

## Re-considerations on *Senecio oxyriifolius* DC. and *S. tropaeolifolius* MacOwan ex F. Muell. (Asteraceae: Senecioneae)

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*Summary:* Analyses of *ITS1-2* data from a comprehensive sample of African succulent species of *Senecio* and related genera reveals that *Senecio tropaeolifolius*, though closely related to *S. oxyriifolius*, should be treated as a separate species. According to our results, it may be one of the parental species to *S. kleiniiformis*, a widely cultivated ornamental of uncertain hybrid origin.

*Keywords:* Asteraceae, Senecioneae, taxonomy, systematics, *Senecio kleiniiformis*, *ITS1-2*

*Senecio tropaeolifolius* MacOwan ex F. Muell. is a widely cultivated succulent ornamental (BRICKELL 2003) whose taxonomic rank has remained uncertain so far. Its similarity to *S. oxyriifolius* DC. was mentioned in its first description (MUELLER 1867) and ROWLEY (1994, 2002) rendered it as a subspecies of the latter one. However, JEFFREY (1986, 1992) treated these allopatric (Fig. 1) taxa, *S. tropaeolifolius* and *S. oxyriifolius*, as two separate species in the section Peltati.

According to their descriptions, these two species differ mainly in their growth form, the number of involucre bracts of the capitula, the number of florets in the capitula, the presence/absence of ray florets and bristles on cypselae. All these characters are rather variable among *Senecio* L. s. latiss. and their taxonomic value is questionable.

Molecular data drastically changed the understanding of taxonomy and phylogeny of *Senecio* and related genera (PELSE et al. 2007, 2010). The two species under consideration were also studied using molecular markers (STÄHELI 2006; PELSE et al. 2007). According to the results obtained, these species belong to the same clade as the genus *Curio* Heath and some other succulent species of *Senecio* s.l. (species of sect. Kleinioidei, *S. abbreviatus* S. Moore, *S. macroglossus* DC., etc.). However, they have never been analyzed together in a single data set, so it has remained unclear yet, whether *S. tropaeolifolius* and *S. oxyriifolius* are really conspecific entities or not.

We analyzed the relationships of *S. tropaeolifolius* and *S. oxyriifolius* in a broader phylogenetic context of succulent *Senecio* s.l. groups than it has been done so far (STÄHELI 2006; PELSE et al. 2007, 2010), because the tree topology may be strongly influenced by taxon sampling (RYDIN & KÄLLERSJÖ 2002; DEGTJAREVA et al. 2004; SOLTIS & SOLTIS 2004; PAVLINOV 2005; BATEMAN et al. 2006). Our study is based on sequence analyses of the *ITS* (*ITS1-5.8S RNA-ITS2*) nuclear DNA region.

### Materials and methods

We used the plants of 35 species of *Senecio* s.l., including *S. tropaeolifolius*, cultivated in the greenhouse collection of the Main Botanical Garden of Russian Academy of Sciences, Moscow

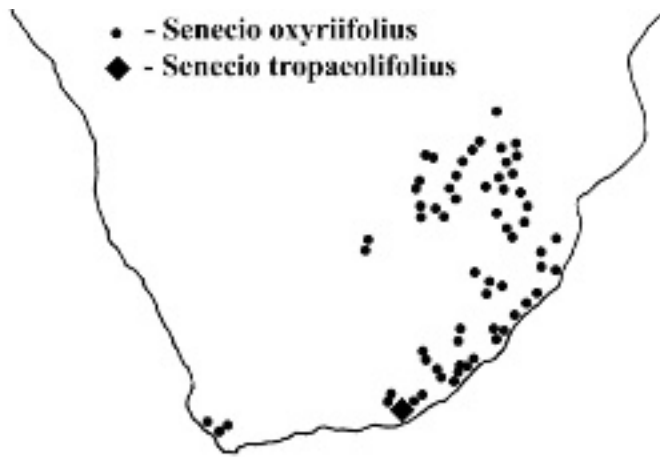


Figure 1. Natural distribution of *Senecio oxyriifolius* and *S. tropaecolifolius* (compiled from AFRICAN PLANT DATABASE).

(MBG Greenhouse) or available as herbarium specimens at the Herbarium of the same Garden [MHA]. The plants of *S. oxyriifolius* were received from the collection of Kirstenbosch National Botanical Garden (South Africa) in 2011. 66 additional data on *ITS* sequences of *Senecio* s.l. and related genera were obtained from GenBank (Appendix 1).

DNA was extracted from fresh or dry leaf tissue using the NucleoSpin® Plant II Kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. The complete nuclear *ITS* region was amplified using primers 5'-ACCTGCGGAAGGATCANNG--3' and 5'-GATATGCTTAAACTCAGCGG -3'. Polymerase chain reactions (PCR) were conducted in 20 µl reaction volumes containing 4 µl of Ready-to-Use PCR MaGMix (200 µM of each dNTP, 1.5 mM MgCl<sub>2</sub>, 1.5 U SmarTaqDNA Polymerase and reaction buffer; Dialat Ltd., Moscow, Russia), 15 µl deionized water, 3.4 pmol of each primer and 1 µl of template DNA of unknown concentration. PCR cycling was performed with a MJ Research PTC-220 DNA Engine Dyad Thermal Cycler (BioRad Laboratories, USA) with the following parameters: initial denaturation for 2 min 30 s at 95°C followed by 35 cycles of 30 s at 95°C, 1 min at 55°C and 2 min at 72°C, ending with 4 min extension at 72°C. Double-stranded PCR products were checked on agarose gels and purified with the GFX PCR Purification kit (Amersham Biosciences, USA) according to manufacturer's recommendations. Sequencing was performed in both directions using ABI PRISM BigDye™ Terminator v. 3.1 Kit (Applied Biosystems) according to the manufacturer's manual and further analyzed on ABI PRISM 3730 Genetic Analyzer (Applied Biosystems) at the facilities of the 'Genome' Centre at the Institute of Molecular Biology of Russian Academy of Sciences. GenBank accession numbers of the *ITS* sequences are KJ561175 to KJ561210 (Appendix 1).

DNA sequences were aligned using MAFFT (KATOHI et al. 2002) under an accurate L-INS-I strategy (KATOHI et al. 2005) with a final manual alignment in BioEdit 7.0.1. (HALL 1999). We performed separate analyses treating indels as missing data or with all indels included. In the latter case, the indels were coded using simple method of indel coding (SIMMONS et al. 2001) as implemented in the GapCoder software (YOUNG & HEALY 2003).

We analyzed the aligned sequences with the T.N.T. program (GOLOBOFF et al. 2003) using both the traditional Wagner and the New Technology approaches of Maximum Parsimony (MP)

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searches with the TBR algorithm. Maximum parsimony analysis (traditional search) parameters: Wagner trees, swapping algorithm TBR, 50 trees saved per replication, keep all trees found, collapse trees after the search, replace existing trees. *Tussilago farfara* L. (Asteraceae: Tussilagineae) was used as an outgroup. Branch support was assessed with 100 replicates. Nodes with less than 50% support were regarded as unresolved and collapsed.

## Results

The *ITS1–5.8S RNA–ITS2* region varied from 576 (*Senecio abbreviatus* S. Moore) to 630 b.p. (*Kleinia galpinii* A. Berger) among the species studied. The final alignment was 688 positions long and included 103 sequences. 534 most parsimonious trees of 1515 steps long were revealed. The NT search with varying parameters revealed 175 trees of the same 1515 steps long.

The length of the alignment was enlarged to 785 positions with gaps coded. Traditional MP search with the same parameters as above returned 926 trees of 1743 steps long; NT search with varying parameters revealed 60 to 179 trees of the same 1743 steps long.

The bootstrap consensus tree from the last analysis is shown in Fig. 2, nodes below 50% support are collapsed. Since the detailed analysis of *Senecio* and related taxa is beyond the scope of present paper and has already been done elsewhere (STÄHELI 2006; PELSER et al. 2007; TIMONIN et al. 2014), we have just focused on relative positions of *S. trophaeolifolius* and *S. oxyriifolius*. The target group of these two species invariably appeared in a Curio–Othonna clade where species of *Curio* Heath, *Othonna* L. and several others still regarded as *Senecio* are nested. This clade is poorly resolved in the consensus tree. However, in separate trees, the species under consideration appear either as sister groups or one of them may appear in a basal position of a clade the other belongs to (not shown).

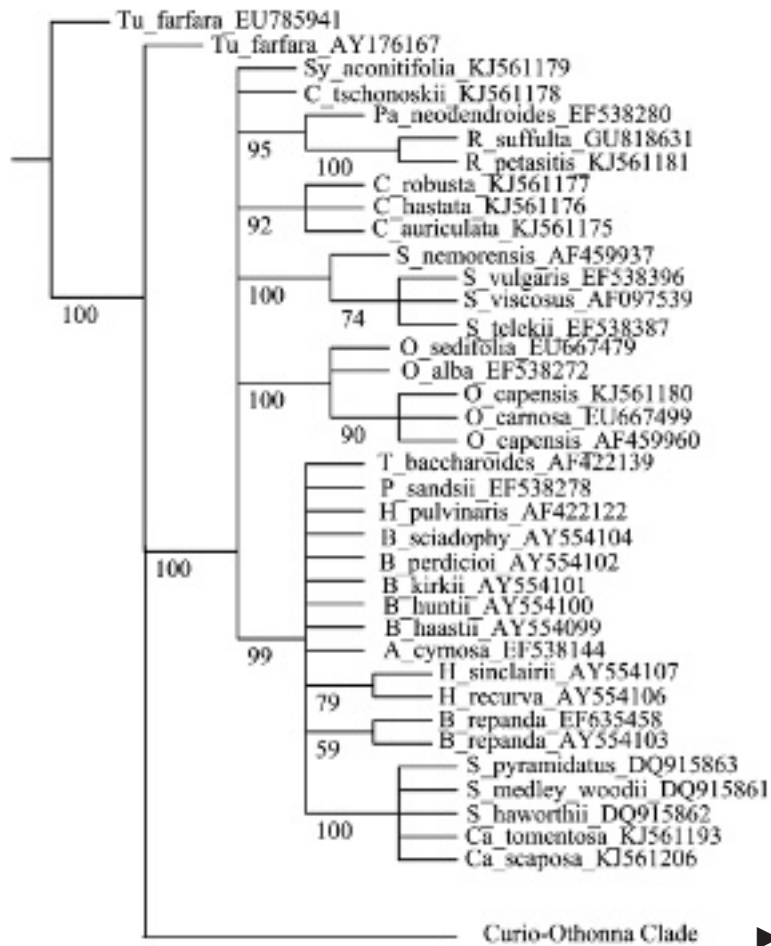
Since they are never nested outside the Curio–Othonna clade, we reduced the dataset to that clade, with sequences of *Iranecio* B. Nord. species used as an outgroup. This, however, has not improved the resolution of the Curio–Othonna clade in subsequent analyses (not shown). The resulting trees (20 trees under the traditional MP search, 4 trees under the NT search) were 518 steps long and of similar topologies as those resulted from the complete data set.

In all analyses the three sequences of *S. oxyriifolius* grouped together form a separate highly supported clade. In the same manner, the two sequences of *S. trophaeolifolius* grouped with each other and with the sequence of *S. kleiniiformis* Suss. form a well supported unresolved clade.

## Discussion

Our results generally coincide with those of STÄHELI (2006) and PELSER et al. (2007, 2010) that *S. trophaeolifolius* and/or *S. oxyriifolius* appear in the same Curio clade despite the differences in taxon sampling. However, our results do not support *S. oxyriifolius* as the sister species of *S. junceus* (Less.) Harvey as shown by PELSER et al. (2007).

Unexpectedly close relationship of *S. trophaeolifolius* and *S. kleiniiformis* may be explained by the putative hybrid origin of the latter, which probably happened in cultivation. Such an origin has already been hypothesized by ROWLEY (1994, 2002). However, Rowley hypothesized that one of the progenitors could be *Curio articulatus* (L.) P. V. Heath, whereas another one could be either the diploid *S. talinoides* Sch. Bip. subsp. *cylindricus* (A. Berger) G. D. Rowley and/or the



**Figure 2.** Bootstrap consensus tree of 175 MP trees revealed after New Technology searching. Bootstrap values above 50 are indicated below branches.

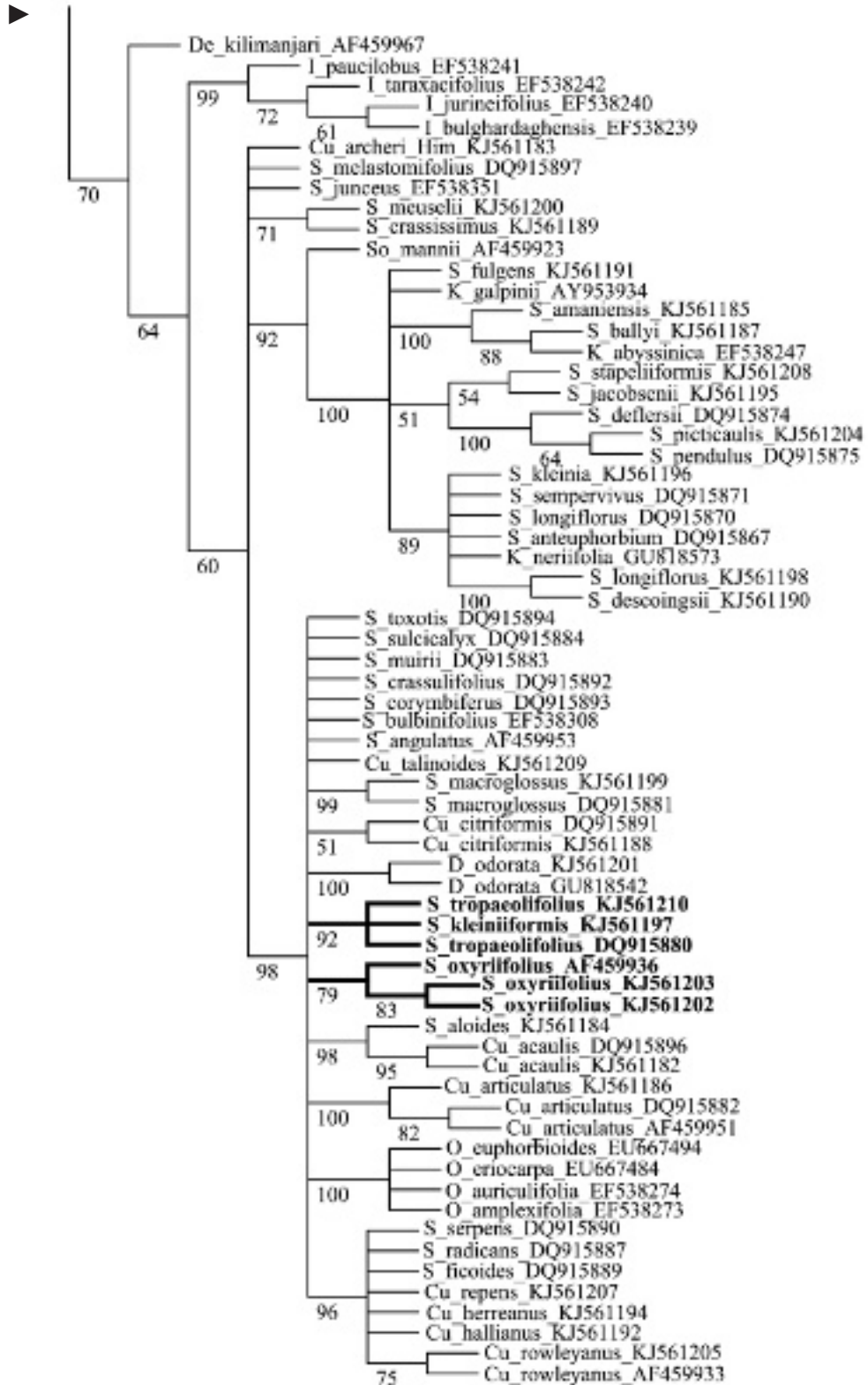
Abbreviations of generic names: A – *Acrisione*; B – *Brachyglottis*; C – *Cacalia*; Ca – *Caputia*; Cu – *Curio*; D – *Delaireia*; De – *Dendrosenecio*; H – *Haastia*; I – *Iranecio*; K – *Kleinia*; O – *Othonna*; P – *Papuacalia*; Pa – *Paragnoxycs*; R – *Roldana*; S – *Senecio*; So – *Solanecio*; Sy – *Syneilesis*; T – *Traversia*; Tu – *Tussilago*. The target group of species is marked in bold.

polyploid *S. ficoides* Sch. Bip. or *S. serpens* G. D. Rowley. Nevertheless, molecular analyses show that neither of these species is closely related to *S. kleiniiformis*. Our results show *S. troepaeolifolius* to be probably one of the parental species of *S. kleiniiformis*.

Our data corroborate rather close relationships between *S. troepaeolifolius* and *S. oxyriifolius*, either they are sister species or not. Nevertheless, the sequences of both species always form two separate monophyletic groups in our cladograms. Besides, both manifest nearly the same relationships with other species (Fig. 2). Equally related taxa should be interpreted as equally ranked. If *S. troepaeolifolius* and *S. oxyriifolius* are considered conspecific, rather many other senecios should be included into the same species. The latter have never been thought to be closely related with each other and with the species under consideration. On the contrary, they were arranged into separate sections, subgenera or even genera (Fig. 2). There is certainly no reason to combine them as infraspecific units of one species. Keeping these taxa as separate ones, an inclusion of

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Figure 2. cont.



*S. tropaeolifolius* into *S. oxyriifolius* under any infraspecific rank would be illogical. Therefore, *S. tropaeolifolius* must reasonably be treated as a separate species, as evidenced by the available molecular data.

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Appendix 1. Species included in the analysis. Names in bold indicate species sequenced for the first time in present study. MHA = Herbarium of the Main Botanical Garden of the Russian Academy of Sciences; MBG = Greenhouse of the Main Botanical Garden of the Russian Academy of Sciences.

Species	NCBI number	Source
<i>Cacalia auriculata</i>	KJ561175	MHA
<i>Cacalia hastata</i>	KJ561176	MHA
<i>Cacalia robusta</i>	KJ561177	MHA
<i>Cacalia tschonoskii</i>	KJ561178	MHA
<i>Caputia scaposa</i>	KJ561206	MBG
<i>Caputia tomentosa</i>	KJ561193	MBG
<i>Curio acaulis</i>	KJ561182	MBG
<i>Curio archeri</i> cult. 'Himalaya'	KJ561183	MBG
<i>Curio articulatus</i>	KJ561186	MBG
<i>Curio citrifolius</i>	KJ561188	MBG
<i>Curio hallianus</i>	KJ561192	MBG
<i>Curio herreanus</i>	KJ561194	MBG
<i>Curio repens</i>	KJ561207	MBG
<i>Curio rowleyanus</i>	KJ561205	MBG
<i>Curio talinoides</i>	KJ561209	MBG
<i>Delairea odorata</i>	KJ561201	MBG
<i>Othonna capensis</i>	KJ561180	MBG
<i>Roldana petasitis</i>	KJ561181	MBG
<i>Senecio aloides</i>	KJ561184	MBG
<i>Senecio amaniensis</i>	KJ561185	MBG
<i>Senecio ballyi</i>	KJ561187	MBG
<i>Senecio crassissimus</i>	KJ561189	MBG
<i>Senecio descoingsii</i>	KJ561190	MBG
<i>Senecio fulgens</i>	KJ561191	MBG
<i>Senecio jacobsenii</i>	KJ561195	MBG
<i>Senecio kleinia</i>	KJ561196	MBG
<i>Senecio kleiniiiformis</i>	KJ561197	MBG
<i>Senecio longiflorus</i>	KJ561198	MBG
<i>Senecio macroglossus</i>	KJ561199	MBG
<i>Senecio meuselii</i>	KJ561200	MBG
<i>Senecio oxyriifolius</i>	KJ561202	MBG
<i>Senecio oxyriifolius</i>	KJ561203	MBG
<i>Senecio picticaulis</i>	KJ561204	MBG
<i>Senecio stapelieformis</i>	KJ561208	MBG
<i>Senecio tropaeolifolius</i>	KJ561210	MBG
<i>Syneilesis aconitifolia</i>	KJ561179	MBG
<i>Acrisione cymosa</i>	EF538144	GenBank
<i>Brachyglottis haastii</i>	AY554099	GenBank
<i>Brachyglottis huntii</i>	AY554100	GenBank
<i>Brachyglottis kirkii</i>	AY554101	GenBank
<i>Brachyglottis perdicoides</i>	AY554102	GenBank
<i>Brachyglottis repanda</i>	AY554103	GenBank
<i>Brachyglottis repanda</i>	EF635458	GenBank
<i>Brachyglottis sciadophila</i>	AY554104	GenBank
<i>Curio articulatus</i>	AF459951	GenBank
<i>Curio articulatus</i>	DQ915882	GenBank
<i>Curio rowleyanus</i>	AF459933	GenBank
<i>Delairea odorata</i>	GU818542	GenBank
<i>Dendrosenecio kilimanjari</i>	AF459967	GenBank
<i>Haastia pulvinaris</i>	AF422122	GenBank
<i>Haastia recurva</i>	AY554106	GenBank
<i>Haastia sinclairii</i>	AY554107	GenBank
<i>Iranecio bulghardaghensis</i>	EF538239	GenBank
<i>Iranecio jurineifolius</i>	EF538240	GenBank
<i>Iranecio paucilobus</i>	EF538241	GenBank
<i>Iranecio taraxacifolius</i>	EF538242	GenBank
<i>Kleinia abyssinica</i>	EF538247	GenBank
<i>Kleinia galpinii</i>	AY953934	GenBank
<i>Kleinia neriifolia</i>	GU818573	GenBank
<i>Othonna alba</i>	EF538272	GenBank
<i>Othonna amplexifolia</i>	EF538273	GenBank
<i>Othonna auriculifolia</i>	EF538274	GenBank
<i>Othonna capensis</i>	AF459960	GenBank
<i>Othonna carnosa</i>	EU667499	GenBank
<i>Othonna eriocarpa</i>	EU667484	GenBank
<i>Othonna euphorbioides</i>	EU667494	GenBank
<i>Othonna sedifolia</i>	EU667479	GenBank
<i>Papuacalia sandsii</i>	EF538278	GenBank
<i>Paragymoxys neodendroides</i>	EF538280	GenBank
<i>Roldana suffulta</i>	GU818631	GenBank
<i>Senecio acaulis</i>	DQ915896	GenBank
<i>Senecio angulatus</i>	AF459953	GenBank
<i>Senecio anteuphorbium</i>	DQ915867	GenBank
<i>Senecio bulbiniifolius</i>	EF538308	GenBank
<i>Senecio citrifolius</i>	DQ915891	GenBank
<i>Senecio corymbiferus</i>	DQ915893	GenBank
<i>Senecio crassulifolius</i>	DQ915892	GenBank
<i>Senecio deflersii</i>	DQ915874	GenBank
<i>Senecio ficoides</i>	DQ915889	GenBank
<i>Senecio haworthii</i>	DQ915862	GenBank
<i>Senecio junceus</i>	EF538351	GenBank
<i>Senecio kilimanjari</i>	AY953933	GenBank
<i>Senecio longiflorus</i>	DQ915870	GenBank
<i>Senecio macroglossus</i>	DQ915881	GenBank
<i>Senecio medley-woodii</i>	DQ915861	GenBank
<i>Senecio melastomifolius</i>	DQ915897	GenBank
<i>Senecio muirii</i>	DQ915883	GenBank
<i>Senecio nemorensis</i>	AF459937	GenBank
<i>Senecio oxyriifolius</i>	AF459936	GenBank
<i>Senecio pendulus</i>	DQ915875	GenBank
<i>Senecio pyramidatus</i>	DQ915863	GenBank
<i>Senecio radicans</i>	DQ915887	GenBank
<i>Senecio sempervivus</i>	DQ915871	GenBank
<i>Senecio serpens</i>	DQ915890	GenBank
<i>Senecio sulcicalyx</i>	DQ915884	GenBank
<i>Senecio telekii</i>	EF538387	GenBank
<i>Senecio toxotis</i>	DQ915894	GenBank
<i>Senecio tropaeolifolius</i>	DQ915880	GenBank
<i>Senecio viscosus</i>	AF097539	GenBank
<i>Senecio vulgaris</i>	AF459923	GenBank
<i>Solanecio manni</i>	AF459923	GenBank
<i>Traversia baccharoides</i>	AF422139	GenBank
<i>Tussilago farfara</i>	EU785941	GenBank
<i>Tussilago farfara</i>	AY176167	GenBank



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