtually no seed (< 1%) (M. Coleman, personal observation, 1999), indicating self-incompatibility. This result is consistent with previous work on the breeding systems of closely related diploid species (Alexander, 1975; Abbott and Forbes, 1993). Although not rare in numerical terms, this taxon is a localized endemic that may be regarded as vulnerable due to its restricted distribution and specialized ecology. The original description of the variety records a distribution between Salé in the north and Cap Beddouza in the south (~300 km) (Alexander, 1979). *Senecio leucanthemifolius* as a whole is widespread in the Mediterranean. Alexander (1979) divided the species into eight varieties, but one of these is generally treated as a distinct species *S. vernalis* Waldst. and Kit. The taxonomy of the species is complicated by intergradation between

some varieties (Alexander, 1979). However, *S. leucanthemifolius* var. *casablancae* is well differentiated from other varieties.

Three atypical populations displaying unusual leaf morphology have been discovered south of the previously known range of var. *casablancae* (M. Coleman and R. J. Abbott, personal observation, 1999). Typical var. *casablancae* has oblong rhomboid rarely shallowly lobed leaves (Fig. 1A). This morphology appears to be stable across the originally described range. In contrast, the three southern populations each display distinct leaf morphologies: (i) variably lobed, (ii) coarsely toothed, and (iii) extremely slender and variably lobed (Fig. 1B–D). Leaf morphology is largely uniform within a given population, with the exception of the degree of lobing, but variable between populations as little as 30 km apart. *Senecio* is known to exhibit high levels of leaf-shape plasticity (Alexander, 1975), but a strong genetic basis can be assumed in this case as differences are maintained under cultivation, at least between lobed and unlobed forms. We refer to the atypical populations collectively as 'atypical-casablancae'.

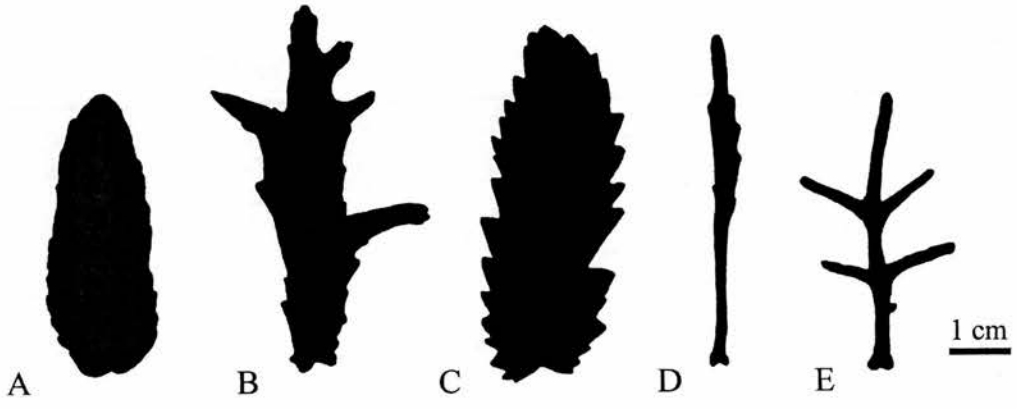

Fig. 1. A-D, Middle cauline leaf silhouettes of Moroccan *Senecio*. A, *S. leucanthemifolius* var. *casablancae*; B, 'atypical-casablancae' from Souira Kédima (SK); C, 'atypical-casablancae' from El-Mehattat (ME); D, 'atypical-casablancae' from Essaouira (ES); E, *S. glaucus* subsp. *coronopifolius*. All material wild collected.

Senecio glaucus subsp. *coronopifolius* seems the most plausible introgressant as this taxon is diploid, has dissected leaves (Fig. 1E) and can occur in maritime dune habitats. In general, *S. glaucus* subsp. *coronopifolius* is associated with more xeric habitats in southern Morocco, occurring widely in inland as well as coastal locations. The minimum distance between *S. glaucus* subsp. *coronopifolius* and 'atypical-casablancae'

in this study was ~100 km, and it remains unclear whether their ranges overlap. Circumstantial support for an introgressive origin is provided by the apparent high fertility of many artificial *Senecio* hybrids (Alexander, 1975). In addition, both putative parental taxa flower concurrently and have unspecialized pollen vectors such as solitary bees (*Halictus*, *Andrena*), syrphid flies and other diptera (Abbott and Irwin, 1988; Comes and Kadereit, 1990). Further, the role of introgression in the origin of other *Senecio* taxa has been supported by molecular evidence (Abbott, Ashton and Forbes, 1992; Abbott, Irwin and Ashton, 1992; Abbott et al., 2000, 2002). Unproven co-occurrence does not by itself rule out hybridization because hybrids can occupy geographic ranges that exclude the parental taxa. For example, where hybrids display higher fitness than one or both of their parents a broad hybrid zone lacking the parental taxa would be expected (Barton and Hewitt, 1985). Alternatively, hybrids may occupy novel habitat (Cruzan and Arnold, 1993) or expand into extreme habitat (Lewontin and Birch, 1966). In addition, species distributions change over time, and currently allopatric species may have been in former contact.

The possibility of restricted gene flow must also be considered as a cause of 'atypical-casablancae'. Indirect estimates of gene flow in terms of number of migrants (*Nm*) exchanged among populations using the infinite island model (Wright, 1951) have been made in three Mediterranean *Senecio* species: *S. gallicus* Vill. from the Iberian Peninsula, and *S. glaucus* and *S. vernalis* Waldst. and Kit. from the Near East (Comes and Abbott, 1998, 1999b). These species provide a useful comparison with *S. leucanthemifolius* var. *casablancae* as all are obligate outcrossers with pappose achenes (fruits) apparently suited to dispersal by wind (Small, 1919). Based on isozyme loci all three display sufficient gene flow to counteract drift and selection (Comes and Abbott, 1998, 1999b). However, *Nm* calculated for the haploid chloroplast genome has shown insufficient gene flow to counteract drift and selection in both *S. gallicus* and *S. glaucus* (Comes and Abbott, 1998, 1999b). Further investigation of *S. gallicus* using an independent nuclear data set composed of randomly amplified polymorphic DNAs (RAPDs) has revealed significant geographical subdivision and isolation by distance (Comes and Abbott, 2000). The failure of isozymes to detect this nuclear variation has been ascribed to low mutation rates and/or artefactual uniformity due to small sampling of the genome (Comes and Abbott, 2000).

Characteristic patterns of molecular variation provide a means of distinguishing hybridization from divergence. Hybridization would be expected to result in an additive

pattern of biparentally inherited markers in at least some individuals. In addition, taxon-specific markers would be expected to show clinal variation across a hybrid zone (Barton and Hewitt, 1985). Further, an increase in genetic variation is expected in introgressed populations, due to recombination of divergent nuclear genomes (Arnold, 1997) and the generation of mixed populations of non-recombining organelle genomes (Ennos et al., 1999). In contrast, divergence of populations would be expected to result in unique molecular markers and great differences in marker frequencies.

We have assessed genetic variation in this study with two DNA-based marker systems: (i) restriction fragment length polymorphism (RFLP) of PCR-amplified cpDNA fragments, and (ii) RAPDs (Williams et al., 1990). The chloroplast genome is a valuable source of markers for studying hybridization due to uniparental inheritance and its relatively low variability. *Senecio*, in common with most angiosperms, shows maternal inheritance of the chloroplast genome (Harris and Ingram, 1992). In contrast, RAPDs represent a random sample of the entire genome (Williams et al., 1990). However, due to the small size of the organelle genomes, RAPDs primarily reflect the nuclear genome (Lorenz, Weihe and Börner, 1994). The predominant biparental inheritance of RAPDs means that additive patterns of markers are expected in hybrids, and consequently they have been widely used to examine hybridization and introgression (e.g., Sale et al., 1996; Martin and Cruzan, 1999).

The aim of this study was to use cpDNA PCR-RFLP and RAPDs to: (i) test the competing causal hypotheses that 'atypical-casablancae' is either the product of introgression between *S. glaucus* subsp. *coronopifolius* and *S. leucanthemifolius* var. *casablancae*, or a product of divergence within *S. leucanthemifolius*; and (ii) quantify genetic variation across the range of the restricted endemic *S. leucanthemifolius* var. *casablancae* including 'atypical-casablancae'.

4.2 MATERIALS AND METHODS

4.2.1 Plant material—Seed was collected from nine wild populations, with *S. leucanthemifolius* var. *casablancae*, 'atypical-casablancae' and *S. glaucus* subsp. *coronopifolius* each represented by three populations (Table 1). Collection localities were as far as possible evenly distributed along the Moroccan Atlantic coast, and reflect the entire known range of *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae'. A single inland population of *S. glaucus* subsp. *coronopifolius* was also

included. The distance to the nearest populations was on average 81 km (range 31–165 km). In all cases high plant densities enabled each population to be sampled from a radius of less than 1 km. Ten individuals were grown from each population to provide leaf material for DNA extraction. Germination was generally sufficient to allow ten separate maternal plants to be used from each population. Herbarium specimens for all but two populations were made at the time of seed collection (Table 1).

TABLE 1. Locations of sampled populations of *Senecio leucanthemifolius* var. *casablancae*, 'atypical-casablancae' and *S. glaucus* subsp. *coronopifolius*.

Species/Population	Pop. code	Latitude	Longitude	Voucher[a]
S. leucanthemifolius var. *casablancae*				
Sehb-Eddheb – dune	SE	33°56'N	06°56'W	Forbes cult. 79-10
El-Jadida – forested dune	JA	33°15'N	08°30'W	Coleman and Abbott 01/99
Cap Beddouza – sandy cliff top	BE	32°36'N	09°13'W	Coleman and Abbott 05/99
'atypical-casablancae'				
Souira Kédima – dune	SK	32°02'N	09°21'W	Coleman and Abbott 07/99
El-Mehattat – sandy gullies	ME	31°50'N	09°34'W	Coleman and Abbott 08/99
Essaouira – dune	ES	31°30'N	09°46'W	Coleman and Abbott 09/99
S. glaucus subsp. *coronopifolius*				
Oued Sous – sandy estuary	OS	30°21'N	09°37'W	Coleman and Abbott 11/99
Tizi Mlil – open sandy woodland	TM	29°43'N	09°00'W	Forbes cult. 71-1
Sidi Ifni – sandy cliff top	SI	29°23'N	10°11'W	Coleman and Abbott 13/99

[a] All herbarium vouchers made from wild material collected by M. C. and R. J. A in 1999 except SE and TM, which are cultivated material grown from seed collected by R. J. A in 1996. Vouchers deposited at the herbarium of the Royal Botanic Garden, Edinburgh (E) except SE and TM, which are at the herbarium of the University of St Andrews (STA).

4.2.2 DNA extraction—Young leaf material was harvested from the greenhouse and total genomic DNA was isolated from ~100 mg of tissue using a modified hexadecyltrimethylammonium bromide (CTAB) miniprep method (Doyle and Doyle, 1990). DNA concentrations were determined, relative to uncut lambda DNA, on 1% agarose gels.

4.2.3 Chloroplast DNA PCR-RFLP—Chloroplast DNA fragment amplifications were carried out in 25 μL total volumes containing 1 unit Biotaq^{TM} polymerase (Bioline, London, UK), 10% volume 10× Biotaq buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl, 0.1% Tween-20), 2 mM $MgCl_2$, 0.1 mM dNTPs, 0.2 μM of each of the primers and approximately 5 ng of template DNA. Five primer pairs HK, CS, SM, AS and ML (Demesure, Sodzi and Petit, 1995) were used. The thermal cycle was as described by (Demesure, Sodzi and Petit, 1995) with annealing temperatures and extension times as follows: HK, 62°C and 2 min; CS, 54°C and 2 min; SM, 55°C and 2

min; AS, 55°C and 4 min; and ML, 55°C and 4 min. Amplification products were digested with eight restriction endonucleases: *Rsa*I, *Pst*I, *Ssp*I, *Hae*III, *Msp*I, *Bam*HI, *Hinf*I and *Stu*I (Promega UK, Southampton, UK) according to the manufacturer's instructions. Digested fragments were separated on 8% polyacrylamide gels (acrylamide: bisacrylamide, 37.5: 1) and TBE buffer (0.5 ×). An electric current (constant voltage 300V, current ~30 mA per gel) was passed through the gels until the loading dye reached the end of the gels (~3 hours) after which they were stained with ethidium bromide, and restriction patterns were visualized by UV transillumination.

4.2.4 RAPD procedure—RAPD reactions were carried out in 25 μL total volumes containing 1 unit Biotaq^{TM} polymerase (Bioline, London, UK), 10% volume 10× Biotaq buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl, 0.1% Tween-20), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM of primer and approximately 5 ng of template DNA. Denaturation at 94°C for 3 min was followed by a thermal cycle of: 30 s denaturation, 94°C; 45 s annealing, 35°C; 1.5 min extension, 72°C; 44 cycles. A final extension step of 4 min at 72°C was carried out. PCR products were separated on 1.5% agarose gels containing 0.5 μg μL^{-1} ethidium bromide and visualized by UV transillumination. Two hundred primers from the University of British Columbia (RAPD sets # one and two, UBC, Vancouver, Canada) were screened using six individuals (two from each of *S. leucanthemifolius* var. *casablancae*, 'atypical-casablancae' and *S. glaucus* subsp. *coronopifolius*). Thirteen primers that gave strong, easily scorable and reproducible bands were selected (UBC-112: GCTTGTGAAC; 141: ATCCTGTTCG; 177: TCAGGCAGTC; 204: TTCGGGCCG; 221: CCCGTCAATA; 226GGGCCTCTAT; 234: TCCACGGACG; 238: CTGTCCAGCA; 240: ATGTTCCAGG; 241: GCCCGACGCG; 248: GAGTAAGCGG; 249GCATCTACCG; 288: CCTCCTTGAC).

4.2.5 Chloroplast DNA analysis—Chloroplast DNA haplotypes were defined by the presence/absence of all detected length and site mutations. All individuals were assigned to a haplotype, and haplotype frequencies were calculated for each population. Differences in haplotype frequency among species were analyzed with an R (taxa) × C (haplotypes) test of independence using the G-test (Sokal and Rohlf, 1995). Differences in haplotype frequency within *S. leucanthemifolius* were examined in the same way by comparing var. *casablancae* with 'atypical-casablancae'. Nei's (1987) unbiased estimates of haplotypic diversity ($\hat{h}$) and their standard errors and the effective number

of haplotypes (n_e) were calculated for each population. The significance of among population haplotypic differentiation was assessed by *t*-test following Nei (1987).

4.2.6 RAPD analysis—A binary data matrix was produced by scoring each RAPD fragment as present or absent for each individual. Inter-individual relationships in multidimensional space were examined by principal coordinate (PCO) analysis using PCO3D (Adams, 1995). The PCO analysis used a similarity matrix derived from Jaccard's (1908) coefficient: $Dj = n_{xy}/n$, where n_{xy} is the number of shared markers and n is the total number of markers scored in each pairwise comparison excluding shared absences. PCO analysis is particularly well suited to identifying cases of recent and ongoing hybridization, as hybrids are generally located between their parents in PCO plots (e.g., Sale et al., 1996).

The hierarchical partitioning of genetic variation was estimated using analysis of molecular variance (AMOVA) of squared Euclidean distance between individuals (Excoffier, Smouse and Quattro, 1992). AMOVA analyses were conducted using ARLEQUIN 2.001 (Schneider, Roessli and Excoffier, 2000) thus enabling extraction of variance components and calculation of analogs of *F*-statistics (Wright, 1978) called Φ-statistics. ARLEQUIN was also used to calculate Pairwise population Φ_{ST} as a measure of genetic distance among populations, and to conduct Mantel tests (Mantel, 1967) to test if genetic distance (Φ_{ST}) among pairs of populations was significantly correlated with geographical distance. Significance levels for all analyses implemented with ARLEQUIN were calculated using a non-parametric permutation procedure with 10000 random permutations.

Shannon's diversity index was chosen to examine further the distribution of diversity within and between species as it does not rely on any assumption of Hardy-Weinberg equilibrium (Chalmers et al., 1992; Yeh, Chong and Yang, 1995). Shannon's diversity index was calculated as: $H_O = -\Sigma P_i \log_2 P_i$, where P_i is the frequency of the ith RAPD band. Diversity was calculated at several hierarchical levels: H_O was calculated for each population and then averaged over populations to give H_{POP}, whereas H_T was calculated as the corresponding value across all populations. Diversity was partitioned into a within population component as H_{POP}/H_T, and into an among population component as $(H_T-H_{POP})/H_T$. This provides both a comparison for results generated by AMOVA and a means of comparing the level of diversity found in individual populations, which is useful from a conservation perspective for identifying areas of particular diversity.

4.3 RESULTS

4.3.1 Chloroplast DNA variation—Length variation was most clearly revealed in two digestions: HK digested with *Hinf*I and AS digested with *Rsa*I. In each case a single digestion fragment displayed length variation. The variable HK fragment occurred as four length variants: 225, 230, 245 and 265 bp. The variable AS fragment occurred as three length variants: 370, 385 and 395 bp. Combining these length variants for all individuals gave a total of five cpDNA haplotypes, designated *A*, *B*, *C*, *D* and *E* (Table 2).

A marked division in haplotype frequency according to species was seen. The *E* haplotype was fixed in the two coastal populations of *S. glaucus* subsp. *coronopifolius* and nearly so (0.9) in the single inland population. In contrast, this haplotype was absent from five out of six populations of *S. leucanthemifolius* (Fig. 2, Table 2). The single occurrence of haplotype *E* in *S. leucanthemifolius* was in the population (ES) geographically closest to *S. glaucus* subsp. *coronopifolius*, suggesting that this may result from introgression. Similarly, haplotype *A* was found to occur at high frequency in both *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae' but was absent from *S. glaucus* subsp. *coronopifolius*. This apparent difference in haplotype frequencies among species was highly significant ($G = 89.66$, d.f. = 4, $P < 0.001$, *G*-test). Haplotypic structuring was not significant between *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae' ($G = 8.67$, d.f. = 4, $P > 0.05$, *G*-test).

Haplotypic polymorphism was observed in all six populations of *S. leucanthemifolius* and one of three populations of *S. glaucus* subsp. *coronopifolius*. The effective number of haplotypes (n_e) within polymorphic populations ranged from 1.471 to 2.174, and haplotypic diversity ranged from 0.189 to 0.568 (Table 2). Haplotypic diversity was greatest in the two most southerly populations (ME, ES) of 'atypical-casablancae', followed by the most northerly population (SE) of *S. leucanthemifolius* var. *casablancae*. Pairwise comparisons of haplotypic diversity among populations of *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae' revealed that the two most diverse populations (ME, ES) were significantly different from the two least diverse populations (BE, SK) ($t = 2.74$ and 2.96, $P < 0.01$, *t*-test). Otherwise differences in diversity were non-significant.

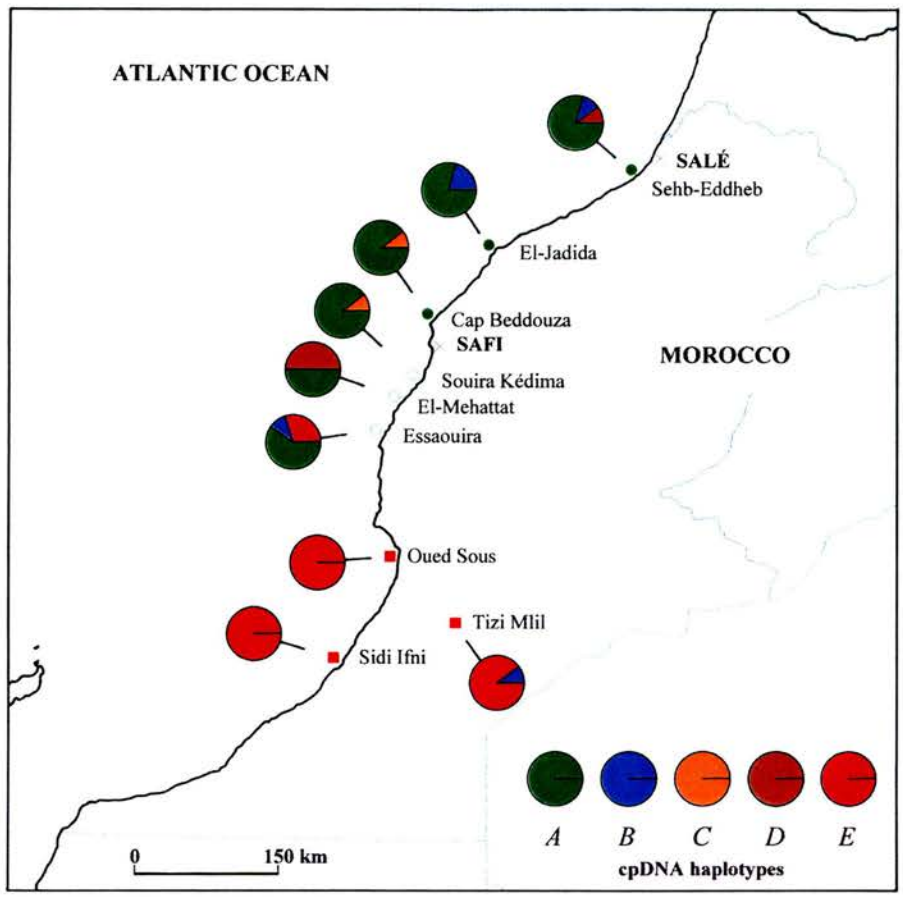

Fig. 2. Map of frequencies of five cpDNA haplotypes observed in *Senecio leucanthemifolius* var. *casablancae* (filled green circles), 'atypical-casablancae' (open green circles) and *S. glaucus* subsp. *coronopifolius* (filled red squares).

TABLE 2. Frequencies of cpDNA haplotypes among populations of *Senecio leucanthemifolius* var. *casablancae*, 'atypical-casablancae' and *S. glaucus* subsp. *coronopifolius*. *N* is the number of individuals surveyed. Measures of haplotypic diversity ($\hat{h}$), their standard errors (SE_h), and effective number of haplotypes (n_e) are also indicated.

		Haplotype[a]							
Species/Population	*N*	*A*	*B*	*C*	*D*	*E*	$\hat{h}$	SE_h	N_e
S. leucanthemifolius var. *casablancae*									
SE	10	0.8	0.1	—	0.1	—	0.358	0.127	1.515
JA	10	0.8	0.2	—	—	—	0.337	0.110	1.471
BE	10	0.9	—	0.1	—	—	0.189	0.108	1.220
'atypical-casablancae'									
SK	10	0.9	—	0.1	—	—	0.189	0.108	1.220
ME	10	0.5	—	—	0.5	—	0.526	0.036	2.000
ES	10	0.6	0.1	—	—	0.3	0.568	0.086	2.174
S. glaucus subsp. *coronopifolius*									
OS	10	—	—	—	—	1.0	0.000	0.000	1.000
TM	10	—	0.1	—	—	0.9	0.189	0.108	1.220
SI	10	—	—	—	—	1.0	0.000	0.000	1.000
Total	90	0.500	0.055	0.022	0.066	0.355			

[a] Haplotypes defined by the following fragment sizes: *A*, HK 245 bp AS 385 bp; *B*, HK 245 bp AS 395 bp; *C*, HK 230 bp AS 395 bp; *D*, HK 265 bp AS 370 bp; *E*, HK 225 bp AS 395 bp.

4.3.2 RAPD variation—Twenty-eight polymorphic RAPDs were amplified using 13 primers and scored in 90 individuals. The number of fragments scored per primer ranged from one (UBC-141, 221) to three (UBC-234, 238, 241, 288). All 90 individuals possessed unique RAPD phenotypes. Only a single diagnostic marker (defined as occurring in all individuals of taxon A and none of taxon B) was identified. This marker (UBC-288/1) was fixed in both *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae' and absent in *S. glaucus* subsp. *coronopifolius*. Taxon specific markers (defined as occurring in some individuals of taxon A and none of taxon B) were more numerous. Three markers (UBC-112/2, 234/2, 240/2) were specific to *S. glaucus* subsp. *coronopifolius*, although all occurred at low frequency (≤ 0.3). All three were also absent from 'atypical-casablancae'. Three markers (UBC-141/1, 234/3, 288/2) were specific to *S. leucanthemifolius* var. *casablancae*, ranging in frequency from 0.3 to 0.6. All three occurred in 'atypical-casablancae' at almost identical frequency to that observed in *S. leucanthemifolius* var. *casablancae*. Two markers (UBC-226/1, 241/1) displayed high frequency (defined as > 0.75) in *S. glaucus* subsp. *coronopifolius* and low frequency (defined as < 0.25) in *S. leucanthemifolius* var. *casablancae*. Both occurred in 'atypical-casablancae' at identical, or nearly so, frequency to that observed in *S. leucanthemifolius* var. *casablancae*.

PCO analysis was used to examine the relationships among individuals in multidimensional space. The first three axes of the PCO analysis accounted for 21.23, 8.09, and 5.16% of the total variance respectively. Plotting PCO1 against PCO2 clearly distinguished two discreet clusters corresponding to *S. leucanthemifolius* and *S. glaucus* subsp. *coronopifolius* (Fig. 3), indicating that these species represent distinct genetic entities. A division was also evident between *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae' (Fig. 3). There was only slight overlap between these sub-clusters, clearly indicating the presence of genetic differentiation. Plotting PCO1 against PCO3 revealed no additional relationships (not shown).

Genetic differentiation between species and within *S. leucanthemifolius* was also revealed by estimation of variance components and Φ-statistics with AMOVA (Table 3). Three analyses were conducted in which alternative hierarchical groupings were used to evaluate genetic structure (Table 3). Based on the three clusters identified by PCO the variance component was large and significant within populations (52.67%, $P < 0.001$; Table 3) and among clusters (39.70%, $P = 0.004$; Table 3). Among populations the variance component was relatively small, but significant (7.63%, $P < 0.001$; Table

3). Defining the top level of the hierarchy as the two species produced an almost identical result (Table 3). However, defining *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae' as the top level of the hierarchy produced an increase in the variance component within populations (69.94%, $P < 0.001$; Table 3) but little change among populations (10.90%, $P < 0.001$; Table 3). In addition, the variance component at the top level of the hierarchy was reduced by about half and not significant (19.16%, $P = 0.10$; Table 3). Individuals of both species were significantly differentiated from each other ($\Phi_{CT} = 0.41$, $P = 0.01$; Table 3), whereas differentiation of *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae' was only significant at the 10% level ($\Phi_{CT} = 0.19$, $P = 0.10$; Table 3).

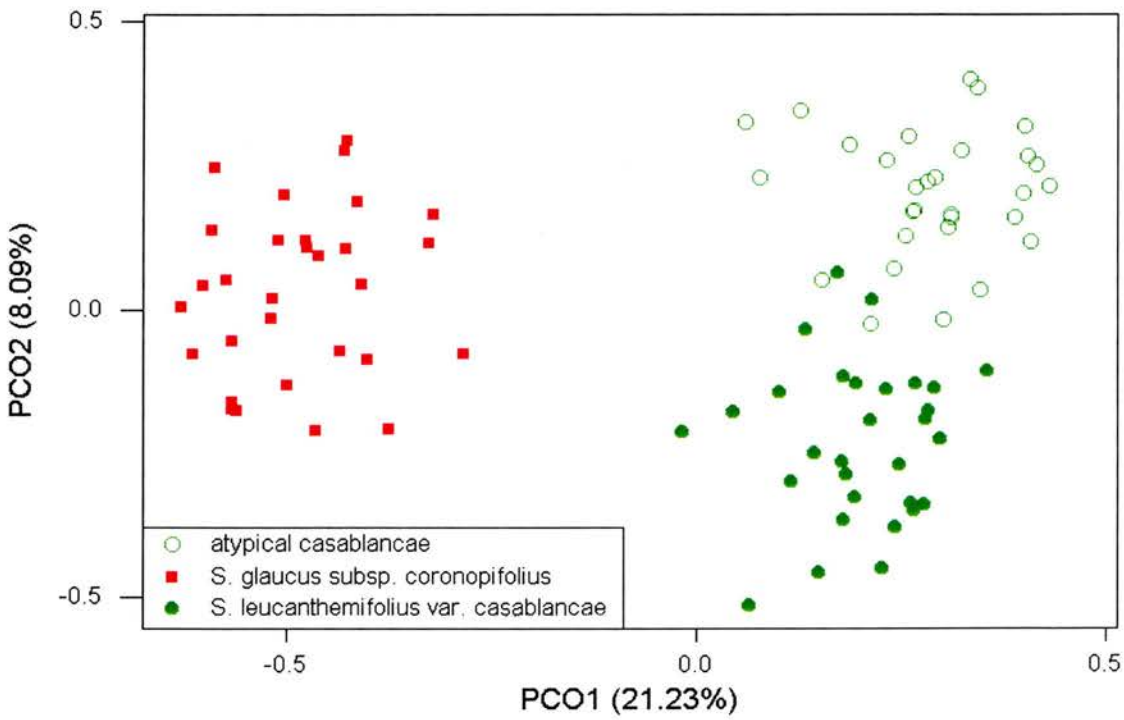

Fig. 3. Principal coordinates (PCO) analysis plot of the first two axes calculated using Jaccard's (1908) distance based on 28 random amplified polymorphic DNA (RAPD) fragments in *Senecio leucanthemifolius* var. *casablancae*, 'atypical-casablancae' and *S. glaucus* subsp. *coronopifolius*.

Thirty-four of the 36 pairwise genetic distances (Φ_{ST}) between populations were significant (Table 4). In both cases where the genetic distance was not significant the geographic distances between populations were relatively low [71 km between Souira Kédima (SK) and Essaouira (ES), and 93 km between Oued Sous (OS) and Tizi Mlil (TM)]. In general, genetic distances and significance levels were lowest for pairwise comparisons within each of the three groups (Table 4).

Testing the hypothesis of isolation by distance for the complete data set showed that the correlation between genetic distance (Φ_{ST}) and geographic distance between pairs of

populations was positive and highly significant ($r = 0.64$, $P < 0.001$, Mantel-test). However, the clear differentiation of *S. leucanthemifolius* and *S. glaucus* subsp. *coronopifolius* may have biased this result. Nevertheless, in the data set consisting of *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae' the correlation between genetic and geographic distances was also positive and significant ($r = 0.68$, $P = 0.01$, Mantel-test).

TABLE 3. Analysis of molecular variance (AMOVA) for RAPD phenotypes in *Senecio leucanthemifolius* var. *casablancae*, 'atypical-casablancae' and *S. glaucus* subsp. *coronopifolius*. "Clusters" in analysis 1 refer to the three clusters identified by principal coordinate (PCO) analysis (see Fig. 2). "Groups" in analysis 3 refer to *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae'. Analyses 1 and 2 were based on 28 RAPD fragments. Analysis 3 was based on 24 RAPD fragments (due to exclusion of *S. glaucus* subsp. *coronopifolius*).

Source of variation	d.f.[a]	SSD[b]	Variance component	% of total	Fixation indices	*P* value
Analysis 1						
Among clusters	2	163.91	2.46	39.70	$\Phi_{CT} = 0.40$	= 0.004
Among populations	6	48.07	0.47	7.63	$\Phi_{SC} = 0.13$	< 0.001
Within populations	81	264.90	3.27	52.67	$\Phi_{ST} = 0.47$	< 0.001
Analysis 2						
Among species	1	127.69	2.89	41.08	$\Phi_{CT} = 0.41$	= 0.01
Among populations	7	84.23	0.88	12.46	$\Phi_{SC} = 0.21$	< 0.001
Within populations	81	264.90	3.27	46.46	$\Phi_{ST} = 0.54$	< 0.001
Analysis 3						
Among groups	1	36.22	0.92	19.16	$\Phi_{CT} = 0.19$	= 0.10
Among populations	4	34.40	0.52	10.90	$\Phi_{SC} = 0.13$	< 0.001
Within populations	54	181.50	3.36	69.94	$\Phi_{ST} = 0.30$	< 0.001

[a] Degrees of freedom, [b] sum of squared deviations and the significance (*P*) of the variance components and fixation indices are shown. Levels of significance are based on 10000 permutations.

TABLE 4. Pairwise genetic distances (Φ_{ST}) among populations of *Senecio leucanthemifolius* var. *casablancae* (SE, JA, BE), 'atypical-casablancae' (SK, ME, ES) and *S. glaucus* subsp. *coronopifolius* (OS, TM, SI).

Pop.	SE	JA	BE	SK	ME	ES	OS	TM
SE	—							
JA	0.27***	—						
BE	0.17***	0.11**	—					
SK	0.35***	0.31***	0.26***	—				
ME	0.35***	0.31***	0.30***	0.10*	—			
ES	0.33***	0.26***	0.20***	0.06	0.07*	—		
OS	0.48***	0.54***	0.46***	0.55***	0.57***	0.56***	—	
TM	0.49***	0.53***	0.44***	0.51***	0.52***	0.52***	0.04	—
SI	0.53***	0.59***	0.54***	0.56***	0.57***	0.59***	0.18**	0.11**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. *P*-values indicate the probability that a random genetic distance (Φ_{ST}) is larger than the observed distance and are based on 10000 permutations.

Shannon's diversity index calculated for *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae' ranged from 0.32 for Essaouira to 0.37 for Cap Beddouza and Souira Kédima, with a mean value (H_{POP}) of 0.35 (Table 5). No significant differences in diversity level were found between populations. The within population diversity

component was 70%, whereas 30% was maintained among populations (Table 5). These figures are identical to those derived from AMOVA (Table 3, analysis 3).

TABLE 5. Shannon's diversity index estimated in *Senecio leucanthemifolius* var. *casablancae* (SE, JA, BE) and 'atypical-casablancae' (SK, ME, ES). Diversity partitions within (H_{POP}/H_T) and between (H_T-H_{POP}/H_T) populations are indicated.

Pop.	H_O	H_{POP}	H_T	H_{POP}/H_T	H_T-H_{POP}/H_T
BE	0.37				
SK	0.37				
SE	0.36				
JA	0.35				
ME	0.35				
ES	0.32				
		0.35	0.50	0.70	0.30

4.4 DISCUSSION

4.4.1 Hybridization and introgression—No evidence was obtained from RAPD analysis that hybridization occurs between *S. leucanthemifolius* var. *casablancae* and *S. glaucus* subsp. *coronopifolius* along the Moroccan Atlantic coast. Additivity of RAPD taxon markers was not observed, and diversity levels were uniform across the range of var. *casablancae* and the morphologically divergent 'atypical-casablancae'. Moreover, PCO analysis neither revealed a genetic continuum between the two species, nor located 'atypical-casablancae' between the putative parents as might be expected if it were of hybrid origin. The distinct genetic identities of *S. leucanthemifolius* var. *casablancae sensu lato* and *S. glaucus* subsp. *coronopifolius* were also highlighted by the significant differentiation between species found by AMOVA ($\Phi_{CT} = 0.41$, $P = 0.01$; Table 3). The RAPD analysis therefore indicates that either there are strong barriers to reproduction or that these taxa are allopatric. The latter situation is possible as the minimum distance observed between 'atypical-casablancae' and *S. glaucus* subsp. *coronopifolius* in the present study was ~100 km.

A possible concern with interspecific studies using RAPDs is that co-migrating fragments from different species are not always homologous (Rieseberg, 1996). In the present study this problem should be minimized by the close phylogenetic relationship between *S. leucanthemifolius* and *S. glaucus* (Comes and Abbott, 2001). However, false homologies would only reduce the genetic distance between taxa, thereby strengthening our conclusion that *S. leucanthemifolius* var. *casablancae sensu lato* and *S. glaucus* subsp. *coronopifolius* represent distinct genetic entities.

The cpDNA analysis (Fig. 2, Table 2) largely supports the genetic isolation of the species. However, limited sharing of haplotypes *B* and *E* was observed. This could be interpreted as evidence of past hybridization. However, shared haplotypes may also arise by convergence or incomplete lineage sorting as many plant species are thought to be paraphyletic shortly after speciation (Rieseberg and Brouillet, 1994).

The *E* haplotype occurred in 'atypical-casablancae' at Essaouira (ES) at moderate frequency (0.3) but was otherwise restricted to *S. glaucus* subsp. *coronopifolius*. Convergence seems an unlikely explanation because this would require two independent mutations. Incomplete lineage sorting could explain haplotype sharing in this case, particularly as divergence time estimates for these taxa are relatively low at 0.44 to 0.88 million years ago (Comes and Abbott, 2001). However, we favour hybridization as an explanation based upon the geographic distribution of the *E* haplotype. In a situation of incomplete lineage sorting the *E* haplotype in *S. leucanthemifolius* would not be expected to show any geographic relationship with *S. glaucus* subsp. *coronopifolius*. The clear clustering of the *E* haplotype (Fig. 2) means that hybridization provides the simplest explanation. In contrast, the *B* haplotype shows no obvious clustering. This haplotype occurs at low frequency (≤ 0.2) in two populations (SE, JA) of *S. leucanthemifolius* var. *casablancae*, one population (ES) of 'atypical-casablancae' and the only inland population (TM) of *S. glaucus* subsp. *coronopifolius*. Consequently, incomplete lineage sorting may best explain haplotype sharing in this case.

If hybridization is the cause of the observed sharing of the *E* haplotype, *S. glaucus* subsp. *coronopifolius* would have acted as maternal parent in past hybridization events, given the maternal inheritance of the chloroplast genome in *Senecio* (Harris and Ingram, 1992). The apparent absence of nuclear markers of *S. glaucus* subsp. *coronopifolius* in the Essaouira (ES) population indicates that backcrossing to *S. leucanthemifolius* would have occurred repeatedly and produced a nuclear genome largely characteristic of the latter. Such "chloroplast capture" has been found to be widespread in plants (Rieseberg and Soltis, 1991).

The absence of detailed distribution data means that the circumstances under which chloroplast capture could have occurred remain speculative. If these taxa are parapatric, with little range overlap, the most likely cause of this pattern is rare establishment of *S. glaucus* subsp. *coronopifolius* in *S. leucanthemifolius* populations, possibly via long-distance seed dispersal. In this situation, it is likely that genetic swamping by the numerically larger *S. leucanthemifolius* would lead to the elimination of the *S. glaucus*

nuclear genome. The alternative of high levels of long-distance pollen flow from *S. leucanthemifolius* seems unlikely as a study of pollinator foraging in North American *Senecio* has indicated pollen will not easily move more than 50 to 100 m (Schmitt, 1980). Alternatively, if range overlap is large and mixed populations exist (or have existed), hybrid swarms containing a mixture of the two nuclear genomes may occur. Although no evidence for hybrid swarms has been found, this possibility cannot be ruled out as many areas of coastline proved to be largely inaccessible. It is possible that a *S. glaucus* haplotype could be captured in the manner outlined above by long-distance dispersal from a hybrid zone.

An important conclusion from these results is that, although past hybridization may have occurred, the RAPD data indicate that introgression from *S. glaucus* subsp. *coronopifolius* is not a potential threat to the genetic integrity of *S. leucanthemifolius* var. *casablancae*.

4.4.2 Genetic structure of *Senecio leucanthemifolius* var. *casablancae*—Various life history traits in plants such as the vagility of pollen and seeds and breeding system are strongly linked to the development of genetic structure in allozymes (Hamrick and Godt 1989, 1996) and RAPDs (Nybom and Bartish, 2000). Generally, long-lived, outcrossing, late successional taxa retain the majority of genetic variation within populations, while annual, selfing, early successional taxa allocate the majority of variation among populations. As an obligate outcrosser with apparently high potential for seed dispersal *S. leucanthemifolius* may be expected to have a relatively homogenous distribution of variation among populations, with the majority of variation maintained within populations. This study supports this in as far as the majority of variation is maintained within populations (69.94%, $P < 0.001$; Table 3). However, genetic variation does not show a homogenous distribution, and population differentiation is evident. A small but significant amount of variation is maintained among populations (10.90%, $P < 0.001$; Table 3), whereas variation maintained among *S. leucanthemifolius* var. *casablancae* and 'atypical-casablancae' is significant at the 10% level (Table 3). Genetic distances as assessed by pairwise Φ_{ST} were significant in all but one of the 15 comparisons in *S. leucanthemifolius* (Table 4). In addition, the significant positive correlation of genetic and geographical distances ($r = 0.68$, $P = 0.01$) indicates that gene flow is restricted, with populations fitting the isolation by distance model. The overall value of Φ_{ST} was 0.30, suggesting a high level of

population structure. This value is close to the average value of 0.28 reported for outcrossing taxa by Nybom and Bartish (2000) in their review of AMOVA-derived Φ_{ST}. It is also close to the value of 0.34 recorded in the related outcrosser *S. gallicus* (Comes and Abbott, 2000).

These results indicate that restricted gene flow has resulted in significant population differentiation. Small-scale genetic differentiation has been widely documented in plants (see Linhart and Grant, 1996, for a review). However, taxa with seeds ingested by animals or dispersed by wind or water have been found to display significantly lower population differentiation than taxa with gravity or adhesive dispersal (Nybom and Bartish, 2000). Because the pappose achenes in *S. leucanthemifolius* are assumed to aid wind dispersal, the highly localized nature of population differentiation is unexpected. Indeed, experimental work on the closely related *S. vulgaris* L. has indicated that under favourable conditions there is effectively no limit to seed dispersal (Small, 1919). Our data contradict this view, indicating instead that long-distance seed dispersal is rare in *S. leucanthemifolius*.

Restricted gene flow is consistent with the remarkable level of morphological differentiation displayed by the three populations of 'atypical-casablancae' (Fig. 1). The apparent rarity of long-distance seed dispersal is also understandable on a more critical examination of the likely dispersal benefits of the pappus. The achenes of both species in this study readily shed their pappus at maturity. This characteristic is common to most members of sect. *Senecio* from the Mediterranean that we have examined. Consequently, the pappus may not be a particularly effective means of long-distance dispersal. This finding may be relevant to understanding the complicated pattern of morphological variation in *S. leucanthemifolius* as a whole. Restricted gene flow may also be an important factor in the evolution of other members of the Mediterranean species complex. It is notable that a similar, but morphologically less marked, pattern of intraspecific differentiation has been found in *S. gallicus* in the Iberian Peninsula and southern France (Comes and Abbott, 2000). Nevertheless, isolation by distance in this study indicates that some long-distance dispersal has taken place, as without it a significant correlation between genetic and geographical distance would not exist.

The coast between Cap Beddouza and Safi is largely rocky and lacking suitable dune habitats. This apparently unsuitable habitat separates var. *casablancae* from 'atypical-casablancae'. In light of the seemingly limited dispersal ability of achenes, this may present a natural barrier to gene flow that has favoured divergence.

It should be stressed that environmental variation between sites may be partly responsible for the morphological variation observed in the field. There is a marked rainfall gradient running along the coast and exposure varies due to topography and shrub cover. The leaf morphology differences between populations of 'atypical-casablancae' appear to be reduced under cultivation, with lobing of leaves becoming more pronounced. However, the unlobed condition of *S. leucanthemifolius* var. *casablancae* is not lost under cultivation, indicating that this characteristic has a large component of genetic control.

4.4.3 Taxonomic implications and future work—The morphological and genetic distinction of 'atypical-casablancae' from *S. leucanthemifolius* var. *casablancae* means that consideration should be given to the value of formally recognizing this variation. In the most recent revision of the Mediterranean members of sect. *Senecio* Alexander (1979) noted that for *S. leucanthemifolius* "It is difficult to make satisfactory infraspecific groups in this extremely variable species". A particular problem is that some varieties appear to lack clear boundaries due to continuous variation inter-connecting them (Alexander, 1979). This is not the case with 'atypical-casablancae', which instead simply represents undescribed variation within a distinct entity, *S. leucanthemifolius* var. *casablancae*.

Briefly, considering the species-wide problem of complex and intergrading variation, one solution would be to recognize *S. leucanthemifolius* as a polymorphic species with no infraspecific taxa. We do not advocate this approach because it would obscure valuable biological information. For instance, an important division exists between erect forms of *S. leucanthemifolius* in the western Mediterranean and procumbent fleshy-leaved forms, which include var. *leucanthemifolius*, in the central and eastern Mediterranean.

Based purely on morphology *S. leucanthemifolius* var. *casablancae* may be closely allied to the Canary Island endemic *S. bollei* Sunding and Kunkel. Both exhibit an erect habit of growth and more or less simple rhomboid leaves. The relationship between these taxa should be investigated as a closer link to *S. bollei* than *S. leucanthemifolius sensu stricto* may indicate that species status is more appropriate for var. *casablancae*. Regardless of the relationship to *S. bollei*, the morphological and ecological distinctness of *S. leucanthemifolius* var. *casablancae* from other varieties of *S. leucanthemifolius* by itself suggests that species rank may be appropriate. We advocate species status but

acknowledge that it is a somewhat subjective distinction between specific and infraspecific rank. An advantage of treatment at specific rank is that the variation encompassed by 'atypical-casablancae' could be recognized at infraspecific level.

Taxonomic changes should await a revision of *S. leucanthemifolius* and closely allied species. This is necessary because our field observations and examination of herbarium material indicate that in addition to the variation examined in this study Morocco supports further variation that is not encompassed by Alexander's (1979) varietal classification. Most importantly, none of the Moroccan material we have seen corresponds to the procumbent fleshy-leaved form of *S. leucanthemifolius sensu stricto*. This view is contrary to Alexander (1979), who recorded var. *leucanthemifolius* along the North African coast from Tunisia to Morocco and down the Moroccan Atlantic coast. The apparent geographic separation of the two growth forms suggests that they may represent genetically distinct groups. Consequently, the taxonomic rank of the other two erect growing western Mediterranean varieties, var. *major* Ball and var. *fradinii* (Pomel) Batt., should also be reassessed. These varieties would also benefit from revision as they intergrade with each other.

In conclusion, further morphological and molecular work is required to clarify relationships within the *S. leucanthemifolius* complex as a whole. Most importantly, the significance of the division of erect and procumbent growth forms between western and central/eastern Mediterranean populations respectively needs to be assessed. These different growth forms are particularly marked in cultivated material. By focussing on variation in a morphologically discreet variety of *S. leucanthemifolius* the present study has highlighted the importance of restricted gene flow in promoting localized divergence. It seems likely that localized divergence is also a factor responsible for the wider taxonomic problems of *S. leucanthemifolius*. Finally, an interesting comparison may be made with related taxa farther north. *Senecio rupestris* Waldst. and Kit., for instance, occupies a much larger range than *S. leucanthemifolius* var. *casablancae* and yet shows comparatively little variation in morphology (Abbott et al., 2002). This difference may reflect the relatively recent colonization of more northern latitudes after the last glacial period ended ~10,000 years ago. In light of this, it would be interesting to establish the timeframe of divergence both in var. *casablancae* and in *S. leucanthemifolius* as a whole.

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TAXONOMIC REASSESSMENT OF THE WIDESPREAD MEDITERRANEAN SPECIES *SENECIO GLAUCUS* (COMPOSITAE): EVIDENCE FROM MORPHOLOGY AND RAPDS

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ABSTRACT

Morphological and DNA (RAPD) variation was surveyed in *Senecio glaucus* from the western, central and eastern Mediterranean. *Senecio hesperidium*, a localized coastal endemic from Morocco, was included due to uncertain affinity with *S. glaucus*. Principal component analysis of 11 continuous morphological characters produced varying degrees of overlap between taxa and regions. Canonical variate analysis showed that mean phenotypes of western, central and eastern Mediterranean populations of *S. glaucus* subsp. *coronopifolius* were differentiated from each other. However, eastern Mediterranean populations were not clearly differentiated from *S. glaucus* subsp. *glaucus*, an eastern Mediterranean coastal subspecies. *Senecio hesperidium* showed close relationships with both *S. glaucus* subsp. *glaucus* and nearby populations of *S. glaucus* subsp. *coronopifolius*. ANOVA identified seven characters that differed significantly among taxa and regions. In *S. glaucus* subsp. *coronopifolius* the most differentiated region was the eastern Mediterranean. Principal coordinate analysis of RAPD data showed a clear separation into west, central and east Mediterranean clusters. *Senecio glaucus* subsp. *glaucus* and *S. hesperidium* were intermixed with eastern and western Mediterranean *S. glaucus* subsp. *coronopifolius* respectively and showed no close relationship to each other. A UPGMA tree indicated that genetic distances were smaller between western and central Mediterranean populations. These results may reflect greater habitat continuity via a corridor of habitat associated with the Atlas Mountains. Total genetic diversity declined from east to west, suggesting spread from the east associated with founder effects. However, variation maintained among populations was lowest in the central region. Denser sampling is required to draw firm conclusions on the colonization history of *S. glaucus*. The absence of a close relationship between *S. hesperidium* and *S. glaucus* subsp. *glaucus* based on RAPDs suggests morphological similarities are coincidental. Instead, these coastal taxa appear to be independently derived from inland subsp. *coronopifolius*. The need to assess promising taxonomic characters against variation across the entire Mediterranean basin is highlighted.

Key words: Asteraceae; genetic diversity; infraspecific differentiation; Mediterranean; morphometrics; random amplified polymorphic DNA; *Senecio*.

5.1 INTRODUCTION

Senecio glaucus L. (Compositae) is one of the most widespread members of *Senecio* sect. *Senecio*, with a distribution including Tenerife in the Canary Islands, North Africa, Sicily, Southwest Asia and the western Himalayas (Alexander, 1979). However, morphological variation across this range and unclear infraspecific taxonomy highlight the need for taxonomic reassessment. A broad concept of *S. glaucus* was adopted by Alexander (1979) based on the high fertility of F_1 hybrids generated from crosses between eastern and western Mediterranean plants (Alexander, 1975, 1979). An alternative narrower concept revolves around the fact that the type material of *S. glaucus* (herb. L. 996.24) only closely matches plants from coastal locations in Egypt and Israel (Alexander, 1979). Plants close to the type are characterized by a generally robust habit, large capitula, fleshy leaves and distinctive trifurcate tips to the leaf lobes. To reflect the morphological differences Alexander (1979) recognized the widespread plant as *S. glaucus* subsp. *coronopifolius* (Maire) Alexander.

Morphological examination of *S. glaucus* subsp. *coronopifolius* within its Mediterranean distribution (Fig. 1) has revealed some consistent differences between eastern Mediterranean plants and those from central and western localities. In terms of gross morphology, eastern Mediterranean plants are generally smaller in all parts. Eastern Mediterranean plants also appear to display longer and more numerous calyculus bracts which are distinctly hairy, whilst central and western Mediterranean plants have glabrous bracts or rarely bracts with a few inconspicuous hairs. Consequently, a single widespread subspecies may not adequately reflect the variation present.

A further question concerns the relationship between *S. glaucus* subsp. *glaucus* (coastal plants from Egypt and Israel) and *S. hesperidium* Jahandiez, Maire and Weiller, a highly restricted Moroccan coastal endemic (Fig. 1). Both taxa have large capitula and fleshy leaves, possibly reflecting recent disjunction via long-distance dispersal. However, *S. hesperidium* lacks both trifurcate leaf lobes and hairy calyculus bracts. Alexander (1975) suggested that these two coastal taxa could have been independently derived from nearby *S. glaucus* subsp. *coronopifolius*, in which case chance and/or selection under similar conditions has caused convergence in terms of certain gross morphological characters.

Finally, the distinction between the two subspecies of *S. glaucus* is not sharp. Alexander (1979) noted intergradation between typical coastal *S. glaucus* subsp. *glaucus* and "smaller leaved, somewhat arachnoid plants from areas behind the coast". Alexander (1979) referred inland plants displaying trifurcate lobes to subsp. *glaucus*. However, this character does not provide a satisfactory distinction, as trifurcate and acute lobes appear to show continuous variation in some populations.

To assess the extent of morphological differentiation within *S. glaucus* subsp. *coronopifolius* a series of continuous and fixed-state morphological variables were measured in multiple populations from the west, central and east Mediterranean. The study also included multiple populations of *S. glaucus* subsp. *glaucus* and *S. hesperidium* to clarify the relationships of these taxa to *S. glaucus* subsp. *coronopifolius* and to each other.

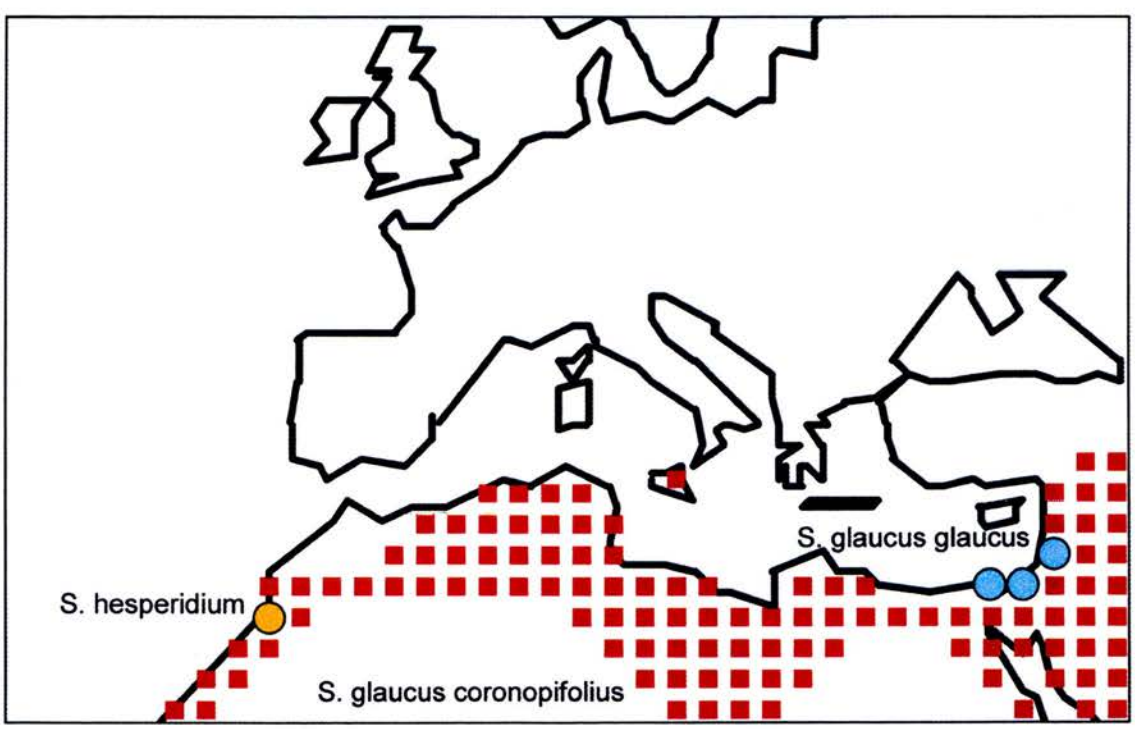

Fig. 1. Distribution of *Senecio glaucus* subsp. *glaucus* and *S. hesperidium*, with squares indicating the approximate Mediterranean distribution of *S. glaucus* subsp. *coronopifolius* (modified from Alexander, 1979).

Because morphological variation in *S. glaucus* subsp. *coronopifolius* suggests that genetic differentiation is present, a comparable data set based on molecular variation was generated to give an independent assessment of relationships. Molecular variation may also provide evidence to distinguish between the alternative hypotheses explaining the similarities between *S. glaucus* subsp. *glaucus* and *S. hesperidium*. Recent dispersal

would be supported by these taxa showing high genetic similarity. Alternatively, genetic differentiation, with each coastal taxon showing closest affinity to adjacent inland populations of *S. glaucus* subsp. *coronopifolius*, would indicate convergence.

Random amplified polymorphic DNA (RAPD) analysis (Williams et al., 1990) was used to assess genetic variation. RAPD analysis provides a technically straightforward way of randomly sampling the entire genome without the need of any prior sequence information (Williams et al., 1990). RAPDs have been effectively used to assess genetic variation in widespread species (e.g., Gillies et al., 1999; Lowe et al., 2000). In addition, the technique is sensitive to variation and compares well with alternative protein- and DNA-based methods (Wolff and Morgan-Richards, 1999; Díaz, Muniz and Ferrer, 2001).

The aim of the study was to use morphological and molecular (RAPDs) variation to: (i) assess whether the current concept of *S. glaucus* subsp. *coronopifolius* adequately reflects morphological and genetic variation across the Mediterranean basin; and (ii) establish whether *S. glaucus* subsp. *glaucus* and *S. hesperidium* are closely related disjunct taxa or derivatives of nearby populations currently assigned to *S. glaucus* subsp. *coronopifolius*.

5.2 MATERIALS AND METHODS

5.2.1 Plant material—All plant material was grown from wild collected seed from a total of 22 populations occurring in the western, central and eastern Mediterranean (Table 1). Twenty populations were included in the morphometric study and 19 populations were included in the RAPD study (Table 1). Seventeen populations were common to both studies (Table 1). The RAPD study included two populations (EN and SB) from Tunisia not available when the morphometric study was completed. In addition, to maintain a sample size of 95 (see RAPD analysis below), three populations (AD, AL and ZT) from Israel were excluded from the RAPD study.

5.2.2 Morphometric analysis—Ten plants (in nearly all cases one offspring per mother plant) were grown from each of 20 populations (Table 1). Seven populations of *S. glaucus* subsp. *glaucus* (five from Israel and two from Egypt), 11 populations of *S. glaucus* subsp. *coronopifolius* (five from Israel, two from Sicily and four from Morocco) and two populations of *S. hesperidium* were included. The 200 plants were

grown from seed to maturity as single individuals in 13 cm diameter pots containing a 1:1 mix of Levingtons M2 compost and gravel. Plants were arranged in a fully randomized block design and grown in a greenhouse with natural light supplemented by 400 W mercury vapor lamps with a 16 h day length. At full anthesis of the apical capitulum each plant was measured for 15 characters. Eleven of the characters were descriptors of the capitulum, while two were descriptors of leaf lobing. The remaining two characters were plant height and inflorescence length. Full details of the measurements made are provided in appendix 1.

TABLE 1. Locations of sampled populations of *Senecio glaucus* subsp. *glaucus*, *S. glaucus* subsp. *coronopifolius* and *S. hesperidium*.

Species/Population	Pop. code	Latitude	Longitude	Voucher[a]
S hesperidium				
1. Aglou Plage, Morocco	AP[b, c]	29°50'N	09°50'W	Coleman and Abbott 67-2 (E)
2. Sidi Rbat, Morocco	SR[b, c]	30°07'N	09°40'W	Forbes cult. 66-8 (STA)
S. glaucus subsp. *glaucus*				
3. Ashdod, Israel	AD[c]	31°48'N	34°38'E	Forbes cult. s.n. (STA)
4. Akko, Israel	AK[b, c]	32°52'N	35°05'E	Forbes cult. s.n. (STA)
5. Ashquelon, Israel	AQ[b, c]	31°40'N	34°35'E	Forbes cult. 27-2 (STA)
6. Alexandria, Egypt	AX[b, c]	31°13'N	29°55'E	Forbes cult. 31-5 (STA)
7. Caesarea, Israel	CA[b, c]	32°30'N	34°54'E	Forbes cult. 24-1 (STA)
8. El Quantara, Egypt	EQ[b, c]	30°52'N	32°20'E	Forbes cult. s.n. (STA)
9. Nof Yam, Israel	NF[b, c]	32°11'N	34°48'E	Forbes cult. 25-1 (STA)
S. glaucus subsp. *coronopifolius*				
10. Almog, Israel	AL[c]	31°45'N	35°28'E	Forbes cult. 6-1 (STA)
11. Khirbet Mezin, Israel	KB[b, c]	31°40'N	35°26'E	Forbes cult. 4-1 (STA)
12. Mizpe Ramon, Israel	MR[b, c]	30°36'N	34°46'E	Forbes cult. 10-1 (STA)
13. Zomet Telalim, Israel	ZT[c]	30°57'N	34°47'E	Forbes cult. 8-1 (STA)
14. Bet-She'an, Israel	BS[b, c]	32°30'N	35°30'E	Forbes cult. 13-1 (STA)
15. Khmes ait Ouafka, Morocco	KO[b, c]	29°25'N	09°05'W	Forbes cult. 69-1 (STA)
16. Oued Sous, Morocco	OS[b, c]	30°21'N	09°37'W	Coleman and Abbott 11/99 (E)
17. Sidi Ifni, Morocco	SI[b, c]	29°23'N	10°11'W	Coleman and Abbott 13/99 (E)
18. Tizi Mlil, Morocco	TM[b, c]	29°43'N	09°00'W	Forbes cult. 71-1 (STA)
19. Licata, Sicily	LI[b, c]	37°06'N	13°55'E	Forbes cult. s.n. (STA)
20. Pozzallo, Sicily	PO[b, c]	36°43'N	14°50'E	Forbes cult. s.n. (STA)
21. Enfida Plage, Tunisia	EN[b]	36°08'N	10°27'E	Coleman and Abbott 19/00 (E)
22. Sbeitla, Tunisia	SB[b]	35°13'N	09°07'E	Coleman and Abbott 6/00 (E)

[a] Herbarium vouchers deposited with either the herbarium of the Royal Botanic Garden, Edinburgh (E), or the herbarium of the University of St Andrews (STA). [b] Populations included in molecular analysis. [c] Populations included in morphometric analysis.

Variables that were not normally distributed were transformed into natural logarithms, except in the case of calyculus bract number, where square root transformation was used. Ray floret number and phyllary number were found to deviate strongly from normality after transformation. This was caused by tight clustering around numbers on the Fibonacci series (e.g., 8, 13, 21, and 34). These characters were excluded from morphometric analyses and instead treated as multiple-state characters.

Numbers not on the Fibonacci series were assigned to the nearest Fibonacci number, allowing frequencies to be calculated. Because there are an odd number of integers between 13 and 21 the cutoff was arbitrarily placed below 17. Finally, calyculus bract hairs and trifurcate leaf lobes were treated as presence/absence characters and frequencies were calculated. Trifurcate leaf lobes were recorded as present if any of the lobes of the fifth leaf to emerge displayed this character.

Principal components analysis (PCA) was conducted on the normalized data to examine inter-individual relationships in multidimensional space using NTSYSpc 2.0 (Rohlf, 1998). Canonical variate analysis (CVA) was also conducted using NTSYS to examine differences in mean phenotype between predefined groups. To test the robustness of the taxa each population was defined as a separate group. CVA finds linear combinations of the original variables that maximize the ratio of between-group to within-group sums of squares and product matrices, thereby giving functions of the original variables that can be used to discriminate between the groups. After testing for a significant difference between groups by single classification multivariate analysis (MANOVA), the relationships among groups were displayed by plotting mean canonical scores of each group against each other for the first three canonical variates.

A two-way ANOVA was also conducted using the general linear model (GLM) procedure of SAS 8.2 (SAS Institute Inc., Cary, NC, USA) to detect differences among taxa and among populations within taxa. The three regions from which *S. glaucus* subsp. *coronopifolius* was sampled were treated as separate taxa for the purposes of ANOVA to assess the extent of differences between them.

5.2.3 DNA extraction—Young leaf material was harvested from the greenhouse and total genomic DNA was isolated from ~100 mg of tissue using a modified hexadecyltrimethylammonium bromide (CTAB) miniprep method (Doyle and Doyle, 1990). DNA concentrations were determined, relative to uncut lambda DNA, on 1% agarose gels.

5.2.4 RAPD procedure—RAPD reactions were carried out in 25 μL total volumes containing 1 unit Biotaq^{TM} polymerase (Bioline, London, UK), 10% volume 10× Biotaq buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl, 0.1% Tween-20), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM of primer and approximately 5 ng of template DNA. Denaturation at 94°C for 3 min was followed by a thermal cycle of: 30 s denaturation, 94°C; 45 s

annealing, 35°C; 1.5 min extension, 72°C; 44 cycles. A final extension step of 4 min at 72°C was carried out. PCR products were separated on 1.5% agarose gels containing 0.5 μg μL^{-1} ethidium bromide and visualized by UV transillumination. One hundred RAPD primers from the University of British Columbia (RAPD set # one, UBC, Vancouver, Canada) were screened using six individuals (one *S. hesperidium*, one *S. glaucus* subsp. *glaucus* and three *S. glaucus* subsp. *coronopifolius*). Thirteen primers that gave strong and easily scorable bands were selected (UBC-108: GTATTGCCCT; 112: GCTTGTGAAC; 126: CTTTCGTGCT; 129: GCGGTATAGT; 135: AAGCTGCGAG; 141: ATCCTGTTCG; 145: TGTCGGTTGC; 153: GAGTCACGAG; 158:TAGCCGTGGC; 162: AACTTACCGC; 177: TCAGGCAGTC; 183: CGTGATTGCT; 189:TGCTAGCCTC).

5.2.5 RAPD analysis—By restricting the number of individuals per population to five it was possible to amplify 19 of the possible 22 populations in one polymerase chain reaction (PCR) (Table 1). Scoring bands across a single PCR is preferable because minor changes in reaction conditions, such as different lots of reagents, may alter banding patterns (Staub, Bacher and Poetter, 1996). For this reason three populations (AD, AL and ZT) were excluded from the RAPD study.

RAPD fragment presence/absence was scored in each of the 95 individuals. Inter-individual relationships in multidimensional space were examined by principal coordinate (PCO) analysis using PCO3D (Adams, 1995). The PCO analysis used a similarity matrix derived from Jaccard's (1908) coefficient: $Dj = n_{xy}/n$, where n_{xy} is the number of shared markers and n is the total number of markers scored in each pairwise comparison. The RAPD data were also used to construct a UPGMA phenogram based on Jaccard's (1908) coefficient using NTSYS. Diversity was estimated in each population using Shannon's index calculated as: $H_O = -\Sigma P_i \log_2 P_i$, where P_i is the frequency of the ith RAPD band. Shannon's index of diversity is suitable for use with dominant markers, such as RAPDs, as it does not rely on the assumption of Hardy-Weinberg equilibrium (Chalmers et al., 1992). Diversity was calculated at several hierarchical levels: H_O was calculated for each population and then averaged over populations to give H_{POP}, while H_T was calculated as the corresponding value across all populations. Diversity was partitioned into a within population component as H_{POP}/H_T, and into an among population component as $(H_T-H_{POP})/H_T$.

5.3 RESULTS

5.3.1 Morphometric analysis—The first three axes of the PCA accounted for 38.78, 16.45 and 12.84% of total variance respectively. Plotting PCA1 against PCA2 revealed a series overlapping clusters (Fig. 2). *Senecio hesperidium* was located entirely within the cluster corresponding to *S. glaucus* subsp. *glaucus*, indicating that for the continuous variables measured these taxa are very similar. The two subspecies of *S. glaucus* in the eastern Mediterranean displayed considerable overlap, strongly suggesting that these taxa display continuous variation. Moroccan *S. glaucus* subsp. *coronopifolius* displayed considerable overlap with the same taxon from Sicily, and slight overlap with all other taxa. The overlap of both Sicilian and Moroccan *S. glaucus* subsp. *coronopifolius* with Israeli material was less marked, suggesting that within this taxon central and western Mediterranean populations are most similar.

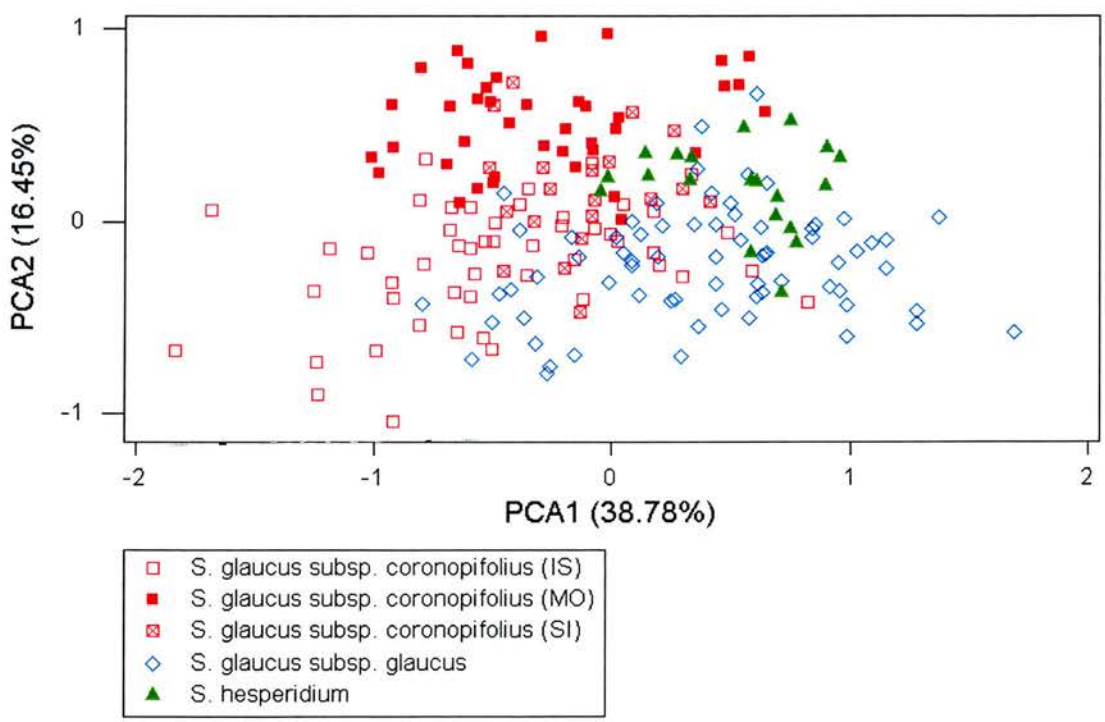

Fig. 2. Principal components analysis (PCA) plot of the first two axes based on 11 morphological variables in *Senecio. glaucus* subsp. *glaucus*, *S. glaucus* subsp. *coronopifolius* and *S. hesperidium*.

The first three axes of the CVA accounted for 41.12, 28.02 and 10.00% of the total variance respectively. A MANOVA showed that differences between group means were highly significant ($P < 0.001$, according to Wilk's Lambda, Pillai's trace, and Hotelling-Lawley trace tests of significance). Plotting mean canonical scores of the groups (populations) for CV1 against CV2 separated eastern and western Mediterranean

populations of *S. glaucus* subsp. *coronopifolius* (Fig. 3), confirming the presence of clear east-west morphological differentiation. Plotting CV1 against CV3 (not shown) revealed further differentiation by separating western and central Mediterranean material into distinct clusters. A close relationship between *S. glaucus* subsp. *glaucus* and *S. glaucus* subsp. *coronopifolius* from the east Mediterranean was still evident by the slight overlap of these two clusters. *Senecio glaucus* subsp. *glaucus* and *S. hesperidium* were slightly separated. This contrasts with the PCA, in which these taxa showed overlap. *Senecio hesperidium* also showed a close relationship to western and central Mediterranean material of *S. glaucus* subsp. *coronopifolius*.

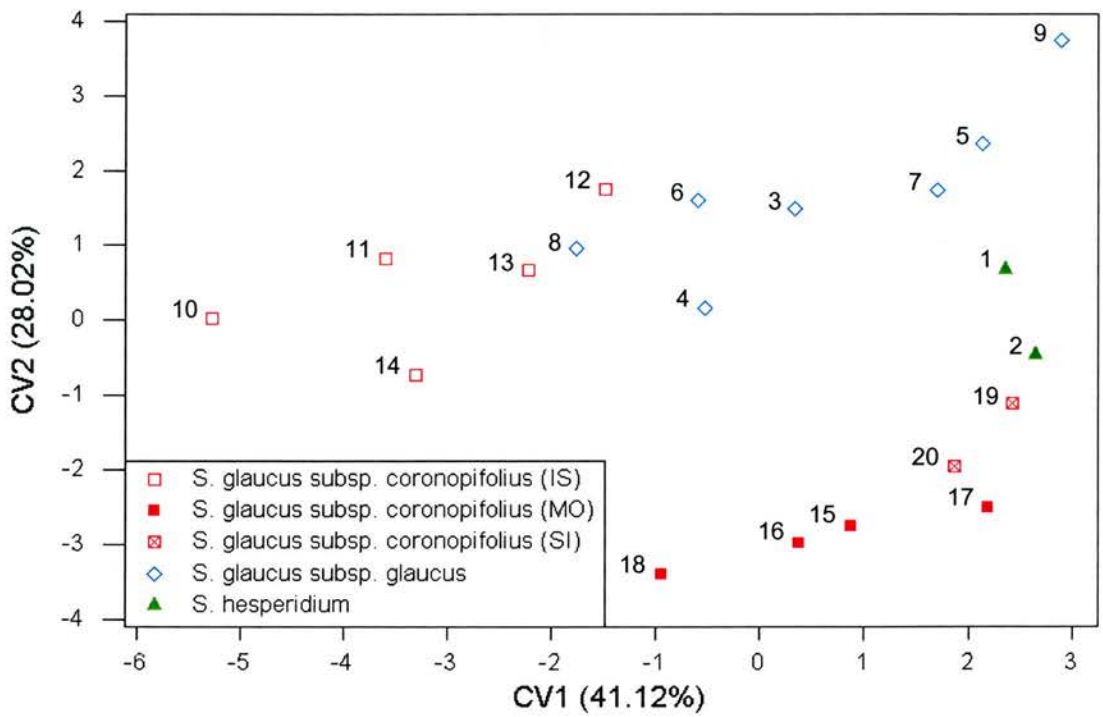

Fig. 3. Canonical variates (CVA) analysis plot of the first two axes based on 11 morphological variables in 20 populations of *Senecio. glaucus* subsp. *glaucus*, *S. glaucus* subsp. *coronopifolius* and *S. hesperidium*. Population numbers are given in Table 1.

The correlation between continuous variables was assessed using NTSYS. The correlation matrix revealed that 39 of 55 character pairings showed significant positive correlation ($r > 0.138$, d.f. = 198, $P = 0.05$). Capitulum width was strongly correlated ($r > 0.7$, $P < 0.001$) with phyllary length, mean ray width and disc floret number. Consequently, not all of these variables may be necessary in future morphometric studies of *Senecio*.

ANOVA revealed that seven of the 11 continuous variables were significantly different among taxa (Table 2). To further examine the nature of the observed

differences pairwise comparison was carried out using Scheffe's test implemented with SAS (Table 2). *Senecio glaucus* subsp. *coronopifolius* from Israel was significantly different from the same taxon in Sicily and Morocco for seven and six of these variables respectively. Significant differences displayed by Israeli plants that may be taxonomically useful included greater inflorescence length, shorter phyllaries, narrower capitula and more numerous and longer calyculus bracts. Moroccan and Sicilian material was less differentiated, showing significant difference for only four of these variables. Similarity between Israeli *S. glaucus* subsp. *coronopifolius* and *S. glaucus* subsp. *glaucus* was indicated by no significant difference in inflorescence length, calyculus bract number and calyculus bract length. *Senecio glaucus* subsp. *glaucus* and *S. hesperidium* revealed no significant difference for four variables, but were significantly different in capitulum width, number of calyculus bracts and ray floret length/width ratio. Differences among populations within taxa were significant ($P < 0.001$) in all cases.

Frequency differences between taxa and populations for phyllary number and ray floret number were small and appeared to be linked to capitulum size. Twenty-one phyllaries was the most frequent state (≥ 0.7) in all populations. The larger capitula of *S. glaucus* subsp. *glaucus* and *S. hesperidium* gave low frequencies (0.1) of 34 phyllaries (populations AQ, AX, CA and AP). This state was also present at low frequency (0.1) in one population (SI) of *S. glaucus* subsp. *coronopifolius* from Morocco. The smaller capitula of *S. glaucus* subsp. *coronopifolius* gave low frequencies (≤ 0.3) of 13 phyllaries in three populations [AL and KB (Israel) and PO (Sicily)]. A similar pattern was observed with ray floret number. In 18 out of 20 populations 13 rays was the most frequent state (≥ 0.7). In two populations [AL (Israel) and TM (Morocco)] of *S. glaucus* subsp. *coronopifolius* the frequency of eight rays equaled or exceeded 13 rays. In three other populations [KB (Israel), PO (Sicily) and KO (Morocco)] of *S. glaucus* subsp. *coronopifolius* eight rays were found at low frequency (≤ 0.3). Individuals with 21 rays were present at low frequency (≤ 0.3) in six populations (AD, AK, AQ, AX, CA and NF) of *S. glaucus* subsp. *glaucus*.

Presence/absence of calyculus bract hair and trifurcate leaf lobe both showed potential as taxonomically useful characters. Eastern Mediterranean material of both subspecies possessed obvious hairs on the calyculus bracts. In contrast, central and western Mediterranean material usually lacked such hairs. The only exceptions were three populations (KO, OS and SI) of *S. glaucus* subsp. *coronopifolius* from Morocco

TABLE 2. Morphological character means and standard errors of *Senecio glaucus* subsp. *glaucus*, *S. glaucus* subsp. *coronopifolius* and *S. hesperidium*. *N* is the number of individuals measured. The analysis of variance *F* ratios are indicated.

	Taxon/regional sample							
	S. glaucus subsp. *coronopifolius* Israel	*S. glaucus* subsp. *coronopifolius* Sicily	*S. glaucus* subsp. *coronopifolius* Morocco	*S. glaucus* subsp. *glaucus*	*S. hesperidium*	*F* ratio	*P* value	
Plant height mm	292.4 ± 12.5	330.2 ± 23.4	320.5 ± 10.2	350.1 ± 11.5	344.1 ± 16.3	0.78	0.554	n.s.
Inflorescence length mm	52.90 ± 3.09^{a}	28.35 ± 1.80^{c}	36.95 ± 1.86$^{b, c}$	60.74 ± 2.58^{a}	46.90 ± 3.29$^{a, b}$	8.16	0.001	**
Phyllary length mm	5.89 ± 0.13^{c}	6.96 ± 0.10^{a}	6.51 ± 0.09^{b}	7.22 ± 0.11^{a}	7.38 ± 0.15^{a}	3.16	0.045	*
Capitulum width mm	4.30 ± 0.09^{d}	5.28 ± 0.10^{c}	5.21 ± 0.12^{c}	5.74 ± 0.11^{b}	6.60 ± 0.14^{a}	5.47	0.006	**
No. of calyculus bracts	7.70 ± 0.29$^{a, b}$	5.05 ± 0.40^{c}	4.13 ± 0.22^{c}	8.57 ± 0.28^{a}	6.55 ± 0.33^{b}	13.17	< 0.001	***
Mean calyculus length mm	3.08 ± 0.10^{a}	1.97 ± 0.08^{c}	2.45 ± 0.07^{b}	3.29 ± 0.07^{a}	3.37 ± 0.15^{a}	10.10	< 0.001	***
Mean ray-floret length mm	10.00 ± 0.26	11.59 ± 0.28	11.96 ± 0.28	11.03 ± 0.24	13.54 ± 0.27	2.77	0.066	n.s.
Mean ray-floret width mm	2.43 ± 0.04^{a}	3.51 ± 0.13^{b}	2.57 ± 0.06^{a}	3.39 ± 0.09^{b}	3.57 ± 0.08^{b}	7.32	0.002	**
Ray-floret length/width ratio	4.12 ± 0.08^{b}	3.39 ± 0.17^{c}	4.70 ± 0.11^{a}	3.31 ± 0.06^{c}	3.82 ± 0.09^{b}	10.23	< 0.001	***
No. of disc-florets	88.12 ± 3.73	93.45 ± 3.53	105.83 ± 4.83	114.33 ± 2.76	117.70 ± 4.70	2.05	0.139	n.s.
No. of leaf lobes	6.94 ± 0.25	7.35 ± 0.36	8.70 ± 0.28	8.09 ± 0.24	7.50 ± 0.30	2.53	0.084	n.s.
N	50	20	40	70	20			

Level of significant difference indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant. Shared superscript letters indicate no significant difference in pairwise comparisons using Scheffe's test.

which contained individuals with hairy bracts at low frequency (0.3), although here the hairs were inconspicuous. Calyculus bract hairs were absent in *S. hesperidium*.

Trifurcate leaf lobes were absent in *S. hesperidium* and all central and western Mediterranean material of *S. glaucus* subsp. *coronopifolius*. In contrast, this character was strongly associated with *S. glaucus* subsp. *glaucus*, being fixed in four populations (AD, AQ, AX and NF) and present at high frequency (≥ 0.8) in the remaining three (AK, CA and EK). In eastern Mediterranean *S. glaucus* subsp. *coronopifolius* trifurcate leaf lobes were absent in one population (MR) and present at low frequency (0.1) in two (AL and KB). However, populations from Zomet Telalim (ZT) and Bet-She'an (BS) contained trifurcate leaf lobes at a frequency of 0.4 and 0.6 respectively, providing further evidence that subsp. *coronopifolius* and subsp. *glaucus* intergrade in the east Mediterranean.

5.3.2 RAPD variation—Forty polymorphic RAPD markers were amplified using 13 primers and scored in 95 individuals. The number of fragments scored per primer ranged from two (UBC-129, 153, 177, 189) to five (UBC-108). Two cases of shared RAPD phenotypes were found. The Bet-She'an (BS) population of Israeli *S. glaucus* subsp. *coronopifolius* contained a phenotype shared by two individuals, while another phenotype shared by two individuals was present in the Sidi Rbat (SR) population of *S. hesperidium*. Otherwise, all individuals possessed unique RAPD phenotypes.

The first three axes of the PCO analysis accounted for 27.63, 9.44 and 4.03% of total variance respectively. Plotting PCO1 against PCO2 clearly distinguished three clusters corresponding to western, central and eastern Mediterranean individuals (Fig. 4a), indicating marked genetic differentiation across the range of *S. glaucus* subsp. *coronopifolius*. *Senecio glaucus* subsp. *glaucus* and *S. hesperidium* were not clearly defined within the east and west Mediterranean clusters respectively, being instead intermixed with *S. glaucus* subsp. *coronopifolius*. However, some indication of clustering of *S. hesperidium* as a distinct entity was seen when PCO1 was plotted against PCO3 (Fig. 4b).

Greater similarity between central and western Mediterranean taxa was highlighted by the UPGMA phenogram (Fig. 5). The basal dichotomy of the UPGMA tree separated Egypt and Israel on the one hand from Sicily, Tunisia and Morocco on the other. Nevertheless, the same three clusters identified by PCO analysis were revealed as central and western plants represent the next most basal dichotomy (Fig. 5).

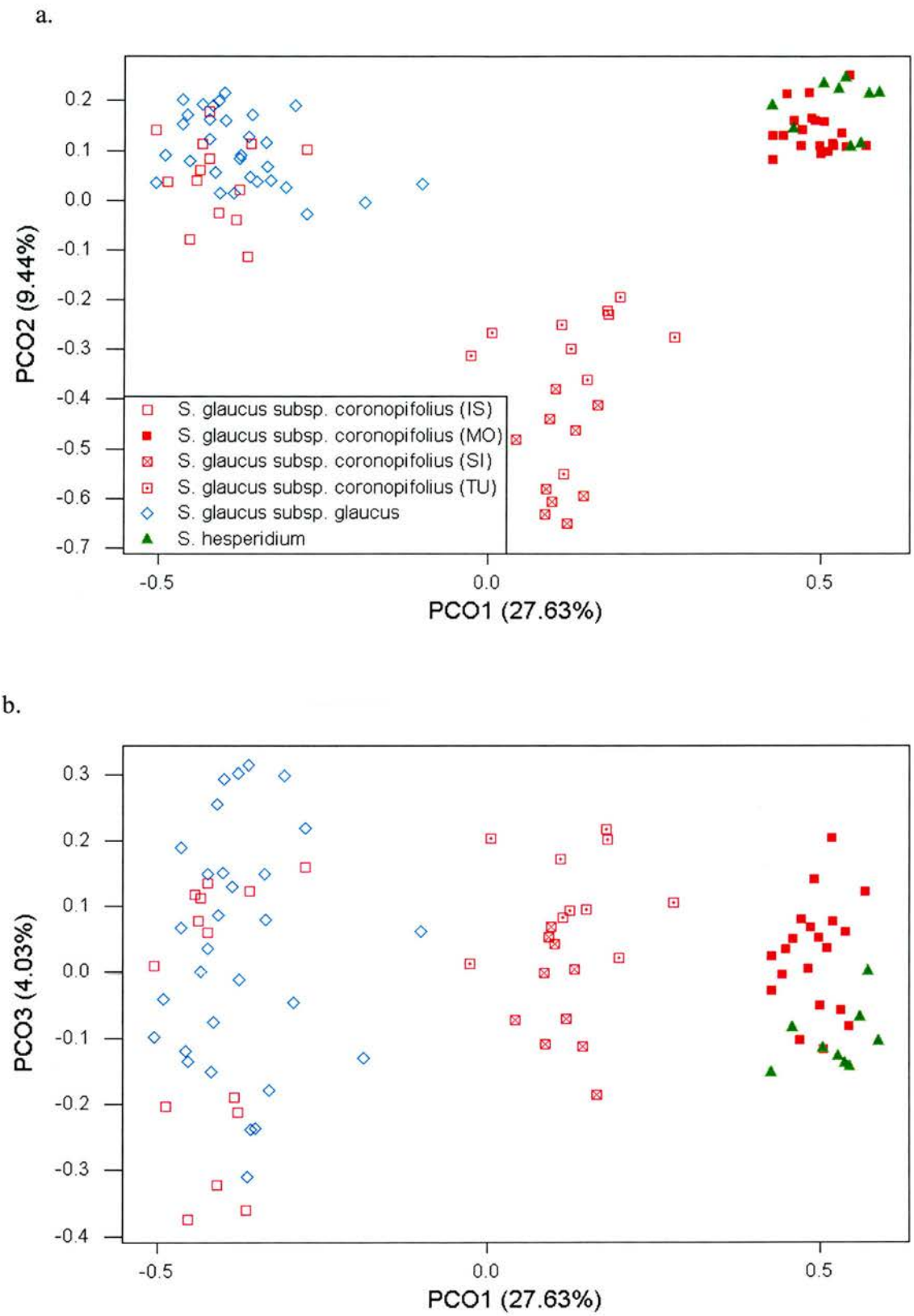

Fig. 4. Principal coordinates (PCO) analysis plots of the first three axes calculated using Jaccard's (1908) distance based on 40 random amplified polymorphic DNA (RAPD) fragments in *Senecio glaucus* subsp. *glaucus*, *S. glaucus* subsp. *coronopifolius* and *S. hesperidium*.

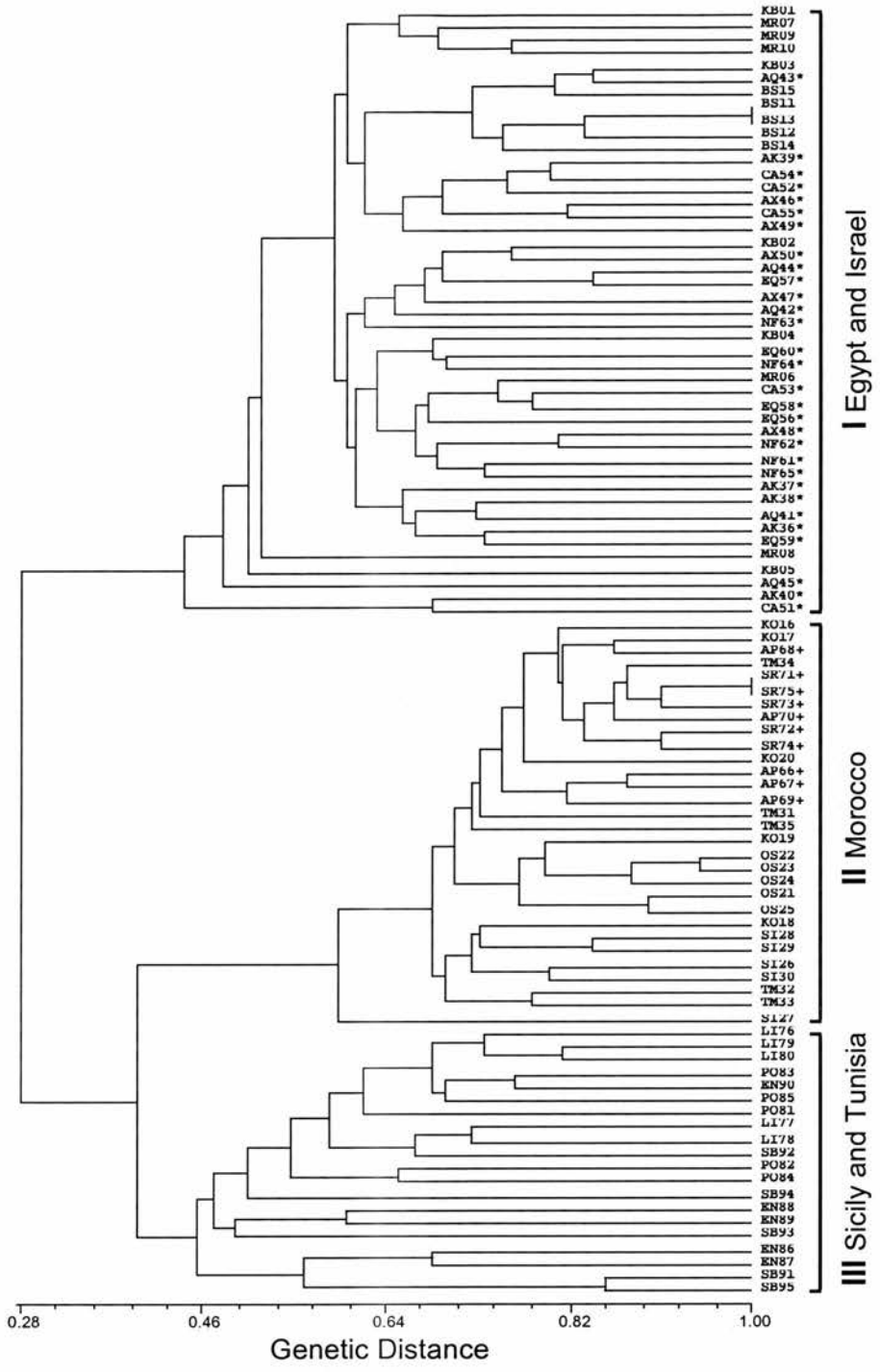

Fig. 5. UPGMA phenogram calculated using Jaccard's (1908) distance based on 40 random amplified polymorphic DNA (RAPD) fragments in *Senecio glaucus* subsp. *glaucus* (*), *S. glaucus* subsp. *coronopifolius* and *S. hesperidium* (+). Population abbreviations are given in Table 1.

As with the PCO analysis, no clear clustering of *S. hesperidium* or *S. glaucus* subsp. *glaucus* as distinct taxa was seen.

Variation in diversity levels revealed some consistent patterns among taxa and regions (Table 3). For individual populations the lowest diversity (0.07) was recorded in *S. hesperidium* from Sidi Rbat (SR), whilst the highest (0.27) was in *S. glaucus* subsp.

coronopifolius from Enfida Plage (EN) in Tunisia. Because the PCO analysis indicated no clear separation of either *S. glaucus* subsp. *glaucus* or *S. hesperidium* from nearby populations of *S. glaucus* subsp. *coronopifolius* total diversity was calculated on a regional basis. Total diversity was highest (0.36) in the eastern region, intermediate (0.32) in the central region and lowest (0.26) in the western region (Table 4). Partitioning the diversity within and among populations for each region indicated that the within population component was 58% and among population component 42% for both eastern and western regions. The central region displayed a higher within population component of 69%, with 31% of diversity maintained among populations (Table 4).

TABLE 3. Shannon's diversity index calculated for populations of *Senecio glaucus* subsp. *glaucus*, *S. glaucus* subsp. *coronopifolius* and *S. hesperidium*.

Western region	H_O	Central region	H_O	Eastern region	H_O
KO	0.20	LI	0.16	AK	0.21
OS	0.10	PO	0.22	AQ	0.22
SI	0.15	EN	0.27	AX	0.21
TM	0.20	SB	0.24	CA	0.24
AP	0.16	—	—	EQ	0.21
SR	0.07	—	—	NF	0.21
—	—	—	—	KB	0.21
—	—	—	—	MR	0.23
—	—	—	—	BS	0.16

TABLE 4. Mean diversity values (H_{POP}) for each region and the total values (H_T) for populations of *Senecio glaucus* subsp. *glaucus*, *S. glaucus* subsp. *coronopifolius* and *S. hesperidium* combined within each region. Diversity partitions within (H_{POP}/H_T) and among (H_T-H_{POP}/H_T) populations are indicated.

	Western region	Central region	Eastern region
Mean (H_{POP})	0.15	0.22	0.21
Total (H_T)	0.26	0.32	0.36
H_{POP}/H_T	0.58	0.69	0.58
H_T-H_{POP}/H_T	0.42	0.31	0.42

5.4 DISCUSSION

5.4.1 Infraspecific variation in *S. glaucus* subsp. *coronopifolius*.—The morphological and molecular analyses demonstrate that western, central and eastern Mediterranean populations of *S. glaucus* subsp. *coronopifolius* are clearly differentiated from each other. However, both data sources indicate that the extent of differentiation is less marked between western and central Mediterranean populations. This is an interesting result as the central Mediterranean populations are equidistant from the

western and eastern Mediterranean localities. Assuming distance alone controls the level of gene flow, equivalent levels of differentiation would be expected.

A possible explanation of this skewed pattern of relationships is differences in habitat continuity. *Senecio glaucus* subsp. *coronopifolius* is primarily an inland species associated with rocky deserts and ascends to ~2000 m on limestone (Alexander, 1979). The Atlas Mountains and lower lying mountains in Algeria and Tunisia, which are continuations of the Atlas range, provide a largely unbroken corridor of apparently suitable habitat. Sicily represents the easternmost end of this habitat corridor. In contrast, large expanses of sandy desert in Libya and Egypt in areas of limited relief provide largely unsuitable habitat, which may act as a barrier to gene flow. The present Sahara Desert dates from the Late Pleistocene (Wickens, 1984), and large expanses of sandy desert may have been restricting gene flow with the east Mediterranean since this time.

The appearance and subsequent history of typical summer-dry "Mediterranean" climate in different parts of the Mediterranean basin has also probably influenced present day patterns of variation. Fossil evidence suggests that plants showing adaptation to summer drought first appeared in the south central Mediterranean ~4 Mya (Bertoldi, Domenico and Thunell, 1989). However, such evidence also indicates considerable temporal variation in the establishment of summer-dry climate across the Mediterranean basin (evidence reviewed by Estabrook, 2001). The present Mediterranean flora seems to have been continuously present for about the past million years. During this time it seems likely that Pleistocene climatic fluctuations have fragmented ranges and been an important factor in speciation (Quézel, 1978; Suc, 1984; Pons and Quézel, 1985). The importance of Pleistocene climatic history for Mediterranean *Senecio* has been highlighted by molecular clock estimates (Comes and Abbott, 2001). It has been found that a clade containing most of the diploid species, including *S. glaucus* and *S. hesperidium*, has probably shown near simultaneous diversification at some time in the last million years (Comes and Abbott, 2001). Consequently, it is possible that from an early stage the range of *S. glaucus* was fragmented and hence disposed to divergence.

An indication of how the distribution of *S. glaucus* might have been affected by geography and climate fluctuations may be provided by genetic diversity estimates. In relative terms, the observed trend from high total diversity in the east to low total diversity in the west may indicate spread from the east accompanied by genetic

bottlenecks. Reduced genetic diversity due to founder effects have been found to be common in areas of Europe and North America subject to postglacial colonization (Hewitt, 1996). The present result must be viewed with some caution due to small population sizes, unequal numbers of populations in each region and the large gaps between regions. Nevertheless, the populations sampled in this study should be comparable in as far as siblings were not included in any populations and sampling methods were broadly the same in each case.

The molecular similarity of Sicilian and Tunisian material probably reflects the close geographical proximity of these samples despite their separation by the Mediterranean Sea. Alexander (1979) considered Sicilian plants from the sampled populations at Licata (LI) and Pozallo (PO) to be intermediate between *S. glaucus* subsp. *coronopifolius* and *S. gallicus* Vill. However, no evidence for this was found in a previous morphometric study that included *S. gallicus* and *S. glaucus* subsp. *coronopifolius* from Tunisia (M. Chapman, unpublished data). *Senecio gallicus* is distinguished most readily by three or less calyculus bracts, with bracts often being absent. In the present study, a mean number of 5.05 calyculus bracts were found in Sicilian material, slightly higher than the value of 4.13 for Moroccan material, providing no support for the intermediacy of these populations. Despite the close relationship of Sicilian and Tunisian material, the evidence of slight differentiation (Fig. 4b) indicates that gene flow may be restricted. Limited gene flow is also indicated by the reduced genetic diversity of Sicilian relative to Tunisian material, consistent with a genetic bottleneck following island colonization.

Considering the flora of the Mediterranean as a whole, distinctive groups of eastern and western species are well known (Quézel, 1978; Médail and Quézel, 1997). The greater differentiation from east to west than from north to south probably reflects the linkage of Africa and Europe via the Middle East and via a former land connection across the strait of Gibraltar between 5 to 6 Mya (Quézel, 1978). Very few studies of infraspecific differentiation in Mediterranean plants have been made (reviewed by Thompson, 1999). Of these, the only study of comparable geographic spread has examined allozyme differentiation across the Mediterranean distribution of *Quercus ilex* (Michaud et al., 1995). The *Q. ilex* study provides a useful comparison with the present study as east-west differentiation into three morphotypes is observed, and this has led to a number of different taxonomic treatments. It was concluded that in *Q. ilex* geographical discontinuities were restricting gene flow, but morphological differentiation was not clearly related to allozyme differentiation (Michaud et al., 1995).

5.4.2 Partitioning genetic diversity—For reasons already stated the diversity data in the present study must be treated with caution. However, because so few studies have addressed the question of diversity distribution in widespread Mediterranean taxa some exploration of the data is of value. Partitioning genetic diversity within and among populations for the three regions indicated that a lower proportion of genetic variation is maintained among populations in the central region compared to the eastern and western regions (Table 3). This may indicate lower population differentiation in the central region. On the other hand, it may simply be a sampling artefact. If this is a genuine difference, it might indicate the existence of a western as well as an eastern refugium for *S. glaucus*. Such a conclusion would be consistent with the two main biodiversity "hot-spots" in the Mediterranean, which are split between a western hot-spot in the Iberian Peninsula/Morocco and an eastern hot-spot in Turkey/Greece (Médail and Quézel, 1997). It is possible that the low diversity seen in the western region is not part of a Mediterranean-wide trend, but instead a reflection of localized low diversity in marginal populations on the Atlantic coast. It is also worth noting that the existence of two refugia would fit well with the observed morphological differentiation. Further sampling throughout the range of *S. glaucus* is required to identify likely refugia.

5.4.2 Relationships between taxa—The RAPD analyses show that the localized taxa *S. glaucus* subsp. *glaucus* and *S. hesperidium* are clearly differentiated from each other. In contrast, both are poorly differentiated from adjacent populations of *S. glaucus* subsp. *coronopifolius*. This provides strong evidence to suggest that any morphological similarities between the two are coincidental, although selection under similar maritime conditions cannot be ruled out. This conclusion cannot be drawn from most of the continuous morphological data. Here a bias towards capitulum characters, many of which are positively correlated, has given a misleading picture of close similarity. However, the distance of many populations of *S. glaucus* subsp. *glaucus* from *S. hesperidium* in the CVA (Fig. 3) does highlight differences even in capitulum characters. Interestingly, the population at Nof Yam (Fig. 3 population 9), which in gross morphology most closely resembles *S. hesperidium*, is confirmed to be an extreme morphotype. Among the continuous variables, only the number of calyculus bracts reflects the differentiation of these taxa. However, the presence/absence of calyculus hairs and trifurcate lobes both provide good distinguishing characters.

The poor molecular differentiation of *S. glaucus* subsp. *glaucus* from subsp. *coronopifolius* is in full agreement with the lack of a clear morphological distinction of the subspecies. The apparently negligible postpollination reproductive isolation of coastal and inland material in Israel (95% stainable pollen in an F_1 hybrid: Alexander, 1979) may explain these results. Alexander (1979) speculated that hybridization between *S. glaucus* subsp. *glaucus* and *S. vernalis* Waldst. and Kit may explain the morphology of inland Israeli material. However, this can be largely discounted based upon an isozyme study carried out by Comes and Abbott (1999b), which revealed an almost complete barrier to nuclear gene flow between these species.

In contrast, in *S. hesperidium* there is an indication of greater differentiation from nearby *S. glaucus* subsp. *coronopifolius* (Fig. 4b). This coincides with a higher level of apparent postpollination reproductive isolation (75% stainable pollen in an F_1 hybrid: Alexander, 1979) than seen in the case of *S. glaucus* subsp. *glaucus*. The low diversity of *S. hesperidium* is consistent with it being a relatively recent derivative of *S. glaucus* subsp. *coronopifolius*. This conclusion agrees with an estimated separation of *S. glaucus* and *S. hesperidium* within the last million years based on a nuclear ribosomal DNA molecular clock (Comes and Abbott, 2001).

The failure to support a close relationship between *S. hesperidium* and *S. glaucus* subsp. *glaucus* and the clear differentiation of *S. glaucus* subsp. *coronopifolius* from the western, central and eastern Mediterranean indicates that long-distance seed dispersal is rare. Given the apparent suitability of *Senecio* achenes (fruits) for wind dispersal (Small, 1919), this conclusion is somewhat surprising. However, other studies of Mediterranean members of sect. *Senecio* have concluded that differentiation has occurred over considerably smaller geographical scales (Comes and Abbott, 2000; Coleman and Abbott, unpublished data). Two cases of extreme long-distance dispersal have been established in sect. *Senecio* (Coleman et al., unpublished data), which appear to be associated with novel pappus modifications that may favour dispersal by birds. However, such cases are probably very rare.

The molecular data provide strong support for the suggestion made by Alexander (1975) that *S. hesperidium* and *S. glaucus* subsp. *glaucus* have been independently derived from inland progenitors currently classified as *S. glaucus* subsp. *coronopifolius*. In both cases, adaptation to maritime conditions may have provided sufficient ecological isolation to allow divergence in the absence of strong postpollination reproductive barriers. However, in the case of *S. glaucus* subsp. *glaucus* introgression

between coastal and inland populations appears to be maintaining continuous variation. The poor differentiation of these taxa is consistent with evidence of their recent divergence (Comes and Abbott, 2001).

5.4.3 Taxonomic implications—The ability to draw firm taxonomic conclusions on how best to deal with the variation displayed by *S. glaucus* subsp. *coronopifolius* is hampered by uncertainty regarding the gaps between the sampled populations. Clearly, it is necessary to establish how characters that have shown promise as being taxonomically useful vary across the Mediterranean as a whole. Characters that should be examined in a more comprehensive survey of herbarium material include calyculus bract number, calyculus bract hairiness, calyculus bract length and leaf lobe morphology. Depending on the outcome of such a study, two main alternative taxonomic treatments seem possible.

Firstly, in a situation where continuous variation is the norm and no clear distinction can be drawn between eastern Mediterranean material and the subspecies in the remainder of its range it seems best to leave the current treatment unaltered. Continuous variation already poses taxonomic problems in the east Mediterranean and the usefulness of establishing a new taxon under such circumstances is doubtful.

Secondly, if eastern Mediterranean material can be reasonably clearly distinguished by morphological discontinuities it would be possible to establish a new east Mediterranean taxon. A potential problem with this may be an unclear distinction between the inland and coastal forms in the eastern Mediterranean.

In a situation of clear east-west distinction, it would also be possible to consider raising central and western Mediterranean material to species status. However, this may not be desirable given the broad morphological similarities and apparent reproductive compatibility (Alexander, 1979).

The maintenance of *S. hesperidium* as a distinct species is desirable. This species is well differentiated from Moroccan *S. glaucus* subsp. *coronopifolius* based on morphology (Table 2), with some evidence of molecular differentiation (Fig. 4b). Nevertheless, these taxa appear to be closely related.

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6.1 GENERAL CONCLUSION

The main conclusions from the four studies carried out on different aspects of the taxonomy, biogeography and evolution of *Senecio* sect. *Senecio* are outlined below and areas of possible future work are highlighted.

Chapter 2—Chromosome counts of *S. flavus* subsp. *breviflorus* from Southwest Asia and *S. mohavensis* from southwest North America revealed that both are tetraploid ($2n = 40$). In contrast, *S. flavus* subsp. *flavus* from northern Africa is diploid ($2n = 20$). The two tetraploid taxa were successfully crossed to give a fertile F_1 hybrid. However, the two subspecies of *S. flavus* were not successfully crossed. A close link between the two tetraploid taxa is also reflected in their morphological similarity, whereas *S. flavus* is morphologically distinct. Based upon these results *S. flavus* subsp. *breviflorus* is transferred to *S. mohavensis*. The unusual Old World–New World disjunction in *S. mohavensis* is presumed to result from natural colonization, as there are minor differences in floral and pappus morphology, and F_1 hybrids display significantly lower fertility than both parental taxa.

Chapter 3—A phylogenetic analysis of nrDNA ITS sequences provides support for sect. *Senecio* including widely disjunct species in southern Africa and southwestern North America in addition to the "core" species from Europe and the Mediterranean. A southern African origin of the section is indicated by two cladistic methods for inferring ancestral areas, although bootstrap percentages for the spine of the tree are low, thus making this result tentative. *Senecio flavus* is not supported as a member of sect. *Senecio*. Instead, this species represents a distantly related lineage, also probably of southern African origin based upon its only close relative, *S. englerianus*, having a southern African distribution. The position of *S. flavus* in the genus remains unclear and further sampling of southern African taxa is required to clarify this. Section *Senecio* is estimated to have arisen during the Early Pliocene based on an ITS molecular clock calibrated with rates established in other Compositae. This result is consistent with the emergence of Mediterranean summer-dry climate having been influential in the evolution of the group. However, diversification during the Pleistocene suggests that climatic fluctuations have also promoted speciation.

The spread of the section from southern African to northern Africa may have utilized an East African arid corridor. Clarification of the role of this arid corridor in dispersal of the section requires inclusion of montane tropical African taxa that have been assigned

to the section (Jeffrey, 1986). Two distinct lineages are represented in the New World, indicating two separate dispersal events: (i) during the Mid-Pliocene, and (ii) during the last 0.15 million years. Both of these considerably postdate the last plausible land bridge across the North Atlantic during the Miocene and must therefore represent long-distance dispersal. The historical biogeography of the first dispersal remains unclear due to poor internal branch support in the phylogeny. This problem may be resolved by more detailed sampling. The second dispersal is reflected in the relatively recent disjunction of *S. mohavensis*. Recent long-distance dispersal is also supported as an explanation of the disjunction of *S. flavus* between southern and northern Africa. Novel hooked pappus hairs are described in both disjunct species that may assist dispersal by birds. This explanation is more plausible for *S. flavus* as the novel pappus type called "connate fluked pappus" is better developed, and bird migrations fit the distribution pattern.

Senecio mohavensis is taxonomically isolated in the New World but is nested within a clade of Mediterranean diploid species. Reticulate evolution of *S. mohavensis* subsp. *breviflorus* has been inferred based on incongruence between morphology and molecular data. Both ITS and cpDNA variation implicate a member of a clade composed of diploid Mediterranean species, whereas *S. flavus* is a likely progenitor based on morphology. The presence of connate fluked pappus in *S. mohavensis* provides further evidence for the involvement of *S. flavus*, as this type of pappus has so far only been found in *S. flavus*. Whether *S. mohavensis* represents an allotetraploid or an introgressed autotetraploid remains unresolved, although the Mediterranean diploid must have acted as the maternal parent based on maternal plastid inheritance (Harris and Ingram, 1992).

Potential benefits of polyploidy include increased heterozygosity, reduced inbreeding depression and increased colonizing ability (Stebbins, 1950; Soltis and Soltis, 2000). Studies using genome *in situ* hybridization and chromosome mapping techniques are indicating that genome rearrangements can occur following polyploidization and may be a source of novel genotypes (Wendel, 2000; Soltis and Soltis, 2000). It may therefore be possible that the reticulate origin of *S. mohavensis* and its polyploid condition have contributed to its successful colonization of habitats in Southwest Asia not occupied by *S. flavus*. The same applies to the successful adaptation to conditions in southwestern North America.

The phylogenetic isolation of *S. flavus* found in this study suggests that an allopolyploid origin of *S. mohavensis* is more likely based upon the likely high level of

dissimilarity between the genomes of the putative progenitors. This is also supported by the strong reproductive isolation of *S. flavus* from all of the Mediterranean species (Alexander, 1975). Confirmation of this requires further study with suitable molecular markers. Due to potentially rapid genome evolution (Wendel, 2000), the use of supposedly neutral molecular markers may not provide the expected additive pattern of an allopolyploid species. Comes and Abbott (1999a, 2001) found RAPD markers placed *S. mohavensis* subsp. *breviflorus* (in their study referred to as *S. flavus* subsp. *breviflorus*) closest to *S. flavus*. Support for an allopolyploid origin may be drawn from a reinterpretation of the evidence for duplicated isozyme loci and fixed heterozygosity found by Liston, Rieseberg and Elias (1989). Genomic *in situ* hybridization could be used to identify the presence of divergent genomes, but *Senecio* chromosomes are small and may be difficult to work with. Another approach would be to examine highly conserved low or single copy nuclear genes. Under an allopolyploid origin an additive pattern of parental genes would be expected, whilst under autopolyploidy deviations from such a pattern may occur. The cycloidea (*cyc*) gene family, concerned with floral development, may be a suitable candidate for study. It has already been found that the *cyc* genes in *S. flavus* are divergent from those of the Mediterranean diploids (A. Gillies, personal communication, 2001).

Chapter 4—Variation in RAPD markers indicates that atypical leaf morphology in three populations south of the known range of *S. leucanthemifolius* var. *casablancae* is most likely the result of divergence due to restricted gene flow. Due to the general uniformity of achene and pappus morphology among the Mediterranean species, this result indicates that seed dispersal in sect. *Senecio* is more restricted than might be expected based on the presence of a well-developed pappus. Consequently, localized divergence due to drift and/or selection may be a general process affecting the evolution of the section. Evidence for past hybridization with *S. glaucus* subsp. *coronopifolius* was provided by cpDNA variation, but this appears to have been a localized phenomenon that has not significantly altered the nuclear genome of the atypical populations.

Senecio leucanthemifolius, as a whole, is taxonomically complex due to intergradation of varieties. It is suggested that *S. leucanthemifolius* var. *casablancae* may be best treated as a distinct species due to its clear morphological differentiation from other Moroccan varieties of this species. Further examination of the relationships

between the varieties of *S. leucanthemifolius* and some potentially allied species is required along with a taxonomic revision of the group.

Chapter 5—ANOVA identified significant variation in seven characters across the range of the Mediterranean-wide *S. glaucus* subsp. *coronopifolius* sampled from western, central and eastern populations. Morphometric analysis only provided separation of regional population groups based on population mean phenotypes (canonical variate analysis). In contrast, RAPD data clearly separated populations into western, central and eastern clusters. Slightly lower genetic distance between western and central populations than between central and eastern populations may reflect greater habitat continuity associated with the Atlas Mountains. The nesting of the localized coastal taxa *S. glaucus* subsp. *glaucus* and *S. hesperidium* within eastern and western population clusters of *S. glaucus* subsp. *coronopifolius*, respectively, indicates these taxa are independent derivatives of the widespread subspecies. Consequently, morphological similarities between the coastal taxa are most likely coincidental, although convergence under similar maritime conditions cannot be excluded.

Understanding the phylogeography of *S. glaucus* will require much denser population sampling. The distinction of the two subspecies of *S. glaucus* in the east Mediterranean is unclear. Given the almost complete lack of reproductive barriers (Alexander, 1975) the morphological extremes are presumably maintained by selection under different ecological conditions. Consequently, the zone between the two subspecies would repay detailed examination to establish the evolutionary processes at work.

Examination of morphological variation across the Mediterranean is required before any conclusions can be drawn regarding the infraspecific taxonomy of *S. glaucus*.

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APPENDICES

Appendix 1: Details of morphometric measurements

1. Plant height: Distance in mm from cotyledons to tip of apical capitulum.
2. Inflorescence length: Distance in mm from apical node to tip of apical capitulum.
3. Phyllary length: Distance in mm from the point of junction of the phyllaries to their tip, measured with digital calipers. This was taken as a single measurement as length variation was very small.
4. Capitulum width: Distance in mm at the point of junction of the phyllaries, measured with digital calipers.
5. Phyllary number: Number of phyllaries, corrected to the nearest Fibonacci number.
6. Calyculus bract number: Number of calyculus bracts, taken to include only those bracts above the point of stem swelling that constitutes the beginning of the capitulum base.
7. Calyculus length: Mean length in mm of the calyculus bracts, measured with digital calipers.
8. Ray number: Number of ray florets, corrected to the nearest Fibonacci number.
9. Ray length: Mean length in mm of the ray florets, measured with digital calipers.
10. Ray width: Mean width in mm of the ray floret, measured with digital calipers.
11. Ray length/width ratio: Ray length divided by ray width.
12. Disc number: Number of disc florets.
13. Lobe number: Number of lobes on the fifth leaf to emerge.
14. Bract hairs: Presence/absence of hairs on the calyculus bracts.
15. Trifurcate tips: Presence/absence of trifurcate tips in any of the lobes on the fifth leaf to emerge.

Appendix 2: Solutions made in the laboratory

Solution name	Chemical	Concentration
2 × CTAB	Tris[1]	0.1M
	EDTA[2]	20mM
	NaCl	1.4M
	CTAB[3]	3%
	PVP[4]	2%
TE	Tris[1]	10mM
	EDTA[2]	1mM
	HCl to set *pH*7.5	as required
0.5 × TBE	Tris[1]	0.045M
	Orthoboric acid	0.02M
	EDTA[2]	1.25mM
Loading buffer	Glycerol	50%
	EDTA[2]	0.125M
	SDS[5]	0.1%
	Bromophenol blue	2g/L
DNA size marker	Gibco 1Kb DNA ladder	10%
	Loading buffer	20%
	Sterile distilled water	70%

[1]Tris is tris(hydroxymethyl)methylamine
[2]EDTA is ethylenediaminetetra-acetic acid
[3]CTAB is hexadecyltrimethylammonium bromide
[4]PVP is polyvinyl pyrolidine
[5]SDS is sodium dodecyl sulphate

Appendix 3: DNA extraction protocol for fresh material

1. Place ~100 mg of fresh leaf material in a 2 mL eppendorf.
2. Preheat 1 mL of 2 × CTAB plus 20 µL of 2 B-mercaptoethanol per sample to 55°C in a water bath placed in a fume hood.
3. Dip sample into liquid nitrogen until fully frozen and grind to a powder with a mini-pestle (gently at first). Once ground the sample, with mini-pestle, should be stored in the freezer until ready for the next step.
4. Add 0.5 mL of preheated CTAB buffer and mix with mini-pestle. Add a further 0.5 mL of buffer and mix well. Incubate for 1 hr at 55°C.
5. Remove from water bath and cool for 10 mins.
6. Centrifuge at 13,000 rpm for 10 mins.
7. Gently remove the supernatant with a medium bore pipette (e.g., cut off blue tip) and place in a clean eppendorf.
8. Add 600 µL of dichloromethane (in fume hood) and mix by rotation for 20 mins. Note mixing should be sufficient to prevent the separation of aqueous and organic phases.
9. Centrifuge at 13,000 rpm for 10 mins.
10. Gently remove the supernatant with a medium bore pipette (e.g., cut off blue tip) and place in a clean eppendorf. Pour waste into appropriate organic waste bottle.
11. Repeat steps 8 to 10.
12. Precipitate DNA by adding 2/3 volume (approximately 600 µL) freezer cold isopropanol. Mix by gentle inversion until oily appearance has gone.
13. Centrifuge at 13,000 rpm for 10 mins. Pour off supernatant, invert and dry for 30 mins (DNA pellet should be visible).
14. Dissolve pellet in 0.5 mL TE buffer.
15. Add 3 µL Rnase (10mg/mL) and incubate at 37°C for 1 hr.
16. Add 50 µL of 3M sodium acetate and mix. Precipitate DNA by adding 2/3 volume (approximately 600 µL) freezer cold 96% ethanol. Mix by gentle inversion until the oily appearance has gone.
17. Centrifuge at 13,000 rpm for 10 mins. Pour off supernatant, invert and dry for 30 mins.
18. Dissolve pellet in 200 µL of TE buffer.
19. Quantify DNA by running 5 µL of sample against standards on a 1% agarose gel.

Appendix 4: DNA extraction protocol for herbarium material

The DNA extraction protocol most successful with herbarium material was the same one used with fresh material with two minor modifications. The brown colour of many herbarium DNA extracts may be due to a higher proportion of polymerase chain reaction (PCR) inhibitors such as tannins, polyphenols and quinones. To counteract this an additional pinch of PVP was added at the grinding stage. The second modification was adopted as a result of trials using different quantities of herbarium material. It was found that drastically reducing the volume of plant material dramatically improved results. Leaf fragments as small as 0.5 cm^2 or less gave far better results than using quantities of herbarium material roughly equivalent to the amount of fresh material used. Small amounts of leaf material gave amplification in the majority of cases, whilst larger amounts of the same specimens generally gave no amplification. This observation may also be due to the presence of inhibitors, which are reduced below a critical concentration if smaller amounts of leaf material are used.

Appendix 5: Chromosome counting protocol

1. Harvest active root tips and place in fresh 2mM 8-hydroxyquinoline for 24 hrs.
2. Wash root tips in water and fix in fresh Farmer's solution (three parts ethanol to one part glacial acetic acid) for at least two hours.
3. Wash root tips in water and hydrolyse in 1N HCl for approximately 20 mins.
4. Wash root tips in two changes of water and digest with 4% pectinase for approximately 20 mins.
5. Place terminal 2 mm of root on a microscope slide and stain with lacto propionic orcein for at least two mins.
6. Tap tissue with metal rod to break cells apart.
7. Apply cover slip and press down evenly but firmly.
8. Gently heat slide over a spirit lamp for a few seconds.
9. Cool and seal slide.
10. View with phase contrast on a light microscope.