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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

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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 20µl reaction volumes containing 4µl of Ready-to-Use PCR MaGMix (200µM 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 30s at 95°C followed by 35 cycles of 30s 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<sup>TM</sup> 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

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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

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Figure 2. cont.
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*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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NCBI Species Source number Cacalia auriculata KJ561175 MHA Cacalia hastata KJ561176 MHA Cacalia robusta KJ561177 MHA Cacalia tschonoskii KJ561178 MHA KJ561206 MBG Caputia scaposa MBG Caputia tomentosa KJ561193 Curio acaulis KJ561182 MBG Curio archeri cult. KJ561183 MBG 'Himalaya' Curio articulatus KJ561186 MBG Curio citriformis KJ561188 MBG Curio hallianus KJ561192 MBG Curio herreanus KJ561194 MBG KJ561207 Curio repens MBG Curio rowleyanus KJ561205 MBG Curio talinoides KJ561209 MBG Delairea odorata KJ561201 MBG MBG Othonna capensis KJ561180 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 MBG KJ561196 Senecio kleiniiformis KJ561197 MBG Senecio longiflorus KJ561198 MBG Senecio macroglossus KJ561199 MBG Senecio meuselii KJ561200 MBG Senecio oxyriifolius KJ561202 MBG Senecio oxyriifolius KJ561203 MBG MBG Senecio picticaulis KJ561204 Senecio stapelieformis KJ561208 MBG MBG Senecio tropaeolifolius KJ561210 MBG Syneilesis aconitifolia KJ561179 Acrisione cymosa GenBank EF538144 Brachyglottis haastii AY554099 GenBank Brachyglottis huntii AY554100 GenBank Brachyglottis kirkii AY554101 GenBank Brachyglottis perdicioides AY554102 GenBank Brachyglottis repanda GenBank AY554103 GenBank Brachyglottis repanda EF635458 Brachyglottis sciadophila AY554104 GenBank Curio articulatus AF459951 GenBank Curio articulatus GenBank DQ915882 Curio rowleyanus AF459933 GenBank Delairea odorata GU818542 GenBank Dendrosenecio kilimanjari GenBank AF459967 Haastia pulvinaris AF422122 GenBank GenBank Haastia recurva AY554106

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	DO915896	GenBank
Senecio angulatus	AF459953	GenBank
Senecio anteuphorbium	DO915867	GenBank
Senecio hulbinifolius	EF538308	GenBank
Senecio citriformis	DO915891	GenBank
Senecio corvmhiferus	DO915893	GenBank
Senecio crassulifolius	DO915892	GenBank
Senecio deflersii	DO915874	GenBank
Senecio ficoides	DO915889	GenBank
Senecio haworthii	DO915862	GenBank
Senecio junceus	FF538351	GenBank
Senecio filimaniari	AV953933	GenBank
Senecio longiflorus	DO915870	GenBank
Senecio macroalossus	DQ915881	GenBank
Senecio medlev-woodii	DQ915861	GenBank
Senecio melastomifolius	DQ915897	GenBank
Senecio muirii	DO915883	GenBank
Senecio nemorensis	AE450027	GenBank
Senecio ovvriitalius	AF450036	GenBank
Senecio pendulus	DO915875	GenBanlz
Senecio penunius Senecio puramidatus	DO915863	GenBank
Senecio radicano	DO915887	GenBank
Senecio semperainas	DO915871	GenBanlz
Sanacio sempervivus	DO015800	CenBank
Senecio sulcicalmo	DQ913090	GenBank
Senecio suundiyx	FE538287	CenBanl
Senecio telekil	DO015004	ConPanl
Senecto ioxolis	DQ913894	ConPan <sup>1</sup>
Senecio tropaeolijolius	1007520	Genbank ConPaul
Senecio viscosus	AFU9/339	Genbank
Senecio vulgaris	AF439923	Genbank
Solanecio mannii	AF459923	GenBank
Traversia baccharoides	AF422139	GenBank ConPaul
Tussilago farjara	EU/85941	GenBank
1ussilago țarțara	AT1/616/	GenBank

**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.

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