

# Resolving the native provenance of invasive fireweed (*Senecio madagascariensis* Poir.) in the Hawaiian Islands as inferred from phylogenetic analysis

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## ABSTRACT

Accurate identification of weedy species is critical to the success of biological control programs seeking host-specific control agents. Phylogenetic relationships based on internal transcribed spacer region (ITS1, ITS2) DNA sequence data were used to elucidate the most likely origin and taxonomic placement of *Senecio madagascariensis* Poir. (fireweed; Asteraceae) in the Hawaiian archipelago. Putative *S. madagascariensis* populations from Madagascar, South Africa, Swaziland, and Hawaii were included in the analysis. Different phylogenetic models (maximum parsimony and maximum likelihood) were congruent in suggesting that Hawaiian fireweed is most closely related to populations from the KwaZulu-Natal region in South Africa. Phylogenetic divergence and morphological data (achene characteristics) suggest that the *S. madagascariensis* complex is in need of revised alpha-level taxonomy. Taxonomic identity of invasive fireweed in Hawaii is important for finding effective biological control agents as native range populations constitute different biotypic variants across a wide geographical area. Based on our phylogenetic results, research directed at biological control of Hawaiian infestations should focus on areas in the KwaZulu-Natal region in South Africa where host-specific natural enemies are most likely to be found. Our results show that phylogeographical analysis is a potential powerful and efficient tool to address questions relevant to invasion biology of plants.

## Keywords

Biological control, biological invasions, invasive species, ITS, *Senecio madagascariensis*, taxonomy.

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## INTRODUCTION

*Senecio madagascariensis* Poir. (fireweed) was accidentally introduced to Hawaii in the early 1980s and became naturalized over a wide range of different habitats within 20 years. On the island of Hawaii, it has infested pastureland in the north eastern and western sides of the island and from there expanded its range towards the southern areas (Motooka *et al.*, 1999). On Maui, fireweed infests roadsides and pastures on eastern parts of the island with naturalized populations found from sea level up to 1600 m a. s. l. *Senecio madagascariensis* competes strongly with existing pasture flora for light, moisture, and soil nutrients (notably phosphorus and nitrogen), leading to the ultimate deterioration of pastures (Watson *et al.*, 1994). In addition, like many other *Senecio* species, fireweed produces pyrrolizidine alkaloids that reduce growth and in severe cases cause mortality when

ingested by livestock (McBarron, 1976: 83–86). In Australia annual losses amounting up to US\$ 2 million directly linked to fireweed infestations have been reported (Motooka *et al.*, 1999).

Prioritizing the control of weeds in Hawaii remains problematic with the estimated number of introduced plant species to the archipelago now nearing 5000. Coupled with the large distribution ranges of some of these species, biological control is in many instances the only sustainable method for control over the long run. *Senecio madagascariensis*'s extensive spread and the lack of closely related relatives in the Hawaiian Islands would make biological control a feasible option. Even though several herbicides proved successful against fireweed, the extent of Hawaiian infestations would make such control uneconomical. A biological control program aimed at *S. madagascariensis*, like any other biological pest control initiatives, would require sound systematics. It would be of utmost importance to identify the most likely

native origin of invasive Hawaiian fireweed, the possible existence of cryptic species and/or intraspecific variants if the introduction of natural enemies is being considered.

The generalization that invasive species often have large native ranges is well documented (Lodge, 1993). This phenomenon implies that it is not always easy to determine the native origin of such species. *Senecio madagascariensis*, an Afro-Madagascan native, is widely distributed from southern Madagascar and the Mascarene islands through coastal southern Natal and Transkei and inland in the Swaziland and Cape regions of South Africa. This geographical range represents an equally impressive array of climatological habitats, e.g. in these areas, precipitation varies from approximately 500–3000 mm annually and mean annual temperatures from 18.0 °C to 27.0 °C. Considerable morphological variation has also been reported for specimens identified as *S. madagascariensis* between Madagascar and regions in southern Africa (Radford *et al.*, 2000). Coupled with the large native range distribution, this would imply that determining the native origin of Hawaiian *S. madagascariensis* to be problematic. The native origin of Hawaiian *S. madagascariensis* remains elusive though it supposedly reached the islands from Australia, where it is also considered a serious invasive pasture weed, through the importation of contaminated fodder.

The taxonomic identity of fireweed in Australia has been the subject of considerable attention after it became apparent that it was not part of the native *Senecio lautus* complex as was traditionally thought (Sindel, 1986). Numerous studies attempting to differentiate Australian fireweed from the *S. lautus* complex suggest that fireweed in Australia is more closely related to South African *S. madagascariensis* and *Senecio inaequidens* than to *S. madagascariensis* from Madagascar (Radford *et al.*, 1995; Radford, 1997; Scott *et al.*, 1998). *Senecio inaequidens* is one of several closely related and widespread species in southern Africa (others include *Senecio skirrhodon*, *Senecio burchellii*, and *Senecio pellucidus*) sharing overlapping morphological characteristics and frequently mistaken for *S. madagascariensis* (Hilliard, 1977). Genetic analysis by Scott *et al.* (1998) did not differentiate between Australian fireweed, *S. madagascariensis* and *S. inaequidens*, from South Africa, leading them to conclude that these encompass a single species that differed from putative *S. madagascariensis* specimens from Madagascar. A more recent study by Radford *et al.* (2000) differentiated *S. inaequidens* and *S. madagascariensis* on the basis of achene morphology and chromosome counts. Genetic distances as inferred from isozyme polymorphisms and morphological data suggested that a considerable amount of variation exists within the current *S. madagascariensis* species complex and its close relatives, and concluded that the taxonomy of the complex needs to be revised (Radford *et al.*, 2000).

Fireweed populations in Hawaii show a high degree of morphological variation in plant size and leaf shape (J.J. Le Roux, pers. obs.). Phenotypic plasticity could explain these variations, but with the known variation that exists within the fireweed species complex, Hawaiian infestations could potentially represent more than one *S. madagascariensis* variant or even different *Senecio* species. Determining the taxonomic position of fireweed in Hawaii is

important as the Hawaii Department of Agriculture is currently assessing the possibility of biological control for fireweed in Hawaii. Correct taxonomic placement and phylogeographical analysis of Hawaiian *S. madagascariensis* would contribute significantly to identify area/s where foreign exploration for parasites and/or pathogens is likely to be most productive. Biological control agents against fireweed have been tested in Australia but proved to be unacceptable there, due to their low host-specificity, potential non-target effects on the closely related *S. lautus* complex, and the temporal and spatial coincidence of acceptable host plants (Radford, 1997). Many of these problems can be circumvented when seeking possible biological control agents for Hawaiian infestations since no closely related *Senecio* species are native to the archipelago, and thus non-target impacts are unlikely.

To this end, a phylogenetic study was undertaken in an attempt to elucidate the taxonomic placement and geographical origin of invasive fireweed in Hawaii. The internal transcribed spacer regions (ITS1 and ITS2) DNA sequences were used to construct a phylogeny to infer relationships between putative *S. madagascariensis* populations from Hawaii, South Africa, Swaziland, and Madagascar. ITS demonstrates a unique pattern of evolution, featuring interspersed of relatively rapidly evolving sequences with some of the most highly conserved sequences known (Hillis *et al.*, 1996), and has previously been used to infer relationships within other *Senecio* species complexes (Bain & Jansen, 1995; Scott *et al.*, 1998). In addition, morphological features of achenes were used as supplemental data to infer taxonomic relationships as these characteristics were previously reported as useful characters for interspecies and intraspecific discrimination for the *S. madagascariensis* complex and closely related species (Radford *et al.*, 2000).

## METHODS

### Population sampling

Leaf and seed (achene) material were collected during March 2005 in the known geographical ranges of *S. madagascariensis* in South Africa, Swaziland, and Madagascar. Field collections were made during a survey and collection of potential natural enemies for biological control by M. Ramadan (Hawaii Department of Agriculture). Flower material was placed in containers containing 95% ethanol in the field and subsequently stored at –80 °C. Leaf material was placed and dehydrated in plastic containers containing Drierite™ (WA Hammond Drierite Co., Ltd, Xenia, Ohio) Locality name, region, latitude, longitude, and elevation were recorded for all populations sampled (Table 1). Specimens were classified according to Hilliard (1977) and current herbarium practices in South Africa.

### Morphological characteristics

Achenes of putative *S. madagascariensis* populations were characterized by recording the surface hairiness and lengths of at least 10 achenes per population. In addition, the number of involucre bracts was identified and leaf morphology recorded for each

**Table 1** Location data for putative *S. madagascariensis* populations used in this study

Specimen number/s	Region	Locality	Latitude/longitude	Elevation (m)
North America				
SM149/SM150	Maui	Haleakela hwy	20°51.449' N/156°21.570' W	303
SM323	Hawaii	Parker ranch	19°59.121' N/155°33.04' W	1140
SM462	Hawaii	Parker ranch	19°54.436' N/155°20.577' W	1766
SM553	Hawaii	Mamalahoa hwy	19°50.502' N/155°45.496' W	227
South Africa				
SM153/SM155	KwaZulu-Natal	Hilton, Pietermaritzburg	29°34.015' S/30°19.059' W	980
SM165/SM170	KwaZulu-Natal	Murray Road	29°39.409' S/30°23.751' E	15
SM172*	Kwazulu-Natal	Park Ryne beach	30°18.839' S/30°44.576' W	14.5
SM193*	KwaZulu-Natal	Blythedale beach	29°22.596' S/31°20.807' W	26
SM182/SM191	KwaZulu-Natal	Umhlanga	29°42.812' S/31°04.729' E	135
SM222	KwaZulu-Natal	Camperdown	29°43.867' S/30°33.748' E	745
SM231/SM232	KwaZulu-Natal	Durban City	29°51.570' S/31°2.326' E	14
Madagascar				
SM241/SM242	Saint Luce	Azafady	24°57.992' S/47°5.437' E	16
SM261/SM263/SM264/SM267	Fort Dauphin	Fort Dauphin airport	25°3.05' S/46°56.73' E	33
SM275/SM276	Tulear	Belebika village	23°19.706' S/43°41.288' E	10
Swaziland				
SM255/SM259	Motshane	Motshane	26°14.508' S/31°3.179' E	1334

\*Putative *Senecio inaequidens* specimens.

population. Achene lengths were measured for each population ( $n = 10$ ) with an electronic micrometer and a phase contrast microscope.

### DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted according to the manufacturer's protocol with the Wizard® genomic DNA purification kit (Promega, Madison, USA) from 40 mg to 50 mg dried leaf material that was frozen in liquid nitrogen, ground by hand, and stored at  $-80^{\circ}\text{C}$ . In total, three nuclear regions (ITS1, 5.8S, and ITS2) were sequenced. These genes were polymerase chain reaction (PCR) amplified and sequenced using primers described elsewhere (White *et al.*, 1990). A thermocycle of 35 cycles: denaturation for 1 min at  $94^{\circ}\text{C}$ , annealing for 1 min at  $50^{\circ}\text{C}$ , and extension for 1 min at  $72^{\circ}\text{C}$ , was used for PCR amplification. Purified PCR products were sequenced in both directions and were run on an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA, USA) using standard dye-terminator chemistry following the manufacturer's protocol.

### DNA sequence alignment and phylogenetic analysis

DNA sequences were aligned using the CLUSTAL-X program (Thompson *et al.*, 1997), followed by manual editing of the alignment. In addition to DNA sequences generated in this study, DNA sequences for other relevant *Senecio* species were obtained from GenBank: *S. inaequidens* (accession numbers AF459943 and AF097537) and *S. lautus* (accession numbers AY554113 and AF097540). Species from the more distantly related sister group (Senecioneae, section Rowleyani), *Senecio serpens* and *Senecio*

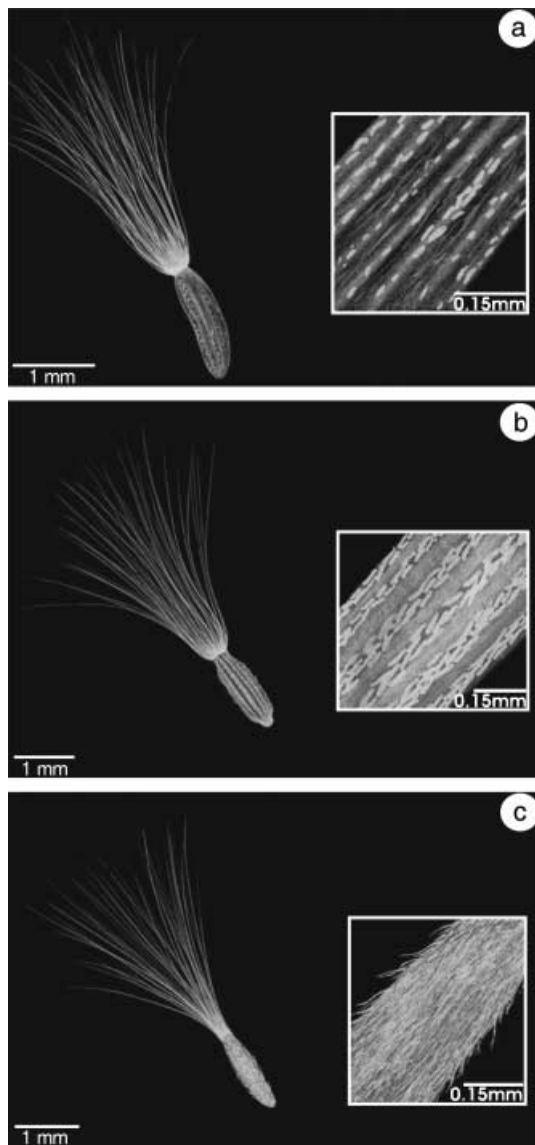
*rowleyanus* (accession numbers AF459930 and AF459933 respectively), were chosen as outgroup taxa.

Phylogenetic analyses were conducted on the ITS1 and ITS2 regions (excluding the non-variable 5.8S rDNA region) using PAUP\* 4.0b10 (Swofford, 2001). Maximum-parsimony (MP) analysis was performed with the option 'heuristic search' with TBR, MULTREES, and COLLAPSE (max) options in effect. Deletion and insertion events (gaps [indels]) were treated as missing data. Confidence in tree topologies was assessed using bootstrap analysis (Felsenstein, 1985) of 1000 replicates. The partition homogeneity test, as implemented in PAUP\*, was used to test for incongruence between the phylogenetic signals of the separate ITS1 and ITS2 data sets. In addition to MP, a 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 & Crandall, 1998). Heuristic searches were carried out with TBR, MULTREES, and COLLAPSE options in effect. Confidence in tree topology was assessed using bootstrap analysis of 100 replicates with the same heuristic settings used for tree construction.

## RESULTS

### Morphology

Achene surface morphology appeared to be of three distinct types (Fig. 1). Two of these types showed definite grooves on the surfaces of achenes, differing in the pattern of papillose (mixogenic) hair coverage. One of these types was sparsely covered with hairs on separate grooves, with only a few hairs on each groove, whereas the second type had intermediate hair coverage



**Figure 1** Illustrations of the three achene types identified for taxa used in this study. (a) Sparsely covered achene surfaces with few mixogenic hairs on individual grooves, (b) intermediately covered achene surfaces with mixogenic hairs concentrated on individual grooves, and (c) fully covered achenes having total surface coverage by mixogenic hairs. For each morphological type a higher resolution illustration of the surface hairiness is included.

on all grooves. A third type showed complete hair coverage and the apparent absence of grooves or very slightly grooved. The papillose hairs of this type also appeared to be longer and thinner. For these three achene types the average lengths and associated standard deviations (SD) were: sparsely covered (1.35 mm, SD = 0.6); intermediately covered (1.66 mm, SD = 0.19); totally covered (1.75 mm, SD = 0.37), representing significantly different length classes ( $P = 0.001498$ , one-way ANOVA).

Leaf morphology varied between and in some instances within populations from slender oblanceolate, broad oblanceolate, to pinnately lobed (Fig. 2). The number of involucre bracts ranged

consistently from 19 to 22 for all the populations collected. Table 2 summarizes morphological data for all populations included in this study.

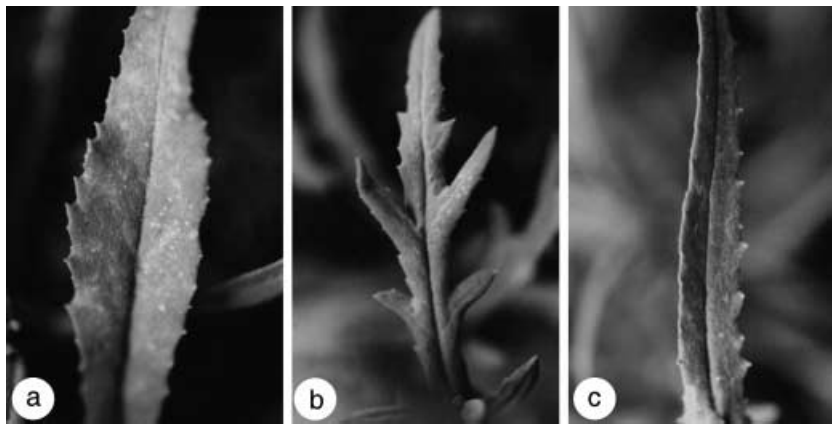
### Sequence variation

The amplified ITS1 and ITS2 regions were between 189 and 236 base pairs (bp) and 229–230 bp, respectively. All DNA sequences were submitted to GenBank (accession numbers DQ322598–DQ322621).

The alignment matrices for ITS1 and ITS2 gene sequences constructed using data generated in this study and additional sequence data obtained from GenBank (<http://www.ncbi.nlm.nih.gov>) required 53 and 7 gaps, respectively, ranging from 1 to 44 bp in size. Individual gaps for all ingroup taxa never exceeded 3 bp. The combined data sets comprised 474 characters, which included 384 (81.0%) constant characters. Sequence divergence (measured as uncorrected  $P$  distance) ranged from 0.0% (e.g. putative *S. madagascariensis* individuals SM182–SM232) to 5.8% (*S. madagascariensis*–*S. lautus*) between ingroup taxa and from 11.59% (*S. rowleyanus* — SM255) to 13.18% (*S. serpens*–*S. inaequidens*) between ingroup and outgroup taxa.

### Phylogenetic analysis of ITS sequences

Partition homogeneity analyses showed that the phylogenetic signal for the two separate internal transcribed spacer regions were congruent ( $P = 1.0$ ). Parsimony analysis of the combined ITS1 and ITS2 regions yielded 572 most parsimonious trees with a length of 101 steps, a consistency index (CI) of 0.9307, a retention index (RI) of 0.9598, and a rescaled consistency (RC) index of 0.8933 (Fig. 3). For ML, the TrNef + G substitution model was selected. This uses a general time-reversible model and gamma-distributed (G) among-site rate variation. The ML tree ( $-\ln$  likelihood = 1209.30) was similar in topology to the MP tree. Overall, both trees were well supported by bootstrap. Both ML and MP analysis placed *S. inaequidens* and two of the putative *S. madagascariensis* populations (Blythedale and Park Ryne Beach populations [100% and 99% bootstrap support respectively]) as a sister group to the otherwise monophyletic *S. madagascariensis*-only clade (Fig. 3). Both ML and MP analysis indicated that a strong geographical relationship exists among putative *S. madagascariensis* populations. Populations from Swaziland (86% and 90% bootstrap support, respectively) and Madagascar (99% and 100% bootstrap support, respectively) formed individual monophyletic clades. With the exception of the Blythedale and Park Ryne Beach populations all the South African east coast populations were unresolved sharing 100% DNA similarity within the monophyletic *S. madagascariensis*-only clade. Both ML and MP analyses placed *S. lautus* as basal to *S. madagascariensis* and *S. inaequidens*. The phylogram generated by ML indicated that the closest phylogenetic relationship between Hawaiian *S. madagascariensis* populations was shared with the South African east coast populations (Durban City, Umhlanga, and Camperdown [1 bp substitution, 0.21% divergence]) (Fig. 4). More distant



**Figure 2** Photographs illustrating the variation observed in leaf morphology for putative *Senecio madagascariensis* populations in the Hawaiian islands. (a) broad oblanceolate, (b) pinnately lobed, and (c) slender oblanceolate.

**Table 2** Morphological data (achene, leaf, and involucre bract) recorded for all populations used in this study

Specimen number/s	Region	Achene type*	Leaf morphology	Involucre bract number/s
North America				
SM149/SM150	Maui	Intermediate	Pinnately lobed	21
SM323	Hawaii	Intermediate	Slender oblanceolate	21
SM462	Hawaii	Intermediate	Broad oblanceolate	21
SM553	Hawaii	Intermediate	Pinnately lobed	21
South Africa				
SM153/SM155	KwaZulu-Natal	Intermediate	Slender oblanceolate	19–22
SM165/SM170	KwaZulu-Natal	Intermediate	Slender oblanceolate	19–22
SM172†	KwaZulu-Natal	Sparsely	Broad oblanceolate	19–22
SM193†	KwaZulu-Natal	Sparsely	Broad oblanceolate	19–22
SM182/SM191	KwaZulu-Natal	Sparsely	Slender oblanceolate	19–22
SM222	KwaZulu-Natal	Intermediate	Pinnately lobed	19–22
SM231/SM232	KwaZulu-Natal	Intermediate	Slender oblanceolate	19–22
Madagascar				
SM241/SM242	Saint Luce	Fully	Broad oblanceolate	19–22
SM261/SM263/SM264/SM267	Fort Dauphin	Fully	Slender oblanceolate	19–22
SM275/SM276	Tulear	Intermediate	Slender oblanceolate	19–22
Swaziland				
SM255/SM259	Motshane	Intermediate	Slender oblanceolate	19–22

†Putative *Senecio inaequidens* specimens.

\*Refers to hair coverage of achenes as discussed in the text.

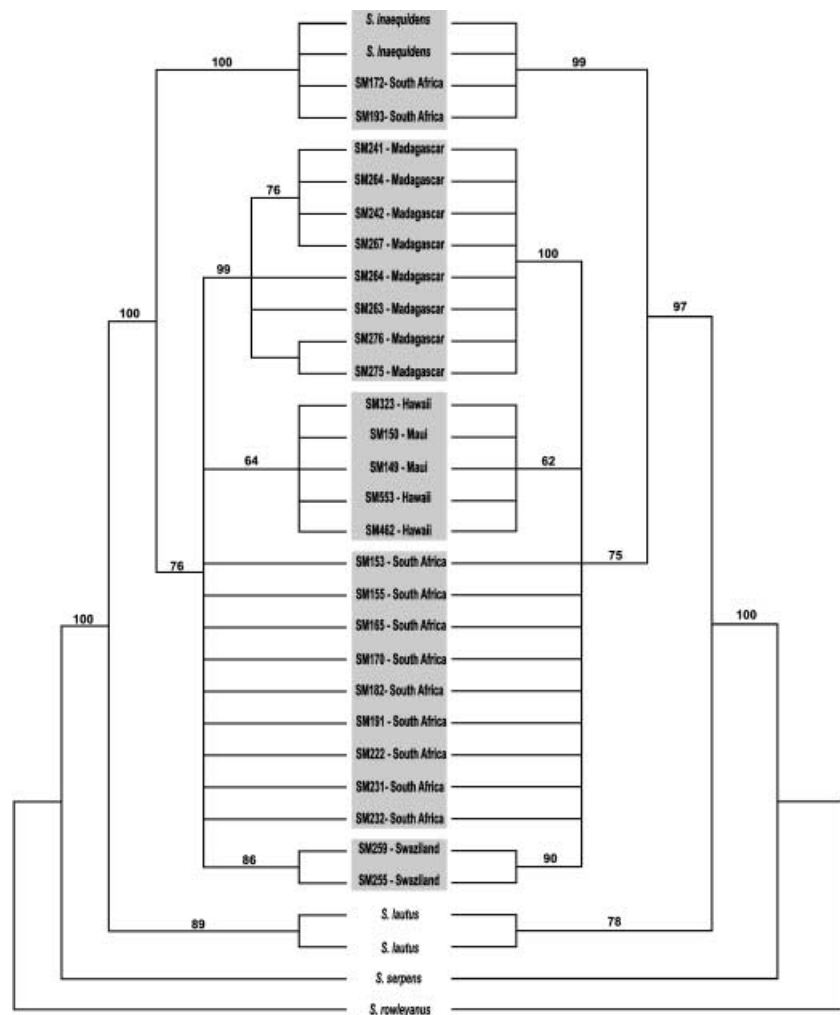
relationships were between Swaziland (0.86% divergence) and Madagascar (1.29% divergence). Within the Madagascan-only clade sequence divergence varied between 0.22% (between Tulear and Fort Dauphin Airport populations) and 0.65% (between Tulear and Saint Luce populations). The two South African populations (Park Ryne Beach and Blythedale Beach) within the monophyletic *S. inaequidens* clade formed a sister group to the monophyletic *S. madagascariensis* clade (1.50% sequence divergence).

## DISCUSSION

Successful invasive species often have large native ranges (Lodge, 1993), complicating efforts aimed at determining their origin. The identification of the region/s of origin, cryptic species, and/or intraspecific variants is especially important when seeking

host-specific natural enemies for biological control purposes. Where morphological or physiological traits fail to reveal taxonomic relationships, phylogenetic studies of plant and/or herbivore taxa can give clues to the evolution of host choice in control agents and/or the most likely region of origin.

*Senecio madagascariensis* is currently considered a serious pasture weed in Hawaii. Its supposed introduction from Australia via contaminated fodder remains to be verified. *Senecio madagascariensis*'s native range spans Madagascar, its surrounding islands, and a great part of southern Africa. Not surprising, as mentioned, this species shows a vast amount of biotypic variation such as plant habit and leaf morphology and is considered a species complex (Radford *et al.*, 2000). The occurrence of several closely related, very similar, and widespread *Senecio* species further complicates this group's taxonomy. For example, *S. inaequidens* is frequently mistaken for *S. madagascariensis* in the field. In



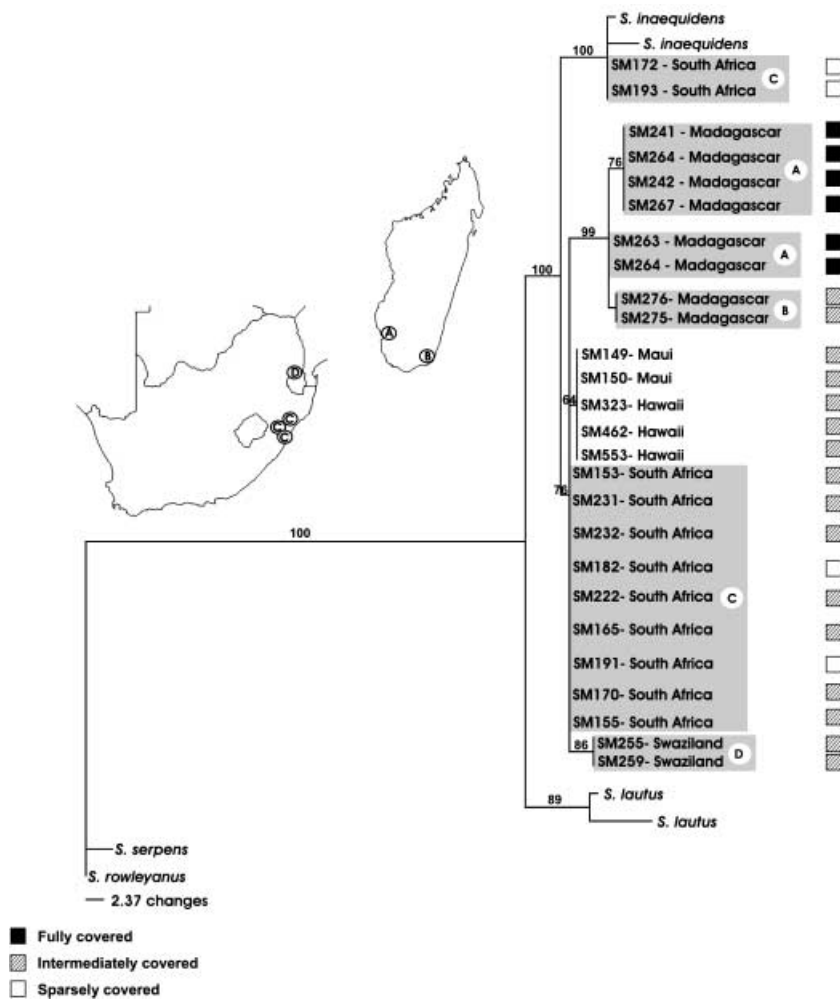
**Figure 3** The maximum-likelihood (ML) tree ( $-\ln$  likelihood = 1209.30) on the left-hand side and a strict consensus maximum-parsimony tree (CI = 0.9307, RC = 0.8933) on the right-hand side constructed by the combined ITS1 and ITS2 data sets. Confidence in tree topologies is indicated as bootstrap values on branches (100 replicates for ML and 1000 replicates for MP).

addition Scott *et al.* (1998) have showed that no genetic differences existed between *S. inaequidens* and *S. madagascariensis* based on ITS 1 DNA sequence data. Indeed, closely related species such as *S. burchellii*, *S. skirrhodon*, and *S. pellucidus* are frequently considered to be part of the *S. madagascariensis* complex when observed in the field (P.A. Muller, pers. comm.).

Understanding attributes such as identity and number of species involved in biological invasions and their native origin/s will contribute valuable insights into predicting potential range expansions, impacts, invasiveness, etc., as well as suggesting prevention, control, and management strategies (Courtenay & Stauffer, 1984). Such information is especially vital to the success of biological control programs. Failure to correctly identify invasive species and/or their native origin could lead to unsuccessful natural enemy establishment, poor host-specificity, and/or incomplete control, especially when dealing with biotypes of a single weed species (e.g. Chaboudez, 1994), weed species complexes (e.g. *S. madagascariensis* [Scott *et al.*, 1998]), or natural enemy host-races (e.g. skeleton weed rust, *Puccinia chondrillina* [Espiau *et al.*, 1998]). A survey of successful biological programs showed that the most effective natural enemies are usually host-specific (Rosen, 1986). Therefore, understanding the phylogenetic relationships of target species and their close relatives will

greatly contribute to improving biological control, particularly given the current concern for non-target impacts of biological control agents (Louda *et al.*, 1997).

In an ongoing biological control program targeting fireweed in Hawaii, it is necessary to elucidate the taxonomic placement of Hawaiian infestations. Given the known variation within the *S. madagascariensis* complex and the amount of morphological variation that we observed in Hawaiian populations, it might seem reasonable to suspect that more than one species/biotypes are responsible for these infestations. Our phylogenetic results, however, showed otherwise, indicating that all Hawaiian populations included in this study share 100% DNA sequence similarity regardless of morphological and ecological differences. Phylogenetic relationships furthermore suggest that the most likely origin of Hawaii's fireweed populations is the KwaZulu-Natal region on the east coast of South Africa, excluding populations sampled in the Blythedale and Park Ryne beach areas. The latter two populations formed a monophyletic group with *S. inaequidens*. Even though achene and flower morphology overlapped among and within different native range regions, Hawaiian fireweed furthermore also had morphological characteristics that were observed within the South African east coast populations. Achene hairiness and leaf and flower morphology appear to be uninformative



**Figure 4** The maximum-likelihood phylogram ( $-\ln$  likelihood = 1209.30) indicating phylogenetic distances between taxa. The approximate regions for all putative native range *Senecio madagascariensis* populations are shown on the inserted maps and indicated accordingly on the phylogram; (A) Western Madagascar (Tulear), (B) Eastern Madagascar (Fort Dauphin), (C) South Africa (KwaZulu-Natal), (D) Swaziland. Achene morphology (full, intermediate, and sparse hair coverage) is indicated for all studied taxa on the right-hand side of the phylogram. Tree topology confidence is indicated as bootstrap values at nodes.

for taxonomical inferences within the *S. madagascariensis* complex and there seem to be no distinct regional or species-specific patterns evident for these traits. Hawaiian *S. madagascariensis* was constantly invariable for involucre bract number (19) and achene hairiness (intermediate) across all populations, whereas the South African east coast populations showed variation in these traits. These differences could be indicative of a single introduction event and/or a founder effect coupled with reduction in genetic diversity in these populations.

Precise elucidation of the invasion route/s of *S. madagascariensis* to Hawaii cannot be determined with 100% certainty from our results; however, phylogenetic results support the notion that fireweed might have reached Hawaii secondarily from Australia rather than directly from any of the native range regions. Even though we did not include Australian fireweed populations in our analysis, the fact that Australian fireweed is more closely related (1 bp substitution) to KwaZulu-Natal region populations while only distantly related to Madagascan populations (Scott *et al.*, 1998; Radford *et al.*, 2000) is very similar to the relationships between Hawaiian fireweed and native range populations observed in this study. While the existing trade market for agricultural products, especially animal fodder, between Hawaii and Australia would create an opportunity for fireweed to easily

hitch a ride to the Hawaiian Islands, no such pathways exist between Hawaii and any of the native range countries.

Populations sampled in Swaziland and Madagascar clearly had a more distant relationship to Hawaiian populations. We excluded populations from the western Cape Province in South Africa since putative fireweed populations from there have previously been shown to be genetically only distantly related to other South African and Madagascan populations (Radford *et al.*, 2000). Populations identified as fireweed from this region are most likely *S. burchellii* since they normally possess 13 involucre bracts instead of the 19–22 characteristic of *S. madagascariensis* (P.A. Muller, unpublished report). *Senecio madagascariensis* populations are more common and localized within the KwaZulu-Natal region than other parts of the native range regions and are thus more likely to be associated with highly adapted and co-evolved predators and/or pathogens. Prati & Bossdorf (2004) recently reported that invasive populations of *S. inaequidens* showed a reduced incidence of parasitizing insects, suggesting that escape from natural enemies may play a role in the invasion success of this species (now considered a part of the *S. madagascariensis* species complex [Radford *et al.*, 2000]). Madagascan control agents failed in Australian fireweed populations due to low host-specificity (Marohasy, 1989; Holtkamp & Hosking,

1993; McFadyen & Sparks, 1996; Sindel *et al.*, 1998). Given the relationship between host-specificity and phylogenetic distance, these failures could be explained by the phylogenetic evidence of this study and other inferences (Radford *et al.*, 1995; Radford, 1997; Scott *et al.*, 1998) that proved Madagascan populations to be only distantly related to Hawaiian/Australian fireweed populations.

Individuals from the two South African coastal populations collected at Blythedale and Park Ryne beaches formed a monophyletic group with *S. inaequidens*, and a sister group to all other *S. madagascariensis* populations. The identification key of the *S. madagascariensis* complex described by Radford *et al.* (2000) suggests that *S. madagascariensis* and *S. inaequidens* are differentiated based on chromosome counts ( $n = 20$  and  $n = 40$ , respectively), and achene hairiness, with only *S. inaequidens* having complete mixogenic hair coverage. On the contrary, even though our study did not include any chromosome counts, we can confirm that populations identified as *S. inaequidens* based on ITS1 and ITS2 DNA sequence data had achenes of the sparsely hairy type (Fig. 1a). Mapping of achene morphology types onto the phylogeny (Fig. 4) illustrates that no relationship exists between geographical regions or even between species and the observed variances for this trait. The only observation worth mentioning is that complete achene hairiness, that is markedly different from the other two achene hairiness types, seems to have evolved only in eastern Madagascan populations that are most likely reproductively isolated from the western (Tulear) population having intermediately covered achenes (Fig. 1b,c). South African east coast populations also varied having achenes of the sparse and intermediate types. These observations reiterate the futility to use this trait for taxonomic inferences. Also, the amount of DNA sequence divergence between different taxa within the monophyletic *S. madagascariensis* clade is surprisingly high considering the relatively short length of DNA sequence (474 bp) used in the phylogeny construction. Coupled with the variation observed for achene morphology and length, we strongly recommend a revised alpha-level taxonomy for the *S. madagascariensis* species complex. We also suggest a need for a more 'total evidence' approach, making use of all available data, to overcome confusion resulting from the numerous morphological overlaps.

In this study, a DNA-based phylogeny proved to be a powerful tool to better understand the system in which biological control of *S. madagascariensis* is being considered. Future biological control research for *S. madagascariensis* in Hawaii should focus on those natural enemies collected from the South African east coast populations (excluding Blythedale and Park Ryne Beach areas) rather than those collected from Madagascar and Swaziland.

Until recently, most plant genetic studies typically have turned to genome-wide markers such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), but these data cannot be ordered in a historic sequence, which precludes the construction of gene trees and subsequent phylogeographical inferences (Schaal *et al.*, 2003). This lag in molecular markers suitable for plant phylogenetics led to phylogeographical studies primarily being dominated by animal taxa, far outnumbering plant taxa (Avice, 2000). However,

recent developments in this field have seen an increase in the identification of molecular markers in plants that present variation at the intraspecific level, in the nuclear genome, and in the chloroplast and mitochondrial genomes. This will undoubtedly warrant phylogeography to become a popular practice when closely related plants are being studied in the range between geographical races and cryptic species, as is often the case in research efforts involving biological control of invasive plants. Indeed, recent studies using this approach for plant invasions revealed aspects concerning cryptic species, hybridization events, and source populations that would otherwise have been difficult or even impossible to detect (see McIvor *et al.*, 2001; Gaskin & Schaal, 2002; Saltonstall, 2002; Gaskin *et al.*, 2005). The natural enemies collected during this study are currently being tested in quarantine and, once behavioural data are available, pose an excellent opportunity to infer correlations between phylogenetic relationships of plant taxa and the corresponding effectiveness of their associated herbivores on Hawaiian fireweed populations.

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