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The phylogenetic position of East Asian *Sedum* species (Crassulaceae) based on chloroplast DNA trnL (UAA)–trnF (GAA) intergenic spacer sequence variation

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SUMMARY

Phylogenetic relationships of 27 Crassulaceae species, including a broad sample of 19 *Sedum* species, were inferred from sequence variation of the chloroplast trnL (UAA)–trnF (GAA) intergenic spacer to assess the position of alkaloid containing Asian *Sedum* species. The overall topology of the phylogeny fully agrees with results based on chloroplast DNA restriction-site variation. The five alkaloidal, Asian *Sedum* species are included in the *Acre* lineage. These results support Steven's and co-workers (1995) hypothesis on the distribution of alkaloids in the Crassulaceae which states that the occurrence of alkaloids is restricted to the species of the *Acre* lineage. Although monophyly of the *Acre* lineage is strongly supported, relationships within this clade are only partially resolved, probably as a result of incomplete systematic sampling.

Key-words: Crassulaceae, *Sedum*, *Acre* lineage, phylogeny, chloroplast DNA intergenic spacer.

INTRODUCTION

The Crassulaceae comprise some 1300–1500 species of succulent herbs divided over six subfamilies and 33 genera (Berger 1930). The family has an almost cosmopolitan distribution, but is most prominent on the northern hemisphere and in southern Africa. Berger's subfamilies are largely confined to a single continent each, except for the Sedoideae which occur on the tree (sub-)continents of the northern hemisphere. The core of subfamily Sedoideae is the genus *Sedum* L. which with about 300–500 species is by far the largest genus of the Crassulaceae. It occurs mainly in the subtropical and temperate zones of the northern hemisphere, but is also present in tropical East Africa and with a few little-known species in South America (NW Argentina, Bolivia, Ecuador). Schönland (1891) placed *Sedum* in the centre of the family, and it contains some morphologically and phytochemically highly specialized taxa as well as less

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derived forms ('t Hart & Koek-Noorman 1989; Stevens 1995). Chloroplast DNA restriction-site analysis indicates paraphyly of *Sedum* (Van Ham 1994).

Opinions about the delimitation and infrageneric classification of *Sedum* are highly divergent and often conflicting, because the genus can not be defined unequivocally by a unique set of morphological characters ('t Hart 1991; Van Ham 1994). Although there is now some kind of general agreement about the generic position of *S.* sect. *Rhodiola* (L.) Scop. (≡*Rhodiola* L.; ≡*S.* subgen. *Rhodiola* (L.) Ohba) and *S.* sect. *Telephium* Gray (≡*Hylotelephium* Ohba; ≡*S.* subgen. *Telephium* (Gray) Clausen) the taxonomy of the major part of the genus, i.e. *Sedum* s.s. (≡*S.* sect. *Sedum*, ≡*S.* sect. *Seda genuina* Koch) which comprises about 300 species, is still badly understood ('t Hart 1995; Eggli *et al.* 1995). Chloroplast DNA restriction-site analysis of over 100 Crassulaceae, including 56 *Sedum* species, indicated a strongly supported monophyletic clade, the *Acre* lineage (Van Ham 1994, 1995), which comprises Asian, Eurasian and American *Sedum* species with a reticulate testa as well as the American subfamily Echeverioideae. In a recent survey of the distribution of alkaloids in Crassulaceae the presence of alkaloids proved to be restricted to species of the *Acre* lineage (Stevens *et al.* 1992, 1993, 1995; Stevens 1995).

The Asian *Sedum* flora comprises some 80–100 species (Berger 1930; Fröderström 1931), but relationships among these taxa are still badly understood, especially among species of *Sedum* s.s. The two Asian species of *Sedum* s.s. included in chloroplast DNA restriction-site analyses, *S. sarmentosum* Bunge and *S. morrisonensis* Hayata, belong to the *Acre* lineage of *Sedum* (Van Ham 1994, 1995). Both species have reticulate seeds and contain alkaloids (Marion & Chaput 1949; Beyerman *et al.* 1972; Kim 1988; Kim *et al.* 1996).

Recently chloroplast DNA spacer sequence variation has been used in a variety of evolutionary studies including infrafamilial relationships of Crassulaceae (Aldrich *et al.* 1988; Wolfson *et al.* 1991; Morton & Clegg 1993; Van Ham *et al.* 1994; Mes & 't Hart 1994). Evolutionary relationships inferred from sequence variation of the trnL (UAA)–trnF (GAA) intergenic spacer of 12 Crassulaceae (all six subfamilies, nine genera) proved to be largely consistent with phylogenies based on chloroplast DNA restriction-site variation (Van Ham *et al.* 1994; Van Ham 1995).

In this study the phylogenetic position is assessed of some East Asian species of *Sedum* s.s. using sequence variation of the trnL–trnF intergenic spacer. The species all have reticulate seeds and are considered to belong to the *Acre* lineage because they were also shown to contain alkaloids in a previous study (Kim *et al.* 1996).

MATERIALS AND METHODS

Plants included in this study were all cultivated in the Botanic Garden of Utrecht and voucher specimens are deposited in the herbarium of the Botany department at Utrecht (U). Origin and accession number of the plants are presented in Table 1.

Total DNA was isolated from leaf tissue according to the procedure of Guillemaut & Marechal-Drouard (1992), as slightly modified by Mes & 't Hart (1994). The universal primers B49873 and A50272 (Taberlet *et al.* 1991) were used to amplify the chloroplast DNA trnL (UAA) 3′ exon–trnF (GAA) intergenic spacer. Sequences were determined by the method of Sanger *et al.* (1977) using the *Taq* Dye Terminator Cycle Sequencing Kit (ABI) and the 373 Automated DNA Sequencer. Alignment of sequences was performed with PCGENE (release 6.70) with final manual editing.

Table 1. Origin and accession number of the Crassulaceae species examined

Species	Origin and accession number
Aeonium castello-paivae Bolle[1]	Spain, Canary Is., Gomera, Bosque del Cedro, 30484.
Cotyledon obiculata L.[2]	S. Africa (exact provenance unknown), 32688.
Crassula multicava Lem.[2]	S. Africa (exact provenance unknown), 30474.
Echeveria venezuelensis Rose[2]	Venezuela (Merida), S. Nevada, Loma Redonda, 30445.
Hylotelephium telephium (L.) Ohba	France (Vienne), Charroux, 31028.
Pachyphytum compactum Rose	Mexico (Queretaro), Cerro Mexicano, near Colon, 30620.
Sedum acre L.[2]	Turkey (Bolu), W of Mengen, 30869.
Sedum aetnense Tineo	Turkey (Elazig), Harput NE of Elazig, 31712.
Sedum album L.[2]	Spain (Teruel), SE of Albarracin, 29329.
Sedum alpestre Vill.	Turkey (Bursa), Uludag, 30915.
Sedum bourgaei Hemsley	Mexico (Guanajuato), near San Filipe, 21588.
Sedum bulbiferum Makino	Korea (Cheonnam), Mt Paegyang, 32769.
Sedum dasyphyllum L.	Italy (Liguria), Rocco, 17579.
Sedum dendroideum Moc. & Sesse	Mexico (Puebla), San Hipolito, 28227.
Sedum fusiforme Lowe	Portugal, Madeira, Pico de Rancho W of Camara, 29011.
Sedum litoreum Guss.	Israel, Coastal Galilee, 33129.
Sedum meyeri-johannis Engler	Keyna, Mt Kenya, Naro Moru Route, 15515.
Sedum moranii R. T. Clausen	USA (Oregon), Rogue River Canyon, 31480.
Sedum morrisonensis Hayata	Taiwan (Nantou), Kum Yang, 31048.
Sedum oryzifolium Makino	Korea, Gojae Is., Haegumgang, 32771.
Sedum polytrichoides Hemsley	Korea, Jeju Is., Mt Halla, 32797.
Sedum reptans R. T. Calusen	Mexico (San Luis Potosi), near Alameda, 30661.
Sedum sarmentosum Bunge	China (Zhejiang), Hangzhou Botanic Garden, 30671.
Sedum sediforme (Jacq.) Pau[2]	Portugal (Beira Litoral), Vaguira Praia, 15429.
Sedum ursi 't Hart	Turkey (Mugla), Sandras Dagi, 30396.
Sempervivum armenum Boiss. & Huet[2]	Turkey (Bolu), Koroglu Daglari, 30411.
Villadia battessi (Hemsley) Rose	Mexico (Pedregal), San Angel, 29606.

[1] Sequence published by Mes (1995).
[2] Sequences published by Van Ham *et al.* (1994).

For the phylogenetic analyses of the sequence data (base substitutions only) we employed Wagner and generalized parsimony (Farris 1970; Albert *et al.* 1992) using PAUP version 3.1.1 (Swofford 1993). Heuristic searches for most parsimonius unweighted trees were performed with Tree-Bisection-Reconnection (TBR) and Nearest-Neighbor-Interchange (NNI) branch swapping, Steepest descent and Mulpars in conjunction with random addition sequences. Acctran was used as character optimization option. Weighted parsimony analyses used the TBR heuristic search option and the stepmatrix option of PAUP with increasing weights for transversions (Tv) over transitions (Ts). *Crassula multicava* Lem. was used as an outgroup because of high sequence divergence with the ingroup taxa (Van Ham 1994) and sister group relationship of *Crassula* L. to *Kalanchoe* Adan., *Sedum* and *Dudleya* Britton & Rose in cladistic analyses based on rbcL sequence variation (Chase *et al.* 1993). In addition to *Crassula multicava* eight functional outgroup species were added to the analysis because of uncertain relationships among some of the major lineages within the Crassulaceae (Van Ham 1994). Reliability of monophyletic groups was estimated using bootstrap analyses with 500 replicates (random addition sequence and NNI swapping, Felsenstein 1985) and decay analyses for trees 1–5 steps longer than the shortest Wagner tree

Table 2. Aligned sequences of the chloroplast DNA trnL(UAA)-trnF(GAA) intergenic spacer for 27 taxa of the Crassulaceae. Spacer length is indicated in the last row. Length of the alignment is 341 bp. Dashes indicate alignment gaps.

	1				50
Crassula multicava	AATCCC----	-CTCA-AAAG	GCTCCTTTGA	ATAACTAGAT	ATTCTAGTTC
Cotyledon orbiculata	AATCCC----	-CTAA-AAAG	TCTCATTTGA	TGACCTA---	-----AATTA
Hylotelephium telephium	AATCCC----	-CTAA-AAAG	TCTTCTTTGA	TTACCT----	-----AATTC
Sedum sediforme	AATCCC----	-CT-A-AAAG	TCTCATTTGA	TTACCT----	-----AATTC
Sempervivum armenum	AAGCCC----	-CTAA-AAAG	TATCATTTGA	TTACCT----	-----AATTC
Aeonium castello-paivae	AATCCC----	-CTAA-AAAG	TCTCATTTGA	TTGCCT----	-----AATTC
Sedum album	AATCCCCTAA	TCTAATAAAG	TATCCTTTTT	TTACCT----	-----AATTC
Sedum dasyphyllum	AATCCCCTAA	TCTAATAAAG	TATCATTTGA	TTACCT----	-----AGTTC
Sedum moranii	-ATCCC----	-CTAATTAAG	TATCATTTGA	TTACCT----	-----AATTC
Sedum polytrichoides	AATCCC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTC
Sedum morrisonensis	AATCCC----	-CTAATAAAG	TAGCATTTGA	TTACCT----	-----AATTC
Sedum sarmentosum	AATCTC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTC
Sedum oryzifolium	AATACC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTC
Sedum acre	AATCCC----	-CTAACAAAG	TAGCATTTGA	TTCCCT----	-----AATTC
Sedum bulbiferum	AATCCC----	-CTAA-AAAG	TAGCATTTGA	TTTCCT----	-----AATTC
Sedum meyeri-johannis	AATCCC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTA
Sedum aetnense	AATCCC----	-CTAA-AAGG	TAGCATTTGA	TTACCT----	-----AAT--
Sedum ursi	AATCCC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----ACTTC
Sedum litoreum	AATCCC----	-TTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTC
Sedum alpestre	AATCCC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTC
Sedum fusiforme	AATCCC----	-CTAA-AAAA	TAGCATTTGA	TTACCT----	-----AATTT
Sedum reptans	ACTCCC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTT
Sedum bourgaei	AATCCC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTT
Villadia battesii	AATCCC----	-CTTA-AAAG	TAGCATTTGA	TTACCTA---	-----AATTT
Sedum dendroideum	AATCCC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTT
Echeveria venezuelensis	AATCCC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTT
Pachyphytum compactum	AATCCC----	-CTAA-AAAG	TAGCATTTGA	TTACCT----	-----AATTT
	51				100
Crassula multicava	TAT-------	-----CATAC	TCTCTTTTCT	TTTCCTTAGT	GGTTCCAAGT
Cotyledon orbiculata	TTTAG-----	---GTCATAC	TCTCGTTTCT	ATTCATTAGT	GGTTTCAAGC
Hylotelephium telephium	TTTTT-----	-----CATAC	TCTCCTTTCT	TTTCATTAGT	GGTTTCAAGC
Sedum sediforme	TTTTT-----	---CTCATAC	TCCCGTTTCT	TTTCATTAGT	AGGTTCAAGC
Sempervivum armenum	TTTTT--CTC	ATACTCATAC	TCTCGTTTCT	TTTCATTAGT	CGTTTCAAGC
Aeonium castello-paivae	TTTTT-----	---TTCATAC	TCTCGTTTCT	TTTCATTAGT	AGTTTCAAGC
Sedum album	TTTTT-----	---CTCATAT	TCTCGTTTCT	TTTCA-TAGT	AGTTTCAAGC
Sedum dasyphyllum	TTTT------	--------AG	TCTCGTTTCT	TTTCA-TAGT	AGTTTCAAGC
Sedum moranii	TTTTT-----	---CTCATAC	TATCATTTCT	TTTCA-TAGT	AGTTTCCAGC
Sedum polytrichoides	TTTTT-----	---CTCAAAC	TCTTGTTTCT	TGGCA-TAGT	ATTTTCCAGC
Sedum morrisonensis	TTTTT-----	---CTCATAC	TCTTGTTTCT	TGGCA-TAGT	ATTTCCAAGC
Sedum sarmentosum	CTTTT-----	---CTCATAC	TCTGGTTTCT	TGGAA-TAGT	ATTTTCAAGC
Sedum oryzifolium	GTTTT-----	---CTCATAC	TCTCGTTTCT	TGGCA-TAGT	ATTTTAAAGC
Sedum acre	TTTTT-----	---CTCATAC	TCTCGTTTCT	TGGCA-TAGT	ATTTTCAAGC
Sedum bulbiferum	TTTTT-----	---CTCAAAC	TCTCGTTTCT	TGACA-TAGT	ATTTTGAAGC
Sedum meyeri-johannis	TTTTT-----	---ATCATAC	TCTCGTTTCT	TGGCA-TAGT	ATTTTCAAGT
Sedum aetnense	TTTTT-----	---CTCATCC	TCTCGTTTCT	TGGCA-TAGT	ATTTTCAAGC
Sedum ursi	TTTTT-----	---CTCAAAC	TCTCGTTTCT	TGGCA-TAGT	ATTTTCCAGC
Sedum litoreum	TTTTT-----	---CTCATAC	TCTCGTTTCT	TGGCA-TAGT	ATTATCCAGC
Sedum alpestre	TTTTT-----	---CTCAAAC	TCTCGTTTCT	TGGCA-TAGT	ATTTTCAAAC
Sedum fusiforme	TTT-------	---ATCATAC	TCTCGTTTCT	TGGCA-TAGT	ATTTTCAAAC
Sedum reptans	TTT-------	---CTCATAC	TCTCGTTTCT	TGACA-TAGT	ATTTTCAAAC
Sedum bourgaei	TTTTT-----	---CTCATAC	TCTCGTTTCT	TGGCA-TAGT	ATTTTCAAAC
Villadia battesii	TTTTTTT---	---CTCATAC	TCTCGTTTCT	TGGCA-TAGT	ATTTTCAAAC
Sedum dendroideum	TTTTT-----	---CTCATAC	TCTCGTTTCT	TGGCA-TAGT	ATTTTCAAAC
Echeveria venezuelensis	TTTTT-----	---CTCATAC	TCTCGTTTCT	TGGCA-TAGT	ATTTTCAAAC
Pachyphytum compactum	TTTTT-----	---CTCATAC	TCTCGTTTCT	TGGCA-TAGT	ACTTTCAAAC

Table 2. *Continued*

	101				150
Crassula multicava	TTGTT----A	TGTTTTTCAT	TCACTCTATT	----------	-ACTTCACAA
Cotyledon orbiculata	TTCTT----A	TGTTTCTCAT	TCACCCTATT	----------	-ATTTCACAA
Hylotelephium telephium	TTGTT----A	TGTTTCTCAT	TCACCCTATT	----------	-TTTTTACAA
Sedum sediforme	GTGTT----A	TGCTTCTCAT	TAACCCTATT	----------	-TTTTTACAA
Sempervivum armenum	TTGTT----A	TGTTTATCAT	TCACCCGATT	T---------	-TTTTTACAA
Aeonium castello-paivae	TTGTT----A	TGTTTCTCAT	TTACCCTATT	----------	-TTTTTACAA
Sedum album	TTGTT----A	TGTTTCGCAT	TCGACCAATT	TT--------	-TTTTTACAA
Sedum dasyphyllum	TTGGT----A	TGTTTCTCAT	TTAACCCATT	----------	-TTTTAAAAA
Sedum moranii	TTGTT----A	TCTTTCTCAT	TCAACCTATT	----------	-TTTTTACAA
Sedum polytrichoides	TTGTT----A	TGTTTCTCAW	TCCACCTATT	TTT-TTTTTA	CTTTTTACAA
Sedum morrisonensis	TTGTT----A	TGTTTCTCAT	TCAACCTATT	TTTTTTTTTA	CTTTTTACAA
Sedum sarmentosum	TTGTT----A	TGTTTCTCAT	TCAACCTATT	TTT-TTTTTA	CTTTTTACAA
Sedum oryzifolium	TTGTT----A	TGTTTCTCAT	TCAACCTATT	T---TTTTGA	CTTTTTACAA
Sedum acre	TTGTT----A	TGTTTCTCAT	TCAACCTATT	TT--ATCTTA	CTTTTTACAA
Sedum bulbiferum	TTGTT----A	TGTTTCTCAA	TCCACCTATT	TTTTTTTTTA	CTTTTTACAA
Sedum meyeri-johannis	TTGTT----A	TGTTTCTCAT	TCAACCTCTT	T---TTTTTA	CTTTTTACAA
Sedum aetnense	TTGTTTGGTA	TGTTTCTCAT	TCAAAC----	----------	----------
Sedum ursi	TTTTT----A	TTTTTCTCAA	TCCACCCATT	TTT-TTTTTA	CTTTTTACAA
Sedum litoreum	TTGTT----A	TGTTTCTCAT	TCAACCT---	--------TA	CTTTTTACGA
Sedum alpestre	TTGTT----A	TGTTTCTCAT	TCAACCTATT	TTTTTTTTTA	CTTTTTACAA
Sedum fusiforme	TTGTT----A	TGTTTCTCAT	TCAACCTATT	TT--GTTTTA	CTTTTTACAA
Sedum reptans	TTATT----A	TGTTTCTCAT	TCAACCTATT	TT--TTTTGA	CTTTTTACAA
Sedum bourgaei	TTGTT----A	TGTTTCTCAT	TCAACCTATT	TTT-TTTTTA	CTTTTTACAA
Villadia battesii	TTTTT----A	TGTTTCTCAT	TCAACCTATT	TTTTTTTTTA	CTTTTTACAA
Sedum dendroideum	TTGTT----A	TGTTTCTCAT	TCAACCTATT	TTTTTTTTTA	CTTTTTACAA
Echeveria venezuelensis	TTGTT----A	TGTTTCTCAT	TCAACCTATT	TT--TTTTTA	CTTTTTACAA
Pachyphytum compactum	TTGTT----A	TGTTTCTCAT	TCAACCTATT	TTT-TTTTTA	CTTTTTACAA

	151				200
Crassula multicava	A-------TC	GATCTGAGT-	------AAAA	TTGGATTCTC	TTATCACAGA
Cotyledon orbiculata	A-------GA	GATCCGATA-	------AAAA	TTGGATTCTC	TTATCACAAA
Hylotelephium telephium	A-------GA	GATCCTAGA-	------AAAA	TTGGATTATC	TTATTACAAA
Sedum sediforme	A-------GA	GATCCTAGA-	------AAAA	TTAGATTCTC	TTATCACAAA
Sempervivum armenum	A-------AA	AATGGTATA-	------AAAA	TTGGATTCTC	TTATCAAAAA
Aeonium castello-paivae	C-------GA	AATCCTATA-	------AAAA	TTGGATTCTC	TTATCACAAA
Sedum album	A-------AA	AATCCTAAA-	------TAAA	TTTGATTCTA	TTATCACAAA
Sedum dasyphyllum	A-------TA	AATCCTCTCA	AAATTAAAAA	TTGGACGCCC	TTCTCAAAAA
Sedum moranii	A-------GA	AATCCTATC-	------AAAA	TTGGATTCCC	TTATCAAAAA
Sedum polytrichoides	A-------GA	AATCCTATA-	------AAAA	TAGGATTCCC	TTATCCCAAA
Sedum morrisonensis	A-------GA	AATCCTATA-	------AAAA	TAGGATTCCC	TTATCCCAAA
Sedum sarmentosum	A-------GA	AATCCTATA-	------AAAA	TTGGATTCCC	TTATCCCAAA
Sedum oryzifolium	A-------GA	AATCCTAGA-	------GGAA	ATGGATTCCC	TTATCCCAAA
Sedum acre	A-------GA	AATCCTAGA-	------AAAA	TTGGATTTCC	TTATCCCAAA
Sedum bulbiferum	A-------AC	AATCCTATA-	------AAAA	TTGAATTCCC	TTATCCCAAA
Sedum meyeri-johannis	A-------GA	AATCCTATA-	------AAAA	TTGGATTCCC	TTTTCCCAAA
Sedum aetnense	----------	----------	----------	----------	----------
Sedum ursi	ATTACCAAGA	AATCCTATA-	------AAAA	TTGGATTCCC	TTATCCCAAA
Sedum litoreum	A-------GA	AATCCTATA-	------AAAA	TTGGATTCCT	TTATCCCAAA
Sedum alpestre	A-------GA	AATCCTATA-	------AAAA	TTGGATTCCC	TTATCCCAAA
Sedum fusiforme	A-------GA	AATCTTATA-	------AAAA	TTGGATTCCC	TTATCCCAAA
Sedum reptans	A-------GA	AATCCAAGA-	------AAAA	TGGGATTCCC	TTATCCCAAA
Sedum bourgaei	A-------GA	AATCCTATA-	------AAAA	TTGGATTCCC	TTATCCCAAA
Villadia battesii	A-------GA	AATCCTATA-	------CAAA	TTGGATTCCC	TTATCCCAAA
Sedum dendroideum	A-------GA	AATCCTATA-	------AAAT	TTGGATTCCC	TTATCCCAAA
Echeveria venezuelensis	A-------GA	AATCCTATA-	------AAAA	TTGGATTCCC	TTATCCCAAA
Pachyphytum compactum	A-------GA	AATCCTATA-	------AAAA	TTGGATTCCC	TTATCCCAAA

Table 2. *Continued*

	201				250
Crassula multicava	CTTAGAAAGT	ATAGGGACTG	GATAAAACTT	TCA-------	---------T
Cotyledon orbiculata	CTTAGAAAGT	CTAGGGACTG	TATCAGACTT	TAA-------	---------G
Hylotelephium telephium	CTTAGAAAGT	CTAGGGACTG	TATAAGACTT	TAA-------	---------T
Sedum sediforme	CTTAGAAAAT	CTCGGGACTG	TATAAGACTT	TAA-------	---------T
Sempervivum armenum	CTTAGAAAGT	ATAGGGGCTG	TATAAGACTT	TA--------	----TTTAAT
Aeonium castello-paivae	CTTAGAAAGT	ATAGGGACTA	TATAAGATTT	TAA-------	---------T
Sedum album	CTTAGAAAGT	CTAGGGACTG	TATACGATTT	TAA-------	---------T
Sedum dasyphyllum	CCTAGAAAGT	CTAGGGACTG	TATAAGACTT	TTT-------	---------A
Sedum moranii	--------GT	CTAAGGACTG	TATAAGACTT	TAA-------	---------G
Sedum polytrichoides	CTTAGAAAGT	CTAGGGTCTG	TATAAGACTT	TAA-------	----TTTAAT
Sedum morrisonensis	CTTAGAAAGT	TTAGGGACTG	TATAAGACTT	TAA-------	----TTTAAT
Sedum sarmentosum	CTTAGAAAGT	CTAGGGACTG	TATAATACTT	TGAATTTACA	A---TTTAAT
Sedum oryzifolium	CTGCGAAAGT	CTAGGGACTG	TATAAGACTT	TAA-------	----TTTAAT
Sedum acre	CTTAGAAAGT	CTAGGCGCTG	TATAAGACTT	TAATTGAATA	AAGATTGAAT
Sedum bulbiferum	CTTAGAAAGT	CTAGGGACTA	TATAAGACTT	TAA-------	----TTTAAT
Sedum meyeri-johannis	CTTAGAAAAT	CTAGGGACTG	TATAAGACTT	TAA-------	----TTTAAT
Sedum aetnense	----------	----------	----------	----------	----------
Sedum ursi	CTTAGAAAGT	CTAGGGACTG	TATAAGACTT	TAA-------	---TTTTAAT
Sedum litoreum	CTTAGAAAAT	CTAGGGACTA	TCTAAGACTT	TAA-------	----TTTAAT
Sedum alpestre	CTTAGAAAGT	CTAGGGACTA	TCTAAGACTT	TCA-------	----TTTAAT
Sedum fusiforme	CTTAGAAAGT	CTAGGGACTA	TCTAAGACTT	TAA-------	----TTTAAT
Sedum reptans	CTTAGAAAGT	CTAGGGAGTA	TCTAAGACTG	TAA-------	----TTTAAT
Sedum bourgaei	CTTAGAAAGT	ATAGGGACTA	TCTAAGACTT	TAA-------	----TTTAAT
Villadia battesii	CTTAGAAAGT	ATAGGGACTA	TCTAAGACTT	TAA-------	----TTTAAT
Sedum dendroideum	CTTAGAAAGT	CTAGGGTCTA	TCTAAGACTT	TAA-------	----TTTAAT
Echeveria venezuelensis	CTTAGAAAGT	CTAGGGACTA	TCTAAGACTT	TAA-------	----TTTAAT
Pachyphytum compactum	CTTAGAAAGT	CTAGGGACTA	TCTAAGACTT	TAA-------	----TTTAAT

	251				300
Crassula multicava	AAATGCCTTT	C---------	-------CAT	CTTATT--AA	TTGACATAGC
Cotyledon orbiculata	AAATACCCTT	TTTAGTAAAT	ACCCTTTCAT	TTTTTG--AA	TTGACATAGC
Hylotelephium telephium	AAATATCCTT	TTTAGTAACT	ACTCTTTCAT	TTTTTT--AA	TTGACATAGC
Sedum sediforme	AAATACCCTT	T---------	-------CAT	TTTTTTT-AA	TTGACATAGC
Sempervivum armenum	AAATACCCTT	T---------	-------CAT	TTTTTTTTAA	TTGACATAGC
Aeonium castello-paivae	AACTACCATT	T---------	-------CAT	TTTTTTTTAA	TTGACATAGC
Sedum album	AAATACCCTT	T---------	-------CAT	TTTTTT---A	TTGACATAGC
Sedum dasyphyllum	AAATACCCTT	G---------	-------CAT	TTTATT---A	TTGACATAGC
Sedum moranii	AAATACCCTT	T---------	-------CAT	TTTTTT---A	GTGACATAGC
Sedum polytrichoides	AAATACCCTT	T---------	-------CAT	TTTTTTT--A	TTGACATAGC
Sedum morrisonensis	AAATACCCTT	T---------	-------CAT	TTTTTTT--A	TTGACATAGC
Sedum sarmentosum	AAATACCCTT	T---------	-------CAT	TTTTTTT--A	TTGCCATAGC
Sedum oryzifolium	AAATACCCTT	T---------	-------CAT	TTTTTTT--A	TTGACATAGC
Sedum acre	AAATACCCTT	T---------	-------TAT	TTTTTTTT-A	TTGACATAGC
Sedum bulbiferum	AAATACCCTC	T---------	-------CAT	TTTTTTT--A	TTGACATAGC
Sedum meyeri-johannis	AAGGACCCTT	T---------	-------CAT	TTTTTTTT-A	TTGACATAGC
Sedum aetnense	----------	----------	-------CAT	TTTTTTTT-A	TTGACATAGC
Sedum ursi	AAATACCCTT	T---------	-------CAT	TTTTTTTT-A	TTGACATAGC
Sedum litoreum	AAATACCCTT	T---------	-------CAT	GTTTTTTT-A	TTGACATAGC
Sedum alpestre	AAATACCCTT	T---------	-------CAT	GTTTTTTT-A	TTGACATAGC
Sedum fusiforme	AAATACCCTT	T---------	-------CAT	GTTTTTT--A	TTGACATAGC
Sedum reptans	AAATACCCTT	T---------	-------CGT	GTTTTTTT-A	TTGACATAGC
Sedum bourgaei	AAATACCCTT	T---------	-------CAT	GTTTTTTT-A	TTGACATAGC
Villadia battesii	AAATACCCTT	T---------	-------CAT	GTTTTTTT-A	TTGACATAGC
Sedum dendroideum	AAATACCCTT	T---------	-------CAT	GTTTTTTT-A	TTGACATAGC
Echeveria venezuelensis	AAATACCCTT	T---------	-------CAT	TTTTTTTT-A	TTGACATAGC
Pachyphytum compactum	AAATACCCTT	T---------	-------CAT	GTTTTTTT-A	TTGACATAGC

Table 2. *Continued*

	301				341	
Crassula multicava	CCCGAGTCAT	CTAGTAAAAT	GAGACTGATG	CGTAAGGGGT	G	(260 bp)
Cotyledon orbiculata	CTCAAGTCAT	ATAGTAAAAT	GAGACTGATA	CGTAGGGGAT	G	(272 bp)
Hylotelephium telephium	CTCAAGTCAT	ATAGTAAACT	TAGACTGATA	CGGAGAGGAT	G	(269 bp)
Sedum sediforme	CTCAAGTCCT	ATAGTAAAAT	TGAACTGATA	CGCAAAGGGT	G	(255 bp)
Sempervivum armenum	CTCAAGTCAT	ATAGTAAAAT	TAGACTGATA	CGGAAAGGAT	G	(268 bp)
Aeonium castello-paivae	CTCAAGTCAT	ATAGTAAAAT	TAGACTGATA	TGGAAAGGAT	G	(257 bp)
Sedum album	CTCAAGTCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGAT	A	(261 bp)
Sedum dasyphyllum	CTCAAGTCAT	ATTGTAAAAT	TAGACTGATA	TGGAAAGGAT	G	(260 bp)
Sedum moranii	CTCAAGTCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGAT	G	(245 bp)
Sedum polytrichoides	CTCAAGTCAT	ATAGTCCAAT	TAGACTGATA	CGGAAAGGAT	G	(269 bp)
Sedum morrisonensis	CTCAAGTCAT	ATAGTAAAAT	TAGACTGATA	CGGAAAGGAT	G	(271 bp)
Sedum sarmentosum	TTCAAGTCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGAT	G	(277 bp)
Sedum oryzifolium	CTGCAAGTCG	TACGTAAAAT	TAGACTGATA	CGGAAAGAAT	?	(267 bp)
Sedum acre	CTCGCGTCAT	ATCGTAAAAT	TAAAGTGATA	CGGAAAGGAT	G	(281 bp)
Sedum bulbiferum	CTCAAGTCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGGT	G	(270 bp)
Sedum meyeri-johannis	CTCAAGTCAT	ATCGTAAAAT	TAGACTGATA	GGGAAAGGAT	G	(268 bp)
Sedum aetnense	CGCAAGTCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGAT	G	(163 bp)
Sedum ursi	CTCAAGTCAT	ATCGTAAAAT	TAGACTGATA	CAGAAAGGCT	G	(278 bp)
Sedum litoreum	CTC?AGTCAT	ATCATAAAAT	TAGACTGATA	CGGAAAGGAT	G	(260 bp)
Sedum alpestre	CTCAAGTCAT	TTCGGAAAAT	TAGACTGATA	CCGAAAGGAT	G	(271 bp)
Sedum fusiforme	CTGAAGTCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGGT	G	(266 bp)
Sedum reptans	CTCAAGTCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGAT	G	(267 bp)
Sedum bourgaei	CTCAAGTCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGAT	G	(270 bp)
Villadia battesii	CTCAAGTCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGAT	G	(274 bp)
Sedum dendroideum	CTCAAATCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGAT	G	(271 bp)
Echeveria venezuelensis	CTCAAATCAT	ATCGTAAAAT	TAGACTAATA	CGGAAAGGAT	G	(269 bp)
Pachyphytum compactum	CTCAAATCAT	ATCGTAAAAT	TAGACTGATA	CGGAAAGGAT	G	(270 bp)

(Donoghue *et al.* 1992; Johnson & Soltis 1994). Decay values were determined as in Gustafsson & Bremer (1995).

Cluster analysis based on Jukes-Cantor distances with pairwise deletion of positions containing gaps, was performed with Neighbor-Joining (Saitou & Nei 1987; Kumar *et al.* 1993).

RESULTS

The aligned sequences of the trnL (UAA)–trnF (GAA) intergenic spacer of 27 Crassulaceae are presented in Table 2. The list comprises seven sequences published by Van Ham *et al.* (1994) and one by Mes (1995). The length of the spacer varies from 163 bp in *Sedum aetnense* Ten. to 281 bp in *S. acre* L. The length of the spacer is within the range of values observed in other Crassulaceae (255–281 bp) investigated to date (Van Ham *et al.* 1994; Mes & 't Hart 1994), except for the spacer of *S. aetnense*, which is much shorter due to a unique deletion of 94 bp (position 127–177).

A major source of the length variation of the trnL–trnF spacer is the short (1–9 bp) indels. In the alignment of the spacer (Table 2) 29 indels (16 insertions and 13 deletions) have been hypothesized for the whole ingroup, using *Crassula multicava* to polarize the indels. The ingroup species are characterized by a 9 bp deletion (position 37–45), except for *Cotyledon* L. and *Villadia* Rose which have only an 8 bp deletion relative to

Crassula, and two 2 bp insertions, position 54–55 (excluding *Sedum fusiforme* Lowe, *S. dasyphyllum* L., and *S. reptans* Clausen), and position 64–65 (excluding *Hylotelephium* and *Sedum dasyphyllum*), respectively. The *Acre* lineage is characterized by two insertions, i.e. a 7 bp duplication (position 135–141), which is completely absent from *Sedum aetnense* and partly absent from *S. litoreum* Guss. probably due to overlapping deletions, and a 5 bp duplication (position 245–249), which is also found in *Sempervivum armenum* Boiss. & Huet.

Sequence divergence ranges from 32·87% (between *Crassula multicava* and *Sedum oryzifolium* Makino) to 1·12% (between *Pachyphytum compactum* Rose and *Sedum bourgaei* Hemsley, *S. dendroideum* Moc. & Sesse, and *Echeveria venezuelensis* Rose, and between *Villadia battesii* (Hemsley) Rose and *Sedum bourgaei*). Within the *Acre* lineage *Sedum bulbiferum* Makino and *S. oryzifolium* represented the most divergent species pair (12·87%).

Excluding alignment gaps 174 base substitutions were observed, of which 60 were phylogenetically informative (synapomorphic). Wagner analyses produced 290 equally most parsimonious trees (length 147 steps, consistency index 0·633, retention index 0·688) excluding uninformative characters. Figure 1 shows the strict consensus tree (SCT) with the character support, bootstrap values and decay values, indicated along the branches. The general topology of the SCT is very similar to phylogenies based on chloroplast DNA restriction-site and intergenic spacer sequence variation (Van Ham 1994; Van Ham *et al.* 1994). *Sedum* s.s. is paraphyletic and scattered over the tree in a series of clades with different support values. As in Van Ham's (1994) more extensive studies the large *Acre* lineage is a strongly supported monophyletic clade (bootstrap and decay values 96% and 4, respectively) defined by four unique base substitutions (position 23, 82, 92, and 196). A clade comprising the European *S. dasyphyllum* and American *S. moranii* R. T. Clausen is a sister group to the *Acre*-clade, although the support for this configuration is not very strong. The *Acre* lineage consists of a large polytomy comprising the five Asian *Sedum* species (*S. bulbiferum*, *S. morrisonensis*, *S. oryzifolium*, *S. polytrichoides* Hemsley and *S. sarmentosum*), the African *S. meyeri-johannis* Engler, and the Eurasian *S. acre*, *S. aetnense*, and *S. ursi* 't Hart and a weakly supported clade (bootstrap and decay values 53% and 1, respectively) defined by one unique synapomorphy (position 222) which comprises the Eurasian *Sedum alpestre* Vill. and *S. litoreum*, the Macaronesian *S. fusiforme* and the Mexican and South American species of *Echeveria* DC., *Pachyphytum* Link, Klotzsch & Otto, *Sedum*, and *Villadia*.

Weighting transversions over transitions resulted only in a few topological changes within the *Acre* lineage of the Wagner tree SCT. Most importantly at tv/ts ratios of 3/1 to 10/1 the terminal clade, which comprises the Mexican taxa and the Macaronesian and Eurasian *Sedum* s.s species, collapsed except for the monophyly of *S. bourgaei* and *Villadia battesii*.

Distance and parsimony methods produced similiar results, except for relationships within the *Acre* lineage (Fig. 2). Neighbor-Joining analysis distinguished three weakly supported groups among the Asian and Eurasian species of the large polytomy at the base of the *Acre* lineage. Of the five Asian *Sedum* s.s. species *S. oryzifolium* is united with *S. acre* whereas *S. bulbiferum*, *S. morrisonensis*, *S. polytrichoides*, and *S. sarmentosum* form a group together with the Eurasian *S. ursi*. Within this group *S. ursi* is most close to *S. bulbiferum* and *S. polytrichoides*.

DISCUSSION

The overall topology and the degree of support of the phylogenies based on the chloroplast DNA trnL–trnF intergenic spacer sequences of 27 Crassulaceae in this study agree with phylogenies of this family based on chloroplast DNA restriction-site variation as well as on the same chloroplast spacer for different samples (Van Ham *et al.* 1994; 't Hart 1995). All major lineages within the family were detected and the largest genetic distance within the family is between *Crassula multicava* and the other taxa which form the *Sedum* lineage. Most notable in this survey is the strongly supported, terminal lineage which is congruent with the *Acre* lineage (clade) in Van Ham's (1994) chloroplast DNA restriction-site variation studies.

The inclusion of the five alkaloid containing Asian *Sedum* s.s. species in the *Acre* lineage supports our hypothesis on the distribution of alkaloids in Crassulaceae (Stevens *et al.* 1995). It states that the occurrence of alkaloids in Crassulaceae is restricted to species of the *Acre* lineage which are characterized by a reticulate testa. Furthermore, these species often have sepals of unequal length (per flower), sometimes basally free sepals (spurred) and petals with quincuncial aestivation (instead of contorted). They are either glabrous or pubescent, but have no glandular hairs ('t Hart 1995). However, so far only *c.* 6% of the species of the Crassulaceae have been examined for alkaloids (about 12% of the *Sedum* species) and phylogenetic relationships of only 60 *Sedum* species (15%) have been determined with molecular methods. Furthermore, the distribution of critical morphological characters such as testa ornamentation and adnation of the sepals is still insufficiently known in *Sedum*, except for the species of Eurasia, Korea and northern North America (Fröderström 1931, 1932, 1936; Clausen 1975; 't Hart & Berendsen 1980; Kim 1988; 't Hart 1991; Knapp 1994). Nevertheless, the present results strengthen our hypothesis that in Crassulaceae seed ornamentation in combination with adanation and size of the sepals, aestivation and the presence of alkaloids are good diagnostic characters to delimit the *Acre* lineage of *Sedum* which is otherwise only characterized by molecular markers.

The results of the Wagner (unweighted and weighted) and the NJ analyses are highly congruent with respect to the monophyly and support of the *Acre* lineage (Figs 1, 2). In particular, the 7 bp insertion (position 135–141) is very significant although it is not a proper synapomorphy because of subsequent, superimposed deletions in *S. aetnense* and *S. litoreum* and numerous base substitutions. Although Van Ham *et al.* (1994) already observed this supposed duplication its significance only became clear in a survey of a large sample which enabled us to assert its general occurrence in this highly variable spacer region.

Within the *Acre* clade the Mexican and South American species of *Sedum*, *Echeveria*, *Pachyphytum*, and *Villadia* form a monophyletic clade together with the Macaronesian and some Eurasian *Sedum* species, although this clade is only weakly supported. The remaining Eurasian species and the Asian species form a large polytomy. Because this polytomy seems to result from homoplasy (multiple shortest topologies which differ only marginally) rather than from complete lack of synapomorphic characters it is regarded as a 'soft' polytomy (Maddison 1989). Consequently the polytomy does not reflect an event of rapid radiation or speciation, which is marked by a lack of synapomorphies, and it may be resolved through extended taxonomic sampling. Walter (1972) postulated that the subfamily Echeveriodeae evolved from a *Sedum*-like ancestor which presumably had reached Central America by way of the Canary Islands. Similar

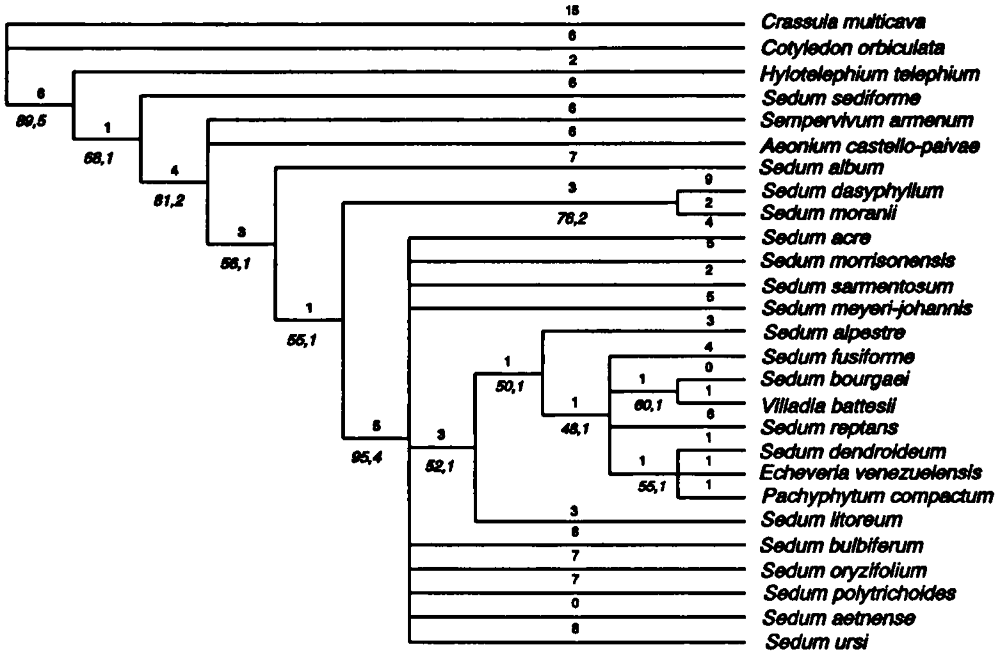

Fig. 1. Strict consensus tree from 290 equally parsimonious trees identified by Wagner parsimony analysis of the trnL (UAA)–trnF (GAA) intergenic chloroplast DNA spacer of 27 Crassulaceae species. The numbers above the branches indicate the number of substitutions; the two figures below the branches indicate bootstrap and decay values, respectively.

to Van Ham's (1994) chloroplast DNA restriction-site analyses our results are compatible with Walter's theory, despite the weakly supported terminal position of the American taxa within the *Acre* lineage and the limited taxonomic sampling (only about 3% of the species).

Distribution patterns of alkaloids in Eurasian *Sedum* s.s. species appeared to be significantly correlated with an infrageneric classification based on biosystematic characters and chloroplast DNA restriction-site analysis ('t Hart 1991; Stevens *et al.* 1992, 1993; Van Ham 1994). In particular, the presence of pyrrolidines and 2,6-disubstituted piperidines proved to be highly significant from a systematic point of view. Alkaloid composition among Asian species of *Sedum* s.s. varies considerably and Kim *et al.* (1996) tentatively distinguished four groups, i.e. (1) *S. oryzifolium* which contains a wide spectrum of alkaloids including the typical *S. acre* alkaloids sedamine and sedinone. Notable is the presence in *S. oryzifolium* of a 2,6-disubstituted piperidine (sedinone), which is only known from *S. acre* (Stevens *et al.* 1992, 1993). (2) *S. morrisonensis* and *S. polytrichoides* in which N-methylpelletierine and pelletierine are the main alkaloids. (3) *S. bulbiferum* which contains only pelletierine. (4) *S. sarmentosum* in which the principal alkaloids are N-methylallosedridine and N-methylpelletierine. Neither the Wagner nor the NJ analysis support the relationships inferred from alkaloid patterns. Notable, however, is the cluster containing *S. acre* and *S. oryzifolium* in the NJ analysis (Fig. 2), because the presence of pyrrolidines and 2,6-disubstituted piperidines in these two species seems to be highly significant from a systematic point of view (Stevens *et al.* 1992, 1993). The link between *S. acre* and *S. oryzifolium* indicated by the

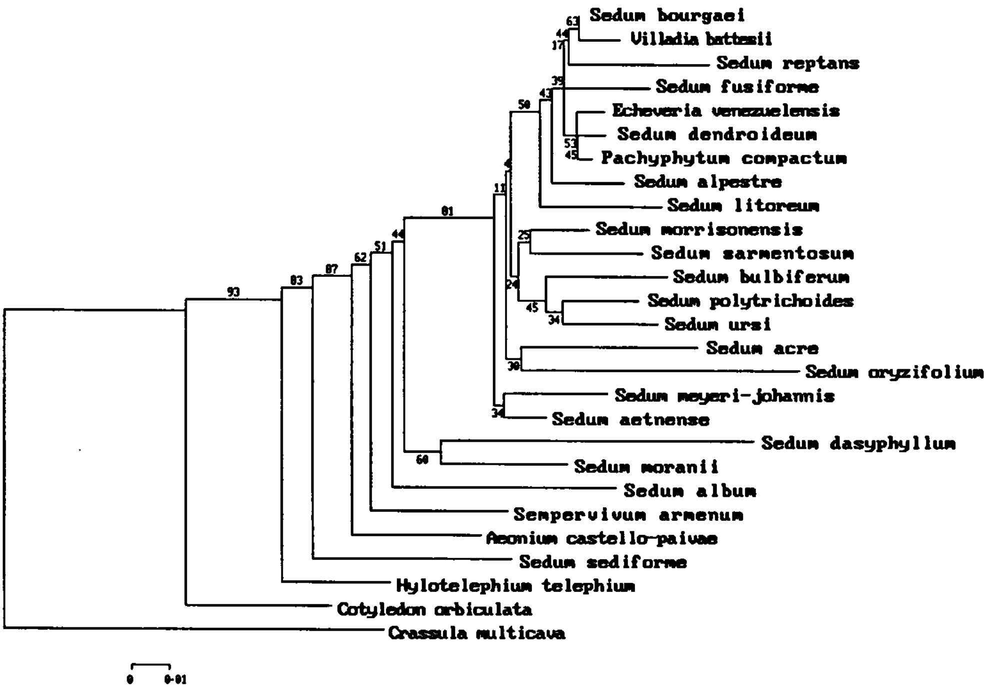

Fig. 2. Neighbour-joining tree based on Jukes–Cantor distances of the trnL (UAA)–trnF (GAA) chloroplast DNA spacer of 27 Crassulaceae species. The numbers above the branches indicate the percentage of occurrence of a monophyletic group in 500 bootstrap samples.

NJ analysis, however, is merely statistical, as it is not supported by a single synapomorphy of the trnL–trnF sequences of the two species. Infrageneric classifications of Asian *Sedum* s.s. by Berger (1930) and Fröderström (1931) differ considerably from infrageneric relationships indicated by molecular and phytochemical studies. Most notably, the intimate relationships between the alkaloidal, Asian *Sedum* species with reticulate seeds and similar Eurasian species, e.g. *S. acre* and the species of *S.* series *Alpestria*, have never before been hypothesized.

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REFERENCES

Albert, V.A., Mishler, B.D. & Chase, M.W. (1992): Character state weighting for restriction site data in phylogenetic reconstruction, with an example from chloroplast DNA. In: Soltis, P.S., Soltis, D.M. & Doyle, J.J. (eds.): *Molecular systematics of plants*, pp. 369–401, Chapman & Hall, New York.

Aldrich, J., Cherney, B.W., Merlin, E. & Christopherson, L. (1988): The role of insertions/deletions in the evolution of the intergenic region between psbA and trnH in the chloroplast genome. *Curr. Genet.* 14: 137–146.

Berger, A. (1930): Crassulaceae. In: Engler, A. & Prantl, K. (eds.): *Die natürlichen Pflanzenfamilien*, 2nd edn, vol. 18a, pp. 436–465, Verlag Wilhelm Engelmann, Leipzig.

Beyerman, H.C., Bordes, B.S.L., Maat, L. & Warnaar, F.M. (1972): Synthesis, optical rotatory dispersion and absolute configuration of

(+)-N-methyl allosedamine [(+)-(2R0-2-{(2S)-2-hydroxypropyl}-1-methylpiperidine] from *Sedum sarmentosum* Bunge and its isomers. *Rec. Trav. Chim.* **91**: 1441–1446.

Chase, M.W., Soltis, D.E., Olmstead, R.G. *et al.* (1993): Phylogenetics of seed plants: and analysis of nucleotide sequences from the plasmid gene rbcL. *Ann. Mo. Bot. Gard.* **80**: 528–580.

Clausen, R.T. (1975): Sedum *of North America north of the Mexican Plateau*, Ithaca, New York.

Donoghue, M.J., Olmstead, R.G., Smith, J.F. & Palmer, J.D. (1992): Phylogenetic relationships of Dipsacales based on rbcL sequences. *Ann. Mo. Bot. Gard.* **79**: 333–345.

Eggli, U., Hart, H. 't & Nyffeler, R. (1995): Toward a consensus classification of the Crassulaceae. In: Hart, H. 't & Eggli, U. (eds.): *Evolution and Systematics of the Crassulaceae*, pp. 173–192, Backhuys Publishers, Leiden.

Farris, J.S. (1970): Methods for computing Wagner trees. *Syst. Zool.* **19**: 100–104.

Felsenstein, J. (1985): Confidence limitation phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Fröderström, H. (1931): The genus *Sedum* L., 2. *Acta Horti Gothob.* **6: (Appendix)**: 1–111.

Fröderström, H. (1932): The genus *Sedum* L., 3. *Acta Horti Gothob.* **7: (Appendix)**: 1–125.

Fröderström, H. (1936): The genus *Sedum* L., 4. *Acta Horti Gothob.* **10: (Appendix)**: 1–262.

Guillemaut, P. & Marechal-Drousard, L. (1992): Isolation of plant DNA: a fast, inexpensive, and reliable method. *Pl. Mol. Biol. Rep.* **10**: 60–65.

Gustafsson, M.H.G. & Bremer, K. (1995): Morphology and phylogenetic interrelationships of the Asteraceae, Calyceraceae, Campanulaceae, Goodeniaceae, and related families (Asterales). *Amer. J. Bot.* **82**: 250–265.

Hart, H. 't (1991): Evolution and classification of the European *Sedum* species (Crassulaceae). *Fl. Medit.* **1**: 31–61.

Hart, H. 't (1995): Infrafamilial and generic classification of the Crassulaceae. In: Hart, H. 't & Eggli, U. (eds.): Evolution and Systematics of the Crassulaceae, pp. 159–172, Backhuys Publishers, Leiden.

Hart, H. 't & Berendsen, W. (1980): Ornamentation of the testa in *Sedum* (Crassulaceae). *Pl. Syst. Evol.* **135**: 107–117.

Hart, H. 't & Koek-Noorman, J. (1989): The origin of the woody Sedoideae (Crassulaceae). *Taxon* **31**: 535–544.

Johnson, L.A. & Soltis, D.E. (1994): MATK DNA sequencing and phylogenetic reconstruction in Saxifragaceae sensu stricto. *Syst. Bot.* **19**: 143–156.

Kim, J.H. (1988): *A taxonomic study of the genus Sedum in Korea*, Ph.D. thesis, Seoul National University, Seoul.

Kim, J.H., Hart, H. 't & Stevens, J.F. (1996): Alkaloids of Asian *Sedum*. *Phytochemistry* **41**: 1319–1324.

Knapp, U. (1994): Skulptur der Samenschale und Gliederung der Crassulaceae. *Bot. Jahrb. Syst.* **116**: 157–187.

Kumar, S., Tamura, K. & Nei, M. (1993): *Molecular Evolutionary Genetics Analysis (MEGA), version 1.0.* Pennsylvania State University, University Park, PA 16802, USA.

Maddison, W.P. (1989): Reconstructing character evolution on polytomous cladograms. *Cladistics* **5**: 365–377.

Marion, L. & Chaput, M. (1949): A new occurrence of *dl*-methylisopelletierine. *Can. J. Res.* **27B**: 215–217.

Mes, T.H.M. (1995): *Origin and evolution of the Macaronesian Sempervivoideae (Crassulaceae)*, Ph.D. thesis, Utrecht University, Utrecht.

Mes, T.H.M. & Hart, H. 't (1994): *Sedum surculosum* and *S. jaccardianum* (Crassualceae) share a unique 70 bp deletion in the chloroplast DNA trnL (UAA)–trnF (GAA) intergenic spacer. *Pl. Syst. Evol.* **193**: 213–221.

Morton, B.R. & Clegg, M.T. (1993): A chloroplast DNA mutational hotspot and gene conversion in a noncoding region near rbcL in grass family (Poaceae). *Curr. Genet.* **24**: 357–365.

Saitou, N. & Nei, M. (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.

Sanger, F., Nicklen, S. & Coulsen, A.R. (1977): DNA sequencing with chain-terminating inhibitors. *Proc. Natl Acad. Sci. USA* **74**: 5463–5467.

Schönland, S. (1981): Crassulaceae. In: Engler, A. & Pranlt, K. (eds.): *Die natürlichen Pflanzenfamilien 3, 2a*, pp. 23–38, Verlag Wilhelm Engelmann, Leipzig.

Stevens, J.F. (1995): Chemotaxonomy of the Eurasian Sedoideae and Sempervivoideae. In: Hart, H. 't & Eggli, U. (eds.): *Evolution and Systematics of the Crassulaceae*, pp. 45–75, Backhuys Publishers, Leiden.

Stevens, J.F., Hart, H. 't, Hendriks, H. & Malingre, T.M. (1992): Alkaloids of some European and Macaronecian Sedoideae and Sempervivoideae (Crassulaceae). *Phytochemistry* **31**: 3917–3924.

Stevens, J.F., Hart, H. 't, Hendriks, H. & Malingre, T.M. (1993): Alkaloids of the Sedum acre-group (Crassulaceae). *Pl. Syst. Evol.* **185**: 207–217.

Stevens, J.F., Hart, H. 't, Van Ham, R.C.H.J., Elema, E.T., Van den Ent, M.M.V.X., Wildeboer, M. & Zwaving, J.H. (1995): Distribution of alkaloids and tannins in the Crassulaceae. *Biochem. Syst. Ecol.* **23**: 157–165.

Swofford, D.L. (1993): *Phylogenetic analysis using parsimony (PAUP), version 3.1.1.* Illinois Natural History Survey, Champaign, Illinois, USA.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991): Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Mol. Biol.* **17**: 1105–1109.

Van Ham, R.C.H.J. (1994): *Phylogenetic implications of chloroplast DNA variation in the Crassulaceae,* Ph.D. thesis, Utrecht University, Utrecht.

Van Ham, R.C.H.J. (1995): Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA variation. In: Hart, N. 't & Eggli, U. (eds.): *Evolution and Systematics of the Crassulaceae,* pp. 16–29, Backhuys Publishers, Leiden.

Van Ham, R.C.H.J., Hart, H. 't, Mes, T.H.M. & Sandbrink, J.M. (1994): Molecular evolution of noncoding regions of the chloroplast genome in the Crassulaceae and related species. *Curr. Genet.* **25**: 558–566.

Walter, E. (1972): *Echeveria,* California Academy of Sciences, San Francisco.

Wolfson, R., Higgins, K.G. & Sears, B.B. (1991): Evidence for replication slippage in the evolution of *Oenothera* chloroplast DNA. *Mol. Biol. Evol.* **8**: 709–720.