

# Invasive *Senecio madagascariensis* Poir. and the *Senecio pinnatifolius* A.Rich. complex (Senecioneae):

## Evolutionary relationships and their implications for biological control research

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### ABSTRACT

Fireweed (*Senecio madagascariensis*, Senecioneae) is native to southern Africa and Madagascar but has become an invasive weed in southern South America, Japan, Australia, and Hawai'i. One strategy for reducing the impact of fireweed is classical biological control (biocontrol), i.e. the release of natural enemies of a weed such as pathogens or insects sourced from its home range. Before release, candidate agents are subjected to rigorous host specificity testing to minimise the risk of collateral damage to non-target species. It is important to include non-target species that are closely related to the target weed in these experiments, because candidate biocontrol agents are more likely to attack them than distantly related species. However, Australian biocontrol research on fireweed has for a long time suffered from confusion about the taxonomy and evolutionary relationships of fireweed and its presumed closest Australian relatives from the *Senecio pinnatifolius* complex. We provide an overview of the history of taxonomic and phylogenetic perceptions and relevant studies and illustrate that although *S. pinnatifolius* belongs to the Australasian clade of *Senecio* most closely related to fireweed, the two are not closely related in the context of the overall evolutionary history of the genus. We conducted a phylogenetic analysis of Senecioneae incorporating new sequence data for 38 specimens including all seven extant varieties of the *S. pinnatifolius* complex. The varieties were placed in different clades, suggesting that the species as currently circumscribed does not constitute a natural group, and that the varieties cannot be used interchangeably in biocontrol research. Further research into the complex is needed to arrive at a more appropriate taxonomy. *Senecio skirrhodon* was sequenced for the first time, confirming it to be closely related to fireweed, *S. harveianus*, and *S. inaequidens*.

**Keywords:** Australia, biological control, centrifugal phylogenetic method, fireweed, phylogenetics, *Senecio*

### INTRODUCTION

Fireweed, *Senecio madagascariensis* Poir. (Senecioneae), is a short-lived perennial herb native to southern Africa and Madagascar that has become successfully established as an invasive weed

in southern South America, Japan, Australia, and Hawai'i (Julien et al., 2012). In Australia, where it is widespread in open pastures across the south-eastern part of the continent, fireweed is recognised as one of 32 Weeds of National Significance (<https://weeds.org.au/weeds-profiles/>, accessed 22 April

2022) due to its adverse effects on pasture quality and toxicity to livestock, especially cattle, that reduce agricultural productivity (McFadyen & Morin, 2012).

Australia and Hawai'i have initiated research into the potential deployment of classical biological control (biocontrol) agents to help manage fireweed – i.e., the introduction of co-evolved 'enemies' (fungal pathogens, insects, mites) sourced from the weed's native range to reduce its competitive performance and invasion potential in the introduced range (Ramadan et al., 2011; Sheppard et al., 2011).

In weed biocontrol research, highly specialised biocontrol agents with native host ranges that are restricted to the target weed are prioritised for further host-specificity experimentation (Egli et al., 2020; Zuma et al., 2021), with the aim of minimising the risk of inadvertent, non-target damage to native or useful plants within the weed's introduced range. All else being equal, non-target plants that are closely related to the target weed are more at risk from candidate biocontrol agents, because herbivores or pathogens are more likely to find plants palatable that are similar to their co-evolved host (McFadyen, 1998; Thomas & Willis, 1998; Briese, 2003, 2005).

This observation has informed the Centrifugal Phylogenetic Method (Wapshere, 1974; Briese, 2003, 2005) that prescribes testing the biocontrol agent on a representative sample of non-target species drawn from lineages of increasing evolutionary distance to the target weed, with those closest to the target weed given highest priority. This means that an accurate understanding of the relationships between a target weed and other species in its introduced range is critical for robust and reliable risk assessment of biocontrol work, especially in the development of a 'host test list' of non-target plant species prioritised for host-specificity testing with the candidate biocontrol agent.

Unfortunately, research into the potential biocontrol of fireweed in Australia has often been impeded by taxonomic confusion around both fireweed itself and its presumed closest Australian relatives, and by misunderstandings of their phylogenetic relationships as discussed below. Uncertainty persists in the literature to this day (Wijayabandara et al., 2022).

In this contribution we summarise the history of taxonomic and phylogenetic (mis)understanding of fireweed and the species frequently considered most closely related to it in Australia, the *Senecio pinnatifolius* A.Rich. complex (previously *S. lautus* G.Forst. ex Willd.). We also comment on the phylogenetic position and status of another Australian species that has been of concern to biocontrol research in fireweed, *S. condylus* I.Thomps. Finally, we present new sequence data for all varieties of the *S. pinnatifolius* complex except one and incorporate these new sequences into a revised phylogenetic analysis of *Senecio* L. to inform the refinement of species selection for ongoing biocontrol risk assessment in Australia and weed management research on fireweed more generally.

## **TAXONOMIC PERCEPTION OF FIREWEED IN AUSTRALIA**

It is assumed that fireweed was introduced to Australia's southeast before 1918, but it was for decades considered to be a member of the native *Senecio pinnatifolius* complex (Julien et al., 2012). It was recognised as the South African and Madagascan *S. madagascariensis* only in 1980, when specimens were sent to the South African taxonomist O.M. Hilliard for identification (Michael, 1981). Chromosome counts confirmed the segregation, with fireweed having  $2n = 20$ , and *S. pinnatifolius*  $2n = 40$  (Radford et al., 1995), as did genetic data (Scott et al., 1998; Radford et al., 2000).

However, even as the evidence was accumulating, controversy and confusion persisted. Marohasy (1993) argued against the introduced status of fireweed in Australia, writing that "it appears the change of status, from native to exotic, was based essentially on a single identification by a foreign Compositae expert". Sindel (1986) and Radford & Cousens (2000) continued to assume that fireweed was "closely related" to Australian *Senecio pinnatifolius*. McFayden & Sparks (1996) cited then unpublished genetic data showing that fireweed was not part of the native Australian *S. pinnatifolius* complex but nonetheless stated that it was "closely related", that hybridisation between the two had been observed, and that the latter species should be prioritised for inclusion in a host test list for biocontrol research. Sheppard et al. (2011) considered the two species to be in "taxonomic close proximity". Most

# Fireweed

Southern African *Senecio madagascariensis* Poir. (fireweed) has been introduced in South America, Japan, Australia, and Hawai'i. It reduces agricultural productivity by invading pastures.

*Senecio madagascariensis*, Dunmore, New South Wales, Australia.  
Photo by Ben Gooden

recently, Wijayabandara et al. (2022) wrote that “the taxonomic position of Australian *S. madagascariensis* is undetermined”, thus underlining the need for a re-examination of the phylogenetic placement of fireweed in the context of native Australian *Senecio* species.

Confusingly, most of these publications cited one of the earliest genetic studies that demonstrated Australian fireweed to be part of the southern African *Senecio madagascariensis/Senecio inaequidens* DC. complex instead of the Australian *S. pinnatifolius* complex (Scott et al., 1998) in support of their close relatedness and/or continuing taxonomic uncertainty. That, however, appears to be a misreading of Scott et al.’s results and intentions, who only argued that the taxonomy inside the two complexes requires further work, not that the closeness of their relationship to each other remains unclear. Since that publication, formal phylogenetic studies have further clarified relationships, as discussed in the following section.

### **PHYLOGENETIC RELATIONSHIPS OF FIREWEED AND AUSTRALIAN SENECIOS**

*Senecio* is a large genus, with over 1,000 species distributed across all continents except Antarctica (Nordenstam et al., 2009), and because of changes in its taxonomic circumscription, it was even larger in the past (Pelser et al., 2007). This means that phylogeneticists needed to pick their battles, focusing either on shallow sampling across a large study group or deep sampling limited to a subgroup of the genus or tribe. This has complicated the interpretation of relevant phylogenetic studies by weed scientists, as partial phylogenies with few overlapping taxa were scattered across the literature without providing a comprehensive overview of all relationships.

As discussed in the previous section, two early genetic studies could have been expected to already have settled the question of a “close relationship”, in the absolute sense, between fireweed and Australian *Senecio* (Scott et al., 1998; Radford et al., 2000). A partial explanation of continuing confusion may be that the seminal phylogeny of Senecioneae produced by Pelser et al. (2007) showed the Australian clade 3, which included *Senecio pinnatifolius*, as, in a sense, “closest” to fireweed. However, that closeness is only relative. While Australian clade 3 was indeed topologically closest of all Australian *Senecio* to the

clade including fireweed, this latter clade including fireweed was itself not only very species-rich but also composed of southern African and New World subclades. This suggested a divergence very deep in time, followed by numerous speciation events, and consequently a very distant relationship between fireweed and Australian clade 3.

A subsequent study focused on Australasian species of *Senecio* related to *S. pinnatifolius*, sampling many more species and informally naming this group of species the Lautusoid group (Liew et al., 2018). The study resolved four distantly related lineages of Australian *Senecio* and identified several species potentially derived from hybridisation between different lineages. Three Australasian ribosomal sequence clades were found: clade 1 (Disciform group, Odoratus group), clade 2 (Quadridentatus group), clade 3 (Lautusoid group); as were three chloroplast clades: clade A (Odoratus group), clade B (Disciform group, Quadridentatus group), clade C (Lautusoid group). Liew et al. (2018) did not include fireweed in their analyses explicitly because they knew it to be unrelated to their study group. They did, however, include its close relative *S. inaequidens* DC., which was consistently placed outside of any of the Australasian groups.

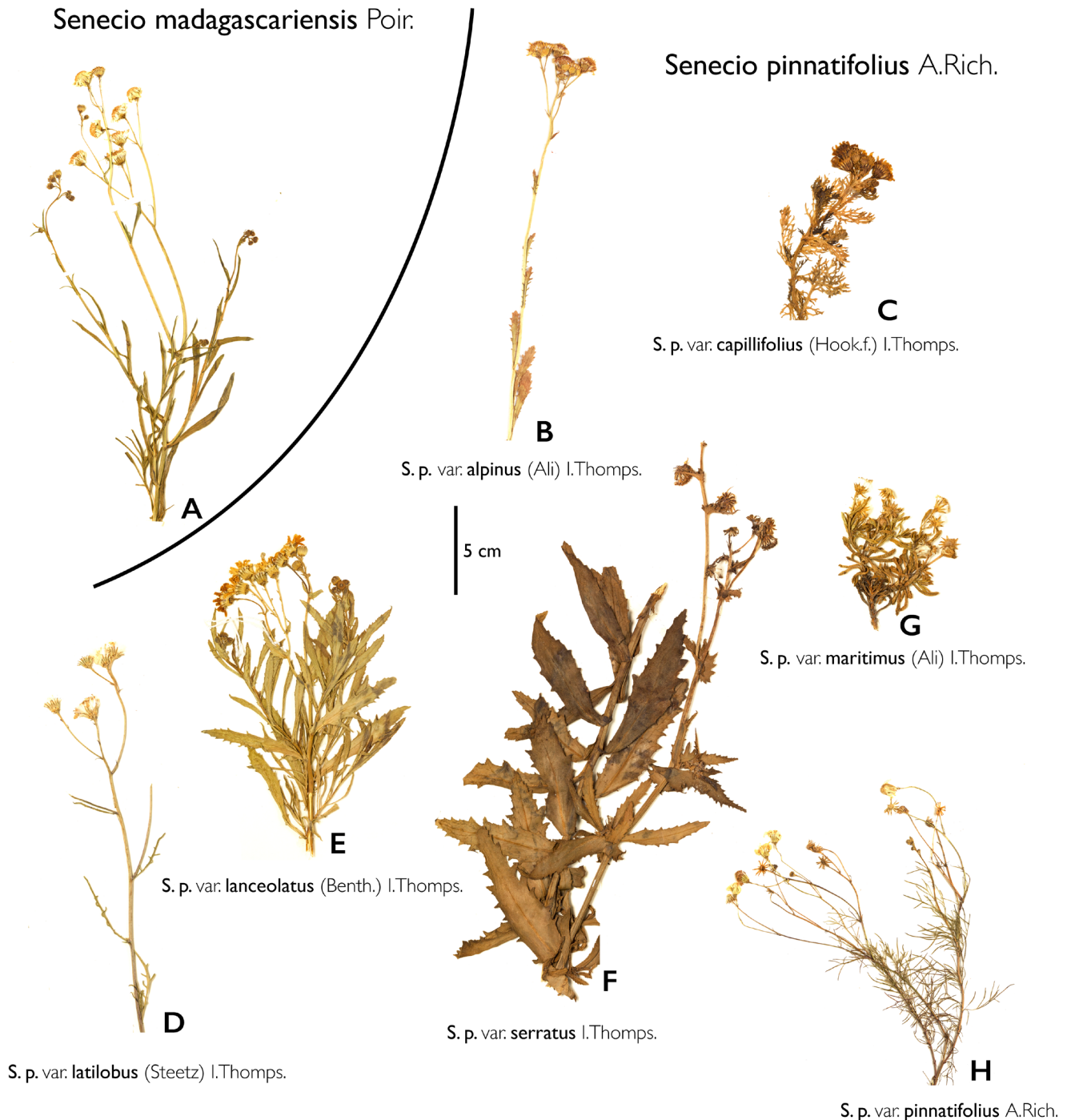
To assist in the prioritisation of native Australian *Senecio* species for inclusion on the biocontrol host test list and deployment in subsequent host-specificity experimentation, we recently conducted an analysis covering all species of Senecioneae for which ribosomal and chloroplast Sanger data were available, plus new data for previously neglected Australian species (Schmidt-Lebuhn et al., 2020). Our analysis therefore united both the broad backbone sampling of, e.g., Pelser et al. (2007) and deep sampling such as that of Liew et al. (2018). Our results confirmed the Australasian clades, including *S. pinnatifolius*, to be phylogenetically very distant from fireweed, and fireweed as part of a large African clade that also included *S. inaequidens* (Schmidt-Lebuhn et al., 2020).

### **THE *Senecio pinnatifolius* COMPLEX**

In addition to the changing status of fireweed itself in Australia, the taxonomic complexity of the *Senecio pinnatifolius* complex has contributed to confusion. For decades, this Australian taxon was named *S. lautus* G. Forst ex Willd., until Belcher

# MORE THAN A SINGLE ENTITY

a species complex with enormous morphological and ecological diversity



**Figure I.** Comparison of fireweed and the morphological diversity of the *Senecio pinnatifolius* complex. **A.** Fireweed, *S. madagascariensis*, C. Burgess s.n. (CBG). **B.** *S. pinnatifolius* var. *alpinus*, J. Pena 1 (CBG). **C.** *S. pinnatifolius* var. *capillifolius*, J.S. Whinray 488 (CANB). **D.** *S. pinnatifolius* var. *latilobus*, N.S. Lander 1025 (CANB). **E.** *S. pinnatifolius* var. *lanceolatus*, P.C. Heyligers 80012 (CANB). **F.** *S. pinnatifolius* var. *serratus*, I. Radford s.n. (CBG). **G.** *S. pinnatifolius* var. *maritimus*, P.C. Heyligers 79078 (CANB). **H.** *S. pinnatifolius* var. *pinnatifolius*, L.W. Cayzer 828 (CBG).

(1994) demonstrated it to be distinct from the New Zealand species of that name. Since then, weed researchers have shifted to using the name *S. pinnatifolius*, but the outdated name *S. lautus* is still used in some research fields, e.g. (Richards et al., 2019; James et al., 2021).

As implied by the term “complex”, *Senecio pinnatifolius* is a morphologically and ecologically diverse species of eight currently recognised varieties (Thompson, 2005b). *Senecio pinnatifolius* var. *alpinus* (Ali) I.Thomps. is an alpine taxon (Figure 1B); *Senecio p.* var. *capillifolius* (Hook. f.) I.Thomps. occurs on islands in the Bass Strait and has unusually finely divided leaves (Figure 1C); *Senecio p.* var. *lanceolatus* (Benth.) I.Thomps. occurs along the coasts of southeastern South Australia, Victoria, and northern Tasmania (Figure 1E); *Senecio p.* var. *latilobus* (Steetz) I.Thomps. occurs along the coast of southwestern Western Australia (Figure 1D); *Senecio p.* var. *leucocarpus* I. Thomps. is known from a single locality in Western Australia and presumed extinct; *Senecio p.* var. *maritimus* (Ali) I. Thomps. occurs along the southern coasts of Australia and Tasmania (Figure 1G); the most widespread and common *Senecio p.* var. *pinnatifolius* (Figure 1H) shares much of its distribution in eastern Australia with fireweed; *Senecio p.* and var. *serratus* I. Thomps. is found in southeastern Queensland and north-eastern New South Wales (Figure 1F) (Thompson, 2005b).

The delineation of varieties and the circumscription of the species complex as a whole remain controversial (Radford et al., 2004; Thompson, 2005b) and have never been comprehensively studied using genetic data, although some ecotypes serve as model organisms for research into incipient speciation (Roda et al., 2013). This means that if the complex is not a natural group but includes disparate lineages that potentially belong to different clades, the phylogenetic position of single samples may be misleading when preparing a host test list for fireweed biocontrol in Australia. Given the highly homoplasious nature of many characters traditionally used for species delimitation in *Senecio*, such a possibility should not be rejected out of hand, and indeed a recent study from New Zealand found what was previously treated as a single species of this genus

to fall into two different clades (Liew et al., 2021).

## HYBRIDISATION

Of particular interest in this context are claims that fireweed and *Senecio pinnatifolius* can be crossed. Although seemingly unlikely given the large phylogenetic distance between the two taxa, the possibility has been of great concern to invasion biology in Australia, sparking fears that hybridisation would drive native populations to genetic extinction (Johnston, 2008).

Claims of genetic intermediates observed in the field appear to trace back to an honours thesis (Scott, 1994), although no such claim was made in the subsequent publication of its results (Scott et al., 1998). Similarly, a Ph.D. thesis (Radford, 1997) is frequently cited as evidence for the risks of hybridisation, although it demonstrated artificially created hybrids to be sterile and did not find any hybrids in the field (Sindel et al., 1998).

The most widely cited study of hybridisation (Prentis et al., 2007) used Amplified Fragment Length Polymorphism markers to study gene flow and crossing experiments to test for hybrid formation. It was able to produce hybrid seed, which did not show reduced viability, but found no adult hybrid individuals in the wild, concluding possible selection against hybrids at later life stages. The main risk factor discussed by Prentis et al. was that fireweed was more successful in fathering hybrid seed than *Senecio pinnatifolius*, which may skew seed set towards fireweed in contact zones.

Dormontt et al. (2017), after clarifying that Prentis et al. (2007) had conducted their experiments on *Senecio pinnatifolius* var. *serratus*, repeated the experiment with *S. p.* var. *pinnatifolius*. They did not detect any adult hybrids in any population of *S. p.* var. *pinnatifolius* and found low hybrid seed set, concluding that the native taxon was unlikely to be at risk from introgression.

In summary, there is little concrete evidence to suggest that significant gene flow takes place between fireweed and *Senecio pinnatifolius*.

## *Senecio condylus*

Of all species presumed native to Australia, *Senecio condylus* was placed closest to fireweed in previous

# *Senecio pinnatifolius*

*Senecio pinnatifolius* A.Rich. has historically been considered the closest Australian native relative of fireweed. Its type variety and introduced fireweed share a large part of their ranges, and there have been concerns about the two species hybridizing.

*Senecio pinnatifolius*, Nerriga, New South Wales, Australia.  
Photo by Ben Gooden.

phylogenetic analyses (Liew et al., 2018; Schmidt-Lebuhn et al., 2020). It was described as new to science only as recently as 2005 and considered endemic to southwestern Western Australia (Thompson, 2005a). However, its placement as part of an otherwise entirely South African clade raised the question of whether it represented an isolated but natural introduction to Australia or whether it was a misunderstood invasive (Schmidt-Lebuhn et al., 2020). Independently, its occurrence in disturbed, near-urban areas has led to doubts about its status as a native species (Keighery & Keighery, 2017). The Western Australian Department of Biodiversity, Conservation and Attractions is conducting research into the provenance of *S. condylus* in collaboration with a South African taxonomist (Williamson & Balkwill, unpublished data).

## MATERIALS AND METHODS

To add to our understanding of the placement of taxa of relevance to fireweed biocontrol research, we generated new Sanger sequence data for 38 samples representing some taxa for which no data were previously available, species whose phylogenetic placement we wanted to confirm independently, and all taxonomic varieties of *Senecio pinnatifolius* except *S. pinnatifolius* var. *leucocarpus*, which is presumed extinct. The targeted sequence regions and primers used were the same as in our previous study (Schmidt-Lebuhn et al., 2020), i.e., the nuclear ETS and ITS as well as chloroplast psbA-trnH and trnL regions, so that the new data could be added to the existing dataset. For the purposes of the results presented here, we focus on ribosomal data (ETS, ITS), because they provide stronger phylogenetic resolution and confidence than the chloroplast regions.

DNA was extracted from herbarium specimens at CANB and NU. Laboratory work and sequencing were outsourced to the Australian Genome Research Facility. Contigs were produced using Geneious ([www.geneious.com](http://www.geneious.com)). Genbank accession numbers and voucher information for sequences newly generated for this study are listed in Appendix I. We removed the sequences of *Senecio pinnatifolius* from the data matrices used in Schmidt-Lebuhn et al. (2020), because their varietal affiliation was not always known and may have been chimeric, and added the new sequences.

ETS and ITS sequences were concatenated using a custom Python script, and alignments were produced using MAFFT 7.453 (Kato & Standley, 2013). A Maximum Likelihood phylogeny was inferred with IQ-TREE 2.1.2 (Minh et al., 2020), with both gene partitions under the substitution model GTR+F+I+G4 chosen by automatic model and partition testing, and 1,000 UltraFast Bootstrap (UFB) replicates as branch support values (Minh et al., 2013). Chloroplast psbA-trnH and trnL sequences were likewise concatenated and analysed with the same approach; model testing favoured K3Pu+F+G4 for psbA-trnH and TIM+F+G4 for trnL. Concatenated data matrices and phylogenetic trees are available on the CSIRO Data Access Portal (<https://doi.org/10.25919/hf5f-6e62>).

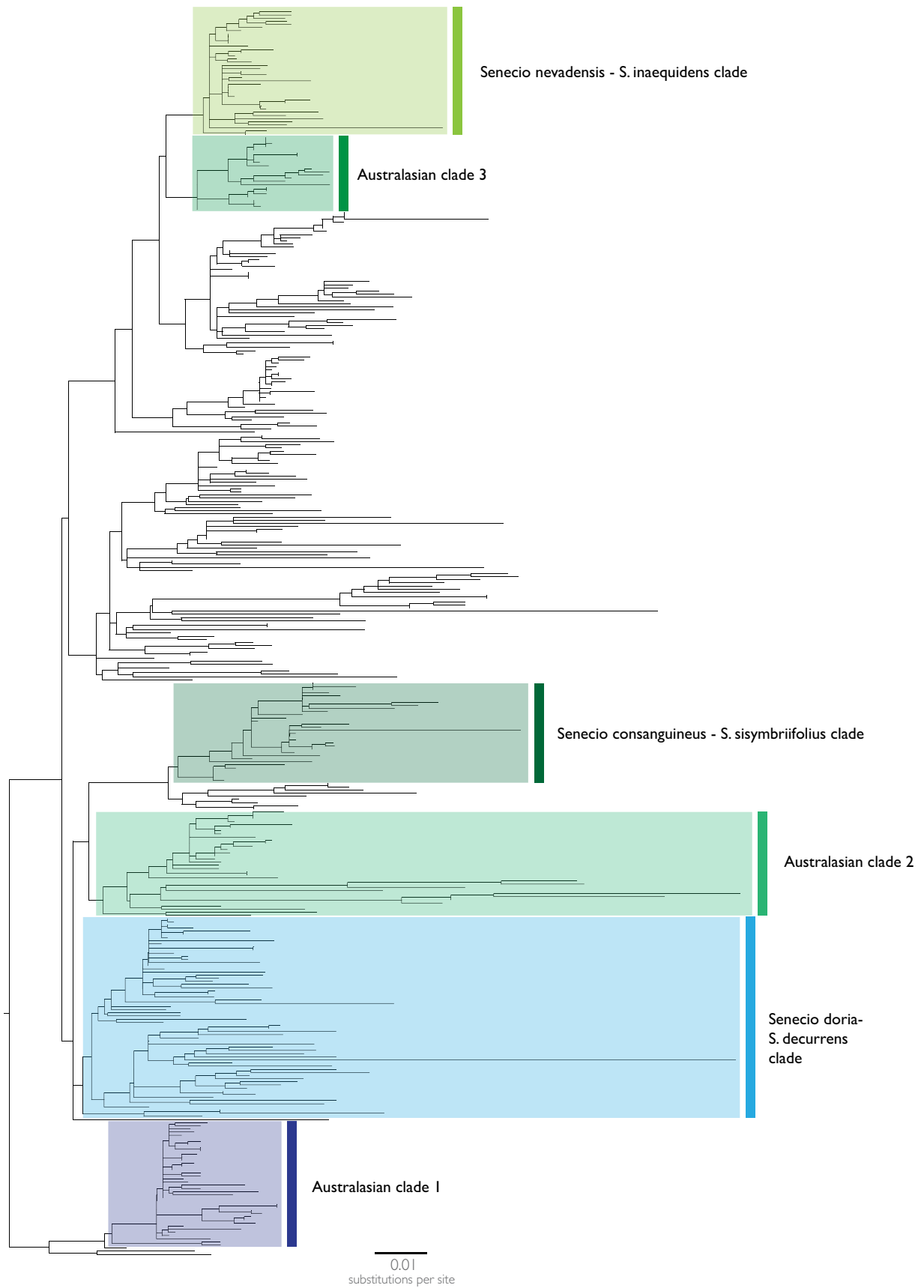
## RESULTS

We focus on the placement of fireweed and newly sequenced specimens, because the ribosomal phylogeny is otherwise not significantly different from Schmidt-Lebuhn et al. (2020). Where possible, clades are subsequently referred to by names used by Liew et al. (2018), as introduced above, Kandziora et al. (2017), and Pelsner et al. (2007) (Figure 2).

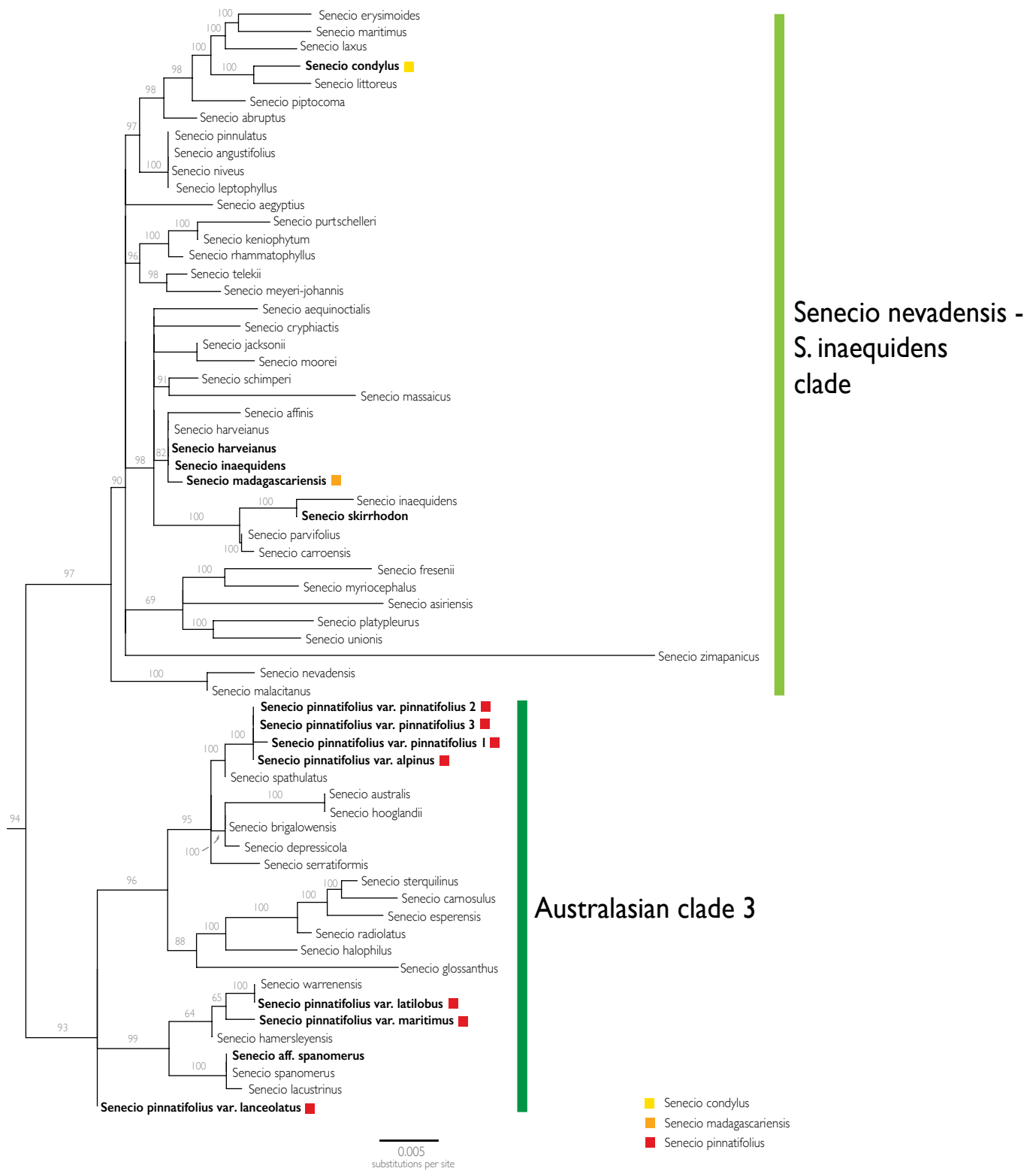
The phylogenetic position of several species we re-sequenced was confirmed, in that they were placed close to older sequence data ascribed to the relevant species. These included new samples of *Senecio inaequidens* and *S. harveyanus* MacOwan in the *S. nevadensis* Boiss. & Reut. – *S. inaequidens* clade that also contains fireweed (Figure 3); new samples of *S. coronatus* Harv., *S. inornatus* DC., and *S. panduriformis* Hillard in the *S. doria* L. – *S. decurrens* DC. clade; and a new sample of *S. distalilobatus* I.Thomps. in Australasian clade 1.

Other newly sequenced samples were placed in the expected clades but not necessarily close to older, conspecific sequences within those clades: new samples of *Senecio retrorsus* DC., *S. scitus* Hutch & Burt Davy, and of doubtful identification (*S. aff. adnatus* N.P.Balacr., *S. aff. conrathii* N.E.Br.) in the *Faujasia* Cass. – *Bethencourtia* Choisy clade outside of *Senecio* s.str.; a new sample of *S. polyodon* in the *S. consanguineus* DC. – *S. sisymbriifolius* DC. clade; and new samples of *S. prenanthoides* A.Rich. in Australasian clade 2. *Senecio skirrhodon* DC., for which no data were previously available, was part of the *S. nevadensis* – *S. inaequidens* clade (Figure 3).

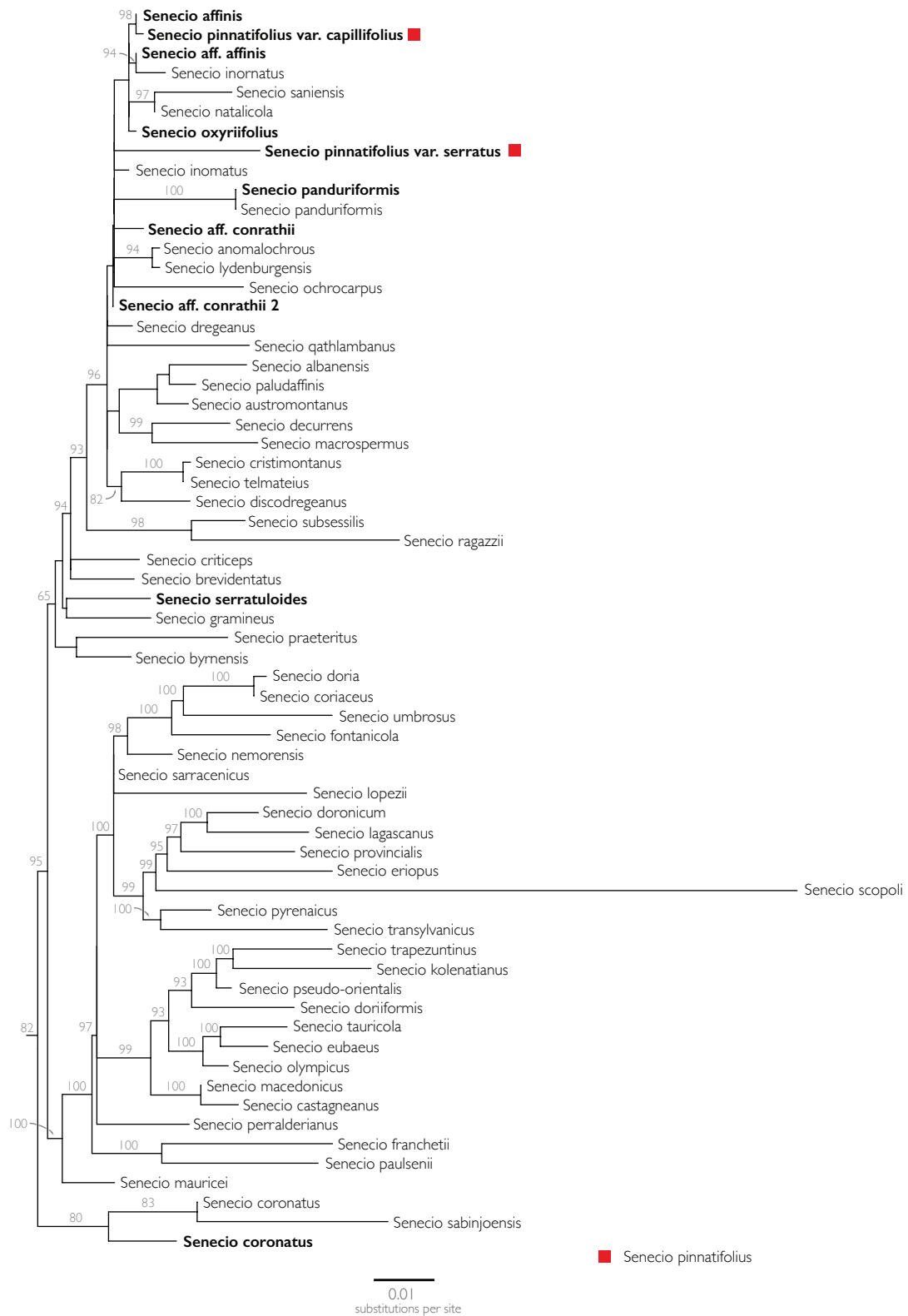




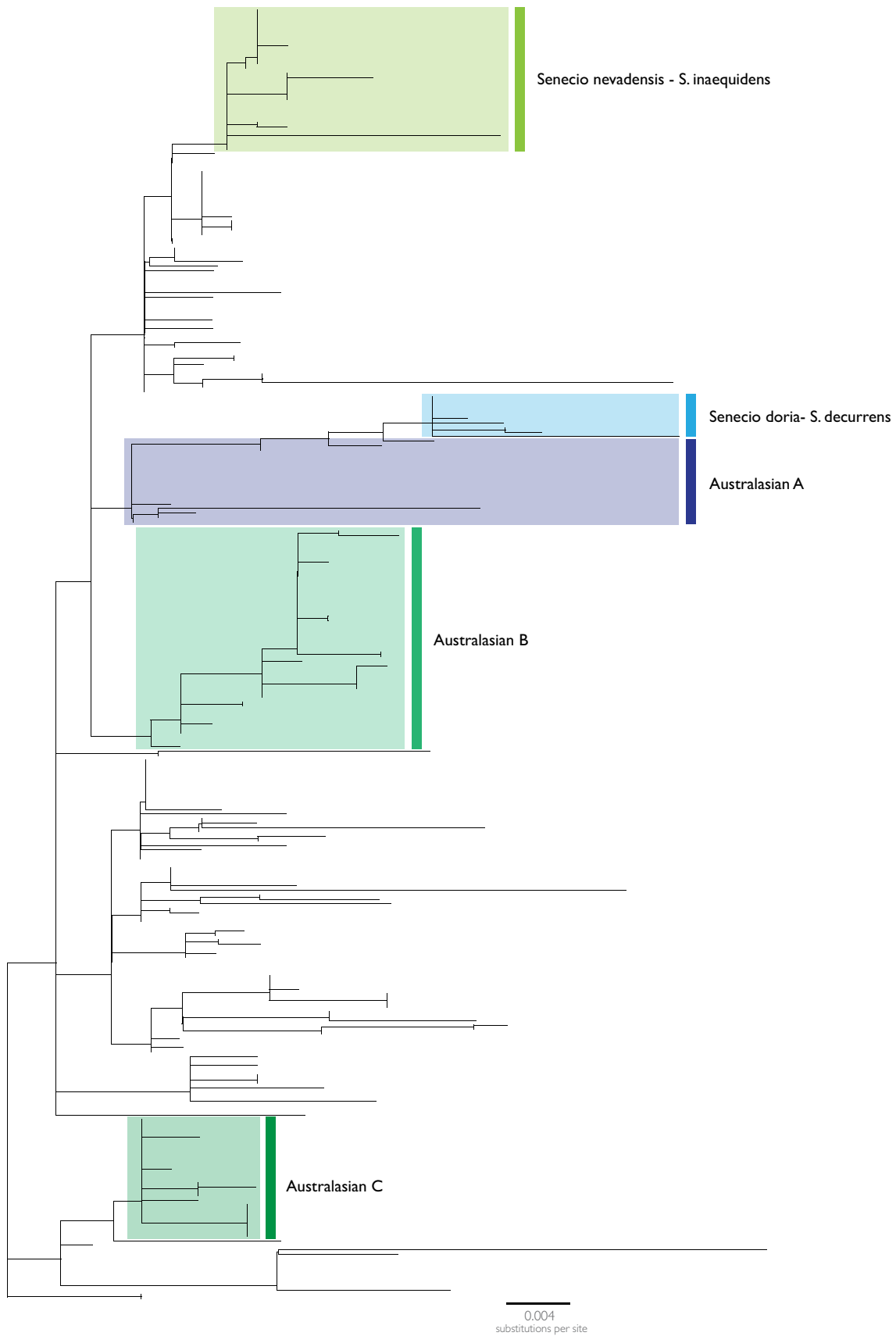
**Figure 2.** Part of ribosomal likelihood phylogeny showing the genus *Senecio* s.str. Coloured and labelled boxes indicate clades mentioned in main text.



**Figure 3.** Detail of ribosomal phylogeny showing *Senecio nevadensis* – *S. inaequidens* clade and Australasian clade 3. Terminals marked with bold font indicate new sequences produced for this study. Numbers above branches are UltraFast Bootstrap values.



**Figure 4.** Detail of ribosomal phylogeny showing *Senecio doria* – *S. decurrens* clade. Terminals marked with bold font indicate new sequences produced for this study. Numbers above branches are UltraFast Bootstrap values.



**Figure 5.** Part of chloroplast likelihood phylogeny showing the genus *Senecio* s.str. Coloured and labelled boxes indicate groups or clades mentioned in main text.

*Senecio pinnatifolius* var. *pinnatifolius*, *S. p.* var. *alpinus*, *S. p.* var. *lanceolatus*, *S. p.* var. *latilobus*, and *S. p.* var. *maritimus* formed part of Australasian clade 3 (Figure 3). Inside this clade they were, however, split into two groups: *S. p.* var. *pinnatifolius* and *S. p.* var. *alpinus* were placed as sister to *S. spathulatus* A.Rich., whereas *S. p.* var. *lanceolatus*, *S. p.* var. *latilobus*, and *S. p.* var. *maritimus* were grouped with *S. hamersleyensis* I.Thomps., *S. lacustrinus* I.Thomps., *S. spanomerus* I.Thomps., and *S. warrenensis* I.Thomps. In contrast, *S. pinnatifolius* var. *capillifolius* and *S. p.* var. *serratus* were unexpectedly placed in the non-Australasian *S. doria* – *S. decurrens* clade (Figure 4).

The chloroplast phylogeny presented in most cases the same placement of samples in clades, with the caveat that, as previously demonstrated (Liew et al., 2018; Schmidt-Lebuhn et al., 2020), its deeper topology was incongruent with that of the ribosomal phylogeny, with Australasian clade C containing *Senecio pinnatifolius* very distant from the *S. nevadensis* – *S. inaequidens* clade (Figure 5). Chloroplasts from species of the *S. doria* – *S. decurrens* clade and *S. pinnatifolius* var. *serratus* formed a clade nested inside Australasian clade A. *Senecio pinnatifolius* var. *capillifolius* was placed in the *S. nevadensis* – *S. inaequidens* clade, and varieties of *S. pinnatifolius* placed in Australasian clade C shared the same chloroplast sequences. However, the resolution provided by chloroplast sequences is overall low, with most species in any of the Australasian clades arranged on zero-branch-length polytomies.

## DISCUSSION

Our results have revealed that within Australasian clade 3, varieties of *Senecio pinnatifolius* did not form a clade and were not even all placed in the same subclade (Figure 3). These results strongly suggest that *S. pinnatifolius* as currently circumscribed is unlikely to be a natural unit, let alone a single species, as suggested by its enormous morphological heterogeneity (Figure 1B-H) and the uncertainty remaining after past studies (Radford et al., 2004; Thompson, 2005b). A dedicated research project with expanded sampling and considering morphology and nomenclature is needed to ascertain a more appropriate taxonomy.

One of the main implications of these results for biocontrol research is that the varieties of *Senecio pinnatifolius* cannot be used interchangeably on test lists, as they are placed at different phylogenetic distances to fireweed. For example, should the placement of *S. pinnatifolius* var. *serratus* be confirmed, it would also have implications for the interpretation of past research on invasive-native plant hybridisation using this variety (e.g. Prentis et al. 2007), and could raise similar questions about its potential status as an introduced species as for *S. condylus* (Keighery & Keighery, 2017). The incongruent position of *S. pinnatifolius* var. *capillifolius* in ribosomal and chloroplast phylogenies is even more unexpected and raises the question of a potential hybridogenic origin of the taxon, as demonstrated for members of the Lautusoid group (Liew et al., 2018).

As summarised in our literature review and reconfirmed in our analysis, the Australasian clade 3 containing (at least most of) *Senecio pinnatifolius* is closest to fireweed of all Australian *Senecio*, but it is nonetheless distantly related to fireweed in absolute terms, when placed in the broader phylogenetic context (Schmidt-Lebuhn et al., 2020). Indeed *S. pinnatifolius* is no more closely related to fireweed than the other members of its clade such as *S. spanomerus*. Contextualising the relatively large evolutionary distance between fireweed and *S. pinnatifolius* in this way reduces the probability that any candidate biocontrol agent will exert undesirable damage to non-target native species in the weed's introduced range. Despite this, the varieties of *S. pinnatifolius* remain important test plants, precisely because of their great diversity, morphological similarities, and overlapping distributions with fireweed across eastern Australia (Briese, 2005). If the candidate biocontrol agent is, for example, an endophagous insect, plants with stems that are too narrow, too short lived, too woody, or too succulent may not be suitable host plants, and therefore varieties of *S. pinnatifolius* morphologically similar to the target weed should be prioritised over those that are less morphologically and ecologically similar.

Conversely, the confirmation of close relationships between *Senecio inaequidens*, *S. harveyanus*, *S. madagascariensis*, and *S. skirrhodon* also has direct implications for ongoing biocontrol research. Candidate biocontrol agents *Gasteroclisus tricostalis*

(Thunberg) (Coleoptera: Curulionidae) and *Metamesia elegans* (Walsingham) (Lepidoptera: Tortricidae) were found on these four species in their native range across southern Africa (Egli & Olckers, 2020; Singh et al., 2022), which could in the absence of phylogenetic data be misinterpreted to mean that these insects have a broad host range and would be unsuitable for ongoing testing as candidate biocontrol agents. However, the very close relationship of these four *Senecio* species indicates that the insects' native host range may indeed be narrow, and that the likelihood of these insects exerting non-target damage to more distantly related native Australian *Senecio* species is low. Previously, a close relationship between these four species was hypothesised based on morphology (Hilliard, 1977) but had never been confirmed with genetic data.

Previous genetic studies have, however, struggled with the delimitation of *Senecio madagascariensis* from *S. inaequidens* (Le Roux et al., 2006), and there is uncertainty in several countries where members of the complex have been introduced about which species is, or are, present. Invasive plants from this complex are called *S. inaequidens* in Europe, *S. madagascariensis* in Australia, and *S. skirrhodon* in New Zealand. Additional research will be required to ascertain if these names are applied consistently across countries and which species have indeed become invasive in each jurisdiction.

Another consideration is that many insects and diseases investigated as candidate biocontrol agents for fireweed were shown to not be host-specific when exposed to *Senecio pinnatifolius* (Holtkamp & Hosking, 1993; McFadyen & Sparks, 1996). This suggests that many species of *Senecio* may have evolved through relatively recent diversification and are therefore biochemically similar.

Most newly sequenced or re-sequenced species were phylogenetically placed as expected. However, the results for the Australian *Senecio pinnatifolius* complex, in particular *Senecio pinnatifolius* var. *capillifolius* and *S. p.* var. *serratus*, and the South African species *S. adnatus* and *S. oxyriifolius* DC. were unexpected. Although not impossible, we currently consider it unlikely that laboratory errors such as sample mix-ups or cross-contamination have caused the observed relationships. In none of the cases would there be a partner for the mix-up, i.e., no species that would have been expected to be placed in the *Senecio dora* – *S. decurrens* clade was placed in Australasian clade 3 containing the other varieties of

*S. pinnatifolius* or in the Gynuroid clade containing older sequences of *S. oxyriifolius* DC. Furthermore, the quality of the sequences was much higher than we would expect contaminated Sanger reads to be.

Nonetheless, the placement of *S. pinnatifolius* var. *capillifolius* and *S. p.* var. *serratus* outside of the Australasian clade 3 should for now be interpreted with caution and requires confirmation. We are in the process of generating more data. Consultation of South African colleagues did not produce a match of *S. p.* var. *capillifolius* with African species (Marinda Koekemoer, pers. comm.).

The analysis presented here highlights the value of applied phylogenetics in weed biocontrol research. It has improved the efficiency of developing host test lists to inform experiments in Australia for a genus with high and phylogenetically disparate native species representation. It improves confidence that host testing is done in a deliberative, well-considered, robust and defensible manner by removing bias in sampling species of 'perceived' closeness (based on superficial morphological similarities) and reinforcing the use of the Centrifugal Phylogenetic Method of selecting species based on evolutionary relatedness (Wapshere, 1974).

Serendipitously, applied phylogenetics in biocontrol research has also thrown up interesting and unexpected taxonomic conundrums that may lead to revisions of accepted taxonomy from genus (Schmidt-Lebuhn et al., 2020) to species level (as potentially for *Senecio pinnatifolius*). Two different fields of research are thus cross-pollinating.

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## APPENDIX

Vouchers and GenBank accession numbers for sequence data newly generated for this study.

Taxon	Voucher	GenBank accession #			
		ETS	ITS	psbA-trnH	trnL
<i>Senecio</i> aff. <i>adnatus</i> DC. #1	D. Egli 142 (NU)	ON758992	ON759065	ON759004	ON759069
<i>Senecio</i> aff. <i>adnatus</i> DC. #2	D. Egli 146 (NU)	ON758991	ON759064	ON759002	ON759070
<i>Senecio</i> aff. <i>adnatus</i> DC. #3	D. Egli 170 (NU)	ON758986	ON759062	ON758999	ON759071
<i>Senecio adnatus</i> DC.	D. Egli 100 (NU)	ON758993	ON759063	ON759000	ON759072
<i>Senecio</i> aff. <i>affinis</i> DC.	D. Egli 188 (NU)	ON758973	ON759045	ON759012	ON759094
<i>Senecio affinis</i> DC.	D. Egli 55 (NU)	ON758970	ON759042	ON759011	ON759091
<i>Senecio bupleuroides</i> DC.	D. Egli 108 (NU)	ON758985	ON759060	ON758998	ON759073
<i>Senecio</i> aff. <i>conrathii</i> N.E. Br. #2	D. Egli 171 (NU)	ON758975	---	ON759013	ON759095
<i>Senecio</i> aff. <i>conrathii</i> N.E. Br.	D. Egli 99 (NU)	ON758976	ON759044	ON759008	ON759087
<i>Senecio coronatus</i> Harv.	D. Egli 128 (NU)	ON758978	ON759049	ON759029	ON759096
<i>Senecio distallobatus</i> I.Thomps.	B. Gooden s.n. (CANB952367) (CANB)	ON758982	ON759055	ON759006	ON759103
<i>Senecio glaberrimus</i> DC.	D. Egli 95 (NU)	ON758990	ON759059	ON758997	ON759077
<i>Senecio harveyanus</i> MacOwan	D. Egli 81 (NU)	ON758958	ON759033	ON759018	ON759098
<i>Senecio hygrophilus</i> Klatt	D. Egli 142 (NU)	ON758984	ON759056	ON758995	ON759076
<i>Senecio inaequidens</i> DC.	D. Egli 84 (NU)	ON758957	ON759032	ON759016	ON759099
<i>Senecio inornatus</i> DC.	D. Egli 53 (NU)	ON758974	ON759046	ON759007	ON759088
<i>Senecio minimus</i> Poir.	B. Gooden s.n. (CANB)	ON758981	ON759054	ON759028	ON759089
<i>Senecio oxyriifolius</i> DC.	D. Egli 90 (NU)	ON758972	ON759043	ON759010	ON759090
<i>Senecio panduriformis</i> Hilliard	D. Egli 198 (NU)	ON758977	ON759050	ON759009	ON759093
<i>Senecio phelleus</i> I.Thomps.	B. Gooden s.n. (CANB952366) (CANB)	ON758979	ON759052	ON759014	ON759101
<i>Senecio pinnatifolius</i> var. <i>alpinus</i> (Ali) I. Thomps.	B. Gooden s.n. (CANB952370) (CANB)	ON758965	ON759035	ON759027	ON759079
<i>S. p.</i> var. <i>capillifolius</i> (Hook. f.) I. Thomps.	J.S. Whinray 488 (CANB)	ON758971	ON759047	ON759030	---
<i>S. p.</i> var. <i>lanceolatus</i> (Benth.) I. Thomps.	P.C. Heyligers 80012 (CANB)	ON758966	---	ON759024	ON759081
<i>S. p.</i> var. <i>latilobus</i> (Steetz) I.Thomps.	N.S. Lander 1025 (CANB)	ON758960	ON759037	ON759026	ON759080
<i>S. p.</i> var. <i>maritimus</i> (Ali) I.Thomps.	M. Carter 155 (CANB)	ON758961	ON759036	ON759020	ON759078
<i>S. p.</i> A. Rich. var. <i>pinnatifolius</i> #1	B. Gooden s.n. (CANB952368) (CANB)	ON758964	ON759039	ON759023	ON759085
<i>S. p.</i> A. Rich. var. <i>pinnatifolius</i> #2	B. Gooden s.n. (CANB952369) (CANB)	ON758963	ON759038	ON759022	ON759084
<i>S. p.</i> A. Rich. var. <i>pinnatifolius</i> #3	B. Gooden s.n. (CANB952365) (CANB)	ON758962	ON759034	ON759025	ON759082
<i>S. p.</i> var. <i>serratus</i> I.Thomps.	I. Radford s.n. (CBG9308770) (CBG)	ON758968	ON759048	---	ON759086
<i>Senecio polyodon</i> DC.	D. Egli 113 (NU)	ON758983	ON759051	ON759019	ON759097
<i>Senecio</i> aff. <i>preanthoides</i> A. Rich.	B. Gooden s.n. (CANB952371) (CANB)	ON758980	ON759053	ON759015	ON759102
<i>Senecio retrorsus</i> DC.	D. Egli 49 (NU)	ON758987	ON759057	ON759003	ON759074
<i>Senecio</i> aff. <i>retrorsus</i> DC.	D. Egli 75 (NU)	ON758988	ON759061	ON759001	ON759068
<i>Senecio scitus</i> Hutch. & Burt Davy	D. Egli 146 (NU)	ON758989	ON759058	ON758996	ON759075
<i>Senecio serratuloides</i> DC.	D. Egli 192 (NU)	ON758969	ON759041	ON759005	ON759092
<i>Senecio</i> aff. <i>serratuloides</i> DC.	D. Egli 194 (NU)	---	ON759066	ON758994	ON759067
<i>Senecio skirrhodon</i> DC.	D. Egli 76 (NU)	ON758967	ON759031	ON759017	ON759100
<i>Senecio</i> aff. <i>spanomerus</i> I.Thomps.	B. Gooden s.n. (CANB952364) (CANB)	ON758959	ON759040	ON759021	ON759083