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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 involucral 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 (Pelser et al. 2007, 2010). The two species under consideration were also studied using molecular markers (Stäheli 2006; Pelser 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; Pelser 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

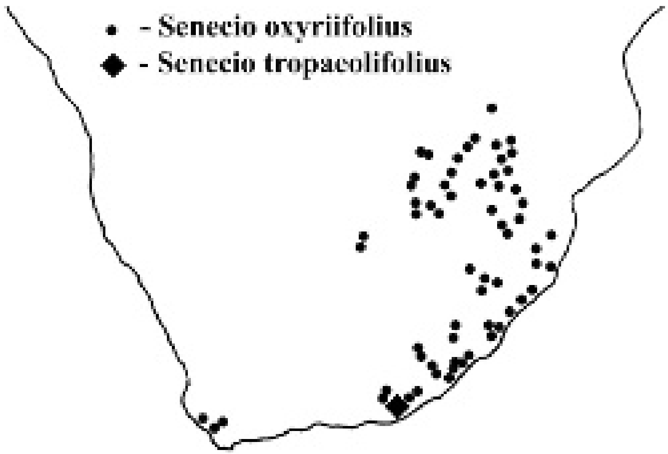


Figure 1. Natural distribution of *Senecio oxyriifolius* and *S. tropaeolifolius* (compiled from African Plant Database).

(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 $MgCl_2$, 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 (Amercham 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 (Katoh et al. 2002) under an accurate L-INS-I strategy (Katoh 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) 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. tropaeolifolius* 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. tropaeolifolius* grouped with each other and with the sequence of *S. kleiniiformis* Suess. form a well supported unresolved clade.

Discussion

Our results generally coincide with those of Stäheli (2006) and Pelser et al. (2007, 2010) that *S. tropaeolifolius* 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. tropaeolifolius* 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

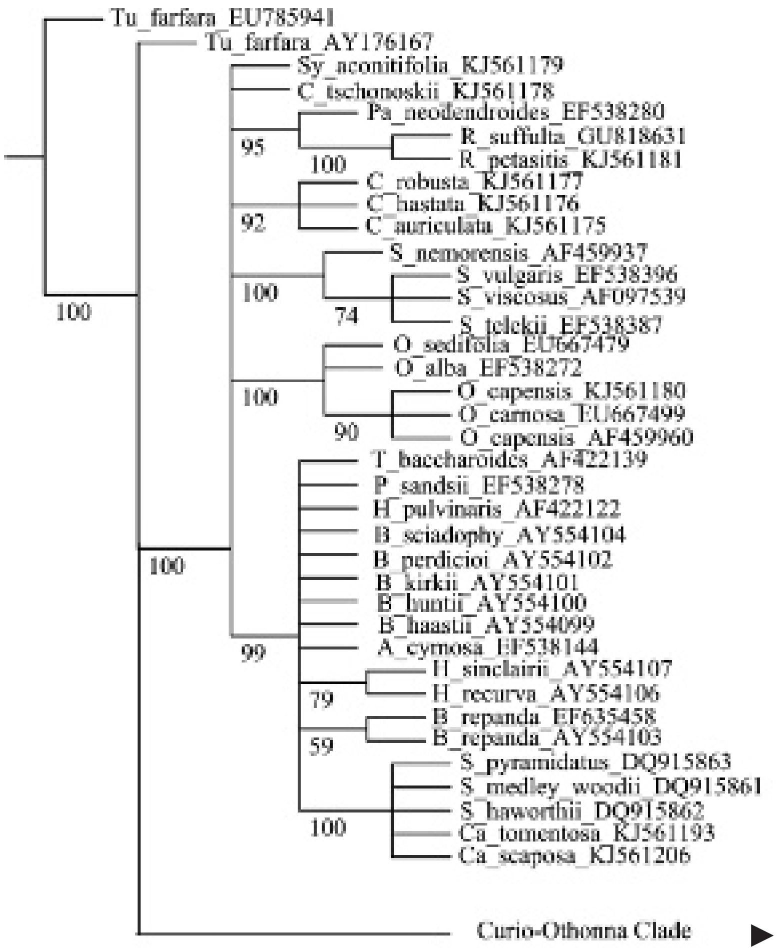
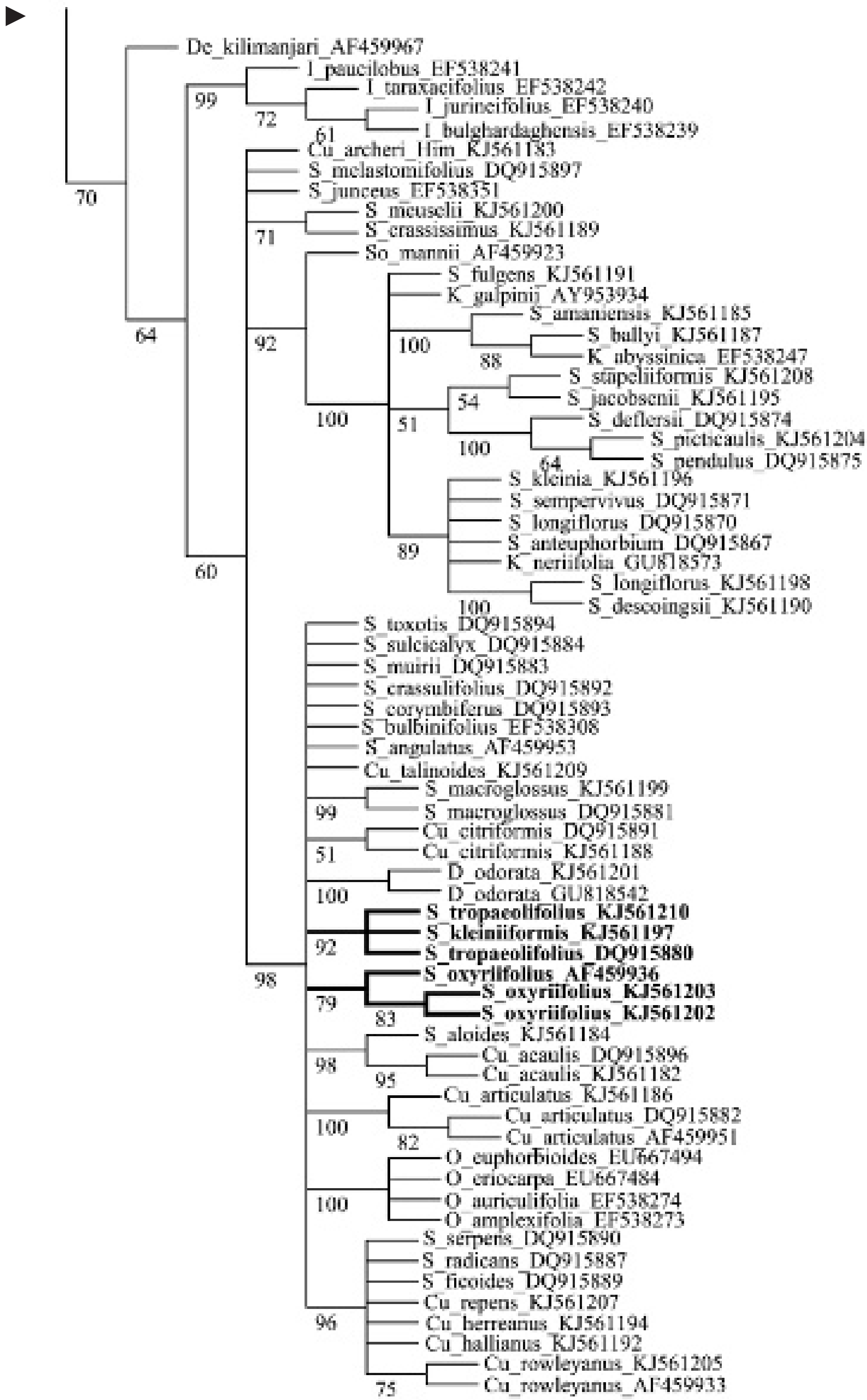


Figure 2. Bootstrap consensus tree of 175 MP trees revealed after New Technology searching. Bootstrap values above 50 are indicated below branches.
Abbreviations of generic names: A – *Acrisione*; B – *Brachyglottis*; C – *Cacalia*; Ca – *Caputia*; Cu – *Curio*; D – *Delairea*; De – *Dendrosenecio*; H – *Haastia*; I – *Iranecio*; K – *Kleinia*; O – *Othonna*; P – *Papuacalia*; Pa – *Paragynoxys*; 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. tropaeolifolius* to be probably one of the parental species of *S. kleiniiformis*.

Our data corroborate rather close relationships between *S. tropaeolifolius* 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. tropaeolifolius* 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

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