Plant Syst Evol (2008) 273:151–167
DOI 10.1007/s00606-008-0007-1

ORIGINAL ARTICLE

Phylogeny of *Androcymbium* (Colchicaceae) based on morphology and DNA sequences

Alberto del Hoyo · Joan Pedrola-Monfort

Received: 17 April 2007 / Accepted: 14 December 2007 / Published online: 14 May 2008

Abstract The inclusion of species of *Colchicum* within *Androcymbium* in a previous cpDNA phylogeny of the Colchicaceae, questioned the monophyly of *Androcymbium*, and it was proposed to unite the two genera in *Colchicum*. Here we expand the previous phylogenetic analyses of *Androcymbium* by increasing the taxon sampling and adding more data. The analysis include 29 of the 57 species of Androcymbium, more cpDNA (*trn*L intron, *trn*L-*trn*F IGS, *trn*Y-*trn*D IGS, and *trn*H-psbA IGS), and nDNA (RNApol2 intron 23) regions, and morphological and life-history traits data. Both parsimony and Bayesian inference were used. According to our data there is no reason to expand *Colchicum* to include *Androcymbium*, but we support the inclusion of *Bulbocodium* and *Merendera* within *Colchicum*. Morphology and life history traits are the main arguments in favor of recognizing *Androcymbium* as a well-circumscribed genus. In the phylogeny two Androcymbium groups are clearly differentiated: (1) one including species from Western and Eastern South Africa, Namibia, and North Africa, and (2) one including species from the north west of South Africa and south of Namibia.

Keywords Androcymbium · Colchicaceae · Colchicum · cpDNA · Morphological data · nDNA · Phylogeny · Southern Africa

A. del Hoyo (✉)
Marimurtra Botanic Garden - Carl Faust Private Foundation, PO box 112, 17300 Blanes, Girona, Spain
e-mail: alberto.delhoyo@marimurtra.cat

J. Pedrola-Monfort
Department of Botany, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Dr. Moliner 50, 46006 Burjassot, Valencia, Spain

Introduction

Since Willdenow (1808) established the genus *Androcymbium* and until the most recent molecular phylogeny (Del Hoyo and Pedrola-Monfort 2006), the taxonomic status of the genus, and its infrageneric classification, has been widely discussed. Schlechtendal (1826) divided the genus in two genera based on tepal morphology: (1) *Androcymbium* (concave tepal blade), and (2) *Erythrostictus* (plane tepal blade). Baker (1874, 1879) grouped the genus in one alone, and divided it into three groups according to the similarity between bracts and leafs. During the following years, the generic status was not questioned, but different infrageneric classifications were made due to description of new taxa (Bentham and Hooker 1862–1883; Krause 1920; Müller-Doblies and Müller-Doblies 1984, 1990, 1998). Recently, Müller-Doblies and Müller-Doblies (2002) have made a thorough morphological and nomenclatural analysis of *Androcymbium* resulting in recognition of a total of 56 species. They pointed out that a selected variation of biometrical characters together with the floral pollination syndromes, considering studies by Vogel (1954), are the key in the taxonomy of *Androcymbium*, and at this point, they established a complex infrageneric division of this genus in five sections, eleven series and eight subseries.

The first molecular phylogeny of *Androcymbium* based on a parsimony analysis of cpDNA–RFLP's (Caujapé-Castells et al. 1999), resulted in a strict consensus maximum parsimony tree with *Androcymbium* as a monophyletic group with high-bootstrap support (BS) of 100%. However, only 12 *Androcymbium* species of one section were included, and consequently more than 80% of the South African species were not included in the analysis (Müller-Doblies and Müller-Doblies 2002). Later, in a phylogenetic study of the Colchicaceae using non-coding cpDNA sequences,

Vinnersten and Reeves (2003) found that *Androcymbium* was not monophyletic, due to the inclusion within this genus of species from *Bulbocodium*, *Colchicum*, and *Merendera*. For this phylogeny, these authors analysed species from the five sections of *Androcymbium* proposed by Müller-Doblies and Müller-Doblies (2002), including those very important for discussing the monophyly of *Androcymbium*, namely species belonging to sections *Dregeocymbium*, *Kunkeliocymbium*, and *Marlothiocymbium*. In the last molecular phylogeny of *Androcymbium* based on non-coding cpDNA sequences (Del Hoyo and Pedrola-Monfort 2006), some of these new and others analysed in Vinnersten and Reeves (2003), it was shown again that *Androcymbium* was non-monophyletic, and the potentially necessary nomenclatural changes are discussed.

From these three molecular phylogenies, several interesting conclusions can be deduced: (1) the solved clades are concordant with the biogeography of the genus, (2) in the two phylogenies based on cpDNA non-coding sequences, the inclusion of more species from the *Androcymbium* section, as well as from species belonging to the previously not analyzed sections *Dregeocymbium* and *Kunkeliocymbium*, put in doubt the monophyly of *Androcymbium*, due to their grouping with species of the genera *Bulbocodium*, *Colchicum*, and *Merendera*. Because of this, Vinnersten and Reeves (2003) and Del Hoyo and Pedrola-Monfort (2006) argued about redefine *Colchicum* to include *Androcymbium* species.

In the present study we increased the taxon sampling in *Androcymbium* trying to analyse taxa from all of its disjunct distribution areas. Additionally, the number of non-coding cpDNA sequences was increased to four, and we have included one nuclear sequence. Finally, we have added morphological data and life-history traits from J. Pedrola-Monfort and A. Del Hoyo (unpublished data) in order to increase resolution (Wortley and Scotland 2006) and support of some clades (Baker et al. 1998; Aagesen and Sanso 2003).

Materials and methods

Taxon sampling

Twenty-nine of the 57 recognized species of *Androcymbium* representing four of the five recognized sections, into which this genus is divided (Müller-Doblies and Müller-Doblies 1998, 2002) were sampled (Table 1). The remaining section (sect. *Marlothiocymbium*) is monotypic. Our taxon sampling represents a wide range of morphological variation in *Androcymbium* and a representative geographic range of the genus across Africa (Fig. 1). Because of their putative affinities with *Androcymbium*, *Baeometra*, *Bulbocodium*, *Colchicum*, *Gloriosa*, *Merendera*, and *Onixotis*, belonging to the family Colchicaceae (Vinnersten and Reeves 2003), were included. Moreover, one taxon from the family Alstroemeriaceae, which is phylogenetically close to Colchicaceae (Bremer 2000; Vinnersten and Bremer 2001; Vinnersten and Reeves 2003) was included in the analysis as outgroup (Table 1). All of the analyzed samples for this study were grown under the same conditions in a greenhouse at the Marimurtra Botanic Garden in Blanes.

DNA extraction and amplification

Genomic DNAs were extracted from fresh leaf tissue, previously dried in silica gel followed by snap freezing in liquid nitrogen, using the procedure of Doyle and Doyle (1987) with the modifications of Li et al. (2001). The isolated DNA was resuspended in T.E. buffer. For each of the samples, the *trn*L intron, *trn*L-*trn*F spacer, *trn*Y-*trn*D spacer, *trn*H-*psb*A spacer, and *RNApol2* intron 23 (*RNApol2*_i23) were separately amplified using the polymerase chain reaction (PCR). The PCR protocol for the *trn*L intron, *trn*L-*trn*F spacer, and *trn*Y-*trn*D spacer was described in Del Hoyo and Pedrola-Monfort (2006). The *trn*H-*psb*A spacer was amplified using the primers trnH (Tate and Simpson 2003) and *psb*A (Sang et al. 1997). The DNA amplification was performed in a 50 µl volume containing 1× PCR buffer (Bioline Ltd., London, UK), 4 mM of $MgCl_2$ (Bioline), 0.1 mM of each dNTP (Bioline), 0.4 µM of each primer (Eurogentec Ltd., Seraing, Belgium), 1 unit of Biotaq (Bioline), and 250 ng of DNA. The *RNApol2*_i23 was amplified using the primers RPB2-P10F (Denton et al. 1998) and RPB2-M11Rr (C. E. Lewis et al., in prepration). The DNA amplification was performed in a 50 µl volume containing 1 × PCR buffer (Bioline), 4 mM of $MgCl_2$ (Bioline), 0.2 mM of each dNTP (Bioline), 0.3 µM of each primer (Eurogentec), 1 unit of Biotaq (Bioline), and 125 ng of DNA. Following and activation step of 3 min at 92°C for the enzyme, the PCR mixture underwent 30 cycles of 30 s at 92°C, an annealing of 30 s for the *trn*H-*psb*A spacer and 20 s for the *RNApol2*_i23 at 62°C, and 30 s at 72°C. All PCR products were purified on GFX PCR DNA columns (Amersham Biosciences Europe GmbH, Cerdanyola, Barcelona, Spain) according to manufacturer's instructions.

DNA sequencing

Sequencing reactions for the *trn*L intron, *trn*L-*trn*F spacer, and *trn*Y-*trn*D spacer were described in Del Hoyo and Pedrola-Monfort (2006). Sequencing reactions for the *trn*H-*psb*A spacer and *RNApol2*_i23 were carried out with the forward primer (*trn*H and RPB2-P10F, respectively) using BigDye Terminator v1.1 Cycle Sequencing (Applied

Table 1 Voucher specimens and GenBank accession for sequences

Taxon	Origin; Voucher	GenBank accession numbers				
		*trn*L intron	*trn*L-*trn*F IGS	*trn*Y-*trn*D IGS	*trn*H-*psb*A IGS	*RNApol*2_i23
Alstroemeria aurea Graham	Ex. cult., Valdivia Botanic Garden, Chile; JBMiM 95095	AY622764	AY622728	AY622773	DQ088276	DQ088364
Androcymbium albanense Schönland	Sidbury, Grahamstown, South Africa; JBMiM ALBASW 2000.0907	AY622733	AY622695	AY611765	DQ088277	DQ088334
A. asteroides J.C.Manning & Goldblatt	Gochas, Stampriet, Namibia; JBMiM ASTEGO 2001.05063	AY622731	AY622693	AY611762	DQ088312	DQ088340
A. austrocapense Hap.1 U.Müll.-Doblies & D.Müll-Doblies	Sardinia Bay, Skoenmakerskop, South Africa; JBMiM AUSTSB 2000.0944	AY136757	AY622696	AY611766	DQ088279	DQ088335
A. austrocapense Hap.2	Good Hope Cape, Simonstown, South Africa; JBMiM AUSTGH 1583	ID Hap.1	ID Hap.1	AY611741	DQ088280	DQ088341
A. bellum Schltr. & K.Krause	Vioolsdrif, South Africa; JBMiM BELLVI 1618	AY622738	AY622700	AY611742	DQ088281	DQ088342
A. burchellii Baker subsp. *burchellii*	Hexrivierpass, Worcester, South Africa; JBMiM BURCHX 1587	AY622739	AY622701	AY611743	DQ088282	ID Hap.1
A. burchellii subsp. *pulchrum* Hap.1 (Schltr. & K.Krause) Pedrola, Membrives, J.M.Monts. & Caujapé	Calvinia, South Africa; JBMiM BURCCA 2242	AY622740	AY622702	AY611744	DQ088283	DQ088343
A. burchellii subsp. *pulchrum* Hap.2	Nieuwoudtville, Calvinia, South Africa; JBMiM BURCNI 2000	AY622741	ID Hap.1	ID Hap.1	ID Hap.1	ID Hap.1
A. capense (L.) K.Krause	Hopefield, Cape Town, South Africa; JBMiM CAPEHO 2027	AY622742	AY622703	AY611745	DQ088284	DQ088344
A. cedarbergense U.Müll.-Doblies & D.Müll-Doblies	Clanwilliam, Wuppertal, South Africa; JBMiM CLANPK 2384	AY622747	AY622708	AY611748	DQ088278	DQ088348
A. ciliolatum Schlt. & K.Krause	Silverhill Seed nursery, Kenilworth, South Africa; JBMiM CILIVS 98335	AY622743	AY622704	AY611746	DQ088285	DQ088345
A. circinatum Baker subsp. *circinatum* Hap.1	Springbok, South Africa; JBMiM CIRCNB 1895	AY622744	AY622705	AY611747	DQ088286	DQ088346
A. circinatum subsp. *circinatum* Hap.2	Springbok, South Africa; JBMiM CIRCSB 1769	AY622745	AY622706	ID Hap.1	DQ088287	DQ088347
A. circinatum subsp. *vestitum* U.Müll.-Doblies & D.Müll-Doblies	Eksteenfontein, Vioolsdrif, South Africa; JBMiM VILLEK 2217	AY622758	AY622719	AY611759	DQ088314	DQ088358
A. cuspidatum Baker	Calvinia, South Africa; JBMiM CUSPCA 2221	AY622746	AY622707	AY611749	DQ088288	DQ088349
A. decipiens N.E.Br.	Saint Lucia Bay, Mtubatuba, South Africa; JBMiM DECISL 1.28800	AY622734	AY622697	AY611767	DQ088289	–
A. dregei C.Presl	Clanwilliam, Wuppertal, South Africa; JBMiM DREGPK 2450	AY622748	AY622709	AY611750	DQ088290	DQ088350
A. eghimocymbion U.Müll.-Doblies & D.Müll-Doblies	Citrusdal, Clanwilliam, South Africa; JBMiM EGHICI 1889	AY622749	AY622710	AY611751	DQ088291	DQ088351
A. gramineum Hap.1 (Cav.) McBride	Barranco de Curriá, Almería, Spain; JBMiM GRBC 545B.1192	AY608517	AY608520	AY608528	DQ088292	DQ088326

Table 1 continued

Taxon	Origin; Voucher	GenBank accession numbers				
		*trn*L intron	*trn*L-*trn*F IGS	*trn*Y-*trn*D IGS	*trn*H-*psb*A IGS	*RNApol*2_i23
A. gramineum Hap.2	Safi, Morocco; JBMiM GRSA 1265.1290	AY608516	AY608521	AY608529	DQ088293	DQ088327
A. hantamense Engl.	Calvinia, South Africa; JBMiM HANTCA 2410	AY622750	AY622711	AY611752	DQ088294	DQ088352
A. henssenianum U.Müll.-Doblies & D.Müll-Doblies	Eksteenfontein, Vioolsdrif, South Africa; JBMiM HENSEK 2161	AY622751	AY622712	AY611753	DQ088295	DQ088353
A. hierrense Santos	El Hierro, Canary Islands, Spain; JBMiM HIHI 563.990	AY608514	AY608523	AY608531	DQ088296	DQ088329
A. huntleyi Hap.1 Pedrola, Membrives, J.M.Monts. & Caujapé	Eksteenfontein, Vioolsdrif, South Africa; JBMiM HUNTEK1 2348	AY622752	AY622713	AY611754	DQ088297	DQ088354
A. huntleyi Hap.2	Eksteenfontein, Vioolsdrif, South Africa; JBMiM HUNTEK3 2313	ID Hap.1	ID Hap.1	ID Hap.1	DQ088298	ID Hap.1
A. irroratum Hap.1 Schltr. & K.Krause	Eksteenfontein, Vioolsdrif, South Africa; JBMiM IRROEK 2239	AY622753	AY622714	AY611755	DQ088299	DQ088355
A. irroratum Hap.2	Eksteenfontein, Vioolsdrif, South Africa; JBMiM IRROEK6 2150	AY622754	AY622715	AY611756	DQ088300	ID Hap.1
A. irroratum Hap.3	Vanrhynsdorp, South Africa; JBMiM IRROVY 1888	ID Hap.2	ID Hap.2	ID Hap.2	DQ088301	ID Hap.1
A. irroratum Hap.4	Bitterfontein, Kamiesberg, South Africa; JBMiM IRROKA 2541	AY622755	AY622716	ID Hap.2	DQ088302	ID Hap.1
A. irroratum Hap.5	Vredendal, Vanrhynsdorp, South Africa; JBMiM IRROKW 1698	ID Hap.4	ID Hap.4	ID Hap.4	DQ088303	ID Hap.1
A. leistneri Hap.1 U.Müll.-Doblies & D.Müll-Doblies	Bloemfontein Botanic Garden, South Africa; JBMiM LEISBG 2000.0959	AY622735	AY622698	AY611768	DQ088304	DQ088336
A. leistneri Hap.2	Bloemfontein Botanic Garden, South Africa; JBMiM LEISBL 2000.0953	AY622736	ID Hap.1	AY611769	ID Hap.1	ID Hap.1
A. longipes Baker	Addo, Port Elizabeth, South Africa; JBMiM LONGZU 2000.0925	AY622737	AY622699	AY611770	DQ088305	DQ088337
A. melanthioides Willd. subsp. *melanthioides* Hap.1	Great Gamsberg, Nauchas, Namibia; JBMiM MELAGA 2001.05018	AY622732	AY622694	AY611763	DQ088306	DQ088338
A. melanthioides subsp. *melanthioides* Hap.2	Okabiruru farm, Otjosondu, Namibia; JBMiM MELAOT 2001.05030	ID Hap.1	ID Hap.1	AY611764	ID Hap.1	ID Hap.1
A. palaestinum Baker	Dimona desert, Israel; JBMiM PADI 595.990	AY136755	AY608527	AY608534	DQ088307	DQ088330
A. poeltianum Hap.1 U.Müll.-Doblies & D.Müll-Doblies	Springbok, South Africa; JBMiM POELCO 2071	AY622756	AY622717	AY611757	DQ088308	DQ088356
A. poeltianum Hap.2	Steinkopf, Springbok, South Africa; JBMiM POELST 1779	AY622757	AY622718	AY611758	DQ088309	DQ088357
A. psammophilum Svent.	Fuerteventura, Canary Islands, Spain; JBMiM PSFU 539.1190	AY136756	AY608524	AY608532	DQ088310	DQ088331

Table 1 continued

Taxon	Origin; Voucher	GenBank accession numbers				
		*trn*L intron	*trn*L-*trn*F IGS	*trn*Y-*trn*D IGS	*trn*H-*psb*A IGS	*RNApol*2_i23
A. rechingeri Greuter	Elafonisis, Crete, Greece; JBMiM REEL 220.691	AY608518	AY608525	AY608535	DQ088311	DQ088332
A. roseum Engl.	Okahandja, Otjimbingwe, Namibia; JBMiM ROSEFB 2001.05047	AY622730	AY622692	AY611761	DQ088313	DQ088339
A. walteri Pedrola, Membrives & J.M.Monts.	Steinkopf, Springbok, South Africa; JBMiM WALTST 1747	AY622759	AY622720	AY611760	DQ088315	DQ088359
A. wyssianum Hap.1 Beauverd & Turrett.	Fonts Bleus, Maski, Morocco; JBMiM WYFB 214.1092	AY608519	AY608526	AY608533	DQ088316	DQ088333
A. wyssianum Hap.2	Essaouira, Morocco; JBMiM WYEA 2002.013	AY608515	AY608522	AY608530	DQ088317	DQ088328
Baeometra uniflora (Jacq.) G.J. Lewis	Simmonstown, South Africa; JBMiM BAEO 1857	AY155494	AY622729	AY622769	DQ088318	DQ088362
Bulbocodium vernum L.	Huesca, Spain; JBMiM BULB 95113208	AY622763	AY622727	AY622767	DQ088319	–
Colchicum lusitanum Brot.	Cadiz, Spain; JBMiM COLCLU 529.1097	AY154475	AY622722	AY622768	DQ088320	DQ088363
Gloriosa superba L.	Ex. cult., Marimurtra Botanic Garden, Blanes, Spain; JBMiM GLSU 96.398	AY154476	AY622721	AY622766	DQ088321	DQ088360
Merendera androcymbioides Valdés	Malaga, Spain; JBMiM MERAND 3.398	AY622761	AY622725	AY622772	DQ088322	–
M. filifolia Cambess.	Mallorca, Balearic Islands, Spain; JBMiM MERFIL 224.991	AY622760	AY622726	AY622771	DQ088323	–
M. montana (L.) Lange	Huesca, Spain; JBMiM MERMON 1432.794	AY154477	AY622724	AY622770	DQ088324	DQ088361
Onixotis stricta (Burm.f.) Wijnands	Silverhill Seed nursery, Kenilworth, South Africa; JBMiM ONIX 1.197	AY622762	AY622723	AY622765	DQ088325	–

Hap. Haplotype, *ID.* idem

Biosystem Inc. Foster City, CA, USA) in a 10 µl volume containing 50 ng of purified DNA and 3.2 pmol of amplification primer, according to the manufacturer's specifications. Sequencing reactions underwent 25 cycles of 30 s at 94°C, 30 s at 50°C, and 4 min at 60°C. Sequencing reactions were electrophoresed on an ABI PRISM 310 DNA sequencer (Applied Biosystems) in the Biology Department of the Girona University, Catalonia. Some individuals needed an extra step of cloning in a pGEM-T plasmid (Promega), according to the manufacturer's protocol, to obtain a clear *RNApol2*_i23 sequence. All sequences were submitted in the GenBank.

Alignment

Sequence information of the five DNA regions were aligned using ClustalW v.1.4 (Thompson et al. 1994), and were tested and corrected manually using Bioedit v. 7.0.5.2 (Hall 1999). Gaps of 2 base pairs (bp) or less were removed, because previous analyses of these regions has demonstrated that insertions/deletions (indels) longer than 2 bp are not too much prone to parallelism and thus may provide important phylogenetic information whereas, homoplasy in indel distribution is almost completely accounted for by indels of 1 or 2 bp (van Ham et al. 1994; Bayer and Starr 1998). Therefore, indels of 3 bp and longer were coded as a binary character data (Simmons and Ochoterena 2000) using the GapCoder program (Young and Healy 2003). Sequence alignments are available from the corresponding author.

DNA data analysis

The DNA sequence from each molecular marker was analysed separately and combined in a final analysis. The ILD test (Farris et al. 1995) was performed to test the

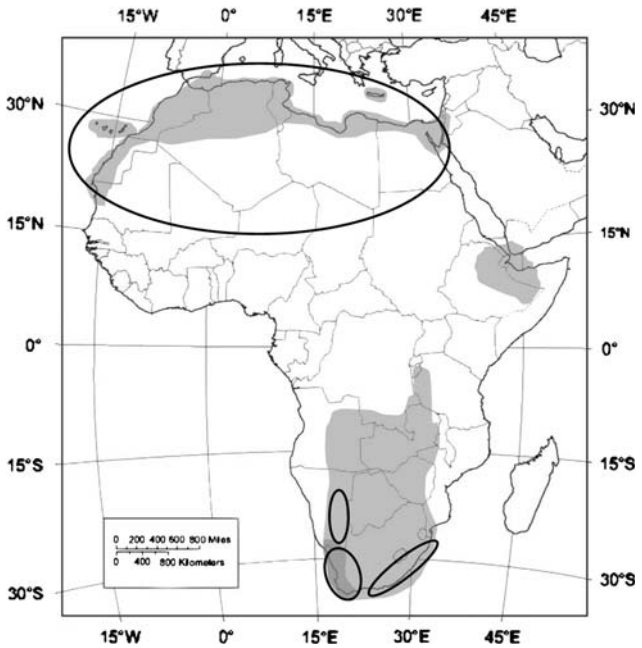

Fig. 1 Distribution of the 57 recognized species of the genus *Androcymbium* (*dark regions*) and region of the 29 sampled species (*ellipse areas*). North Africa: 6 species; Namibia: 3 species; Western South Africa: 16 species; Eastern South Africa: 4 species

combinability of the five DNA sequences region. Each region was analyzed using a Bayesian Markov chain Monte Carlo (B/MCMC) approach, as implemented in MrBayes 3.1 (Huelsenbeck and Ronquist 2001), to test for the presence of "conflicting parts" following Wiens (1998). Models of sequence evolution were selected using the likelihood ratio test (LRT) implemented in Modeltest (Posada and Crandall 1998). The gaps recoded as binary data were analyzed under the F81 evolutionary model (Felsenstein 1981), according to Ronquist et al. (2005). The B/MCMC analyses were conducted using the selected model of evolution (with flat priors) and four chains (one cool and three heated). Chains were allowed to run for 1.0×10^6 generations, and trees were sampled from the cool chain every 100 generations. Following completion, sampled trees were plotted against their likelihood in order to recognize the point where the likelihoods converged on a maximum value, and all trees prior to this convergence were discarded as the "burn-in" phase. The remaining trees were combined in a majority rule consensus.

The five DNA data sets were then combined and analysed together, using parsimony (MP) and B/MCMC methods. For the parsimony analyses, all searches were implemented in PAUP* 4.0b10 (Swofford 2003) with 100 random addition sequence replicates, TBR branch swapping, and MULTREES in effect. The gaps coded as binary data were analysed as presence/absence data. The consistency index (CI) is presented to estimate the amount of homoplasy in the characters. Resolution was measured using the consensus fork index (CFI), calculated by dividing the number of nodes found in a strict consensus tree by the total number of possible nodes (Colless 1980, 1981), and branch support was assessed by both bootstrap (Felsenstein 1985) and posterior probabilities B/MCMC analysis. The bootstrap analysis, implemented in PAUP* 4.0b10, consisted of 1,000 pseudoreplicates, each with 10 random addition sequence replicates, TBR branch swapping, and MULTREES in effect. The B/MCMC analyses of the combined data set was conducted using the same model but separately for each of five molecular marker, flat priors, and four chains. Chains were allowed to run for 1.5×10^6 generations. Following completion, the burn-in trees were discarded and the remaining trees were combined in a majority rule consensus, to determine the posterior probability of each node.

Morphological and other life-history traits data analysis

Various morphological and life-history characters examined in the field, in cultivation, and from herbarium, were coded in J. Pedrola-Monfort and A. Del Hoyo (unpublished data), and used in this paper for cladistic analyses. Patterns of character evolution were explored using the character-evolution reconstruction function of McClade 4.08 (Maddison and Maddison 2005) on one of the B/MCMC phylogenetic trees. Both ACCTRAN (maximizing the proportion of the homoplasy that is accounted by parallelism) and DELTRAN (maximizing that by reversal) resolutions were considered and analyzed.

A total of 22 morphological and life-history traits characters (J. Pedrola-Monfort and A. Del Hoyo, unpublished data) were included in the DNA data matrix to test its effect in the phylogeny of *Androcymbium* with B/MCMC and parsimony analysis. Because some characters have more than two states, these data was analysed under the model HKY + I + Γ (Ronquist et al. 2005).

Morphology, life-history traits and DNA data combined analysis

All characters (morphology, life-history traits and molecular data) were combined and analysed both with parsimony and B/MCMC methods with the aim of getting a better support and resolution than in the individual analyses (Baker et al. 1998; Aagesen and Sanso 2003; Wortley and Scotland 2006).

The parsimony analyses were implemented in PAUP* 4.0b10 (Swofford 2003) with 100 random addition sequence replicates, TBR branch swapping, and MULTREES in effect. The consistency index (CI) and the retention index (RI) are presented to estimate the amount of

homoplasy in the characters. The branch support was assessed by bootstrap analysis in PAUP* 4.0b10, consisting of 1,000 pseudoreplicates, each with 10 random addition sequence replicates, TBR branch swapping, and MULTREES in effect. The B/MCMC analyses of the combined data set was conducted using the same model but separately for each of seven character data sets (four cpDNA, one nDNA, one gap data set, and one morphological and life-history traits data set), flat priors, and four chains. Chains were allowed to run for 2.0×10^6 generations. Following completion, the burn-in trees were discarded and the remaining trees were combined in a majority rule consensus, to determine the posterior probability of each node.

Results

Sequence data

The lengths of the combined data vary between 2,015 bp (northern Africa species) and 1,899 bp (western South Africa species) (Table 2). Because of this, it was necessary insert gaps to align sequences, increasing the total length of the aligned matrix (Table 3). These gaps can provide phylogenetic information but some authors ignore these zones, losing much phylogenetic information when analysing the data. Due to this, the gaps codified as a character data were introduced into the analysis, resulting in a final 3,038 pb matrix. The mean sequence length, alignment length (L), variable characters, parsimony informative characters, indels, PIC's value (potentially informative characters [NS + ID + IV], where NS = number of nucleotide substitutions, ID = number of indels, and IV = number of inversions) and the proportion of observed mutational events (or % of variability) for each region using a modified version of the formula used in O'Donnell (1992), and Gielly and Taberlet (1994) following Shaw et al. (2005) [(no. PIC's/L) × 100], are given for each sequenced region in Table 3.

The region that posses the largest percentage of parsimony informative sites was the nuclear region *RNApol2*_i23 (15.3%), whereas the least informative was the *trn*H-*psb*A spacer.

Table 2 Mean length (in base pairs) and standard deviation (SD) of the different sequenced regions

	*trn*L intron	*trn*L-*trn*F IGS	*trn*Y-*trn*D IGS	*trn*H-*psb*A IGS	cpDNA	*RNApol2*_i23	Combined
North Africa	613 (9)	396 (2)	358 (34)	352 (9)	1,706 (29)	308 (0)	2,015 (29)
Eastern South Africa	596 (2)	399 (5)	329 (56)	349 (0)	1,659 (59)	308 (1)	1,969 (57)
Western South Africa	604 (14)	391 (15)	316 (60)	341 (26)	1,594 (64)	302 (5)	1,899 (67)
A. austrocapense	598	397	368	349	1,712	308	2,020
A. asteroides	597	393	369	356	1,715	308	2,023
A. roseum	625	396	376	560	1,957	308	2,265
A. melanthioides	575	380	370	364	1,689	308	1997
Outgroups							
Colchicaceae	568 (4)	395 (5)	372 (13)	355 (2)	1,677 (12)	306 (0)	1,990 (13)
Total	567 (17)	387 (24)	354 (40)	354 (17)	1,650 (54)	306 (0)	1962 (56)

Table 3 Characterization of the five non-coding regions of DNA of *Androcymbium* sequenced in this study

	*trn*L intron	*trn*L-*trn*F IGS	*trn*Y-*trn*D IGS	*trn*H-*psb*A IGS	*RNApol2*_i23	cpDNA	Combined
Aligned lengths (bp)	774	496	495	798	314	2,563	2,877
Variable sites (%)	153 (19.7)	104 (21.0)	112 (22.6)	117 (14.6)	84 (26.8)	486 (18.9)	570 (19.8)
Parsimony informative sites (%)	57 (7.4)	42 (8.5)	31 (6.3)	44 (5.5)	48 (15.3)	174 (6.8)	222 (7.7)
Autapomorphic sites (%)	96 (12.4)	62 (12.5)	81 (16.4)	73 (9.1)	36 (11.5)	312 (12.2)	348 (12.1)
Number of indels (parsimony informative)	49 (25)	23 (6)	33 (8)	46 (17)	10 (2)	151 (56)	161 (58)
Parsimony informative sites including informative gaps (%)	82 (10.6)	48 (9.7)	39 (7.9)	61 (7.6)	50 (15.9)	230 (9.0)	280 (9.7)
PIC's	202	127	145	163	94	637	731
Percentage of variability	26.1	25.6	29.3	20.4	29.9	24.9	25.4

bp Base pairs

In some cases, we found different DNA sequences within the same *Androcymbium* species. Each different DNA sequence of the same species was identified as haplotype (Hap.) and included as different haplotype of the same specie into the analyses.

Individual DNA analyses

Through comparisons using the likelihood ratio test, a submodel of HKY model (Hasegawa et al. 1985) was selected for each of the molecular regions, except for the *trn*L intron, where the selected was a submodel of F81 model (Felsenstein 1981). A gamma distribution for among-site variation was included for all of the molecular regions and the invariable parameter was also included for the *trn*L intron region. The F81 model was also selected for the binary data from the indels according with Ronquist et al. (2005) (Table 4).

Bayesian inference analyses provided 10,000 trees (1×10^6 generations, sampling each 100 generations) where the first 2,500 trees (250,000 generations) were removed from the analysis, the 25% of the trees according with the percentage of burn-in recommended in Ronquist et al. (2005). The *trn*L intron analysis yielded the best-resolved (CFI = 0.48) and best-supported topology. Twelve nodes received significant support (Posterior probability $\geq$ 0.90). None of the remaining nodes received posterior probability (PP) support greater than 0.82. The analysis of the *trn*L-*trn*F spacer provided significant support for seven nodes and CFI = 0.45, the analysis of the *trn*Y-*trn*D spacer provided significant support for only four nodes and CFI = 0.29, and the analysis of the *trn*H-*psb*A spacer provided significant support for only five nodes and CFI = 0.33. The *RNApol2* analysis provided the least-resolved (CFI = 0.19) and least-supported tree.

Combined DNA analyses

Through comparisons using the likelihood ratio test, a submodel of the HKY model with invariable site proportion and gamma distribution for among-site variation was selected for the combined data set (Table 4). In the B/MCMC analysis under this model, the first 3,750 trees (375,000 generations) of the 15,000 generated trees were removed, the 25% according with Ronquist et al. (2005). With the 11,250 remaining trees, a half-compatible strict consensus tree was constructed, resulted in a single most likely topology (Fig. 2). The parsimony analysis resulted in a strict consensus tree with CI = 0.734, and RI = 0.764 (Fig. 3). The resulting topology was better resolved and better supported by both Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values (BS) in the combined analyses than in the separated ones.

Table 4 Models of sequence evolution for each region

Region	Model
Combined	HKY + I + G
*trn*L intron	F81 + I + G
*trn*L-*trn*F IGS	HKY + G
*trn*Y-*trn*D IGS	HKY + G
*psb*A-*trn*H IGS	HKY + G
*RNApol2*_i23	HKY + G

According to the molecular data *Androcymbium* is not monophyletic as species of *Bulbocodium*, *Colchicum* and *Merendera* are nested within the genus. The tribe Colchiceae, comprising the four genera *Androcymbium*, *Bulbocodium*, *Colchicum*, and *Merendera*, is strongly supported as monophyletic (PP = 1,00; BS = 99). In the parsimony tree (Fig. 3) five clades are clearly differentiated, (1) clade 1 (BS = 69%): *Androcymbium* species from northern Africa, eastern South Africa, 9 of the 15 western South Africa analyzed species, and two of the three species from Namibia; (2) clade 2 (BS = 79%): four *Androcymbium* species from northwestern South Africa; (3) clade 3 (BS = 100%): the two haplotypes of *A. melanthioides* subsp. *melanthioides*, species widely distributed through the high regions of Namibia and northern South Africa; (4) the clade 4 is made by *A. cuspidatum*, species from western South Africa; (5) clade 5 (BS = 63%): made by *A. dregei*, species from western South Africa, plus species of *Bulbocodium*, *Colchicum*, and *Merendera* from the Iberian Peninsula. Within clade 1, two other well-supported subclades (BS $\geq$ 87%) are evident: subclade 1A (BS = 94) composed of *Androcymbium* species from northern Africa, eastern South Africa, two species from Namibia, and *A. austrocapense*, species that occurs in both zones of South Africa (eastern and western), and subclade 1B (BS = 87%) consisting exclusively of western South African species.

The B/MCMC tree has better-resolved topology (CFI = 0.86) than the parsimony tree (CFI = 0.75) (Fig. 2). In this tree, two well-supported clades are clearly differentiated. (1) Clade 1 (PP = 0.83) is the same from the parsimony tree, divided also in two subclades but with better support and resolution (subclade 1A: PP = 1.00; subclade 1B: PP = 1.00); clade 2 (PP = 0.89): made up of the species from the clades 2, 3, 4, and 5 of the parsimony tree.

The ILD test failed to test the combinability of the five regions ($P = 0.01$) (Table 5). Only when the sequences from the *trn*L intron and the *trn*L-*trn*F spacer were combined, the result was not significant ($P = 0.45$). With the method of Wiens (1998), only in few cases conflicting

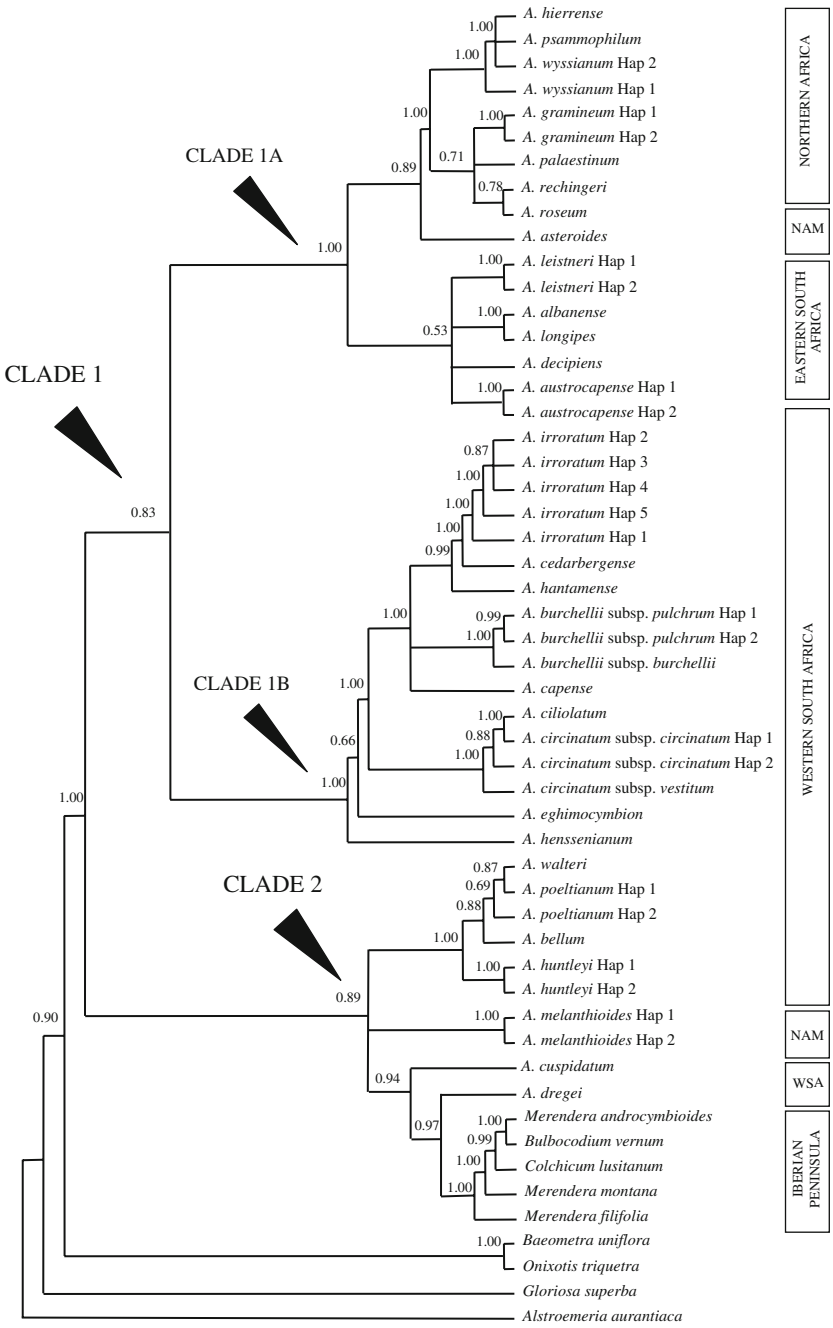

Fig. 2 Fifty percent majority-rule consensus tree derived from Bayesian analysis of five DNA regions analysed separately. *Numbers at nodes* are posterior probability values. Black arrows indicate the main clades. *NAM* Namibia, *WSA* Western South Africa

parts were observed between the single-data region trees and the combined-data tree. Due to it has been demonstrated that the ILD test could fail in testing the combinability of different data sets (Dolphin et al. 2000; Yoder et al. 2001; Barker and Lutzoni 2002; Darlu and Lecointre 2002), that only few conflicting parts were found and then resolved in the combined-data tree, and that this combined-data tree is better-resolved and better-supported than the single-data ones, we decided to discuss the results following the trees generated through the combined-data analyses.

Morphology and life-history traits analysis

A morphological and life-history traits matrix of 41 taxa (all the species without the different molecular haplotypes) and 22 characters was analyzed both with MP and B/MCMC methods. Under the B/MCMC method, these character data were analyzed following the HKY + I + G evolutive model. Both analyses resulted in two consensus trees without resolution, but concluding with *Androcymbium* as a well supported monophyletic group (BS = 82%; PP = 0.96) (results not shown).

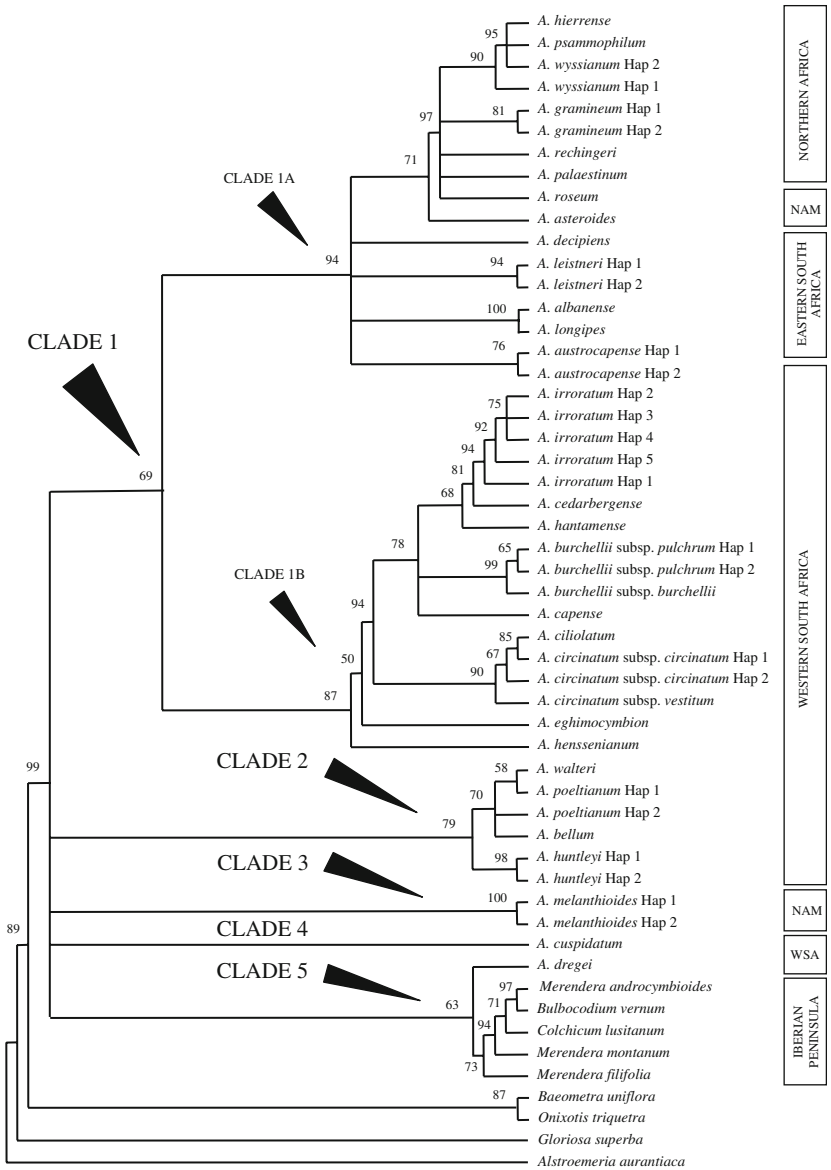

Fig. 3 Bootstrap strict consensus tree resulting from DNA phylogenetic analysis with MP methods. Numbers above the branches represent bootstrap support. *Black arrows* indicate the main clades. Nonparametric bootstrap analysis employed 1,000 pseudoreplicates, limiting the number of trees saved per pseudoreplicate to 5,000. *NAM* Namibia, *WSA* Western South Africa. CI = 0.734; RI = 0.764. *Hap* Haplotype

Morphology, life-history traits and DNA combined analysis

A new phylogeny using the two data sets (DNA + morphology and life-history traits) was made with parsimony and B/MCMC methods to test the effect of the inclusion of morphological and life-history traits characters in the DNA phylogeny.

In the resulting parsimony tree some clades received better support but the tree has less resolution than in the DNA analyses (CFI = 0.73) and lower values of CI and RI (Fig. 4). The inclusion of morphological and life-

Table 5 Results of the ILD test

Region	*P*
*trn*L intron + *trn*L- *trn*F IGS + *trn*Y- *trn*D IGS + *trn*H- *psb*A IGS + *RNApol2*_i23	0.01
cpDNA (*trn*L intron + *trn*L- *trn*F IGS + *trn*Y- *trn*D IGS + *trn*H- *psb*A IGS)	0.01
*trn*L intron + *trn*L- *trn*F IGS + *trn*H- *psb*A IGS + *RNApol2*_i23	0.01
*trn*L intron + *trn*L- *trn*F IGS + *trn*H- *psb*A IGS	0.01
*trn*H- *psb*A IGS + *RNApol2*_i23	0.01
*trn*L intron + *trn*L- *trn*F IGS	0.27

Notes: 100 repetitions with 500 trees each repetition and partition were made

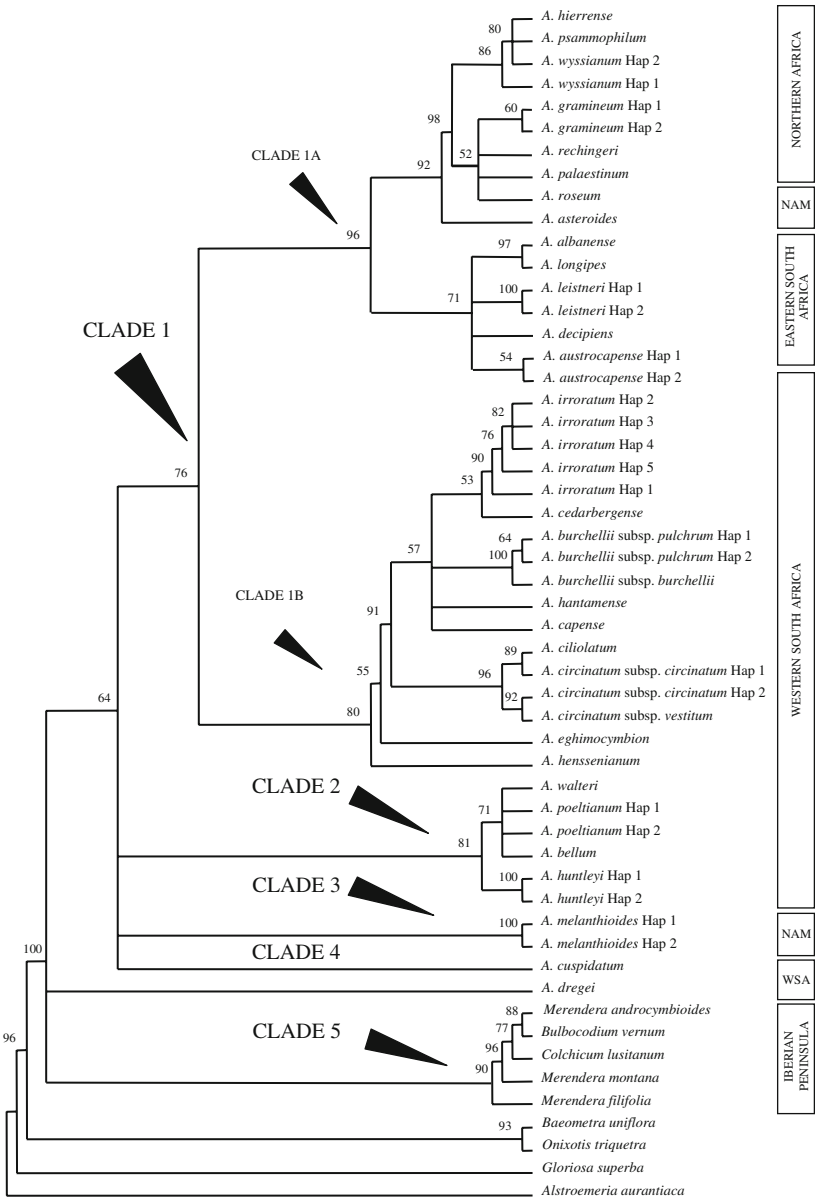

Fig. 4 Bootstrap strict consensus tree resulting from DNA, morphological, and life-traits data phylogenetic analysis with MP methods. *Numbers* above the branches represent bootstrap support. *Black arrows* indicate the main clades defined in the previous MP tree. Nonparametric bootstrap analysis employed 1,000 pseudoreplicates, limiting the number of trees saved per pseudoreplicate to 5,000. *NAM* Namibia, *WSA* Western South Africa. CI = 0.653; RI = 0.723. *Hap* Haplotype

history traits have two effects on the phylogeny of *Androcymbium*. The first one is the grouping in the same clade of Eastern South Africa species and *A. austrocapense*, a species that occurs in both sides of South Africa (BS = 71%). The second is the inclusion of the species from the clades 2, 3, and 4, within the same clade, separated from the *Colchicum* group (BS = 64%). Only *A. dregei* remains outside of the *Androcymbium* main group, in a trichotomy that also involves the *Colchicum* group. Hence, *Androcymbium* is potentially monophyletic.

But the most striking aspect is found in the B/MCMC tree, as it shows *Androcymbium* as a well-supported monophyletic group (PP = 0.96) (Fig. 5). This tree has the highest resolution (CFI = 0.90) and is highly supported (the 87% of the nodes received PP values higher than 0.92). The phylogenetic tree divides *Androcymbium* into two groups. One group is made of the *Androcymbium* species from clade 1 (PP = 1.00). The second group is made of the *Androcymbium* species that appear in the previous molecular trees within or closer to *Colchicum* species. The inclusion in the analyses of the morphological data and life-history traits has produced the grouping within a well-supported clade (PP = 0.96) of all the species of *Androcymbium*, including those that appear in a well-supported group with *Colchicum*.

Because of the B/MCMC tree made with DNA and character data is the most robust and supported one, the character mapping and the final discussion will be made in base of this tree.

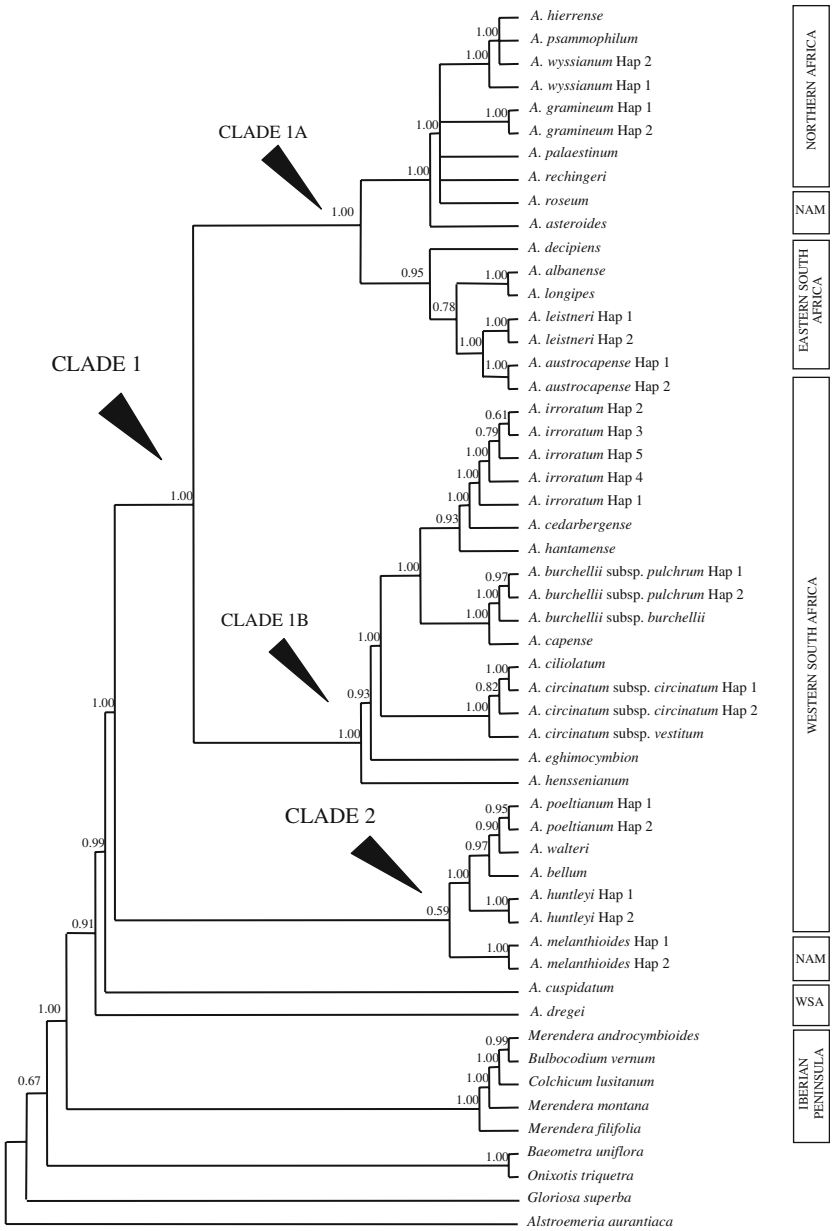

Fig. 5 Fifty-percent majority-rule consensus tree derived from Bayesian analysis of five DNA regions and morphological and life-traits data analysed separately. *Numbers* at nodes are posterior probability values. *Black arrows* indicate the main clades described in the previous B/MCMC tree. *NAM* Namibia, *WSA* Western South Africa

Monophyly test

Because *Androcymbium* is monophyletic in the B/MCMC phylogenetic tree obtained through morphological-life traits-DNA combined analysis, and because under a parsimony framework there are only 13 steps more necessary to constraint *Androcymbium* as a monophyletic group in the combined DNA phylogenetic tree, a monophyly test was carried out in Mesquite v.1.11 (Maddison and Maddison 2006) following Maddison (2004). As a result, a difference of 13 steps between one of the paraphyletic DNA combined tree (unconstrained, 1,120 steps) and the monophyletic DNA combined tree (constrained, 1,133 steps), is not significantly different ($P = 0.16$). Therefore, we may not reject *Androcymbium* as a monophyletic genus.

Character mapping

The dichotomous all-compatible tree of the B/MCMC analyses with DNA and character data were selected to trace the evolution of some morphological characters and life-history traits of *Androcymbium* using McClade 4.08 (Maddison and Maddison 2005). Because a thorough discussion about the character mapping and evolution has been done in a parallel paper (J. Pedrola-Monfort and A. Del Hoyo, unpublished data), here we have put more emphasis in those characters and life traits that support the

Androcymbium monophyly. In general, the morphological characters and life traits presented here showed accentuated homoplasy, but this phenomena need not be problematic because homoplasy may be highly structured in different lineages (Doyle 1996).

Among the mapped characters that support the monophyly of *Androcymbium*, the most important ones are the blade and claw differentiation in the tepal (with or without auricles) (Fig. 6a), the presence or absence of subterranean ovary (Fig. 6b), and the presence or absence of idioblasts on leaves and tepals (Fig. 6c). In case of equivocal characters tracings we have resolved this with ACCTRAN or DELTRAN.

Discussion

Monophyly of *Androcymbium*

Analyses of molecular data together with morphology and life-history traits data, concluded with the genus *Androcymbium* as monophyletic (PP = 0.96) in a well supported and highly resolved B/MCMC phylogenetic tree. This is not surprising, as non-conflicting data set often provide more support and more resolution when combined (Bremer et al. 1999; Hahn 2002; Matheny et al. 2002). Caujapé-Castells et al. (1999) in their cpDNA-RFLP phylogeny, concluded with the same result. It was because very important *Androcymbium* species that put in doubt the monophyly of this genus, like *A. dregei*, *A. huntleyi*, or *A. melanthioides* (Vinnersten and Reeves 2003; Del Hoyo and Pedrola-Monfort 2006), were not included in their analysis, and only two species of *Colchicum* and one of *Merendera* were included and used as outgroup. With the inclusion of more species of Colchicaceae, the genus *Androcymbium* becomes paraphyletic, like it has been demonstrated by Vinnersten and Reeves (2003) and Del Hoyo and Pedrola-Monfort (2006). Manning et al. (2007), following the monophyly criteria proposed by the APG (1998, 2003), argued about expansion of the *Colchicum* s.l. circumscription to include the *Androcymbium* species based on the paraphyly of this genus. These authors also support their decision from the lack of morphological characters that could differentiate *Androcymbium* from *Colchicum*. There are three motives for rejecting their proposal of redefine *Colchicum* to include *Androcymbium*. (1) With the inclusion of more DNA data (5 regions vs. 3 regions) together with morphological and life history traits data into the phylogenetic analyses, we have found that the genus *Androcymbium* becomes monophyletic. If we take into account only the DNA data, the genus *Androcymbium* becomes paraphyletic, but it has been demonstrated with a monophyly test described by Maddison (2004), that a difference of 13 steps between the paraphyletic tree (unconstrained) and the monophyletic tree (constrained) is not significant ($P = 0.16$). (2) The analysis of only nine species of *Colchicum*, a genus with ca. 100 species (Persson 2007), is not enough as basis for making this nomenclatural change. The inclusion of more *Colchicum* species could play a key role for the topology of the tree, keeping both genera monophyletic (A. Del Hoyo et al. unpublished data). (3) Admittedly, it is quite difficult to find clear and easily recognized morphological characters that could differentiate the *Colchicum* species from those of *Androcymbium*. But it is clear that the inclusion of morphological characters in the phylogenetic analysis has played a key role for the proposed monophyly of *Androcymbium*.

Among the 22 morphological and life traits characters included in the analyses, three are very important: (1) blade and claw differentiation in the tepal (with or without auricles); (2) the presence/absence of subterranean ovary; (3) the presence/absence of idioblasts on leaves (Fig. 6). The *Colchicum* s.l. species has the blade and the claw undifferentiated in the tepal, while there are differentiated in all the *Androcymbium* species. Within the *Androcymbium* species two groups can be distinguished: (1) with auricles in the tepal, mainly the South African species, and (2) without auricles in the tepal, made of mainly species from of sections *Erythrostictus* (Fig. 6a). While the *Colchicum* s.l. species have the ovary subterranean previous to its fecundation, the *Androcymbium* species have always the ovary on the ground (Fig. 6b). The presence of idioblasts on leaves and tepals, identifiable as purplish dots (blackish when dry) during the plant senescence is a character exclusive only of *Androcymbium* species (Fig. 6c). We agree with Vinnersten and Reeves (2003) in the difficulty of finding clear morphological characters that could differentiate between these two genera, but some discriminatory characters exist that we cannot obviate that support its. Then, and with the goal of analyzing more *Colchicum* species, we propose to keep *Androcymbium* and *Colchicum* as two independent genera according to our present molecular and character data analyses. Notwithstanding, we agree to include *Bulbocodium* and *Merendera* within *Colchicum*, as has been suggested by several authors based on primarily morphological analyses (Stefanov 1926; Burtt 1968; Dahlgren et al. 1985; Nordenstam 1998; Persson 1992, 1993, 2007).

Phylogeny and taxonomy

In the B/MCMC phylogenetic tree (Fig. 5), we can observe various clades within the *Androcymbium* genus, where two groups are easily distinguished. The first clade (Clade 1)

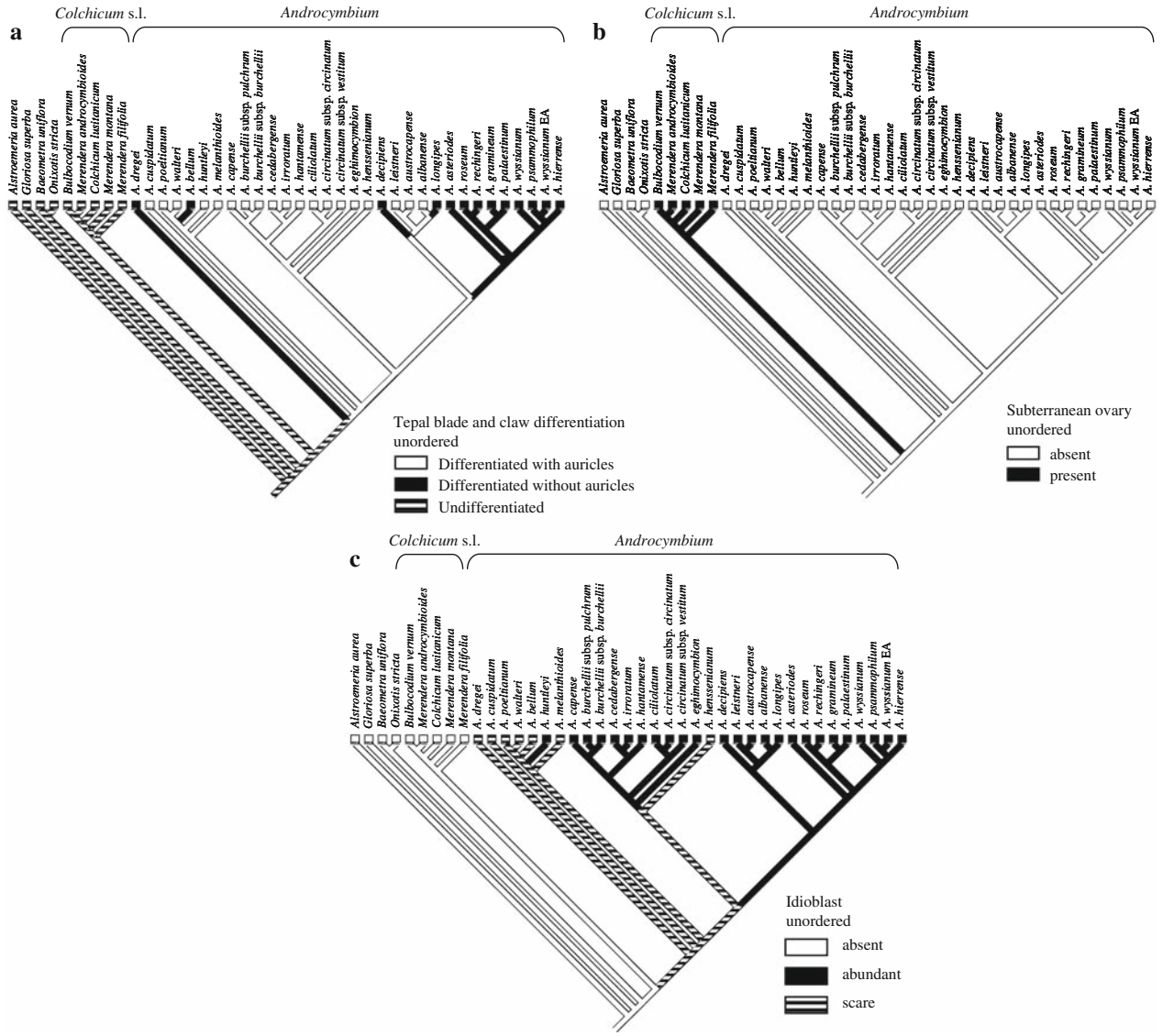

Fig. 6 Morphological character mapping on dichotomous Bayesian inference tree made with molecular, morphological, and life-trait data, showing inferred evolution of tepal blade and claw differentiation (**a**), subterranean ovary (**b**), and idioblast on leaves and tepals (**c**) in *Androcymbium* and outgroups

group 22 species from all of the distribution areas of the genus (western South Africa, eastern South Africa, Namibia, and northern Africa), with a high branch support (PP = 1.00). This clade also was observed in the cpDNA-RFLP phylogenetic tree of Caujapé-Castells et al. (1999, 2001), and in the Vinnersten and Reeves (2003) cpDNA tree. Within this clade, two well-supported clades are clearly differentiates. The first clade (1A) (PP = 1.00) groups the six northern African species, five species from eastern South Africa, and two from Namibia. In this clade also appears *A. austrocapense*, a species that has its populations distributed from Cape Town to Port Elizabeth (South Africa) following the coast line. Despite *A. asteroides* and *A. roseum* were collected in the tropical steppe belt of Namibia, they show a wide distribution in the South of Africa. *Androcymbium asteroides* is distributed from Western and Northern Cape provinces of South Africa to the South of Namibia, joined with the Orange river course (Manning and Goldblatt 2001), and *A. roseum* is centered in the tropical steppe belt of Namibia and Flora Zambesiaca (Zambia, Botswana, and Zimbabwe), radiating to Angola in the Northwest. This species has played and important role in the dispersion of *Androcymbium* towards the North of the continent (Del Hoyo and Pedrola-Monfort 2006). The six north African plus the two species from Namibia, that form a clade (PP = 1.00), belong to the *Erythrostictus* section, and are characterized by having coloured tepals (white or pink), where the floral blade is

plane and longer than the claw (Müller-Doblies and Müller-Doblies 2002). The five analyzed species that inhabit eastern South Africa, within the summer-rainfall area, also form a clade (PP = 0.95). At infrageneric level, *A. austrocapense* belongs to section *Androcymbium* while the other four to section *Kunkeliocymbium*. The second clade (1B) (PP = 1.00) is made up by nine species that habit either in the Cape Floristic Region or in the Nama-karoo Region, in the winter-rainfall area within the Greater Cape Floristic Region of western South Africa (Born et al. 2007). Unlike the northern African species, these South African species shown a very high morphological variation, phenomenon very common in a lot of Southern African genera (Goldblatt 1997; Goldblatt and Manning 2002; Linder 2003; Linder and Hardy 2004). All of these species have, in higher or lower degree, modified colored bracts with pollinator attracting function, and belong at infrageneric level to the section *Androcymbium* (Müller-Doblies and Müller-Doblies 2002). The exception is found in *A. henssenianum*, a species situated at the base of this clade, belonging to the section *Kunkeliocymbium* and without having modified colored bracts.

A second clade (Clade 2) is made up by four species with a narrow distribution in the north-west of the northern Cape Province (South Africa) (PP = 1.00), and close to this clade appears *A. melanthioides*, *A. cuspidatum*, and *A. dregei*. These species that are situated at the base of *Androcymbium*, are distributed in the South of Namibia–northwest of South Africa, in the Nama-karoo and succulent karoo biomes. Only the species *A. melanthioides* has a wide distribution from the Center–South of Namibia, and through the north of South Africa. This group of species is very heterogeneous at morphological level, having species from four of the five sections into which Müller-Doblies and Müller-Doblies (2002) divided the genus. The remaining section, section *Marlothiocymbium*, is monotypic, and its only one species, *A. cruciatum*, is phylogeneticaly very close to one species of this group, *A. dregei* (Vinnersten and Reeves 2003).

In a sister clade to the *Androcymbium* genus, the *Colchicum* s.l. species are situated (PP = 0.99). Both genera create the highly supported tribe Colchiceae (PP = 1.00).

However, more morphological and molecular data, as well as more *Colchicum* s.l. species, are needed to infer with more precision the evolutionary relationships within the Colchiceae tribe. It is necessary also to understand the ancestrality relationships between *Androcymbium* and *Colchicum* to create a reliable scenario about its biogeography and evolutionary history.

Acknowledgments The authors thank the Genetic area of the Department of Biology of the University of Girona, specially J. L. García-Marín, for his help with the sequencing of the samples, G. Petersen and O. Seberg, from the Laboratory for Molecular Systematics of the Copenhagen University, for their help with the final revision of this manuscript, and we are specially indebt with K. Persson, from the Botanical Institute of the Göteborg University, for her comments and suggestions. The authors are indebted to with the Carl Faust Foundation, which provided all the plant material, the maintenance of living collections of *Androcymbium*, and founded this research.

References

Aagesen L, Sanso AM (2003) The phylogeny of the Alstroemeriaceae, based on morphology, *rps*16 intron, and *rbc*L sequence data. Syst Bot 28:47–69

APG [Angiosperm Phylogeny Group] (1998) An ordinal classification for the families of flowering plants. Ann Missouri Bot Gard 83:531–553

APG [Angiosperm Phylogeny Group] (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. Bot J Linn Soc 141:399–436

Baker JG (1874) On the genus *Androcymbium*, with descriptions of seven new species. J Linn Soc Bot X:243–245

Baker JG (1879) A synopsis of Colchicaceae and the aberrant tribes of Liliaceae. J Linn Soc Lond Bot 17:405–510

Baker RH, Yu X, De Salle R (1998) Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. Molec Phylogenet Evol 9:427–436

Barker FK, Lutzoni F (2002) The utility of the incongruence length difference test. Syst Biol 51:625–637

Bayer RJ, Starr JR (1998) Tribal phylogeny of the Asteraceae based on two non-coding chloroplast sequences, the *trn*L intron and *trn*L/*trn*F spacer. Ann Missouri Bot Gard 85:242–256

Bentham G, Hooker JD (1862–1883) Genera plantarum ad exemplaria imprimis in herbariis Kewensibus servata, vol 3. London

Born J, Linder HP, Desmet P (2007) The Greater Cape Floristic Region. J Biogeogr 34:147–162

Bremer B, Jansen RK, Oxelman B, Backlund M, Lantz H, Kim K-J (1999) More characters or more taxa for a robust phylogeny—case study from the coffee family (Rubiaceae). Syst Biol 48:413–435

Bremer K (2000) Early Cretaceous lineages of monocot flowering plants. Proc Natl Acad Sci USA 97:4707–4711

Burtt BL (1968) *Colchicum* and *Merendera*. A lily group discussion opened by Mr. B. L. Burtt, with contributions from Mr. R. D. Meikle and Rear-Admiral J. P. W. Furse. Lily Year Book, vol 31, pp 90–105

Caujapé-Castells J, Jansen RK, Pedrola-Monfort J, Membrives N (1999) Chloroplast DNA restriction site phylogeny of the genus *Androcymbium* (Colchicaceae). Syst Bot 24:581–597

Caujapé-Castells J, Jansen RK, Membrives N, Pedrola-Monfort J, Montserrat JM, Ardanuy A (2001) Historical biogeograpy of *Androcymbium* Willd. (Colchicaceae) in Africa: evidence from cpDNA RFPLs. Bot J Linn Soc 136:379–392

Colless DH (1980) Congruence between morphometric and allozyme data for *Menidia* species: a reappraisal. Syst Zool 29:288–299

Colless DH (1981) Predictivity and stability in classifications: some comments on recent studiees. Syst Zool 30:325–331

Dahlgren RMT, Clifford HT, Yeo PF (1985) The families of the monocotyledons: structure, evolution and taxonomy. Springer, Berlin

Darlu P, Lecointre G (2002) When does the incongruence length difference test fail? Molec Biol Evol 19:432–437

Del Hoyo A, Pedrola-Monfort J (2006) Missings links between disjunct populations of *Androcymbium* (Colchicaceae) in Africa

using chloroplast DNA noncoding sequences. In: Columbus JT, Friar EA, Porter JM, Prince LM, Simpson MG (eds) Monocots: comparative biology and evolution, vol I. Rancho Santa Ana Botanic Garden, Claremont, pp 604–616

Denton AL, McConaughy BL, Hall BD (1998) Usefulness of RNA polumerase II coding sequences for estimation of green plant phylogeny. Molec Biol Evol 15:1082–1085

Dolphin K, Belshaw R, Orme DL, Quicke D (2000) Noise and incongruence: interpreting results of the incongruence length difference test. Molec Phylogenet Evol 17:401–406

Doyle JJ (1996) Homoplasy connections and disconnections: genes and species, molecules and morphology. In: Sanderson MJ, Hufford L (eds) Homoplasy: the recurrence of similarity in evolution. Academic Press, San Diego, pp 37–66

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Farris JS, Källersjö M, Kluge AG, Bult C (1995) Testing significance of incongruence. Cladistics 10:315–319

Felsenstein J (1981) Evolutionary tree from DNA sequences: a maximum likelihood approach. J Molec Evol 17:368–378

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Gielly L, Taberlet P (1994) The use of chloroplast DNA to resolve plant phylogenies: noncoding versus *rcb*L sequences. Molec Biol Evol 11:769–777

Goldblatt P (1997) Floristic diversity in the Cape Flora of South Africa. Biodiversity Conserv 6:359–377

Goldblatt P, Manning JC (2002) Plant diversity of the cape region of Southern Africa. Ann Missouri Bot Gard 89:281–302

Hahn WJ (2002) A molecular phylogenetic study of the Palmae (Arecaceae) based on *atp*B, *rbc*L, and 18S nrDNA sequences. Syst Biol 51:92–112

Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucl Acids Symp Ser 41:95–98

Hasegawa M, Kishino H, Yano T (1985) Dating of the huma-ape splitting by a molecular clock of mitochondrial DNA. J Molec Evol 22:160–174

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Krause K (1920) Revision der Gattung *Androcymbium* Willd. Notizbl Bot Gart Berlin 7:512–526

Li H, Luo J, Kemphill JK, Wang J, Gould JH (2001) A rapid and high yielding DNA miniprep for Cotton (*Gossypium* spp.). Pl Molec Biol Rep 19:183a–183e

Linder HP (2003) The radiation of the Cape flora, southern Africa. Biol Rev 78:597–638

Linder HP, Hardy CR (2004) Evolution of the species-rich Cape flora. Phil Trans R Soc Lond 359:1623–1632

Maddison DR (2004) Testing monophyly of a group of beetles. Study 1 in Mesquite: a modular system for evolutionary analysis, version 1.04, http://www.mesquiteproject.org

Maddison DR, Maddison WP (2005) MacClade 4: analysis of phylogeny and character evolution, version 4.08, Sinauer, Sunderland, MA, USA

Maddison WP, Maddison DR (2006) Mesquite: a modular system for evolutionary analysis. Version 1.11 http://www.mesquiteproject.org

Manning J, Goldblatt P (2001) Notes on African Plants: Colchicaceae. A remarkable new species of *Androcymbium* from Northern Cape, South Africa. Bothalia 31:203–205

Manning J, Forest F, Vinnersten A (2007) The genus *Colchicum* L. redefined to include *Androcymbium* Willd. based on molecular evidence. Taxon 56(3):872–882

Matheny PB, Liu YJ, Ammirati JF, Hall BD (2002) Using RPB1 sequences to imporve phylogenetic inference among mushrooms (Inocybe, Agaricales). Amer J Bot 89:688–698

Müller-Doblies U, Müller-Doblies D (1984) Zur Kenntnis der Gattung *Androcymbium* (Colchicaceae) im südlichen Afrika: Zwei Synonyma und fünf neue Arten. Willdenowia 17:179–197

Müller-Doblies U, Müller-Doblies D (1990) Zur Kenntnis der Gattung *Androcymbium* (Colchicaceae) im südlichen Afrika: 2. *Androcymbium exiguum* hat doch Artwert. Willdenowia 19:456–470

Müller-Doblies U, Müller-Doblies D (1998) De Liliifloris Notulae 6. De decuria prima specierum novarum generis *Androcymbium* sect. *Androcymbium* (Colchicaceae) in Africa Australi s.l. Praeterea novitates de huius sectionis nonnullarum specierum dstributione (praesertim aucta speciminibus STEllenbosensibus i.a. collectis a botanico vero E. G. H. OLIVERO). Feddes Repert 109:551–572

Müller-Doblies U, Müller-Doblies D (2002) De Liliifloris Notulae 7. De decuria altera specierum novarum generis *Androcymbium* (Colchicaceae) in Africa Australi s.l. Feddes Repert 113:545–599

Nordenstam B (1998) Colchicaceae. In: Kubitzki K (eds) The families and genera of vascular plants III flowering plants, monocotyledons. Springer, Berlin, pp 175–185

O'Donnell K (1992) Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). Cur Genet 22:213–220

Persson K (1992) Liliceae subfam. Wurmbaeoideae Buxbaum. Flora Iranica 170:1–40

Persson K (1993) Reproductive strategies and evolution in *Colchicum*. In: Proceedings of the 5th optima meetings. Istambul University, Istambul, pp 394–414

Persson K (2007) Nomenclatural synopsis of the genus *Colchicum* (Colchicaceae), with some new species and combinations. Bot Jahrb Syst 127:165–242

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Ronquist F, Huelsenbeck JP, Van del Mark P (2005) MrBayes 3.1 Manual. http://mrbayes.csit.fsu.edu/mb3.1_manual.pdf

Sang T, Crawford DJ, Stuessy TF (1997) Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). Amer J Bot 84:1120–1136

Schlechtendal DFL (1826) Ueber die Melianthaceen am Vorgebirge der Guten Hoffnung. Linneae 1:78–95

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Amer J Bot 92:142–166

Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. Syst Biol 49(2):369–381

Stefanov B (1926) Monografiya na roda *Colchicum* L. Sborn Balg Nauk Sofiya 22:1–100

Swofford DL (2003) Phylogenetic analysis using parsimony, PAUP*, 4.0b10. Sinauer., Sunderland, MA, USA

Tate JA, Simpson BB (2003) Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. Syst Bot 28:723–737

Thompson JD, Higgins DG, Gibon TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acids Res 22:4673–4680

van Ham RCHJ, T'Hart H., Mes THM, Sandbrink JM (1994) Molecular evolution of noncoding regions of the chloroplast genome in the Crassulaceae and related species. Cur Genet 25:558–566

Vinnersten A, Bremer K (2001) Age and biogeography of major clades in Liliales. Amer J Bot 88:1695–1703

Vinnersten A, Reeves G (2003) Phylogenetic relationships within Colchicaceae. Amer J Bot 90:1455–1462

Vogel S (1954) Blütenbiologische Typen als Elemente der Sippengliederung. Jena

Wiens JJ (1998) Combining data sets with different phylogenetic histories. Syst Biol 47:568–581

Willdenow F (1808) Nähere Bestimmung einiger Liliengewächse besonders aber derjenigen, die zur sechsten Klasse und dritten Ordnung des Linnéschen Systems gehören. Ges Naturforsch Freunde Berlin Magazin 2:14–30

Wortley AH, Scotland RW (2006) The effect of combining molecular and morphological data in published phylogenetic analyses. Syst Biol 55:677–685

Yoder AD, Irwin JA, Payseur BA (2001) Failure of the ILD to determine data combinability for slow loris phylogeny. Syst Biol 50:408–424

Young ND, Healy J (2003) Gapcoder automates the use of indel characters in phylogenetic analysis. BMC Bioinfor 4:6