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Phylogenetic relationships in *Monanthes* (Crassulaceae) based on morphological, chloroplast and nuclear DNA variation

T. H. M. Mes,^{1*} G.-J. Wijers² and H. 't Hart²

¹*Present address: Institut für Pflanzengenetik und Kulturpflanzenforschung, Abteilung Taxonomie, D-06466 Gatersleben, Germany, e-mail: mes@ipk-gatersleben.de*

²*Department of Botanical Ecology and Evolutionary Biology, Section of Population Genetics, Utrecht University, Padualaan 8, NL-3584 CH Utrecht, The Netherlands*

Key words: *Monanthes*; morphology; RAPDs; ITS; trnL – trnF.

Abstract

Phylogenetic relationships of all 10 recognized taxa of the genus *Monanthes* which is endemic to the Canary Islands and Salvage Islands, were investigated using the four data sets: morphology, sequences of the chloroplast DNA trnL (UAA) – trnF (GAA) intergenic spacer, ITS2 sequences of the nuclear ribosomal region and Random Amplified Polymorphic DNAs (RAPDs). In contrast to the molecular data, the morphological data were internally inconsistent which probably resulted from parallel or convergent evolution of morphological characters. The molecular data sets indicated that the genus is not monophyletic due to inclusion of the annual *M. icterica* which is the putative sister taxon of *Aichryson*, that *M. polyphylla* is the sister taxon of the perennial species of the genus, that *M. muralis* from Hierro is of allotetraploid origin and that *M. lowei*, *M. minima*, *M. brachycaulos*, *M. laxiflora*, *M. anagensis* and *M. muralis* from La Palma are closely related. Combined ITS2 sequences, trnL – trnF sequences and morphological data indicated that the relationships among three types of perennial growth forms, i.e. tiny rosettes, small, branched shrubs and diffuse branches shrublets, are highly dependent on the outgroup used. After deletion of the most distant outgroup and a taxon of alleged hybrid origin (*M. muralis*) relationships among the growth forms of *Monanthes* still could not be consistently resolved.

* Author for correspondence.

Introduction

Monanthes comprises about 10 taxa (Praeger, 1932; Nyffeler, 1992). It includes small to minute herbs with globose buds, long, filiform peduncles, 6-8(5-10)-merous, obdiplostemonous flowers, with free, narrow, greenish, yellowish or purplish petals and conspicuously large nectariferous scales (Praeger, 1932; Nyffeler, 1992). The Moroccan *Sedum surculosum* Cosson is often included in *Monanthes* (as *M. atlantica* Ball) because of its large nectariferous scales and a general resemblance in habit and flower morphology (Ball, 1878; Berger, 1930; Uhl, 1961; Galland, 1988; Nyffeler, 1992). Recent molecular studies showed, however, that *Sedum surculosum* shares a unique 70 bp deletion in the chloroplast DNA trnL (UAA) – trnF (GAA) intergenic spacer with the Moroccan *S. jaccardianum* Marie & Wilczek and consequently should be excluded from *Monanthes* (Mes and 't Hart, 1994). *Monanthes* is generally included in the so called Macaronesian Sempervivoideae together with *Aeonium* (including *Greenovia*) and *Aichryson* (Praeger, 1932; Lems, 1960; Lösch, 1990; Mes, 1995). Molecular data further indicate that the Macaronesian Sempervivoideae are monophyletic as are the constituent genera *Aeonium*, *Aichryson*, and *Monanthes* (Mes, 1995; Mes et al., 1996). The sister taxon of the Macaronesian Sempervivoideae is *Sedum* series *Monanthoidea* (Batt.) Mes. It is endemic to Morocco and comprises *S. modestum* Ball, *S. jaccardianum*, and *S. surculosum* (Mes et al., 1996). *Aeonium*, which comprises 41 species, is the putative sister genus of *Monanthes*.

Species of *Monanthes* differ considerably in life-form and growth-form and this diversity has been emphasized in infrageneric classification. There is one annual species, *M. icterica*, whereas the other taxa are perennial and have erect, diffusely branched shoots and elliptic leaves, or form tufts of decumbent (rarely ascending, much branched shoots with dense terminal rosettes. Finally, some species have monopodial, usually compact rosettes and axillary, stoloniferous offsets of variable length. Sventenius (1960) distinguished four sections which correspond with the four life-form and growth-form types. Nyffeler (1992) distinguished only three sections including the annual *M. icterica* in the group of species with monopodial, compact rosettes because of a similar flower morphology.

Cytologically *Monanthes* is very uniform (Tab. 1). Most taxa have a secondary basic number of $x = 18$ and very small chromosomes, except for *M. icterica*, which has a basic number of $x = 10$ and large chromosomes (Uhl, 1961; 't Hart and Nyffeler, pers. comm.). Most taxa are diploid with a chromosome number of $2n = 36$. Tetraploids ($2n = 72$) have occasionally been found in *M. laxiflora* and *M. pallens* and predominate in *M. polyphylla* ssp. *polyphylla* and *M. muralis*. *M. anagensis* is most probably hexaploid ($2n = 108$).

Monanthes occurs on all seven Canary Islands and the Salvage Islands, but its centre of diversity is Tenerife, the largest of the Canary Islands and the centre of the archipelago (Praeger, 1932; Nyffeler, 1992). Seven out of nine species of *Monanthes*, including two one-island endemics, *M. anagensis* and *M. minima*, have been reported for this island alone. The two easternmost islands, Fuerteventura and Lanzarote, have only *M. laxiflora*. For Hierro, the westernmost island, only *M.*

muralis has been reported and *M. lowei* is the only species occurring on the Salvage Islands (North of the Canary Islands). *M. laxiflora* is the most widely distributed species and occurs on the five easternmost islands (except for Hierro and La Palma). *M. polyphylla* occurs on four western islands whereas *M. brachycaulos*, *M. ictERICA*, *M. muralis* and *M. pallens* are restricted to only two of the five western islands each. Morphological differentiation of geographically separated populations has been reported for the widely distributed *M. brachycaulos*, *M. laxiflora*, *M. muralis*, *M. pallens*, and *M. polyphylla* (Nyffeler, 1992). In the latter four species the morphological and geographical differentiation is accompanied by polyploidy.

Extensive interspecific hybridization has been reported in the Macaronesian Sempervivoideae (Praeger, 1929, 1932; Jacobsen and Rowley, 1973; Bañares, 1990; Nyffeler, 1992). In nature hybrids of *Monanthes* are found at many sites where two or more perennial taxa are sympatric (hybrids involving the annual *M. ictERICA* are as yet unknown). The hybrids are of restricted distribution and usually occur only in small numbers or as single plants. The distribution of the perennial species and subspecies of *Monanthes* on the Canary Islands shows that there are about 12 sympatric combinations of two taxa which could produce hybrids, and so far about 10 alleged interspecific hybrids have been reported (Praeger, 1932; Nyffeler, 1992). Some hybrids can be recognized at a glance, in particular all hybrids involving *M. laxiflora*, but more frequently the hybrid nature of a plant is very difficult to determine. Nyffeler (1995) supplied experimental support for the possibility of

Table 1. Chromosome numbers in *Monanthes*.

<i>Monanthes</i>	2n =	Reference
sect. <i>Annua</i> Sventenius		
<i>M. ictERICA</i> (Webb ex Bolle) Christ	20	't Hart and Nyffeler (pers. comm.)
sect. <i>Monanthes</i>		
<i>M. polyphylla</i> Haworth	36, 72	Uhl (1961)
subsp. <i>amydros</i> Nyffeler	36	't Hart and Nyffeler (pers. comm.)
subsp. <i>polyphylla</i>	36	Uhl (1961)
	72	't Hart and Nyffeler (pers. comm.)
<i>M. muralis</i> (Webb ex Bolle) Hooker	72	Uhl (1961)
	36, 72	't Hart and Nyffeler (pers. comm.)
sect. <i>Petrophyllaea</i> Sventenius ex P. V. Heath		
<i>M. lowei</i> (Paiva) Perez and Acebes	36	Uhl (1961)
	36	't Hart and Nyffeler (pers. comm.)
<i>M. brachycaulos</i> (Webb and Berth.) Lowe	36	Uhl (1961)
	36	't Hart and Nyffeler (pers. comm.)
<i>M. minima</i> (Bolle) Christ	36	Uhl (1961)
<i>M. pallens</i> (Webb) Christ	36	Uhl (1961)
	36, 72	't Hart and Nyffeler (pers. comm.)
sect. <i>Sedoidea</i> Sventenius ex Nyffeler		
<i>M. laxiflora</i> (DC.) Bolle ex Bornm.	36	Uhl (1961)
	36, 72	't Hart and Nyffeler (pers. comm.)
<i>M. anagensis</i> Praeger	c. 72	Uhl (1961)
	108	't Hart and Nyffeler (pers. comm.)

hybridization in *Monanthes*. Hybrids among taxa at the same ploidy-level (diploid as well as tetraploid) turned out to be highly fertile. Pollen fertility of triploid hybrids was considerably impaired (less than 25%), but these plants produced extra large pollen grains which most probably are unreduced (or $2n$) gametes. Large pollen grains have also been observed in *Sedum* hybrids which in a few cases produced spontaneous amphiploid offspring ('t Hart, 1987).

We determined the evolutionary relationships among the species of *Monanthes* and the impact of reticulation in the genus by means of phylogenetic analysis of morphological data, sequence variation of the chloroplast *trnL* – *trnF* chloroplast intergenic spacer, Transcribed Spacer 2 (ITS2) sequences of the nuclear ribosomal DNA, and RAPDs.

Materials and methods

Morphological and anatomical data presented by Nyffeler (1992) on eleven taxa of *Monanthes* (including *Sedum surculosum*) were used for a Principal Component Analysis (PCA) and a cladistic analysis. In Table 2, the compiled data set for the 30 characters (unordered, bi- and multistate characters) is presented. The data were transformed to accommodate for Nyffeler's (1992) character states "present in almost all cases" and "occasionally or partly present". For outgroup comparison *Aichryson tortuosum* was added to the data set (Mes, 1995; Mes et al., 1996). For the Principal Components Analysis of the morphological data set we used the program SPSS for Windows with means used for missing values. Morphological data were cladistically analysed using PAUP's (version 3.1.1.) branch-and-bound option. In the cladistic analyses all phylogenetically non-informative characters were excluded. *Aichryson tortuosum* and *Sedum surculosum*, which are both closely related to *Monanthes* (Mes, 1995; Mes et al., 1996) were used as outgroups.

Total DNA was isolated as described by Guillemaut and Marechal-Drouard (1992), although slightly modified (Mes and 't Hart, 1994), from plants collected in nature and cultivated in the greenhouse of the Botanic Garden of Utrecht (Tab. 3).

For amplification of chloroplast and nuclear sequences universal primers were used (Taberlet et al., 1991; White et al., 1990). Sequences were determined by the method of Sanger et al. (1977) using the Taq Dye Deoxy Terminator Cycle Sequencing Kit and the 373 Automated DNA Sequencer. Chloroplast sequences were determined for five species of *Monanthes*, and nuclear sequences were determined for all species currently recognised (Nyffeler, 1992), with multiple accessions for some species. Alignment of sequences was performed with PCGENE (Intelligence) with manual editing. In order to provide the most unambiguous polarisation of mutations for the chloroplast and nuclear sequences we included five North African species of *Sedum*, six species of *Aeonium*, and three species of *Aichryson* to the sequence data, in addition to the outgroup species *Sedum sediforme* and *Sempervivum ciliosum*, which are quite distantly related to the Macaronesian Sempervivoideae (van Ham, 1994).

Table 2. Morphological characters of the species of *Monanthes* used for cladistic analysis and Principal Coordinate Analysis.

	1	3 0
<i>M. anagensis</i>	00?0110?? ? 10110001010111000000	
<i>M. laxiflora</i>	00?1111?? ? 11100001010111000000	
<i>M. lowei</i>	0100000100000000000000001000000	
<i>M. brachycaulos</i>	0110000100011110000000001000010	
<i>M. minima</i>	0110000101000010000000001100110	
<i>M. pallens</i>	0110000201000211000000001100111	
<i>M. ictERICA</i>	10?0110?? ? 0110000?000001000000	
<i>M. polyphylla</i> subsp. <i>amydros</i>	00?110021101101011101011111111	
<i>M. polyphylla</i> subsp. <i>polyphylla</i>	00?210021100121101101011111111	
<i>M. muralis</i>	00?110001001111011101011111111	
<i>Aichryson tortuosum</i>	00?111000? 11101001000011001110	
<i>Sedum surculosum</i>	010000000000?00000000000000000	

Characters

1. life form 0 = perennial 1 = annual
2. growth form 0 = branched 1 = simple
3. offsets 0 = rarely 1 = regular
4. habit of axes 0 = erect 1 = decumbent 2 = trailing
5. shape of axes 0 = short, stout 1 = long, slender
6. internodes 0 = compressed 1 = elongate
7. phylotaxis 0 = alternate 1 = decussate
8. rosette 0 = loose 1 = compact 2 = dense
9. rosette 0 = wider than high 1 = higher than wide
10. orientation of leaves inner rosette 0 = patent 1 = erect
11. leafshape 0 = obovate 1 = ovate elliptic
12. leafshape 0 = ratio 6:1 to 3:1 1 = ratio 2:1 to 3:2
13. leaf base 0 = attenuate 1 = cuneate
14. leaf apex 0 = rounded, acute 1 = acuminate 2 = truncate
15. leaf indumentum 0 = no, few glandular hairs 1 = glandular hairs
16. leaf surface 0 = smooth or upper margin papillose 1 = upper part papillose
17. leaf surface 0 = smooth or upper part papillose 1 = upper margin papillose
18. inflorescence arising from 0 = branched, simple floriferous shoot 1 = tips of vegetative shoot
19. pedicels 0 = evenly arranged 1 = basally clustered
20. indumentum of pedicels, sepals 0 = glandular hairs 1 = few, very short glandular hairs
21. indumentum of pedicels, sepals 0 = glandular hairs < 0.6 mm 1 = glandular hairs > 0.6 mm
22. bud shape 0 = globular 1 = conical
23. number of flower elements 0 = 5, 6, or 7 1 = 8 or 9
24. petal shape 0 = ovate 1 = oblong
25. petal shape 0 = other 1 = narrowly oblong
26. glandular hairs of petal indumentum 0 = < 0.3 mm 1 = 0.3 mm - 1.2 mm
27. nectary lamina 0 = cuneate, rarely attenuate 1 = truncate, cordate, distinctly clawed
28. margin of nectary lamina 0 = crenulate 1 = erose
29. carpel indumentum 0 = no glandular hairs 1 = some glandular hairs
30. carpel surface 0 = smooth 1 = papillose

Table 3. Origin and accession numbers of the plants studied. Accessions indicated with an asterisk were used for sequence determinations.

Monanthes HAWORTH

Monanthes sect. *Monanthoidea*

M. lowi (PAIVA) PÉREZ & ACEBES

Portugal, Selvagens, Island, 31951*.

M. brachycaulos (WEBB & BERTHELOT) LOWE

Spain, Canary Islands, Tenerife, Punta de Teno, 31601. Spain, Canary Islands, Tenerife, near Guimar, Barrance Teguigo near Los Morrenos, 31946*. Spain, Canary Islands, Gran Canaria, Las Lagunetas along the road to Cruz de Tejada, 32180*.

M. minima (BOLLE) CHRIST

Spain, Canary Islands, Tenerife, Las Rosas, 2 km SW of Guimar along the road to Alta Vista, 32175*.

M. pallens (WEBB) CHRIST

Spain, Canary Islands, Tenerife, Barranco del Infierno, 31610*. Spain, Canary Islands, Tenerife, Barranco del Infierno, 31954. Spain, Canary Islands, Tenerife, Adeja, Barranco del Infierno, 32746.

M. icterica (WEBB EX BOLLE) CHRIST

Spain, Canary Islands, Tenerife, below Teno, along footpath across hills towards Las Casas and Punta de Teno, 32737*.

Monanthes sect. *Monanthes*

M. polyphylla HAWORTH

M. polyphylla ssp. *polyphylla*

Spain, Canary Islands, Tenerife, Teno, along the road to Punta de Teno, 31956. Spain, Canary Islands, Tenerife, Teno, 500 m W of the tunnel to Punta de Teno, 32177. Spain, Canary Islands, Tenerife, Barranco de Las Cuevas, about 9 km W of Buenavista, 32187*. Spain, Canary Islands, Tenerife, Santa Ursula, 32744.

M. polyphylla ssp. *amydros*

Spain, Canary Islands, Gomera, E part of the Island, above Puntallana near the village, 31955. Spain, Canary Islands, Gomera, alle Gran Rey, along the road from Vizcaina to El Cercado, 31957. Spain, Canary Islands, Gomera, Barranco del Valle, 1.5 km S of Vallehermosa, 32179. Spain, Canary Islands, Gomera, Degollada de la Cumbre, on rocks NW of the N portal of the tunnel, 32183*. Spain, Canary Islands, Gomera, Barranco de la Villa, 32754.

M. muralis (WEBB EX BOLLE) HOOKER

Spain, Canary Islands, Hierro, laurel forest along the descent to El Golfo, on cliffs along footpath, 31958. Spain, Canary Islands, Hierro, Valverde on a wall, 31959. Spain, Canary Islands, Hierro, Laurisilva, descent to El Golfo, on cliffs, 32176*. Spain, Canary Islands, La Palma, along the road from Los Quemados to Volcán de Teneguia, 32708*. Spain, Canary Islands, Hierro, Taibique, 32750*.

Monanthes sect. *Sedoidea* SVENENIUS EX NYFFELER

M. anagensis PRAEGER

Spain, Canary Islands, Tenerife, Anaga mountains, vicinity of Los Carboneras, 31608*. Spain, Canary Islands, Tenerife, Anaga mountains, S of El Bailadero, along the road to San Andres, 32181.

M. laxiflora (DC.) BOLLE

Spain, Canary Islands, Gomera, Bosque del Cedro, 30486*. Spain, Canary Islands, Gomera, near El Cedro, laurel forest along the road to Carretera del Sur, 31944. Spain, Canary Islands, Tenerife, Anaga region, El Bailadero, 32184.

Table 3. (continued)*Aeonium* WEBB & BERTH.

*A. diplocyclum** (DC.) WEBB EX BOLLE. Canary Islands, Tenerife, 30443. *A. glutinosum** (AITON) WEBB & BERTH. Madeira, valley of the river Machico, 30452. *A. haworthii** SALM-DYCK EX WEBB & BERTH. Canary Islands, Tenerife, 9 km N of Masca, 31487. *A. sedifolium** (WEBB EX BOLLE) PIT. & PROUST. Canary Islands, Tenerife, Barranco de Masca, 31908. *A. simsii** (SWEET) STEARN. Canary Islands, Gran Canaria, 250 m N of Ayacata, 30440. *A. tabuliforme** (HAW). WEBB EX BERTH. Canary Islands, Tenerife, 1 km S of Buenavista del Norte, 31918.

Aichryson WEBB & BERTH.

*A. dichotomum** (DC.) WEBB & BERTH. Canary Islands, Tenerife, 31938. *A. punctata** (CHR. SM. EX BUCH) WEBB & BERTH. Madeira, 30462. *A. tortuosum** (AIT.) WEBB & BERTH. Canary Islands, Lanzarote, 30466.

Sedum L.

S. surculosum COSS. var. *surculosum**. Morocco, 1 km NE of Oukaimeden, 2460 m, Ait Lafkih c.s. 601, 1989 (RNG; Herb. Inst. Argon. Vet. Hassan II, Rabat). *S. jaccardianum** MAIRE & WILCZEK. Morocco, 24 km S of Azrou, 32219. *S. modestum** BALL. Morocco, Taza-Jebel Tazzeke, 32226. *S. caeruleum** L. Tunisia, Cap Serrat, 31621. *S. pubescens** VAHL. Tunisia, Jebel Ichkeul, 31627. *S. sediforme** (JACQ.) PAU. Portugal (Beira Litoral), Vaguira Praia, 15429.

Sempervivum L.

*Sempervivum ciliosum**. Greece, Mt. Siniaski, 31473.

In cladistic analysis of the sequence data, most parsimonious trees (MPTs) were obtained using Tree Bisection-Reconnection (TBR), mulpars, steepest descent and 500 random additions of taxa. Bootstrap analysis (Felsenstein, 1985; 100 addition replicates, 5 random addition replicates per bootstrap replicate; replicate aborted if 32 000 trees were found) and decay analysis (Donoghue et al., 1992; Soltis et al., 1993) were used to assess support for monophyletic groups. In analysis of the chloroplast and nuclear sequence data sets, branches with decay values higher than 2 were very difficult to find due to the highly structured data matrices. Therefore a Strict Consensus Tree (SCT) of all trees up to two steps longer than the MPTs was computed and the converse of this topology was used as a constraint in heuristic searches to determine decay values of the stronger supported branches (Johnson and Soltis, 1994; Morgan et al., 1994). In addition, in decay analyses of the sequence data, non-minimal trees were searched using 10 000 random additions without swapping. SCTs from both strategies were combined to determine decay values. The character state optimization option minF was used to determine branch-lengths (including autapomorphies). Cluster analysis of the chloroplast and nuclear sequences based on Jukes-Cantor distances with pairwise deletions of positions containing gaps, was performed with Neighbor-Joining (Saitou and Nei, 1987; Kumar et al., 1993).

Amplification of RAPDs was according to Yu and Pauls (1992). For amplification of RAPDs of *M. polyphylla*, *M. muralis* and a limited number of accessions of all species of the genus (see Fig. 5), we used "operon primers" F1, F4, F9, F10, F12, F14, C1, C2, and C8. Presence and absence of RAPDs was compiled into a binary

datamatrix and ambiguities were coded as missing (?) in the datamatrix. We did not determine homology of any of the RAPDs with Southern hybridization. For a Principal Coordinate Analysis of the RAPD data, zero-zero combinations were not used to calculate similarity values, i.e. absolute character state differences (Manhattan metric) divided by the maximum value of that characters over all taxa (=Gower metric; Gower, 1971), the latter of which were used as input for PCA using the program PCO3D, kindly provided by Dr. RP Adams (1993). For graphical presentation we used SPSS for Windows. RAPDs were used to determine the distribution of RAPD fragments between *M. icterica* and the perennial species of *Monanthes* and to determine the phylogenetic position of *M. muralis* from Hierro among the other perennial species of *Monanthes*.

MPTs for the RAPD data set were searched for using options TBR branch swapping, steepest descent and mulpars with 500 random additions of taxa. Options used for bootstrap analysis were identical to that of the sequences, except for the use of TBR branch swapping. The cladogram based on RAPDs were midpoint rooted only for presentation and are interpreted as unrooted networks. Cluster analysis was also performed using Neighbor-Joining and I-Jaccard's (1908) coefficient as a distance measure.

Morphological data, chloroplast and nuclear sequences were combined and cladistically analyzed. All characters were treated unordered and unweighted. PAUP's branch-and-bound option was used to calculate MPTs, as well as to determine support values. *Aichryson tortuosum* and *Monanthes icterica* were used as outgroups, either singly or in combination.

Results

Morphology

Principal Component Analysis of the morphological data of *Monanthes* (including *Sedum surculosum*) corroborates Nyffeler's classification in a (more) formal way (Fig. 1). The first two principal axes covered 63.8% of the variation, whereas the third became relatively insignificant (11.4%). The morphological characters which contribute most include growth form, the shape of axes and rosettes, the position of inflorescence, arrangement of pedicels, indumentum of pedicles, and the size of the hairs of the petal indumentum. When the first two axes were plotted, three clusters were found which agree with the three sections distinguished by Nyffeler. The cluster comprising *M. polyphylla* and *M. muralis* is the most cohesive cluster. Similarities among the species are lowest in *M. sect. Monanthoidea* which comprises, in addition to *M. brachycaulos*, *M. lowei*, *M. minima*, and *M. pallens*, the aberrant *S. surculosum* (= *M. atlantica*) and the cytologically distinct *M. icterica* (Tab. 1). *M. laxiflora* and *M. anagensis* form another cluster.

Cladistic analysis of the morphological data (3 unique and 27 informative characters) using both *Aichryson tortuosum* and *Sedum surculosum* as outgroups,

indicated that *Monanthes* is not monophyletic (46 steps, consistency 0.61, retention index 0.75), since both outgroup species provided different root positions. When a monophyly constraint was enforced, sixteen shortest trees (length 58, consistency 0.55) were obtained, which were much longer than the trees generated with the use of either *Aichryson tortuosum* or *Sedum surculosum* as outgroup (46 steps). In the strict consensus tree (Fig. 2a) relationships among the ingroup taxa were unresolved, except for a strongly supported clade comprising both subspecies of *M. polyphylla* together with *M. muralis* and less well-supported monophyly of *M. laxiflora* and *M. anagensis*. The single shortest tree with *Aichryson tortuosum* as an outgroup (length 46, consistency 0.65; Fig. 2b) and the two shortest trees (length 46, consistency 0.67; Fig. 2c) obtained with *Sedum surculosum* as an outgroup also strongly supported monophyly of *M. polyphylla* and *M. muralis*, but either as sister taxon to the other species of *Monanthes* (*Aichryson tortuosum*) or terminal in a weakly supported larger clade which also comprised *M. pallens* and *M. minima* (*Sedum surculosum*). In both cladograms monophyly of *M. laxiflora* and *M. anagensis* is supported with *M. icterica* as a sister species. Both topologies were retained when the morphological characters were weighted according to the rescaled consistency index with base weight 1000 (Farris, 1969).

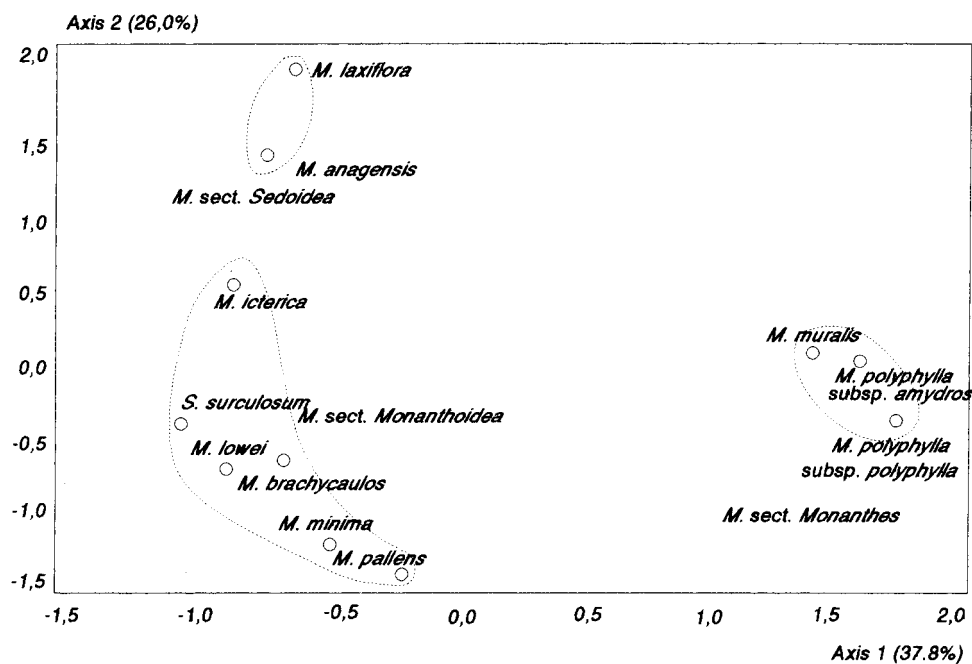


Fig. 1. Principal Component Analysis of the morphological data of the species of *Monanthes* and *Sedum surculosum* (from Nyffeler, 1992). Missing values were treated as intermediate values. The clusters (dotted lines) indicate sectional classification according to Nyffeler (1992).

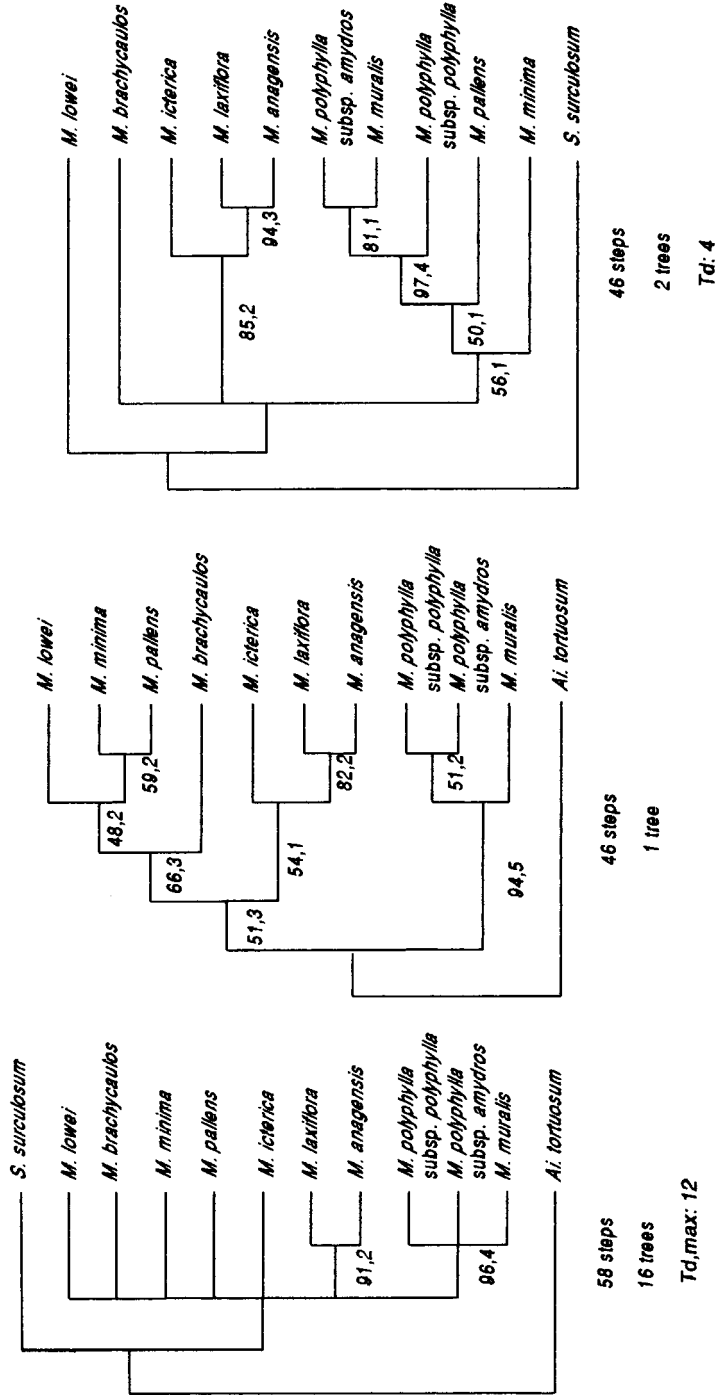


Fig. 2. Left: Strict consensus tree of 16 shortest cladograms of 61 steps of the species of *Monanthus* based on morphological data using *Sedum surculosum* and *Aichryson tortuosum* as an outgroup. *Monanthus* was forced to be monophyletic. Middle: Single shortest cladogram of 49 steps of the species of *Monanthus* based on morphological data using *Aichryson tortuosum* as an outgroup. Right: Strict consensus tree of two shortest cladograms of 49 steps of the species of *Monanthus* based on morphological data using *Sedum jaccardianum* as an outgroup.

Chloroplast DNA

Chloroplast DNA trnL – trnF spacer sequences were determined for diploid *M. icterica*, *M. laxiflora*, *M. brachycaulos*, and *M. polyphylla* ssp. *polyphylla* and a tetraploid accession of *M. muralis* from Hierro. The sequences were aligned along the species of *Aeonium*, *Aichryson*, *Sedum*, and *Sempervivum*. The aligned sequences are 286 positions long. Within *Monanthes*, the length of the spacer varies from 248 (*M. polyphylla* ssp. *polyphylla*) to 256 bp (*M. icterica*). Excluding alignment gaps, 82 variable, and 25 informative base substitutions were found in the entire alignment. Three were outgroup variable positions, one characterized all perennial species of *Monanthes* (excluding *M. icterica*), and one was informative within *Monanthes*. No base substitutions characterized *Monanthes*. The other 20 phylogenetically informative base substitutions were synapomorphic for other combinations of taxa. Sequence divergence within *Monanthes* ranges from 0.00% (between *M. laxiflora* and *M. muralis* from Hierro) to 3.05% (between *M. icterica* and *M. muralis* from Hierro).

In the alignment (obtainable from the authors), 13 indels have been hypothesised (outgroup variable and indels associated with replication slippage excluded). When *Sedum sediforme* and *Sempervivum ciliosum* (Mes, 1995; Mes et al., 1996), were used to polarise the indels, four insertions and nine deletions are present in the whole ingroup. Four indels characterize one or more species of *Monanthes*. A 10 bp deletion [41–50], a 4 bp insertion [217–220] and a putative 1 bp deletion are present in all species examined except for *M. icterica*. Finally, a 7 bp insertion [132–138], present in *M. laxiflora*, *M. muralis*, *M. brachycaulos*, is also found in *Sedum pubescens* and *Aichryson punctata*, albeit with some slight sequence alternations. It is absent from *M. polyphylla* ssp. *polyphylla*. However, the well supported monophyly of *Aichryson* as well as of the perennial taxa of *Monanthes* (excluding *M. icterica*), suggests that this homoplasious indel could nevertheless be phylogenetically informative within the respective genera. We surveyed the length of the trnL – trnF chloroplast spacer in nine plants of both subspecies of *M. polyphylla*. The 7 bp insertion seemed to be absent in all plants, except for accession 32179 (see Tab. 3) which has a spacer approximately 30 bp longer than the others (not shown). This suggests that *M. polyphylla* is the only species of *Monanthes* which lacks the 7 bp insertion. The chloroplast spacer sequence of *M. icterica* differs considerably from the spacer of the other species of *Monanthes*. It is characterized by 10 unique indels, i.e., 7 single bp indels [45, 74, 115, 155, 157, 207, and 274], a 2 bp indel [166] as well as by a 7 bp insertion [170–176].

Cladistic analysis of the chloroplast DNA sequences found eight trees of 36 steps (consistency 0.72, retention index 0.74). The SCT is calculated using only base substitutions. Indels were subsequently fitted onto this topology (Fig. 3). The results indicate that the Macaronesian Sempervivoideae together with the Moroccan *Sedum* series *Monanthoidea* are monophyletic (Mes, 1995; Mes et al., 1996). Due to a lack of sequence variation, the support for this clade as well as resolution among many of the taxa is low. The chloroplast genomes of four species of *Monanthes*, *M. muralis* from Hierro, *M. laxiflora*, *M. brachycaulos* and *M. polyphylla* ssp. *polyphylla*

(the perennial species), are indicated to be monophyletic, whereas *M. icterica* constitutes a monotypic clade. As a result of low levels of sequence variation, the support measures of this analysis, which are based on base substitutions only, are accordingly low. Most notably, the indels further resolve and give extra support to some clades (e.g., monophyly of the perennial taxa of *Monanthes* [217–220 and

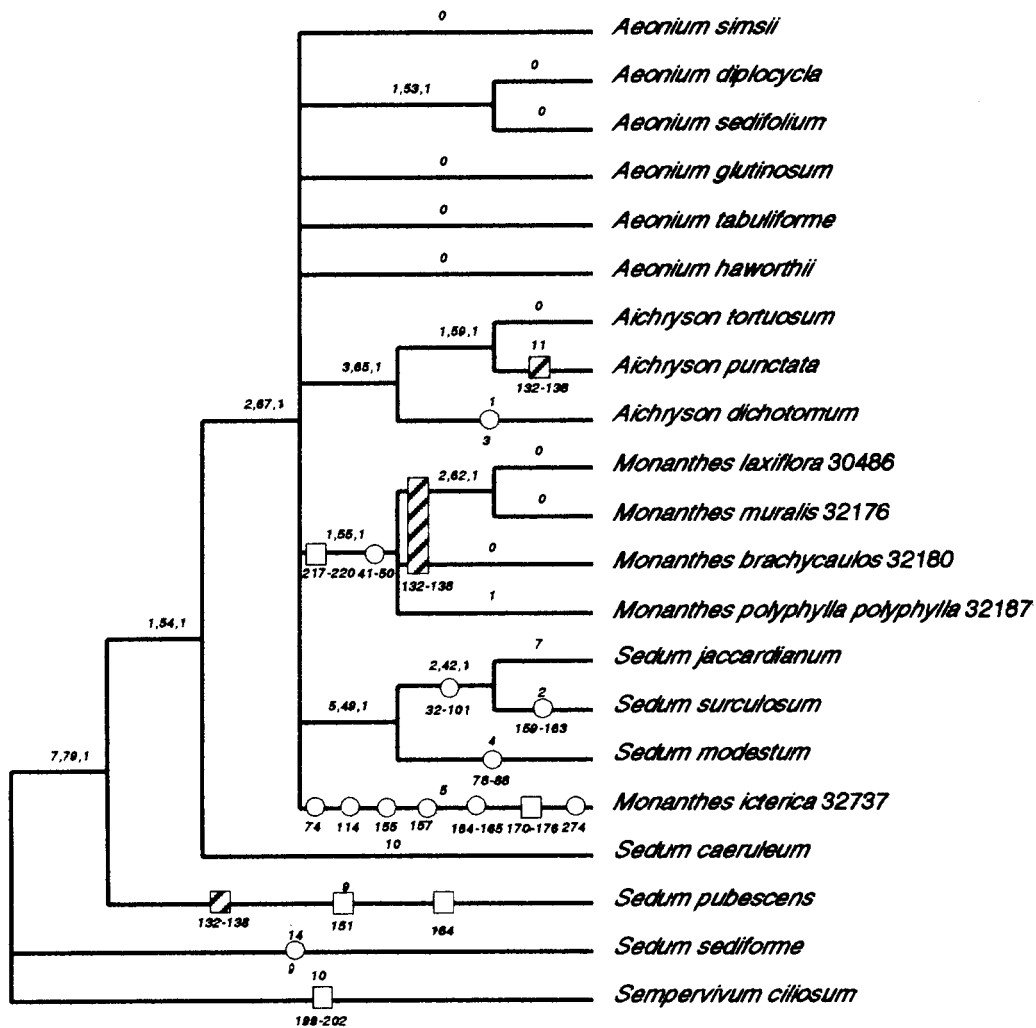


Fig. 3. Strict consensus tree of eight more parsimonious cladograms of 36 steps based on sequence variation of the chloroplast DNA trnL (UAA) – trnF (GAA) intergenic spacer of species of *Aeonium*, *Aichryson*, and North African *Sedums* as additional ingroup species and *Sedum sediforme* and *Sempervivum ciliosum* as outgroups. Alignment gaps were treated as missing data. Indels in regions which contained dubious alignments due to replication slippage [109–111 and 231–235] were not fitted. Numbers above branches indicate branch lengths. Numbers below branches indicate bootstrap-values and decay-values. These were determined using only base substitutions. Indels were fitted onto the topology afterwards.

41–50]). Both the topology and support for individual branches of the Neighbor-Joining dendrogram (not shown) are in agreement with the cladistic analysis.

Nuclear ribosomal DNA

The alignment of the ITS2 sequences (obtainable from the authors) of the species of *Monanthes*, 6 of *Aeonium* and 3 of *Aichryson*, and 5 North African Sedoideae and *Sempervivum ciliosum* is 227 positions long. The length of the ITS2 sequences of *Monanthes* varies from 209 (*M. ictERICA*) to 214 bp (*M. laxiflora*). In the alignment of the ITS2 sequences a large number of short indels (1 or 2 bp) is hypothesized and accordingly a large number of alternative, equally likely alignments can be made. One alignment has been used in all phylogenetic analyses. The entire alignment (including the species of *Sedum*, *Sempervivum*, *Aichryson*, *Aeonium* and *Monanthes*) comprises 133 variable and 87 phylogenetically informative sites. Polarization of the base substitutions in *Monanthes* using the species of *Aeonium* shows that 34 of the 67 variable positions are informative and that 33 are unique (alignment gaps and missing data excluded). Twelve base substitutions are informative among the perennial taxa of *Monanthes*. Fifteen indels are present in the alignment, two of which are synapomorphies for the perennial taxa of *Monanthes*, and one is potentially informative within *Monanthes* (excluding *M. ictERICA*). *M. ictERICA* has a ITS2 region which is very different from the other species of *Monanthes*, given the number of unique indels (6) and the number of unique base substitutions (18). This is also reflected in the sequence divergence which ranges from 18.5% to 23.1%, between *M. ictERICA* and *M. pallens* and between *M. ictERICA* and *M. polyphylla* ssp. *polyphylla*, respectively. ITS2 sequence divergence among the perennial taxa of *Monanthes* ranges from 0.00% to 10.1% among *M. minima*, *M. anagensis*, and one of the plants of *M. brachycaulos* on one hand and between *M. lowei* and *M. polyphylla* ssp. *amydros* on the other.

Divergence of the ITS2 sequence between *M. ictERICA* and the other species of *Monanthes* is within the range of intergeneric divergences in the Macaronesian Sempervivoideae and varies from 12.7% between *M. ictERICA* and *Aeonium* to 17.5% between *M. ictERICA* and *Aichryson*. In contrast, among *M. pallens*, *M. anagensis*, *M. laxiflora*, *M. lowei*, *M. minima*, and *M. brachycaulos*, sequence divergences vary from 0.0% to 2.4% and between the subspecies of *M. polyphylla* and *M. muralis* (Hierro), maximum sequence divergence amounts of 3.4%. Between the two sister-taxa of *Monanthes* (the subspecies of *M. polyphylla* are sister to the other perennial species), minimum genetic divergence is 5.4% (between *M. pallens* and *M. polyphylla*). The Neighbor-Joining dendrogram (not shown) strongly indicates monophyly of both *Aeonium* and *Monanthes* (excluding *M. ictERICA*). Paraphyly is indicated for the two accessions of *M. brachycaulos* since the accession of *M. brachycaulos* from Tenerife is the sister group of *M. pallens* and the accession of *M. brachycaulos* from Gran Canaria is the sister group of *M. lowei*. The relatively low support for a clade comprising *M. muralis* and *M. polyphylla* is probably due to the large amount of missing data for *M. muralis* from Hierro.

The aberrant *M. icterica* and *Aichryson* form a weakly supported clade in the Neighbor-Joining tree.

We attempted to determine the ITS2 sequence of *M. muralis* from Hierro from both directions. In parts of the ITS2 region which could be sequenced, frequently two predominant nucleotides were present at a single position in the chromatogram. In nearly all instances nucleotides were either identical to the homologous positions of the maternal or to the paternal species. The ITS2 sequence of *M. muralis* from Hierro might therefore be merely a reflection of the number of (different) parental copies of ribosomal DNA present in this taxon. It has been suggested that the lack of sequence homogenization may result from recent reticulation (Baldwin, 1995). In such cases additivity can definitely confirm hybridity as reported elsewhere (Campbell et al., 1993; Kim and Jansen, 1994).

Cladistic analysis (positions containing gaps treated as missing data) of the data set which comprised 49 variable and 100 informative positions resulted in 60 MPTs of 208 steps (consistency 0.67, retention index 0.82; SCT in Fig. 4). The results indicate that 1) the perennial taxa of *Monanthes* form a strongly supported monophyletic clade, 2) *Aeonium* is the (putative) sister group of the perennial *Monanthes* clade, 3) annual *M. icterica* is separated from *Monanthes* and forms a weakly supported monophyletic clade with the species of *Aichryson*, 4) *Monanthes* (excluding *M. icterica*), *M. polyphylla* and *M. muralis* form a monophyletic group which has a sister group relationship to the other species of *Monanthes* 5) within the latter group, *M. pallens* is the sister species of a clade comprising *M. brachycaulos*, *M. lowei*, *M. minima*, *M. anagensis*, *M. laxiflora* and the diploid *M. muralis* from La Palma, and 6) the accessions of *M. muralis* from Hierro ($2n = 72$) and La Palma ($2n = 36$) are indicated to be polyphyletic. Similar results were obtained using Neighbor-Joining as a clustering criterion (not shown).

Weighting transitions and transversions equally, *M. icterica* is the sisterspecies of *Aichryson*. However, this topology is only weakly supported, which is probably due to the high number of unique mutations, while only few synapomorphies support this or any other position of *M. icterica*. We examined the relationships among the species of the Macaronesian Sempervivoideae using different weights of transversions over transitions in the ITS2 sequences (from 1:1 to 5:1; Wojciechowski et al., 1993). Except for the position of *M. icterica*, relationships among species of *Monanthes* did not change significantly. At a weight ratio of 2:1 only *M. lowei* and *M. brachycaulos* (from Gran Canaria) as well as *M. muralis* from La Palma and *M. laxiflora* were indicated to be monophyletic, but all other relationships remained intact. The position of *M. icterica* however changed significantly. As already mentioned, a 1:1 ratio found *M. icterica* to be the most likely sisterspecies of *Aichryson*. In the SCT of weight ratio's 2:1 and 3:1 a basal polytomy in the Macaronesian Sempervivoideae appeared, which comprised separate lineages for the species of *Aichryson*, *Aeonium*, *Monanthes*, and a monotypic lineage comprising *M. icterica*. SCTs using weight ratio's of 4:1 and 5:1 indicated monophyly of *Monanthes*, with *M. icterica* as the obviously distantly related sister species of the perennial *Monanthes* taxa. However, at these weight ratio's *Monanthes* was included in *Aeonium*, which renders the latter genus paraphyletic.

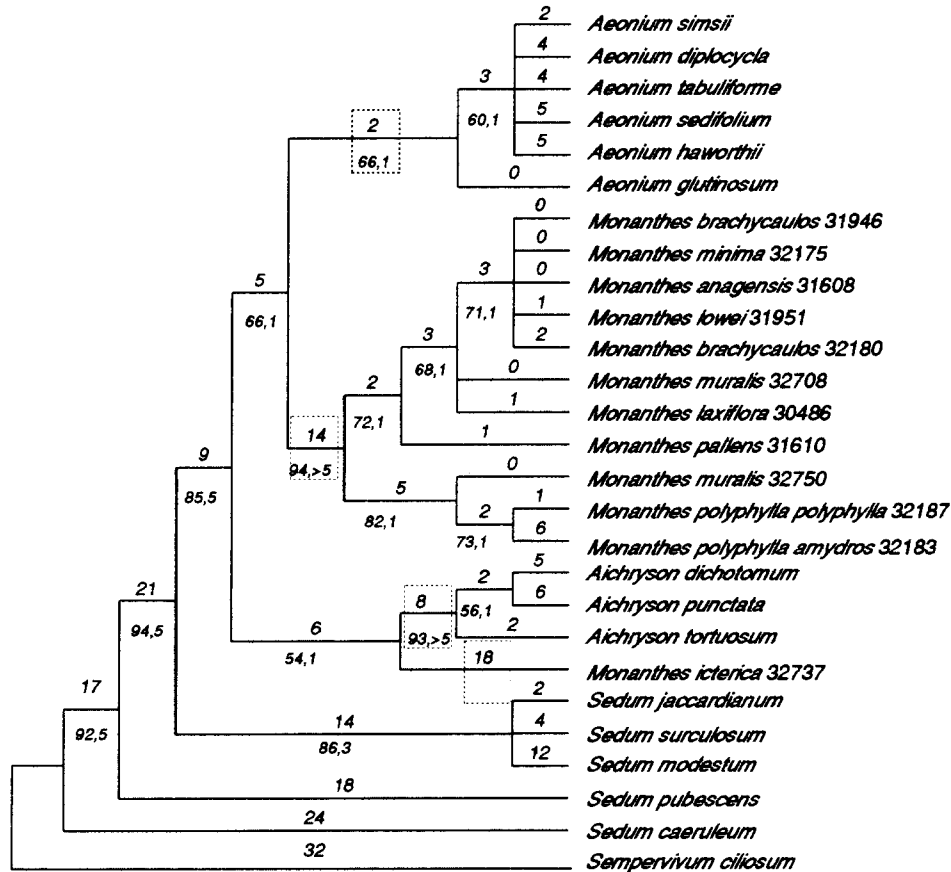


Fig. 4. Strict consensus tree of 60 most parsimonious cladograms of 208 steps based on sequence variation of the nuclear ribosomal Internal Transcribed Spacer 2 (ITS2) of species of *Aeonium*, *Aichryson*, and North African *Sedums* as additional ingroup species and *Sedum sediforme* and *Sempervivum ciliosum* as outgroups. Alignment gaps were treated as missing data. When the option gapmode = "newstate" instead of "missing" was used, a polytomy with four lineages was found. These lineages are indicated by dotted squares. Numbers above branches indicate branch lengths. Numbers below branches indicate bootstrap-values and decay-values.

RAPDs

RAPD patterns of *M. icterica* were very different from all other species of *Monanthes*. Several markers unique to either *M. icterica* or to the perennial species have been detected (Tab. 4). Although many more RAPDs were found, only intense RAPD markers are presented. Since *M. icterica* is only distantly related to the perennial species of *Monanthes*, homology assessment of RAPDs is likely to be compromised. Consequently, this species excluded from the RAPD analyses described below.

Table 4. A RAPD markers found between *M. muralis* from Hierro and the other species of the genus. Markers indicated were either present in *M. muralis* and subspecies *M. polyphylla*, or in *M. muralis* and *M. pallens*, *M. anagensis*, *M. laxiflora*, *M. brachycaulos*, *M. lowei*, *M. muralis* from La Palma, and *M. minima*. Exceptions are indicated. Accessions are identical to those indicated in Figure 5. Markers are indicated by the size (in bp) and the primer used, respectively. B. RAPD markers found between either *M. ictERICA* or in the other species of *Monanthes*. We surveyed 1 plant of *M. ictERICA*, 5 plants of *M. polyphylla* ssp. *polyphylla*, 4 of *M. polyphylla* ssp. *amydros* (POL), 4 of *M. muralis* from Hierro (MUR), one plant of *M. muralis* from La Palma, two plants of *M. laxiflora*, one of *M. anagensis*, two of *M. brachycaulos*, one of *M. minima* and *M. lowei*, and two of *M. pallens* (PAL). Accessions are identical to those indicated in Figure 5.

A	
570F14	MUR Hierro + POL
600F12	MUR Hierro + POL
550F14	MUR Hierro + PAL, except for <i>M. pallens</i> (32746, and 31954)
850F9	MUR Hierro + PAL
507F9	MUR Hierro + PAL
550C1	MUR Hierro + PAL, except for <i>M. muralis</i> from La Palma (32708)
B	
1200F12	<i>M. ictERICA</i>
850F10	<i>M. ictERICA</i>
1900C8	<i>M. ictERICA</i>
1550F12	<i>Monanthes</i> without <i>M. ictERICA</i>
1650F10	<i>Monanthes</i> without <i>M. ictERICA</i>
1800C2	<i>Monanthes</i> without <i>M. ictERICA</i>

The RAPD data set of all perennial species of *Monanthes* contained 151 informative and 64 variable characters. Wagner analysis of the RAPD data found 5 shortest trees of length 337 (consistency 0.45, retention index 0.68, Fig. 5B). Three main clusters were detected. One comprised both subspecies of *M. polyphylla* which were separated from the other species. The second group comprised accessions of *M. muralis* from Hierro. The third group comprised *M. muralis* from La Palma, *M. anagensis*, *M. laxiflora*, *M. lowei*, *M. brachycaulos*, *M. minima* and *M. pallens*. The two species with branched, ascending shoots, *M. laxiflora* and *M. anagensis*, were separated from the rosulate species in PCA and cluster analysis. Wagner analysis, however, excluded *M. laxiflora* and *M. anagensis* from the group of rosulate taxa and combined the two species with *M. muralis* from Hierro.

Accessions of *M. muralis* from Hierro and La Palma are highly divergent genetically. The four plants of *M. muralis* from Hierro were terminal in a strongly supported clade (bootstrap value 100%) which also comprised the other perennial species of *Monanthes*, except for *M. polyphylla* (Fig. 5B). In contrast, in a Neighbor-Joining cluster analysis based on Jaccard distances, the plants of *M. muralis* from Hierro formed a cluster intermediate between the two sister clades in the genus. The position of the plants from Hierro thus differs between cluster

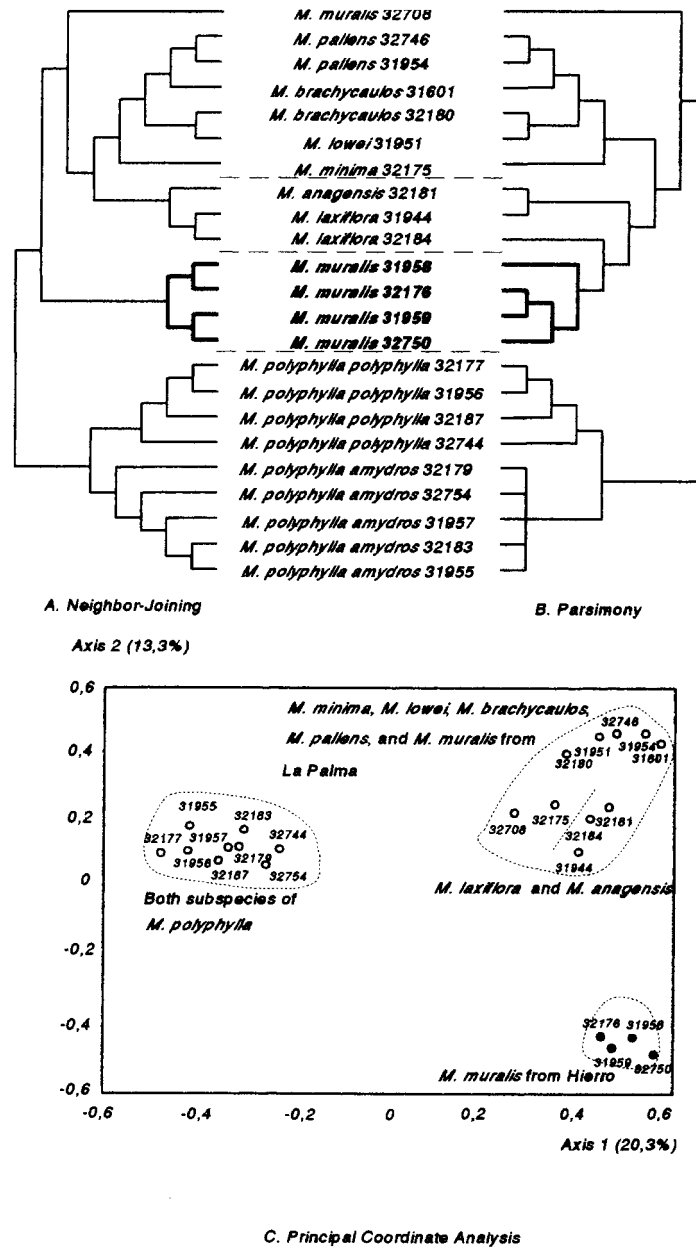


Fig. 5. A. Neighbor-Joining dendrogram based on presence and absence of RAPDs of all species of *Monanthes* except for *M. ictERICA*. The dendrogram was constructed from 1-Jaccard's distance coefficients (Jaccard, 1908). B. Strict consensus tree of five most parsimonious unrooted Wagner cladograms of 337 steps of nine (sub)species of *Monanthes*. C. Scatter plot of the first two Principal Coordinates (covering 20.3 and 13.3% of the variation, respectively) resulting from a Principal Coordinate Analysis (PCO23D; Adams, 1994) of 23 accessions of nine (sub)species of *Monanthes*.

analysis and cladistic analysis (Figs. 5A and B). Their aberrant position is also apparent in a Principal Coordinate Analysis (axis 1 and 2 covered 20.3% and 13.3% of the variation, respectively) in which three well separated clusters can be observed, i.e. one comprising all eight plants of *M. polyphylla* (either subspecies), one comprising *M. brachycaulos*, *M. minima*, *M. laxiflora*, *M. anagensis*, *M. pallens*, and *M. muralis* from La Palma, and a cluster comprising the four plants of *M. muralis* from Hierro. The position of the plants of *M. muralis* from Hierro is therefore dependent upon the method of analysis. Their ambiguous position may be due to the presence of several RAPDs which they share with (nearly) all species of the clade with *M. pallens* as a basal species (Fig. 5) or with the subspecies of *M. polyphylla* (Tab. 4).

Analyses of combined data from morphology, chloroplast DNA and nuclear DNA

The data set comprised 34 informative ITS2 and 27 morphological characters. Three chloroplast DNA characters were available for a subset of the species. Since *M. icterica* is not included in the clade which comprises the perennial species, this annual species is used as an outgroup species, in addition to *Aichryson tortuosum*. *Sedum surculosum* was not used as an outgroup because it is more distant to *Monanthes* than the other two species, which both belong to the Macaronesian Sempervivoideae. The morphological data of *M. muralis* are mainly based on the accessions from Hierro rather than La Palma (Nyffeler, 1992). This taxon is probably a hybrid between representatives of the major lineages within the genus (at least based on molecular data). The plants from Hierro are probably of hybrid origin (see above) and we therefore excluded *M. muralis* from the combined analysis because a hybrid between (relatively) distant lineages may have a profound effect on cladistic analyses (McDade, 1992).

Unconstrained cladistic analysis using both *Aichryson tortuosum* and *Monanthes icterica* as outgroups resulted in a single MPT of 86 steps (CI 0.67, RI 0.70, Fig. 6). In the resulting topology, *M. polyphylla* was sister to the other perennial species and *M. anagensis* and *M. laxiflora* were sister to *M. brachycaulos*, *M. pallens*, *M. lowei*, and *M. minima*. The topology differs from the molecular tree (Fig. 4) with respect to the resolution of the relationships among growth forms. Due to the impact of the morphological characters, *M. sect. Sedoidea* is sister to the other perennial species of *M. sect. Monanthoidea*. Monophyly of *M. polyphylla* is strengthened. However, when *Aichryson tortuosum* and *Monanthes icterica* were singly used as outgroups, very different topologies were obtained (Fig. 6). For example, the species with loose rosettes were paraphyletic when *Aichryson tortuosum* was used as an outgroup.

Discussion

The results of Principal Component Analysis of the morphological data of *Monanthes* agree perfectly well with Nyffeler's (1992) as well as Sventenius'

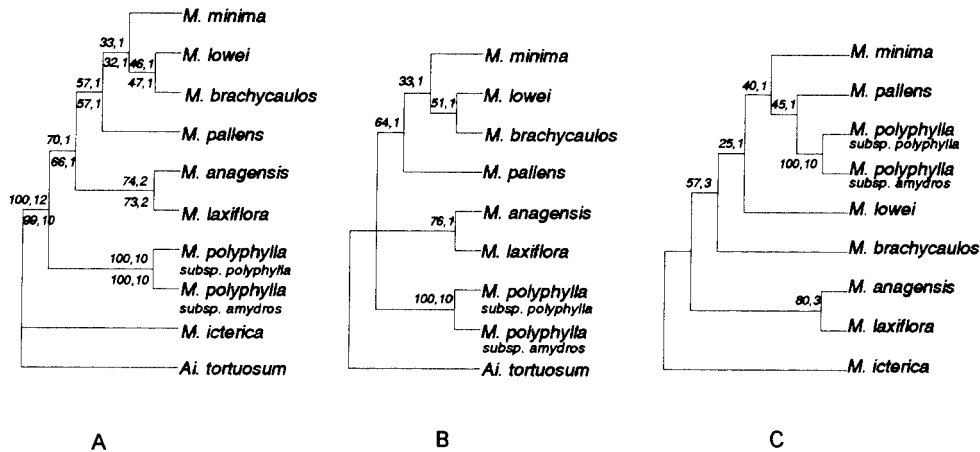


Fig. 6. A. Single most parsimonious tree (86 steps; CI 0.61) of combined data from morphology, chloroplast trnL + trnF sequences and ITS2 sequences of the species of *Monanthes*. Bootstrap- and decay values were determined using PAUP's branch-and-bound option. *Aichryson tortuosum* and *Monanthes ictERICA* were used as outgroups. Support measures above internal branches resulted from analysis without the chloroplast sequences. The strict consensus tree of both analyses was identical. B. Strict consensus tree of two most parsimonious trees (66 steps) of combined data from morphology, trnL + trnF chloroplast sequences and ITS2 sequences of the species of *Monanthes*. *Aichryson tortuosum* was used as an outgroup. C. Single most parsimonious tree (64 steps) of combined data from morphology, trnL + trnF chloroplast sequences and ITS2 sequences of the species of *Monanthes*. *Monanthes ictERICA* was used as an outgroup.

classification (1960). In cladistic analysis however, both outgroup species *Aichryson tortuosum* and *Sedum surculosum* provided different root positions which was indicated by the increase of a large number of steps and homoplasy when *Monanthes* was forced into monophyly. This indicates that on basis of morphology, monophyly of the genus is unlikely. If *M. ictERICA*, which is genetically distinct from the other species of *Monanthes* is used as an additional outgroup, yet another root position is suggested. Although the variable root positions could result from the use of too distantly related outgroups, no more appropriate outgroup is available. Although we did not use the putative sister genus *Aeonium* as an outgroup, *Aichryson* which is genetically closely related (Mes, 1995), is probably equally appropriate as an outgroup (see weighted analysis of ITS2 sequences).

Inclusion of *M. ictERICA* in *Monanthes* is primarily based on the typical flowers with the uniquely enlarged nectariferous scales. However, the phenetic analysis (PCA) of the morphological data (Fig. 1) as well as its annual life form and deviating chromosome number ($2n = 20$) indicate that this species holds a unique position within the genus (Sventenius, 1960; 't Hart and Nyffeler, pers. comm.). Genetically, *M. ictERICA* is also quite distant to the other species of *Monanthes*. Phylogenetic analysis of chloroplast and nuclear sequences as well as RAPDs all indicate a unique position of *M. ictERICA*, without any close ties to either *Monanthes* or any of the other Macaronesian genera and North African Sedoideae. The

cytological and morphological features of *M. ictERICA* as well as its peculiar life form are unique when compared to other taxa of the Macaronesian Sempervivoideae. As *M. ictERICA* does not share any RAPD fragments with *Monanthes*, it is certainly not a hybrid between a species of *Monanthes* and an (extant) species of one of the other genera of the Macaronesian Sempervivoideae. As the inclusion of *M. ictERICA* in *Monanthes* is primarily based on flower morphology, a multiple origin of this flower type with large nectariferous scales could be considered. Large nectariferous scales also occur in the North African *Sedum surculosum* (= *Monanthes atlantica*) and have been described for species of Mexican *Sedum napiferum* group (Fröderström, 1936; Clausen, 1957). Homoplasy of floral characters could also account for the disconcerting results of the cladistic analysis of the morphological data. Removing these characters (20, 21, and 23 through 30) from the morphological data set however still resulted in multiple root positions when using different outgroups. Probably also the vegetative characters of *Monanthes* are not very reliable characters for evolutionary inference.

Although relationships between *M. ictERICA* and the other species of *Monanthes* are rather distant, this species is evidently included in a strongly supported clade comprising the Macaronesian Sempervivoideae and certain North African species of *Sedum* ("Aeonium"-clade; van Ham, 1994) as shown by the cladistic analysis of trnL (UAA) – trnF (GAA) chloroplast DNA spacer sequence variation of a large taxonomic sample, covering all major genera of the Crassulaceae ('t Hart et al., pers. comm.) and phylogenetic analysis of sequence variation of the ITS2 region of the Macaronesian Sempervivoideae (bootstrap value 85%; Fig. 4). In the ITS2 phylogeny its position shifts depending on the differential weighting of transitions and transversions, but none of the topologies is strongly supported. One of the alternative topologies, indicated paraphyly of *Aeonium* by inclusion of *Monanthes* (including *M. ictERICA*) which is unlikely on basis of chloroplast DNA RFLPs. Using these characters, identical root positions were found for *Aeonium* using either *Monanthes laxiflora* or *Aichryson tortuosum* as an outgroup (Mes and 't Hart, 1995). As a result, relationships among the genera *Aichryson*, *Aeonium*, *Monanthes* and a monotypic clade comprising *M. ictERICA* are essentially unresolved. The four lineages differ with respect to basic chromosome numbers (Tab. 1), which range from $2n = 20$ in *M. ictERICA*, to $2n = 30$ to 34 in *Aichryson*, and $2n = 36$ in *Aeonium* and *Monanthes*. It has often been found that after arrival on an oceanic archipelago chromosome numbers increase and remain more constant after final settlement (Stuessy et al., 1992). The variable chromosome numbers of the Macaronesian lineages therefore either indicate the existence of an ancestral and polymorphic population whose distribution area might have been fragmented (e.g. by volcanism) or indicate periods of rapid speciation with subsequent changes of chromosome numbers in the surviving lineages. Both schemes are expected to result in polytymous relationships among taxa.

We found convincing evidence for the allopolyploid origin of *M. muralis* from Hierro. The diploid ($2n = 36$) plant from La Palma is neither genetically nor evolutionary linked to the tetraploid ($2n = 72$) plants from Hierro. Our molecular data from sequences and RAPDs unequivocally support a hybrid origin of *M.*

muralis from Hierro. Although certain regions of the trnL – trnF chloroplast spacer are prone to homoplasy (indels at positions 231–234 and 132–138; Mes et al., 1996; Mes, 1995), the 7 bp insertion in the accessions of Hierro indicates that their chloroplast genomes share a synapomorphy with *M. laxiflora*, *M. brachycaulos* and *M. muralis* from Hierro whereas the morphological and ITS2 data indicate inclusion of *M. muralis* from Hierro in the clade comprising the sister taxon, *M. polyphylla*. The different lineages to which the maternal and paternal donor of *M. muralis* from Hierro belong strongly suggest a hybrid origin for this taxon. Because only a small portion of the ITS2 region of *M. muralis* from Hierro could be sequenced, the clade comprising *M. polyphylla* and *M. muralis* is only weakly supported. The inability to determine the nucleotide sequence is consistent with the multiple ITS types expected in hybrids of recent origin (Kim and Jansen, 1994), “if concerted evolution fails to act across repeat units contributed by different parental species” (Baldwin et al., 1995). Both the distribution of RAPDs (Tab. 4) and the variable position of *M. muralis* from Hierro (Fig. 5) are wholly consistent with a hybrid origin of this taxon. Although no general criteria can be given with respect to the position or identification of hybrid taxa in cladistic analysis (McDade, 1992), a highly variable position of such taxa in evolutionary analysis using multiple data sets can readily be envisioned to result from reticulation. *M. laxiflora* is the most probable maternal donor given the synapomorphy of the chloroplast genome [161] and the identical sequence of the 7 bp insertion [132–138]. Morphological and cytological characters are not conclusive with respect to the paternal species. The number of parastichies of *M. muralis* from Hierro is intermediate between both subspecies of *M. polyphylla*, and the chromosome numbers are not distinctive with respect to the paternal (sub)species either. The more ascending and tufted habit of *M. muralis* indeed suggests that *M. laxiflora* might have been involved in the evolution of this taxon, and this has explicitly been stated for one of the accessions used (32176; Nyffeler, 1995). Interestingly, *M. laxiflora* does neither occur on La Palma nor on Hierro. Gomera is the island nearest to Hierro on which both *M. laxiflora* and one of the subspecies of *M. polyphylla* occur. The genetic similarity of the accessions of *M. muralis* from Hierro which share approximately 94% of the RAPDs (averaged over all pairwise comparisons of accessions), and the distribution of the plants studied over the entire island (see Tab. 3), suggest that this allotetraploid has spread rapidly over the island.

All molecular data agree with respect to the close genetic relationships of the perennial *Monanthes* taxa. Within this monophyletic lineage, three groups can be distinguished which by and large agree with the sections distinguished by Sventenius (1960) and Nyffeler (1992). *M. sect. Monanthes* with *M. polyphylla* (two subspecies) and *M. muralis* from Hierro is the sister taxon of the other perennial species. Two lineages, corresponding with *M. sect. Sedoidea* and *M. sect. Petrophyllae*, respectively (Fig. 5). Relationships among these lineages are not consistently resolved (Fig. 4). Although the perennial species of these sections are genetically very similar, they differ considerably in habit and gross morphology.

The results of cladistic analysis of the combined morphological and sequence data (ITS2 and/or trnL – trnF) are topologically identical (Fig. 6A) because the

chloroplast sequences comprise only 3 ingroup-outgroup variable base substitutions. The topology of the tree of the combined analysis is consistent with the molecular data, but with a higher level of resolution (Fig. 6A). The topology of the combined analysis is in conflict with the molecular data when the outgroup taxa are used singly. Most aberrant is the analysis with *M. icterica* as an outgroup (Fig. 6C) in which the species of *M. sect. Petrophyllae* are basal to *M. polyphylla*. Although the topology using two outgroup species is compatible with previous strongly supported clades, and is compatible with a single origin of the 4 bp insertion in the chloroplast trnL – trnF intergenic region, relationships among the growth forms of *Monanthes* remain weakly supported. Exclusion of *M. icterica* and *M. muralis* from Hierro on the basis of information newly obtained in this study, and a “total evidence” approach results in a single rooting point for the outgroups but does not consistently resolve relationships among growth forms within the genus.

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