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The phylogeny of *Monsonia* L. (Geraniaceae)

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Abstract. The phylogeny of *Monsonia* L. (Geraniaceae) is examined. Analysis of nrDNA ITS and *trn*L (UAA) 5'exon-*trn*F (GAA) chloroplast DNA sequence data of 26 *Monsonia* and two outgroup *Pelargonium* species, suggests monophyly for the genus including the former genus *Sarcocaulon* (DC.) Sweet. The species of *Monsonia* sect. *Sarcocaulon* resolve as two subclades (ITS cladogram 80% and *trn*L-F cladogram 96%) and two additional species in an unresolved basal polytomy, in a clade with species of the sect. *Olopetalum* and *M. senegalensis*. All these species share the basic chromosome number x = 11. The remaining species have derived karyotypes of x = 12, 10, 9 or 8. Chromosome sizes vary considerably between the species, and polyploids are rare. The current infrageneric classification is discussed.

Key words: Geraniaceae, *Monsonia*, *Sarcocaulon*, ITS and *trn*L-F sequences, karyology, phylogeny, Africa.

The family Geraniaceae comprises the genera *Erodium*, *Geranium, Monsonia*, and *Pelargonium*. The formally recognized genus *Sarcocaulon* has been incorporated into the genus *Monsonia* (Albers 1996). The genus *Hypseocharis* has also recently been included in the family based on several shared morphological and molecular characters with the other genera of the Geraniaceae (Albers and van der Walt, in press). Despite the close relationship between *Monsonia* and *Sarcocaulon* reported by various authors, the two taxa were kept separate until recently, chiefly on the basis of differences in the development and morphology of the androecium (Harvey and Sonder 1894, Reiche 1896, Knuth 1912, Moffett 1979). However, these differences could not be verified for the species of *Monsonia*, included in the present study, or for those species previously included in genus *Sarcocaulon* (Klenter and Albers 2004). All species develop an identical pentadelphous androecium. In addition, *Sarcocaulon* and *Monsonia* species share an identical radially symmetrical flower structure. The distinction between *Sarcocaulon* and *Monsonia* is mainly based on differences in macro-morphology. In contrast to the predominantly herbaceous stems of *Monsonia*, those of *Sarcocaulon* form thick, more or less woody to succulent, often spinescent stems (Moffett 1979). In the closely related genus *Pelargonium* species with similar morphological adaptations and growth forms are frequently found in comparable habitats.

The genus *Monsonia* s. str. and the taxonomic position of *Sarcocaulon* have been under discussion since the contributions by

De Candolle (1824). Sweet (1820), Boissier (1867), Harvey and Sonder (1894), Reiche (1896), Knuth (1912), Kers (1968), Venter (1979, 1990), Moffett (1979, 1997), Verhoeven and Venter (1986) and Dreyer et al. (1997) contributed to a better understanding of *Monsonia* and *Sarcocaulon* and proposed taxonomic classifications of the genus.

Aldaroso et al. (2001) proposed the most recent infrageneric classification of *Monsonia* s. str. based on 20 morphological and anatomical characters. Although the authors found that the *Sarcocaulon* species belong to the *Monsonia* clade, they still treated these species as members of a separate genus.

Monsonia has been shown to display a remarkably broad range of morphological variation (Venter 1979, Aldaroso et al. 2001). The inflorescence consists of either a large single flower or several small flowers arranged into cymes. The flowers are bisexual, quinate and actinomorphic. Two mericarp types have been identified: The first type is plumose, with a loosely wound awn beset with long, feather-like hairs. This type is typical of anemochorous species. The second type is barbate with a spiral awn, beset with bristly and durable persisting hairs. This kind of fruit is typical for species that display epizoochory and autochory. Mericarps has been considered as important in several subgeneric classifications (Boissier 1867, Knuth 1912, Kers 1968, Venter 1990). *Monsonia* is also palynologically diverse, displaying four distinct pollen types (Bortenschlager 1967, Verhoeven and Marais 1990).

So far, the DNA-based analyses of *Monsonia* have been very limited. The *rbc*L cpDNA region and mtDNA *nad*1b/c exon of just a few representatives of the genus have been sequenced (Price and Palmer 1993, Bakker et al. 2000a, Soltis et al. 2000). These study showed that *Monsonia* and *Sarcocaulon* are either sister genera or congeneric and sister to the other three (or four) genera in the Geraniaceae. The close relationship between *Monsonia* and the former genus *Sarcocaulon* is also supported by the shared loss of the cpDNA *rp1*2 intron (Price et al. 1990, Downie and Palmer 1992).

In this paper the phylogenetic relationships among 16 of the 24 representatives of the genus *Monsonia* and nine of the 14 species of the former *Sarcocaulo*n were studied using nuclear rDNA ITS and cpDNA *trn*L-F sequences data. One sequence is added from GenBank (*M. vanderietiae*).

To date, about no karyological results have been published for *Monsonia,* except for a few numbers (cf. Albers 1990). In this paper the previous karyological results are confirmed, and new data is added.

The DNA sequence analysis and karyological data are used to establish a phylogeny for the genus *Monsonia*. Along with the available morphological, palynological and phytogeographical data the current infrageneric classification is discussed and emended.

Materials and methods

Plant material. Table 1 lists 16 *Monsonia* species and nine species (+ one species from GenBank [*M. vanderietiae*]) of the former genus *Sarcocaulon* that were included in the molecular analysis, together with accession details. The ingroup taxa also included two duplicate isolates of *M. natalensis* and *M. nivea* for biogeographic comparisons. Most of the plant material was obtained from collections of living plants from the Botanical Garden of the Westfälische Wilhelms-Universität Münster (Germany). Voucher specimens were deposited in MSUN.

Two representatives of the genus *Pelargonium* were selected from GenBank to serve as outgroup taxa. The ITS sequences are from *P. gibbosum* Z95277 and *P. album* Z952580, the *trn*L-F sequences are from *P. gibbosum* Z95298 and *P. album* Z95287.

Table 2 lists 43 individuals out of 25 species for which karyological results were obtained.

DNA extraction, PCR, cloning and sequencing. The same methods and protocols for DNA extraction, rDNA ITS and cpDNA *trn*L-F amplification that were described in Bakker et al. (1998) were followed. The PCR temperature profile used consists of 30 cycles of 1 minute at 94°C, 1 minute at 55°C, followed by two minutes at 72°C. Before the

Table 1. *Monsonia* species included in sequencing

Species	Accession Collector	GenBank numbers *trn*L-F regions	GenBank numbers ITS regions	Locality	Voucher
M. angustifolia E. Mey. ex A. Rich.	Al 3521	AYO36160	AF505632	RSA, 15 km W Potchefstroom	MSUN
M. attenuata Harv.	Al 3542	AY036161	AF505630	RSA, Royal Natal National Park, KwaZulu -Natal	MSUN
M. brevirostrata R. Knuth	Al 4015	AY036159	AF505631	Quacha's Nek, Drakensberge, Lesotho	MSUN
M. ciliata (Moffett) F. Albers	K 862632/11	AY036166	AF505638	Unknown, cultivated plant	MSUN
M. crassicaulis (Rehm) F. Albers	Al 2416	AY036165	AF505637	RSA, 33 km N Steinkopf	MSUN
M. deserticola Dinter ex R. Knuth	NJ 100123	AY036178	AF505652	Namibia, N Aus	MSUN
M. emarginata (L. f.) L'Hérit.	Al 3032	AY036157	AF505628	RSA, Knysna, 54 km S George	MSUN
M. flavescens (Rehm) F. Albers	Al & Me 041086/46	AY036164	AF505636	Namibia, 8 km S Rosh Pinah,	MSUN
M. glauca R. Knuth	Masinde 964	AF 551333	AF505643	Kenya, 3 km after Olepolos township, road to Lake Magadi, alt. ca. 1500 m	MSUN
M. grandifolia R. Knuth	181198 (308)CC	AY036158	AF5056229	RSA, 10 km NE Ixepo, Highland Sourveld	MSUN
M. heliotropioides (Cav.)Boiss.	Al 3644	AY036177	AF505651	Egypt, 24 km W Safaga, Wadi Umm Taghir	MSUN
M. ignorata Merxm. & A. Schreib.	NJ 28393		AF505647	Namibia, Lüderitz, 2615CB	MSUN
M. inermis (Rehm) F. Albers	Al & Me 051086/52	AY036162	AF505634	RSA, 3-4 km N Steinkopf, Northern Cape	MSUN
M. luederitziana Focke & Schinz	NJ 22707	AY036173	AF505646	RSA, Richtersveld, 2817AD, Northern Cape	MSUN
M. marlothii (Engl.) F. Albers	Al 2729	AY036170	AF505642	Namibia, near Groot Welwitschia Vlakte	MSUN
M. mossamedensis (Welw. ex Oliv.) F. Albers	Al 3607	AY036169	????	Namibia, 6 km E Skeleton Coast, S Orupembe, Kaokoveld	MSUN
M. multifida (E. Mey. ex R. Knuth) F. Albers	Al & Me 051086/64	AY036163	AF505635	Namibia, 10 km S Rosh Pinah	MSUN
M. natalensis R. Knuth	280CC	AY036156	AF505626	RSA, 3130 AA, Port Edward Nature Reserve, KwaZulu-Natal	MSUN
M. natalensis R. Knuth	325CC		AF505627	RSA, Oribi Gorge Nature Reserve, KwaZulu -Natal	MSUN
M. nivea (Decne.) Webb	Al 3642	AY036175	AF505649	Egypt, 24 km W Safaga, Wadi Umm Taghir	MSUN

Table 1. (Continued)

Species	Accession Collector	GenBank numbers trnL-F regions	GenBank numbers ITS regions	Locality	Voucher
M. nivea (Decne.) Webb	Al 3645	AY036176	AF505650	Egypt, 27 km S Hurghada, Wadi Umm Uibash	MSUN
M. parvifolia Schinz	Al & Kl 3580	AY036171	AF505644	RSA, Richtersveld, 2817 AC	MSUN
M. patersonii (DC.)	DM 113	AY036168	AF505640	Namibia, S Lüderitz	MSUN
M. salmoniflora (Moffett) F. Albers	Al & Me 061086/86	AY036167	AF505639	Namibia, N Aus, turn off Helmeringhausen	MSUN
M. senegalensis Guill. & Perr.	Al 3591	AY036179	AF505633	Namibia,, Omaruru, 2115 BD	MSUN
M. speciosa L.	Al 2300	AY036174	AF505648	RSA, Gordon's Bay	MSUN
M. umbellata Harv.	Al & Kl 3583	AY036172	AF505645	Namibia, Hardap-Dam, 2417 DB	MSUN
**M. vanderietiae* (L. Bolus) F. Albers	CPG 8471	AF16715		RSA, Cookhouse, E. Cape	BM

first cycle a denaturing step of 4 minute at 94°C was applied, and after the last cycle the extension step at 72°C was prolonged up to 10 minutes.

All sequences were produced by sequencing Quiaquick™ cleaned fragments using the dideoxy chain-termination method (Sanger et al. 1977) with subsequent processing on an automatic sequencer LI-COR 4000 (MWG, Germany). The standard White et al. (1990) primers (ITS1 and ITS4) and the additional primer optimised for *Pelargonium* and *Monsonia* ITS regions FB1: 5′- AAT GGC TTC GGG CGC AAC TTG-3′ and TAS: 5′-CCG GCG ACT CGC GAG AAG TCC-3′ were used. PCR products of the ITS regions from *M. nivea* and *M. heliotropioides* were cloned with the TA TOPO cloning kit (Invitrogen). Only one type of clone from each of the two taxa was identified, sequenced and included in the phylogenetic analyses. The *trn*L-F sequences were produced using the Taberlet et al. (1991) primers and the additional primers optimised for *Monsonia* tamf1: 5′-CTA CCA GCT GAG CTA TCC CGT C- 3′: tamr1: 5′-CTA ACA AAT GGA ATC AGC CAC G-3′. Sequence assembly and alignment for all sequences was performed as described in Bakker et al. (1998). Five nucleotide positions in the *trn*L-F regions could not be aligned unambiguously and were therefore excluded from the analyses.

All taxa were sequenced on both strands for at least 75% of their total length. All sequences have been deposited in GenBank (Table 1) and the ITS and *trn*L-F alignments are available from the authors on request.

Phylogenetic analysis. Phylogenetic analysis and tests for clade support was performed using PAUP* 4.010b (Swofford 2002). Parsimony, as implemented in PAUP*, version 4.010b (Swofford 2002), was used to infer phylogenies based on nucleotide substitutions in aligned sequences. Unweighted parsimony analyses were performed by heuristic search which included tree bisection-reconnection (TBR) branch swapping, MULPARS, ACCTRAN optimisation, collapse branches when maximum length is zero, and 1000 replicates of random-addition. Bootstrap analysis (Felsenstein 1985) was carried out using PAUP with 1000 replications of heuristic search using branch swapping.

Incongruence between ITS and *trn*L-F. Congruence between the two datasets was examined with the partition-homogeneity test (Farris et al. 1995), implemented in PAUP*, version 4.010b. The

Table 2. List of species and karyological results

Species	Locality	Collection No.	Chromos. No. (2n)	Basic Chromos. No. (x)	1	2	3
M. angustifolia E. Mey. ex A. Rich.	RSA, s.loc.	YS 255.1	22	11	1,3	29,5	29,5
M. angustifolia E. Mey. ex A. Rich.	RSA, Cradock	Al 3575	22	11	–	–	–
M. angustifolia E. Mey. ex A. Rich.	Namibia, Waterberg	Al & Kl 3619	22	11	1,6	34,3	34,3
M. attenuata Harv.	RSA, Drakensberge	Al 4043	22	11	–	–	–
M. brevirostrata R. Knuth	Lesotho, Drakensberge	YS 256.2	22	11	1,3	27,7	27,7
M. burkeana Planch. ex Harv.	RSA, s.loc.	Verhoeven s.n.	22	11	–	–	–
M. camdeboensis (Moffett) F. Albers	RSA, Steytlerville	Al 2468	22	11	–	–	–
M. crassicaulis (Rehm) F. Albers	RSA, Steinkopf	Al 2416	22	11	–	–	–
M. crassicaulis (Rehm) F. Albers	RSA, Augrabis	Me & Li 609	22	11	–	–	–
M. deserticola Dinter ex R. Knuth	Namibia, Aus	Al & Kl 3629	16	8	3,7	59,3	81,5
M. emarginata (L. f.) L' Hérit.	RSA, George	Al s.n.	22	11	1,6	34,3	34,3
M. emarginata (L. f.) L' Hérit.	RSA, Knysna	Al 3032	22	11	1,4	30,0	30,0
M. emarginata (L. f.) L' Hérit.	RSA, s.loc.	CR 257.4	22	11	1,5	33,2	33,2
M. heliotropioides (Cav.) Boiss.	Algeria, Tassili N'Ajjer	AA 78-584	18*	9	–	–	–
M. ignorata Merxm. & A. Schreib.	Namibia, Lüderitz	Al 2411	60	10	1,6	98,6	36,2
M. ignorata Merxm. & A. Schreib.	Namibia, Lüderitz	DM 107	40	10	–	–	–
M. inermis (Rehm) F. Albers	Namibia, Rosh Pinah	Al & Me 51	22	11	–	–	–
M. inermis (Rehm) F. Albers	Namibia, Rosh Pinah	Al & Me 52	22	11	–	–	–
M. inermis (Rehm) F. Albers	Namibia, Rosh Pinah	Al & Me 53	22	11	–	–	–
M. marlothii (Engl.) F. Albers	Namibia, Roessing Mts.	Al 2720	22	11	–	–	–
M. mossamedensis (Welw. ex Oliv.) F. Albers	Namibia, Kaokoveld	Al & Kl 3607	22	11	1,5	32,2	32,2
M. multifida (E. Mey. ex R. Knuth) F. Albers	Namibia, Rosh Pinah	Al & Me 55	44	11	1,6	70,1	35,5
M. natalensis R. Knuth	RSA, Oribi George N.P.	Al 4001	22	11	–	–	–
M. nivea (Decne.) Webb	Egypt, Hurghada	Al 3645	36	9	0,9	33,5	20,5
M. patersonii (DC.) F. Albers	Namibia, Lüderitz	Al 2387	44	11	1,7	73,8	36,9
M. patersonii (DC.) F. Albers	Namibia, Lüderitz	Al 2393	44	11	–	–	–
M. patersonii (DC.) F. Albers	RSA, Alexander Bay	Al & al. K1348	44	11	–	–	–
M. patersonii (DC.) F. Albers	Namibia, Rosh Pinah	Al & Me 46	44	11	–	–	–
M. patersonii (DC.) F. Albers	Namibia, Lüderitz	DM 113	44	11	–	–	–
M. patersonii (DC.) F. Albers	Namibia, Rosh Pinah	Al & Me 82	44	11	–	–	–
M. praemorsa E. Mey. ex R. Knuth	RSA, s.loc.	Verhoeven 8780	22	11	–	–	–
M. peniculina (Moffett) F. Albers	s.loc.	s.n.	22	11	2,0	44,2	44,2

Table 2. (Continued)

Species	Locality	Collection No.	Chromos. No. (2n)	Basic Chromos. No. (x)	1	2	3
M. salmoniflora (Moffett) F. Albers	RSA, Springbok	Me 239	22	11	–	–	–
M. salmoniflora (Moffett) F. Albers	Namibia, Aus	Al & Me 86	22	11	1,8	39,5	39,5
M. senegalensis Guill. & Perr.	Namibia, Omaruru	Al & Kl 3626	22	11	1,2	25,4	25,4
M. senegalensis Guill. & Perr.	Namibia, Etosha	Al & Kl 3621	22	11	1,2	26,8	26,8
M. senegalensis Guill. & Perr.	Namibia, Kaokoveld	Al & Kl 3597	22	11	1,2	26,2	26,2
M. speciosa L.	RSA, Gordon's Bay	Al 2300	24	12	1,6	37,9	34,7
M. transvaalensis R. Knuth	RSA, N Drakensberge	Verhoeven 8684	44	11	–	–	–
M. umbellata Harv.	Namibia, Hardap Dam	Al & Kl 3582	18	9	1,6	28,7	35,1
M. umbellata Harv.	Namibia, Karibib	Al & Kl 3627	18	9	1,5	27,1	33,1
M. umbellata Harv.	Namibia, Kaokoveld	Jürgens s.n.	18	9	1,3	24,0	29,3
M. vanderietiae (L. Bolus) F. Albers	RSA, Steynsburg	Al 3563	22	11	–	–	–

ITS-sequence from *M. deserticola* was excluded from the test, because the ITS sequence of this species is highly divergent. For the purposes of this test, datasets of the two genes were reduced so that they shared the same set of 24 *Monsonia* species, and each region was represented by a single species. The tests were performed with 100 replication of heuristic search with TBR branch swapping.

Karyological investigation. Chromosome counts were performed as described in Gibby et al. (1996). The results were obtained from an investigation of mitosis (root tips) and meiosis (flower buds) from plants in cultivation. For mitosis, root tips were pre-treated with 0.002 mol 8-hydroxyquinoline for 4 h at room temperature and fixed in 3:1 absolute ethanol, glacial acetic acid, and kept in 70% ethanol. The root tips were stained in Snow's reagent (Snow 1963). For meiosis, flower buds were fixed in freshly prepared 6:3:1 absolute ethanol, glacial acetic acid, chloroform, and then stained in Snow's reagent. Measurements were mainly taken from camera lucida drawings (see Table 2).

Results

rDNA ITS (Fig. 1A)

Phylogenetic relationships among 25 species of *Monsonia* were investigated using nuclear rDNA ITS and cpDNA *trn*L (UAA) 5′exon-*trn*F (GAA) DNA regions. The rDNA ITS region contains the transcribed spacers ITS1 and ITS2 and the gene coding for 5.8S rDNA (Baldwin et al. 1995), whereas the chloroplast region includes the 5′ *trn*L exon, the *trn*L intron, the 3′*trn*L exon, the intergenic spacer and the *trn*F exon regions (Taberlet et al. 1991). As outgroups, we included two representatives of the genus *Pelargonium*: *P. album* from sect. *Reniformia* (x = 8, chromosome length >1,5 μm) and *P. gibbosum* from sect. *Polyactium* (x = 11, chromosome length <1,5 μm).

The final ITS alignment consists of 689 characters and 28 taxa. A total of 442 characters were constant, 77 positions were variably parsimony-uninformative and 170 were parsimony informative. The heuristic search yielded 30 most parsimonious trees (tree length = 533,

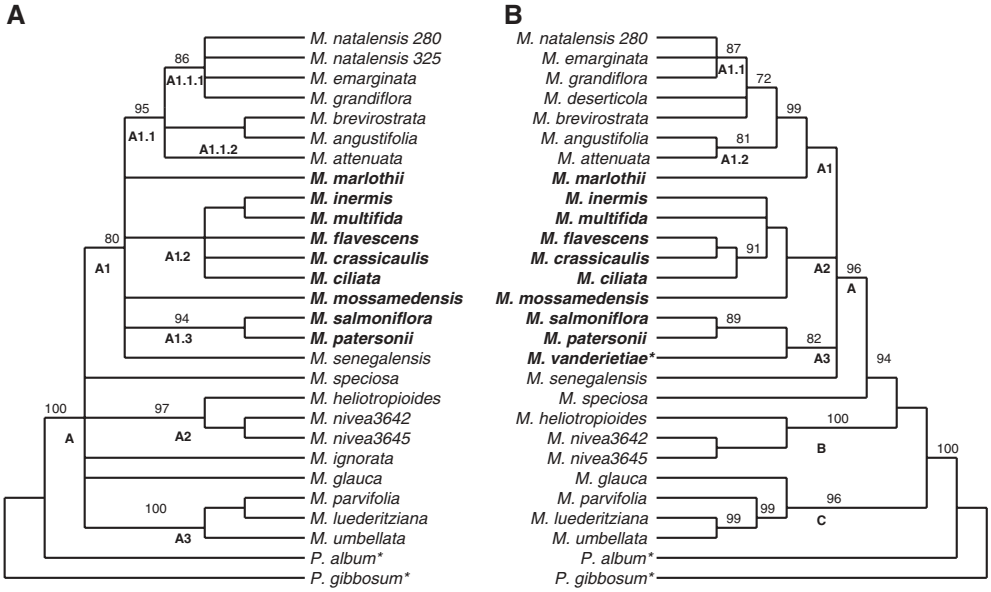

Fig. 1. Bootstrap tree (maximum parsimony analysis) for ITS (**A**) and bootstrap tree (maximum parsimony analysis) for *trn*L-F (**B**). The numbers above the clades are the bootstrap values. The ML bootstrap tree has the same topology. Species of the former genus *Sarcocaulon* in bold letters. *from GenBank

CI = 0.6829, RI = 0.7380). The best-fit evolution model for the maximum likelihood analyses was: TrN + G (Tamura and Nei 1993, Table 3). There was just a single maximum likelihood tree, which had the same topology as the strict consensus topology (Fig. 1A).

The ITS sequence from *M. deserticola* is highly divergent from the other *Monsonia* species and was removed from the analysis in order to prevent possible long branch attraction artefacts.

The genus *Monsonia* s.l. is monophyletic in the ITS tree (Fig. 1A). A large subclade (A1) is identified which contains the subclades A1.1, A1.2 and A1.3. Subclade A1.1 comprises of *M. natalensis*, *M. emarginata*, *M. grandiflora*, *M. brevirostrata*, *M. angustifolia* and *M. attenuata*. All these species belong to the section *Olopetalum* DC. All *Sarcocaulon* species group together in either subclade A1.2 or A1.3, except for *M. marlothii*, which resolves at the base of clade A1. *M. senegalensis* is part of a basal polytomy in clade A1. Clade A2 contains two representative clones of the North African/Arabian species *M. nivea* and *M. heliotropioides*. Clade A3 consists of *M. parvifolia*, *M. umbellata* and *M. luederitziana* from southwestern Africa. The position of *M. ignorata*, *M. speciosa* and *M. glauca* is not well-resolved in the *Monsonia*-clade A.

cpDNA, *trnL-F* (Fig. 1B)

The *trn*L-F alignment (27 taxa and 799 positions) contains 99 variable but phylogenetically uninformative positions, 162 phylogenetically informative positions, and 14 indels (insertion/ deletion) that vary in length from three to fifteen nucleotides. Indel characters, five of which are shared by the two outgroup *Pelargonium* species, were recoded as single binary characters, irrespective of indel length. The PCR of *M. ignorata* failed, and this species could therefore not be included in this analysis.

Excluding the outgroups, the matrix contained 618 constant, 89 parsimony uninformative and 92 parsimony informative characters. The heuristic search of the total matrix yielded 45 equally MPTs (tree length: 337, CI = 0.878, RI = 0.910), ti/tv for the ingroup is 0.79. The model selected for the maximum likelihood

Table 3. Maximum likelihood models for the ITS and *trn*L-F datasets

Model selected for the *trn*L-F dataset		Model selected for the ITS dataset	
TrN + G	Rate matrix	TVM + G	Rate matrix
Base frequencies	R(a)[AC] = 1.0000	Base frequencies	R(a)[A-C] = 1.13368
freqA = 0.2328	R(b)[A-G] = 2.3639	freqA = 0.3187	R(b)[A-G] = 1.0680
freqC = 0.3016	R(c)[A-T] = 1.0000	freqC = 0.2032	R(c)[A-T] = 0.2565
freqG = 0.2701	R(d)[C-G] = 1.0000	freqG = 0.1941	R(d)[C-G] = 0.7877
freqT = 0.1955	R(e)[C-T] = 5.6168	freqT = 0.2839	R(e)[C-T] = 1.0680
Proportion of invariable sites = 0	R(f)[G-T] = 1.0000	Proportion of invariable sites = 0	R(f)[G-T] = 1.0000
Gamma distribution shape parameter = 0.2993		Gamma distribution shape parameter = 1.8831	

analyses was: TVM + G (transversional model, Table 3).

Most indels are synapomorphies. The *trn*L-F bootstrap consensus cladogram also demonstrates that the genus *Monsonia* s.l. is monophyletic (Fig. 1B), although the resolution in the ingroup is not identical to that of the ITS cladogram. The predominately *Monsonia*-containing subclade (A1), the species of the former genus *Sarcocaulon* and the species *M. senegalensis* all arise from an unresolved basal polytomy A. Interestingly the *Sarcocaulon* species again resolve together into one of the two subclades (A2, A3), again with the exception of *M. marlothii*. This species is sister to the *Olopetalum* clade. *M. speciosa* is sister taxon to the *Olopetalum*/ *Sarcocaulon* clade (A).

Clade B is formed by the north African representatives (*M. nivea*, *M. heliotropioides*). Clade C consists of *M. parvifolia*, *M. umbellata* and *M. luederitziana*. The partition-homogeneity test without outgroups (maximum parsimony, TBR, 1000 replicate) was 0.35, which meant that the two data sets could not be combined.

Karyological data and phytogeography

The karyological data of 25 species of *Monsonia* are listed in Table 2. Limited information was available earlier (Albers 1990), and most of the chromosome numbers published before lacked locality data. The data set presented here gives an informative insight of the karyology of the genus.

Five different basic chromosome numbers occur in *Monsonia*. The majority (19 species studied) have x = 11 (sect. *Olopetalum*, sect. *Sarcocaulon*), all belonging to the clade A1 (ITS tree) (Fig.1) and A1, A2 and A3 (*trn*L-F tree) (Fig. 1). Only three species are tetraploid. Species with a basic chromosme number of x = 11 are all distributed in southern Africa, except for one species. *M. senegalensis* occurs in northern Namibia and West Africa.

Six species deviate from this common basic number of x = 11. A higher basic number is only found in one species (*M. speciosa* with x = 12). This taxon is restricted to the southern part of the South African winter rainfall area. The remaining southern African species with the basic numbers x = 10, 9 and 8 occur in extremly dry areas of the Namib Desert and belong to different clades in both trees (Fig. 1). Tetra- and hexaploid specimens within a single species are only known from *M. ignorata* with 2n = 40 and 60. The morphologically very similar diploid *M. heliotropioides* (2n = 18) and tetraploid *M. nivea* (2n = 36) with the derived basic chromosome number of x = 9 are common in the northern hemisphere, from the Sahara desert and adjacent areas of Arabia. These species form a monophyletic group of their own. (ITS tree: A2 clade [Fig. 1A], *trn*L-F tree: B clade [Fig. 1B]).

Not only basic chromosome numbers vary within *Monsonia* but also the length of the karyotypes (Table 2). Within the clade A1 (ITS tree [Fig. 1A]) all species have a basic chromosome number of x = 11, but three different chromosome size classes were recorded (Table 2). *M. senegalensis* has the smallest chromosomes (karyotype length 26.1 μm, average chromosome length 1.2 μm), the remaining species of the section *Olopetalum* has slightly larger karyotypes (31.5 μm, 1.5 μm), which clearly differ from the members of the former genus *Sarcocaulon* (38.0 μm, 1.8 μm). Although they do not form a distinct monophyletic group in either of the analyses, chromosomes of *M. speciosa* (2n = 24; 37.9 μm, 1.6 μm), *M. umbellata* (2n = 18, 26.6 μm, 1. 5μm) and *M. ignorata* (2n = 60, 98.6 μm, 1.6 μm) are all in the same range. *M. nivea*, the North African species, has the smallest chromosomes of the genus, with chromosome lengths of less than 1 μm. The large size of the chromosomes of *M. deserticola* (3.2–4.0 μm) is unique within the genus.

Photographs and drawings of the chromosomes of all taxa are available from the corresponding author on request.

Discussion

The ITS- and the *trn*L-F-regions of the majority of the species of *Monsonia* were employed to disclose infrageneric relationships. Both markers yielded similar but not identical results. The ratio of transition to transversion of the *trn*L-F-sequences is < 1.0 for the *Monsonia* group, which means that more transversions than transitions took place. This agrees with results of recent studies that have shown that the non-coding chloroplast regions have a ti/tv-ratio of < 1 (Bakker et al. 2000b, Sheahan and Chase 2000). The *trn*L-F region of *Monsonia* s.l. shows insertions and deletions (indels) which are not homoplastic and that further support the grouping observed in the phylogenies.

With 100% bootstrap support, the results of both DNA analyses support the monophyly of the genus *Monsonia* s.l. as circumscribed by Albers (1996). Thus, the ITS- and *trn*L-F-data unambiguously show that the former genus *Sarcocaulon* is nested as a paraphyletic assembledge in the genus *Monsonia*. Both *rbc*L-analyses and the fact that the *rp12* intron is lost in *Monsonia* and *Sarcocaulon* have previously already indicated a close affinity between these two taxa within the Geraniaceae (Price et al. 1990, Downie and Palmer 1992, Price and Palmer 1993). Almost identical flavonoid patterns, which have been classified as basal, were described for the *Monsonia* and the former *Sarcocaulon* species (Marschewski 1995). Albers (1996) assigned the *Sarcocaulon* species to the genus *Monsonia* based on flower development, morphology, palynology and karyology.

Although a cladistic analysis of morphological characters lead Aldaroso et al. (2001) to the conclusion that the *Sarcocaulon* species belong to the genus *Monsonia*, they still treated these species as a separate genus. They also found, that based on morphological characters, *Sarcocaulon* is monophyletic and share the following synapomorphies: Succulent stems covered by wax and spines formed from petioles. Although the development of waxy bark is unique to the former *Sarcocaulon* species, the closely related genus *Pelargonium* shows corresponding spine formation from petioles, a character believed to represent xeromorphic adaptations (e.g. *P. spinosum* Willd.).

In both bootstrap consensus cladograms (ITS and *trn*L-F) the former *Sarcocaulon* species are grouped into two clades (Fig. 1). In the ITS cladogram the resolution of the *Sarcocaulon* species is low and they resolve as two subclades and two additional species in an unresolved basal polytomy. In the *trn*L-F cladogram the species are split into two subclades, one of which enjoys reasonable high bootstrap support (82%). The classification of the former *Sarcocaulon* species according to the two gene regions does not match the sections according to Moffett (1979) whose infrageneric classification was mainly based on

leaf morphology. The former *Sarcocaulon* species and those of *Monsonia* section *Olopetalum* share the same basic chromosome number and an identical pollen sculpture (Verhoeven and Venter 1986, 1988; Verhoeven and Marais 1990). The mericarps are barbate, and the histological structure of the leaves is similar to those of *Monsonia* s.str. (Albers and Klenter, unpubl.).

In both cladograms the species *M. natalensis*, *M. emarginata*, *M. grandiflora*, part of the section *Olopetalum*, form a reasonably well-supported subclade (clade A1.1.1 [ITS cladogram] and clade A1.1 [*trn*L-F cladogram]). *M. brevirostrata*, *M. angustifolia* and *M. attenuata* are part of a polytomy in the ITS cladogram (A1.1). These species, together with *M. natalensis*, *M. emarginata* and *M. grandiflora* form a highly resolved clade (99%) in the *trn*L-F cladogram, suggesting possible relationships within the section *Olopetalum*. *M. senegalensis* resolves in the basal polytomy in clades A1 and A in the ITS and *trn*L-F analyses, respectively. This species shares the same basic chromosome number with the discussed species but its chromosome size is smaller.

Both cladograms include two more clades both with fairly high bootstrap support. One clade comprises the species *M. parvifolia*, *M. luederitziana* and *M. umbellata* (ITS: clade A3; *trn*L-F: clade C), which share the same deletions and insertions in the *trn*L-F region. This grouping matches the morphological classification in that all three species have pollen of the *Monsonia* subtype *Drudeana* (Verhoeven and Marais 1990), and similar flower and vegetative morphology (Venter 1990). To date, the chromosome number of only *M. umbellata* is known (x = 9). Its chromosomes are of medium size (< 1.5 μm).

The second clade comprises the species *M. nivea* and *M. heliotropioides* (ITS: clade A2; *trn*L-F: clade B). They share the same basic chromosome number (x = 9) and display the smallest chromosomes within the genus (< 1,0 μm). Both are annuals with small, inconspicuous flowers, a similar habit and pollen of the *Monsonia* subtype *Nivea* (Verhoeven and Marais 1990). Based on their morphological similarity, these species were at times also regarded as one species with two varieties (*M. heliotropioides* (Cav.) Boiss. var. *heliotropioides*, *M. heliotropioides* var. *nivea* (Dec.) Guin.). These species of the Northern Hemisphere and those of the extremely arid region of southern Africa have plumose mericarps, which suggests anemochory. This character has developed at least twice in this genus.

M. ignorata resolves at the base of clade A in the ITS cladogram and was not included in the *trn*L-F analysis. In a total clustering based on morphological characters, this species grouped with *M. luederitziana*, *M. parvifolia* and *M. trilobata* (Venter 1990). The species forms root tubers, has a basic chromosome number of x = 10 and is polyploid. It has pollen of the *Monsonia* type.

The ITS sequence of *M. deserticola* was divergent and was therefore omitted from the analysis. In the *trn*L-F cladogram (Fig. 1B) it resolved at the base of clade A1.1, which also includes the species *M. emarginata*, *M. natalensis*, *M. grandiflora* and *M. brevirostrata*. To date, *M. deserticola* is the only species studied with a basic chromosome number of x = 8 and has by far the largest chromosomes (3.2–4.0 μm) in the genus. According to Verhoeven and Marais (1990) the species displays a pollen type that is unique in the genus (*Erodium* type *Gruinum*). Similary, the nectaries are also unique (Link 1990). The species is only found in a very limited area in SW Namibia. It is definitely not a species of the genus *Erodium*, which is not indigenous in southern Africa.

M. speciosa resolves at the base of clade A in both the ITS and *trn*L-F analyses. The species has the only ascending basic chromosome number in the genus (x = 12) with chromosomes of medium size. The pollen, which belongs to the *Monsonia* type, is easily recognized by the dentate tectum. This species includes both pleisiomorphic and derived morphological characters. It is the only *Monsonia*

species with pinnate leaves and inflorescences consisting of just a single large and conspicuous flower. It populates relatively humid areas of the winter rainfall area of the Cape Floristic Region (CFR) (Goldblatt and Manning 2000) of South Africa (Fynbos).

The majority of the perennial herbs of the section *Olopetalum*, all with a basic chromosome number of x = 11, are rather widely distributed, and are able to grow in a number of habitats on different substrates in moderate climates. The perennials in clade A1 (ITS cladogram) are found in extreme habitats like deserts and semi- deserts, but also in transitional areas where they are represented by the succulent and often woody species of the section *Sarcocaulon* (ITS cladogram, clade A1.2 and A1.3).

In *Monsonia*, annuals have the most derived chromosome numbers (x = 9) and the smallest chromosomes (*M. heliotropioides*, *M. nivea*, *M. umbellata*). The Namibian species *M. umbellata*, however, resolves in a different subclade (Fig. 1A, B) than the two species distributed in the northern hemisphere. This life form has therefore developed at least twice independently. The polyploid *M. nivea* is more widely distributed and more common than the closely related diploid *M. heliotropioides*. The decrease of the basic chromosome number in combination with a reduction of the chromosome size is also found in annual *Pelargonium* species of the section *Peristera* (Albers, unpubl.). In general, such a pattern is associated with a shortened life cycle (Stebbins 1966). The fact that these two annual *Monsonia* species resolve into two different clades, also suggests that plumose awns serving anemochory should have developed independently from each other in at least two instances. We therefore conclude that the taxonomic value of this character, which has been repeatedly stressed by several authors (Boissier 1867; Kers 1968; Venter 1979, 1990), appears to be limited.

M. deserticola and *M. ignorata* from the southern Namib are both perennials with subterranean organs for storing water and nutrients. Both species have derived chromosome numbers. *M. deserticola* has the biggest karyotype of all *Monsonia* species, but the lowest basic chromosome number of x = 8 (2n = 16). *M. ignorata* has a basic chromosome number of x = 10, it also displays the highest polyploidy value recorded in the genus, namely of 2n = 6x = 60. The two species do not resolve anywhere close to one another in the ITS cladogram. It is often true that plants in extreme habitats are polyploid (Ehrendorfer 1980, Gottschalk 1989, Meve 1997), but we propose that in the present study a preferential correlation should be made between genome size and habitat. The tendency for a reduction of the chromosome number in combination with an increasing chromosome length can also be seen in *Pelargonium* species of the section *Reniformia* (Dreyer and Marais 2000). In *Pelargonium* sect. *Hoarea* it is caused by Robertsonian translocation (Gibby et al. 1996).

In the ITS bootstrap cladogram, clade A1 is characterized by diploid species with the basic chromosome number of x = 11, whereas the clades A2 and A3 show polyphyly and consist of species with derived chromosome numbers, some of them with highly diverging chromosome sizes. The development of the different basic chromosome numbers obviously occurred several times independently, a phenomenon already known to be true for *Pelargonium* sect. *Hoarea* (Touloumenidou et al. 2004). In *Monsonia* chromosome evolution is obviously an important character that drove taxon differentiation.

The Saharo-Sindian *M. heliotropioides* and *M. nivea*, which form a section of their own (*Plumosae*) (Boissier 1867), are regarded as highly derived terms of flower characters (e.g. extremely short-lived petals, about 10 pollen per anther). The section *Olopetalum* is represented in the northern hemisphere only by *M. angustifolia*, *M. senegalensis*, and *M. ignea* (not studied). The remaining species of the section are exclusively found in the Southern Hemisphere. The East African land bridge may be interrupted at present, but a north-south

corridor has been postulated on several occasions (Winterbottom 1997). Southern Africa became a secondary species centre for the genus, as seen in several other taxa (Albers and Meve 2001). Herbaceous species like *M. glauca*, *M. angustifolia* and *M. senegalensis* could have spread into this area, while new species could have developed since and are now endemic to the region.

It is proposed that under the conditions of the semi-arid habitats in the Karoo-Namib region the sect. *Sarcocaulon* could have developed. Changing climatic condition may have lead to a segregation into different genetic isolated groups within the section *Sarcocaulon* (ITS cladogram: A1.2 and A1.3, *trn*L-F cladogram: A2 and A3). In both cladograms the most northern distributed *M. mossamedensis* (northern Namibia, southern Angola) is part of a polytomy.

Deserts like the Namib Desert acted not only as refuges for desert plants, but also as evolutionary centres (Verdcourt 1969, Zinderen Bakker 1975). The relatively diverse species, *M. ignorata*, *M. deserticola* and *M. luederitziana,* which Venter (1990) also considers the most modern within the genus, all occur in this region.

M. speciosa (sect. *Monsonia*) is restricted to the CFR under rather moist winter rainfall conditions. Venter (1990) already discussed a possible link between this species and *M. longipes*. Unfortunately no material of this species was available for this study.

Molecular, karyological and recent palynological data support the taxonomic inclusion of *Sarcocaulon* into *Monsonia* as proposed by Albers (1996). *Monsonia* is monophyletic, it would be paraphyletic without the former genus *Sarcocaulon*. The current infrageneric taxonomic classification is supported with regard to *Monsonia* sectt. *Monsonia, Olopetalum* and *Sarcocaulon*. Sect. *Plumosae* Boiss. should be limited to the two north African species *M. heliotropioides* and *M. nivea* sensu Boissier (1867). *M. parvifolia*, *M. luederitziana* and *M. umbellate* from the southern Namib are grouped together. More information is needed on *M. ignorata*, *M. deserticola* and *M. glauca* and the remaining species which could not be studied here before a final taxonomic decision can be taken.

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