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MISSING LINKS BETWEEN DISJUNCT POPULATIONS OF *ANDROCYMBIUM* (COLCHICACEAE) IN AFRICA USING CHLOROPLAST DNA NONCODING SEQUENCES

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ABSTRACT

With the objective of clarifying some aspects of the biogeography, phylogeny, and taxonomy of the genus *Androcymbium,* we sequenced three chloroplastic DNA noncoding regions (*trn*L intron, *trn*L–*trn*F IGS, and *trn*Y–*trn*D IGS). These data were analyzed with maximum parsimony and the ancestral areas methods following Bremer. Results show that *Androcymbium* is not monophyletic and that the origin of its distribution and speciation is situated in western South Africa. Later, it dispersed to North Africa, going first to eastern South Africa. *Androcymbium austrocapense* and *A. roseum* allow us to phylogenetically connect the species of western with eastern South Africa, and the southern species with the northern, respectively. The formation of an arid track in Africa at the end of the Miocene explains the colonization of *Androcymbium* in the Mediterranean basin. *Androcymbium wyssianum* is a key element in understanding colonization of the Canary Islands. The biogeographical pattern of distribution of *Androcymbium* fits with many other genera with similar disjunct distributions. This indicates the importance of the Miocene arid track in understanding the floristic connections between northern and southern Africa. Because of the close relationships of *Bulbocodium, Colchicum,* and *Merendera,* with *Androcymbium* inferred from the chloroplast data, restructuring the taxonomy and nomenclature of the tribe *Colchiceae* may be required.

Key words: *Androcymbium,* arid track, biogeography, Colchicaceae, cpDNA phylogeny, disjunct pattern, Miocene.

INTRODUCTION

Studies of the biogeography of Africa have emphasized that the floristic relationship between arid zones of northern and southern Africa is one of the most intriguing phenomena in plant distribution (De Winter 1971). About 63% of the genera of northern African xerophytic flora, including many monocots, are found in the symmetric austral zone (Monod 1971). To explain this phenomenon, the importance of the role of an arid track (Balinsky 1962), established in the Late Miocene, in the biogeographical history of Africa has often been asserted (Axelrod and Raven 1978). This region may have been a migration corridor from southern to northern Africa (or vice versa) for some groups.

Molecular phylogenetic methods provide great potential for testing this argument and clarifying several aspects of biogeography and evolutionary biology of disjunctions in Africa. We believe that a reasonable understanding of diversification processes within the component taxa of a given flora provides the best basis for generalizations about the diversification of the flora as a whole.

There are recent examples of phylogenetic studies of several genera with similar geographic distributions; *Leucas* R. Br. (Ryding 1998), *Lotononis* (DC.) Eckl. & Zeyh. (Linder 1992), and *Moraea* Mill. (Goldblatt et al. 2002). Some of these authors argued in support of a biogeographical hypothesis of fragmentation for the one pan-African distribution of its taxa, while other authors put forward arguments for long- or short-range dispersals across the arid track. In some studies, these disjunctions have been established during the Miocene. Unfortunately, there is a paucity of molecular phylogenetic investigations of important African groups. Thus, the role of the arid track in the biogeographical history of Africa is still poorly understood.

Androcymbium Willd. (Colchicaceae) consists of 56 hermaphroditic geophytes that exhibit a disjunct distribution between northern and southern Africa, with western South Africa as the center for taxonomic diversity. Previous phylogenetic analyses with morphology, allozymes, and chloroplast DNA restriction fragment length polymorphisms (cpDNA–RFLP) allow us to develop evolutionary hypotheses about relationships of these taxa on some disjunct areas of their distribution (North Africa and western South Africa) (Caujapé-Castells et al. 1999, 2001; Membrives 2000).

Our principal aim in this work is to present one phylogenetic hypothesis, including representative taxa from all areas of its distribution. For this we have considered samples of the four general areas of distribution (western South Africa, eastern South Africa, south-central Africa, and North Africa) of *Androcymbium* (Fig. 1). Based on previous studies of morphology and life traits of *Androcymbium* of south-central Africa and eastern South Africa (never before included in molecular analysis), some of these taxa could be the species that phylogenetically connect the populations of the northern and southern areas of Africa (missing links). Therefore, their inclusion in the phylogenetic tree was considered essential to better understand this disjunction.

MATERIAL AND METHODS

Plant Material

We analyzed 75 populations belonging to 28 taxa from the genus *Androcymbium* (Table 1). Our taxon sampling represents a wide range of variation in *Androcymbium* and the entire geographic range for the genus across Africa.

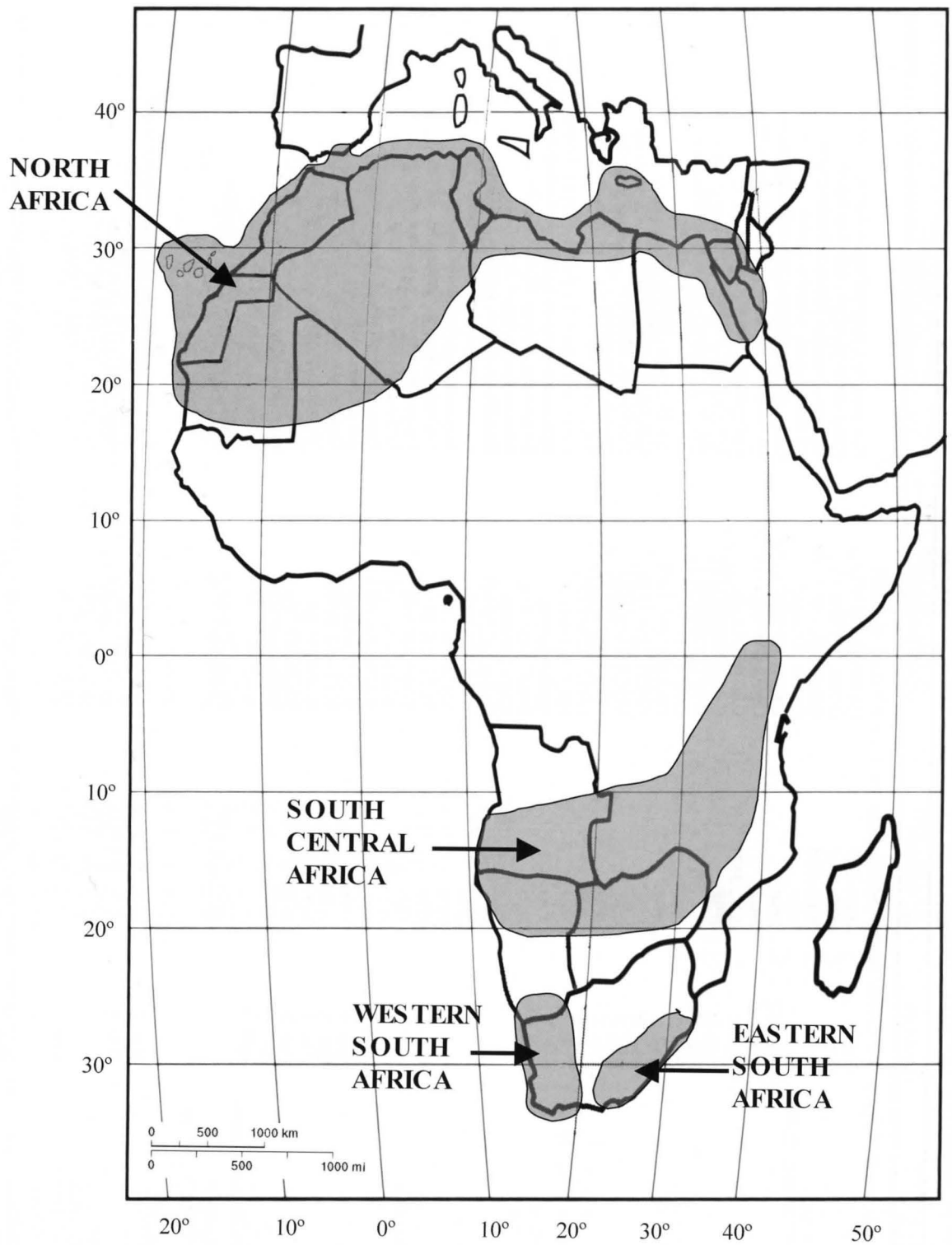

Fig. 1.—Geographical distribution of the genus *Androcymbium*.

Six different taxa of the family Colchicaceae, and one taxon from the family Alstroemeriaceae (Brummitt 1992), which is phylogenetically close to Colchicaceae (Bremer 2000; Vinnersten & Bremer 2001; Vinnersten and Reeves 2003), were used as outgroups (Table 1).

All of the analyzed samples for this study were planted and grown under the same conditions in the investigation greenhouse at the Marimurtra Botanic Garden in Blanes, Spain.

DNA Isolation, PCR Amplification, and DNA Sequencing

Genomic DNAs were extracted from fresh leaf tissue, previously dried in silica gel followed by snap freezing in liquid

Table 1. Species and populations of the genus *Androcymbium* analyzed in this study. The South African and Namibian populations are cited according to the Degree Reference System (Leistner and Morris 1976) widely used by South African biologists. Collector codes are AH: Alberto del Hoyo; JCC: Juli Caujapé-Castells; JG: Jordi Gibert; JMM: Josep María Montserrat; JPM: Joan Pedrola-Monfort; MA: M. Avishai; MV: Magdalena Vicens; YT: Y. Tankus. The collection number belongs to living specimens in culture at Marimurtra Botanic Garden.

Taxon	Haplotype	Collectors	Collection number	Population
Species and populations of Almeria (south of Spain), the Canary Islands (Spain), Israel and the north coast of Africa				
A. gramineum Macbride	Hap. 1	*JCC, JPM*	GRBC 545B.1192	Barranco de Curriá, Almeria, Spain.
A. gramineum	Hap. 1	*JCC, JPM*	GRCP 789.990	Cerro de los Peligros, Almeria, Spain
A. gramineum	Hap. 1	*JCC, JPM*	GRCH 761.990	Charco del Lobo, Almeria, Spain
A. gramineum	Hap. 1	*JPM*	GREB 583.1192	El Barranquete, Almeria, Spain
A. gramineum	Hap. 1–2	*JPM*	GRSC 723.990	Cerro de San Cristóbal, Almeria, Spain
A. gramineum	Hap. 1–2	*JPM*	GRES 744.990	El Solanillo, Almeria, Spain
A. gramineum	Hap. 2	*JCC, JPM*	GRCL 797.1192	Cerro de los Lobos, Almeria, Spain
A. gramineum	Hap. 2	*JPM*	GRLM 1006A.1189	Los Molinos, Almeria, Spain
A. gramineum	Hap. 2	*JCC, JPM*	GRPM 720B.990	Playas de Monsul, Almeria, Spain
A. gramineum	Hap. 2	*JPM*	GRZA 712.990	Zonas Áridas, Almeria, Spain
A. gramineum	Hap. 2	*JPM*	GRAH 1346C.1290	Aïn Harrouda, Morocco
A. gramineum	Hap. 2	*JPM*	GRCB 1219.1290	Cap Beddouza, Morocco
A. gramineum	Hap. 2	*JPM*	GRCA 1281.1290	Casablanca, Morocco
A. gramineum	Hap. 2	*JPM*	GROU 1011.1290	Oualidia, Morocco
A. gramineum	Hap. 2	*JPM*	GRSA 1265.1290	Safi, Morocco
A. hierrense A. S. Guerra		*JPM*	HILP 504.990	Costas del Mazo, La Palma, Canary Islands, Spain
A. hierrense		*JPM*	HIHI 563.990	Dehesa del Sabinar, El Hierro, Canary Islands, Spain
A. hierrense		*JPM*	HIGO 843.1190	La Gomera, Canary Islands, Spain
A. palaestinum Baker		*MA*	PADI 595.990	Dimona desert, Israel
A. palaestinum		*YT*	PABS 1028.1189	Beit Shean Valley, Israel
A. psammophilum Svent.		*JPM*	PSLA 872.1190	Lanzarote, Canary Islands, Spain
A. psammophilum		*JPM*	PSFU 539.1190	Fuerteventura, Canary Islands, Spain
A. rechingeri Greuter		*JPM*	REEL 220.691	Elafonisis, Crete, Greece
A. wyssianum Beauverd & Turrettini	Hap. 1	*JPM*	WYFI 308.1092	Figuig, Morocco
A. wyssianum	Hap. 1	*JCC, JPM*	WYEF 434A.1192	Er Foud, Morocco
A. wyssianum	Hap. 1	*JCC, JPM*	WYFB 214.1092	Fonts Bleus of Maski, Morocco
A. wyssianum	Hap. 1	*JPM*	WYAO 643B.990	Aïn Ouarka, Algeria
A. wyssianum	Hap. 1	*JMM, JPM*	WYTI 627B.990	Taghit–Igli, Algeria
A. wyssianum	Hap. 1	*JCC, JPM*	WYN1 37.191	Nefta1, Tunisia
A. wyssianum	Hap. 1	*JCC, JPM*	WYN2 46.191	Nefta2, Tunisia
A. wyssianum	Hap. 2	*JPM*	WYEA 2002.011	Essaouria, Morocco
Species and populations of western South Africa				
A. albanense subsp. *clanwilliamense* J. Pedrola-Monfort, N. Membrives & J. M. Montserrat		*JCC, JG, JPM*	CLANPK 2384	Pakhuispass, 3219AA (Wuppertal)
A. austrocapense U. Müll.-Doblies & D. Müll.-Doblies	Hap. 2	*JCC, JG, JPM*	AUSTGH 1583D	Cape of Good Hope, 3418BB (Simonstown)
A. austrocapense	Hap. 2	*JCC, JG, JPM*	AUSTWP 2089	Whale Point, 3418BB (Simonstown)
A. bellum Schltr. & K. Krause		*JCC, JG, JPM*	BELLVI 1618E	Villesdorp, 2817DC (Vioolsdrif)
A. burchellii subsp. *burchellii* Baker		*JCC, JG, JPM*	BURCHX 1587	Hexrivier, 3319BC (Worcester)
A. burchellii subsp. *pulchrum* (Baker) J. Pedrola-Monfort, N. Membrives, J. M. Montserrat, & J. Caujapé	Hap. 1	*JCC, JG, JPM*	BURCCA 2242	Calvinia, 3119BD (Calvinia)
A. burchellii subsp. *pulchrum*	Hap. 2	*JCC, JG, JPM*	BURCNI 2000	Nieuwwodtville, 3119BA (Calvinia)
A. capense (Druce) K. Krause		*JCC, JG, JPM*	CAPEHO 2027	Hopefield, 3318AB (Cape Town)
A. circinatum Baker	Hap. 1	*JCC, JG, JPM*	CIRCNB 1895	Nababiep, 2917CD (Springbok)
A. circinatum	Hap. 2	*JCC, JG, JPM*	CIRCSB 1759K	Springbok, 2917CD (Springbok)
A. cuspidatum Baker		*JCC, JG, JPM*	CUSPCA 2221	Around Calvinia, 3119BD (Calvinia)

Table 1. Continued.

Taxon	Haplotype	Collectors	Collection number	Population
A. cuspidatum		*JCC, JG, JPM*	CUSPMO 1529E	Montagu, 3320DA (Montagu)
A. dregei Presl		*JCC, JG, JPM*	DREGPK 2450	Pakhuis, 3219AA (Wuppertal)
A. eghimocymbion U. Müll.-Doblies & D. Müll.-Doblies		*JCC, JG, JPM*	EGHICI 1889	Citrusdal, 3218CA (Clanwilliam)
A. eghimocymbion		*JCC, JG, JPM*	EGHIPK 2358B	Pakhuis, 3219AA (Wuppertal)
A. hantamense Engl. ex Diels		*JCC, JG, JPM*	HANTCA 2410	Around Calvinia, 3119BD (Calvinia)
A. henssenianum U. Müll.-Doblies & D. Müll.-Doblies		*JCC, JG, JPM*	HENSEK 2161	Eksteenfontein, 2817DA (Vioolsdrif)
A. huntleyi J. Pedrola-Monfort, N. Membrives, J. M. Montserrat, & J. Caujapé		*JCC, JG, JPM*	HUNTEK1 2348	Steinkopf, 2917AC (Springbok)
A. huntleyi		*JCC, JG, JPM*	HUNTEK3 2325	Steinkopf, 2917AC (Springbok)
A. irroratum Schltr & K. Krause	Hap. 1	*JCC, JG, JPM*	IRROEK 2339	Steinkopf, 2917AC (Springbok)
A. irroratum	Hap. 2	*JCC, JG, JPM*	IRROEK2 2460	Steinkopf, 2917AC (Springbok)
A. irroratum	Hap. 2	*JCC, JG, JPM*	IRROEK6 2150	Eksteenfontein, 2817DA (Vioolsdrif)
A. irroratum	Hap. 3	*JCC, JG, JPM*	IRROKA 2541	Kliprand, 3018CB (Kamiesberg)
A. irroratum	Hap. 3	*JCC, JG, JPM*	IRROKW 1698	Vredendal, 3118CC (Vanrhynsdorp)
A. irroratum	Hap. 3	*JCC, JG, JPM*	IRROVP 1937	Varhynsdorp, 3119AC (Calvinia)
A. irroratum	Hap. 3	*JCC, JG, JPM*	IRROVY 1875	Vredendal, 3118CC (Vanrhynsdorp)
A. poeltianum U. Müll.-Doblies & D. Müll.-Doblies	Hap. 1	*JCC, JG, JPM*	POELNB 2526	Nababiep, 2917CD (Springbok)
A. poeltianum	Hap. 1	*JCC, JG, JPM*	POELCO 2071	Concordia, 2917CD (Springbok)
A. poeltianum	Hap. 2	*JCC, JG, JPM*	POELST 1779	Steinkopf, 2917AC (Springbok)
A. villosum U. Müll.-Doblies & D. Müll.-Doblies		*JCC, JG, JPM*	VILLEK 2217	Eksteenfontein, 2817DA (Vioolsdrif)
A. villosum		*JCC, JG, JPM*	VILLST 1676E	Steinkopf, 2917AC (Springbok)
A. walteri J. Pedrola-Monfort, N. Membrives & J. M. Montserrat		*JCC, JG, JPM*	WALTST 1747	Steinkopf, 2917BC (Springbok)
Species and populations of eastern South Africa				
A. albanense subsp. *albanense* Schoenl.		*JPM*	ALBASW 2000.0907	Grahamstown, 3326BC (Grahamstown)
A. austrocapense	Hap. 1	*JPM*	AUSTCR 2000.0973	Cap Recife, 3425AC (Shoenmakerskop)
A. austrocapense	Hap. 1	*JPM*	AUSTSB 2000.0944	Sardinia Bay, 342AB (Shoenmakerskop)
A. decipiens N. E. Brown		*JPM*	DECISL	Santa Lucia, 2832BB (Mtubatuba)
A. leistneri U. Müll.-Doblies & D. Müll.-Doblies	Hap. 1	*JPM*	LEISBG 2000.0959	Bloemfontein, 2926AA (Bloemfontein)
A. leistneri	Hap. 2	*JPM*	LEISBL 2000.0953	Bloemfontein, 2926AA (Bloemfontein)
A. longipes Baker		*JPM*	LONGZU 2000.0925	Addo, 3325CC (Port Elizabeth)
A. melanthioides Willd.	Hap. 1	*JPM*	MELAGA 2001.05018	Gramsberg, 2316BA (Nauchas), Namibia
A. melanthioides	Hap. 2	*JPM*	MELAOT 2001.05030	Otjosondu, 2117BD (Otjosondu), Namibia
A. roseum subsp. *albiflorum* U. Müll.-Doblies, Raus, Weiglin & D. Müll.-Doblies		*JPM*	ROSEGO 2001.05063	Gochas, 2418DD (Stampriet), Namibia
A. roseum subsp. *albiflorum*		*JPM*	ROSETW 2001.05039	Tweerivier, 2519BB (Koes), Namibia
A. roseum subsp. *roseum* Engl.		*JPM*	ROSEFB 2001.05047	S Okahanda, 2216AD (Otjimbingwe), Namibia
Outgroup species				
Alstroemeria aurantiaca D. Don		*AH*	ALS.AUR 95095	Valdivia Botanic Garden, Valdivia, Chile
Baeometra uniflora (Jacq.) G. J. Lewis		*JCC, JG, JPM*	BAE.UNI 1857.1194	Simon's Town, 3418AB (Simonstown), South Africa
Bulbocodium vernum Linn.		*MV*	BUL.VER 95113208	Huesca, Spain
Colchicum lusitanum Brot.		*JCC, JG*	COL.LUS 528.1097	Cadiz, Spain
Gloriosa superba Linn.		*AH*	GLO.SUP 96.398	Marimurtra Botanic Garden, Girona, Spain
Merendera montana Lange		*MV*	MER.MON 1432.794	Huesca, Spain
Onixotis triquetra (L. f.) D. J. Mabberley		*AH*	ONIX.TRI 1.197	Silverhill Seeds, Cape Town, South Africa

nitrogen, using the CTAB method (Doyle and Doyle 1987) with some modifications (Li et al. 2001). The isolated DNA was resuspended in TE buffer (TRIS–EDTA).

The *trn*L intron and *trn*L–*trn*F IGS regions were amplified using the "c," "d," and "e," "f" primers of Taberlet et al. (1991), respectively. The *trn*Y–*trn*D IGS was amplified using the trnYf (5′-TCTACGCTGGTTCAAATCCAG-3′) and trnDr (5′-AACCCGCAGCTTCCGCCTT-3′) primers. Double-stranded DNA amplifications were 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 primer (Eurogentec Ltd., Seraing, Belgium) and 1 Unit of Biotaq (Bioline). Following an activation step of 3 min at 92°C for the enzyme, the PCR mixture underwent 30 cycles of 30 sec at 92°C, 20–30 sec at annealing temperature, and 30 sec at 72°C. The annealing conditions for the *trn*L intron, *trn*L–*trn*F IGS, and *trn*Y–*trn*D IGS were 30 sec at 58°C, 20 sec at 64°C, and 20 sec at 63°C, respectively. To remove excess primers and deoxynucleotide triphosphates after amplification, PCR products were purified on GFX™ PCR DNA columns (Amersham Biosciences Europe GmbH, Cerdanyola, Barcelona, Spain) according to manufacturer's instructions. Sequencing was performed using the dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, California, 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 sec at 94°C, 30 sec 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 Girona University, Spain.

Data Analyses

Sequence information of the three noncoding cpDNA regions were aligned using CLUSTAL_W vers. 1.4 (Thompson et al. 1994), and were tested and corrected by hand with Bioedit vers. 5.0.9 (Hall 1999). Gaps 2 base pairs (bp) or less were removed. Previous analyses of these cpDNA regions have demonstrated that insertions/deletions (indels) longer than 2 bp are not too 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, the indels of 3 bp and longer were coded as binary character data (Simmons and Ochoterena 2000) using the GapCoder program (Young and Healy 2003).

The ILD test (Farris et al. 1995), implemented in PAUP* vers. 4.0b10 (Swofford 2002) as *partition homogeneity test,* was carried out to test the combinability of the three data sets.

We analyzed the phylogenetic relationships using maximum parsimony (MP) methods using PAUP*. The analyses were carried out with the heuristic search strategy with tree bisection reconnection (TBR), saving all shortest trees at each step (MULPARS), and branch swapping on all trees saved (STEEPEST descent). Multiple islands of equally most parsimonious trees were searched by the heuristic option with 100 random sequence additions. The consistency index (CI) and the retention index (RI) are presented to estimate the amount of homoplasy in the characters and the relative support for each clade was assessed by bootstrap analysis (Felsenstein 1985) with 1000 pseudoreplicates of the data and TBR branch swapping. In each replicate of bootstrapping, we limited the maximum number of trees to 5000.

The ancestral area analysis of Bremer (1992) was performed to study the geographic origin of *Androcymbium.*

RESULTS

The amplification of noncoding cpDNA sequences using universal primers has been shown successful for phylogenetic reconstructions at low taxonomic levels (Taberlet et al. 1991; Demesure et al. 1995). Phylogenetic studies based in noncoding cpDNA sequences have been successful at both interspecific (Gielly and Taberlet 1994; Bruneau 1996; Asmussen and Liston 1998) and intraspecific level (Dumolin-Lapègue et al. 1997; Petit et al. 1997). For this reason, the sequencing of noncoding cpDNA regions was chosen to create the phylogeny of the *Androcymbium* genus.

Sequence Data

Sequences were obtained from three cpDNA noncoding regions: *trn*L intron, *trn*L–*trn*F IGS, and *trn*Y–*trn*D IGS. The average lengths of the combined cpDNA regions vary between 1267 bp (northern African species) and 1212 bp (western South African species) (Table 2). Because of this, it was necessary to insert gaps to align sequences, increasing the total length of the aligned matrix (Table 3). These gaps can provide phylogenetic information. Some authors ignore these zones, losing much phylogenetic information when analyzing the data. Due to this, the gaps were coded as character data (Simmons and Ochoterena 2000) and then introduced into the analysis, resulting in a final 1690 bp matrix.

The length of sequences is correlated with geography: the western South African species possess the shortest, eastern South African taxa intermediate, and North African species the longest *trn*L intron sequences (Table 2). *Androcymbium austrocapense* populations of western and eastern South Africa have the same nucleotide substitutions and indel pattern as the rest of species from eastern South Africa. *Androcymbium roseum* subsp. *roseum* occurs in south-central Africa, but its sequences have similar length and the same nucleotide substitutions and indel pattern as those of species of North Africa.

The chloroplast region that possesses the largest percentage of parsimony-informative sites is the *trn*L–*trn*F IGS (7.6%). If the gaps are coded as character data and added to the parsimony-informative characters, it is found that the most phylogenetically informative region is the *trn*L intron. The least informative region is the *trn*Y–*trn*D IGS (Table 3).

The *trn*Y–*trn*D IGS region, never used before in phylogenetic studies, has a very unstable 101 bp zone. It is present or absent in different populations of different taxa of *Androcymbium* without any evident biogeographic or phylogenetic pattern. Hence, this unstable zone was removed from the analysis. In the outgroup taxa, this unstable region is always present.

In some cases, we found different DNA sequences within

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

	*trn*L intron	*trn*L–*trn*F IGS	*trn*Y–*trn*D[a] IGS	Combined
North Africa	613 (9)	394 (2)	260 (3)	1267 (10)
Eastern South Africa	597 (2)	397 (5)	260 (1)	1251 (7)
Western South Africa	570 (13)	389 (15)	258 (13)	1212 (23)
Outgroups	567 (18)	383 (27)	252 (25)	1201 (34)
WIDESPREAD SPECIES				
A. roseum subsp. *roseum*	625	394	258	1275
A. roseum subsp. *albiflorum*	579	391	258	1247
A. austrocapense	598	395	260	1248
A. melanthioides	575	378	261	1217

[a] The *trn*Y–*trn*D IGS region has a very unstable zone of 101 bp. This zone is present or absent in different populations and species of *Androcymbium* without any biogeographic or phylogenetic pattern. Due to this, the zone was removed from phylogenetic analyses and from this table. The outgroup does not show this phenomenon.

the same *Androcymbium* species. Each different DNA sequence of the same species was identified as a haplotype.

Incongruence Length Difference Test

The Incongruence Length Difference (ILD) test (Farris et al. 1994) was performed to test for conflicting signal among the three DNA data sets. The result was significant (P = 0.01) pointing out that there is evolutionary heterogeneity among the three data sets. If we test only the *trn*L intron and *trn*L–*trn*F IGS, the result is not significant (P = 0.45), indicating that significant incongruences cannot be detected between these two regions.

It has been pointed out that rejection of the null hypothesis of the ILD test may not be due to incongruence caused by different histories (Dolphin et al. 2000). Wiens (1998) recommends analyzing the data sets separately and making one tree with each data set. If there is no incongruence among the groups found in the trees analyzed separately, and the groups found in a tree made using the combined set, the data should be combined. We did not find incongruence between the tree topology with the separate data and with the combined data. Therefore we decided to combine the three data sets. Moreover, all three regions are linked and part of a nonrecombining chloroplast genome, providing ample justification for combining data sets.

Phylogenetic Analyses

The MP analyses with the data for the three regions combined produced a bootstrap strict consensus tree (Fig. 2). The phylogenetic tree is the result of 1000 resamplings where we limited the number of trees in each replicate to 5000. The tree length was 645 steps, and the consistency index (CI) and retention index (RI) were CI = 0.767 and RI = 0.737. No different islands were found in the phylogenetic analysis.

We can see that *Androcymbium* is not monophyletic in Fig. 2 because *Bulbocodium* L., *Colchicum* L., and *Merendera* Ramond, are nested within *Androcymbium.* These four genera, that form the Colchiceae tribe, are morphologically characterized by having subterranean, tunicate, bulb-like corms, flowers situated on a very short central stem, and long-clawed tepals. All have the alkaloid colchicine (Dahlgren et al. 1985).

In the phylogenetic tree (Fig. 2), four clades are clearly differentiated. Clade 1: North African species with *A. roseum* subsp. *roseum,* with bootstrap support (BS) 93%; Clade 2: North African species—*A. roseum* subsp. *roseum* with eastern South African species—*A. austrocapense* (western South African populations) (BS 98%); Clade 3: western South Africa Clade A species (BS 84%); Clade 4: western South Africa Clade B species (BS 68%).

In Clade 2, made up of North African and eastern South African species, is found *Androcymbium austrocapense* which is distributed from Cape Town, in western South Africa (Hap. 2 populations), to Port Elizabeth, in eastern South Africa (Hap. 1 populations).

Androcymbium roseum subsp. *roseum* is found in Clade 1, formed by northern African taxa. This species is largely distributed from the Orange River, in western South Africa, to Tanzania (south-central Africa), following the river courses. Given this distribution it is possible to connect the two disjunct regions on the African continent. This is also consistent with similarities in micro- and macro-morphological characters of the North African species and *A. roseum* (Martín et al. 1993; Pedrola-Monfort 1993; Membrives 2000). The monophyly of taxa in Clade 1 also provides a phylogeographic connection among species and populations of physically separated regions: the Atlantic coast of Morocco (*A. wyssianum* Hap. 2) with the Canary Islands (*A. psammophilum* and *A. hierrense*).

Nucleotide Substitutions/Indels Patterns

The North African species (Clade 1; BS 93%) possess a set of synapomophic changes at DNA sequence level (Fig. 3; Table 4). These changes are also present in *A. roseum* subsp. *roseum,* distributed in south-central Africa. Within Clade 1, a clade composed of *A. wyssianum* Hap. 2, *A. psammophilum,* and *A. hierrense* (BS 86%), also share several synapomorphies.

The Clade 2 species (BS 98%) of North Africa and eastern South Africa also are characterized by a series of synapomorphies (Fig. 3; Table 4). These are different from western South African species, with the exception of *A. austrocapense.* The eastern and western populations of *A. austrocapense* share identical nucleotide and indels patterns with the eastern South African species of Clade 2.

Table 3. Characterization of the three noncoding cpDNA regions of *Androcymbium* species sequenced in this study.

Character	*trn*L/F IGS		*trn*L intron		*trn*Y/D IGS		Combined		Combined + coded gaps	
Aligned lengths of base pairs	489		743		365		1597		1690	
Variable sites (%)	103	(21.1)	138	(18.6)	75	(20.5)	316	(19.8)	409	(24.2)
Parsimony-informative sites (%)	37	(7.6)	45	(6.1)	20	(5.5)	102	(6.4)	134	(7.9)
Autapomorphic sites (%)	66	(13.5)	93	(12.5)	55	(15.0)	214	(13.4)	275	(16.3)
Number of indels (parsimony-informative)	21	(6)	52	(22)	20	(4)	93	(32)		
Parsimony-informative sites including informative gaps (%)	43	(8.8)	67	(9.0)	24	(6.6)				

Areas of Bremer

Results from Bremer's ancestral area method (1992) show that the highest gain-to-loss ratios (G/L), and their rescaled quotients (AA), are for the western South African region. This is followed by eastern South Africa and North Africa (Table 5). They are more easily compared by rescaling the G/L quotients to a maximum value of 1. Rescaled quotients (AA; for estimating ancestral area) are obtained by dividing each G/L value by the maximum G/L found for each cladogram.

DISCUSSION

The most striking aspect of our results is that *A. austrocapense* and *A. roseum* phylogenetically connect the disjunct areas of *Androcymbium* in Africa. The topological position of these taxa in the phylogenetic tree (Fig. 2) matches the geographical distribution, following the west-east and south-north axes.

Origin of Androcymbium

The ancestral area analysis, following Bremer (Table 5), provides support for western South Africa as the most probable region of origin for the genus. Other morphological (Membrives et al. 2003*a*, *b*, *c*), palynological (Martín et al. 1993; Membrives et al. 2002*b*), reproductive (Membrives et al. 2002*a*), karyological (Margelí et al. 1999; Montserrat et al. 2002), and molecular divergence evidence, both allozymes (Membrives et al. 2001) and cpDNA RFLPs (Caujapé-Castells et al. 1999, 2001), support this hypothesis.

West–East South African Disjunction

In the ancestral zone of western South Africa, we find several lineages (Fig. 2), while all the species that occur in eastern South Africa form a clade, along with the North African species (BS 98%). Within the clade that contains all the eastern South African species, we find the coast species *A. austrocapense* that inhabits both of the west–east disjunction regions. A recent study of polymorphism based on RAPDs (del Hoyo in prep.), indicates that the western populations of *A. austrocapense* have more molecular polymorphism. This can be used to infer a higher probability that western populations are older than the eastern. The ancestral area analysis also suggests that the eastern South African region is more modern than the western, but older than the rest of regions of this disjunction. If we look at the specific diversity of these regions, we find that of 56 *Androcymbium* species, 36 are located in western South Africa, 10 in eastern South Africa, and *A. austrocapense* occurs in both zones of South Africa. This asymmetry of species distribution is similar to many other genera of the African xerophytic flora, i.e., the genus *Haemanthus* L., with 21 species, 15 of which are found almost exclusively in western South Africa and with five in the east. Only the species *H. albiflorus* Jacq. occurs in both regions (Snijman 1984). This disjunction also occurs in other taxa, such as the genus *Erica* L., with 621 in western South Africa and 23 in eastern South Africa (Brown and Lomolino 1998), and in many other genera such as *Crassula* L. (Jürgens 1997), *Ehrharta* Juss. (Verboom et al. 2003), *Leucas* Burm. (Ryding 1998), *Lotononis* (DC.)

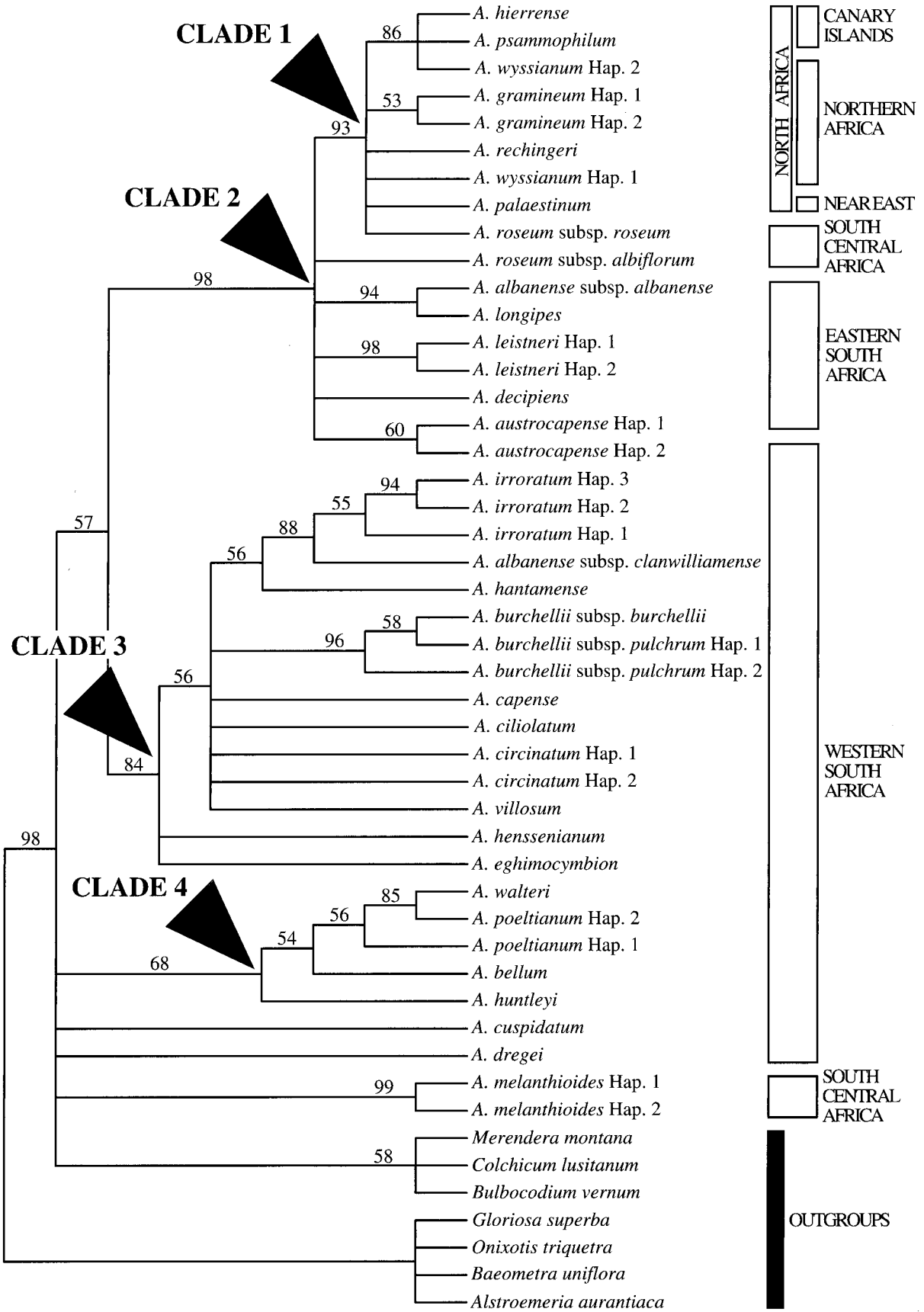

Fig. 2.—Bootstrap strict consensus tree resulting from a phylogenetic analysis with MP methods showing the biogeographic and phylogenetic relationships among the species of *Androcymbium*. Numbers above the branches represent bootstrap support. Black arrows indicate the main clades. Nonparametric bootstrap analysis employed 1000 pseudoreplicates, limiting the number of trees saved per pseudoreplicate to 5000. CI = 0.767; RI = 0.737; tree length = 645. Hap. = haplotype.

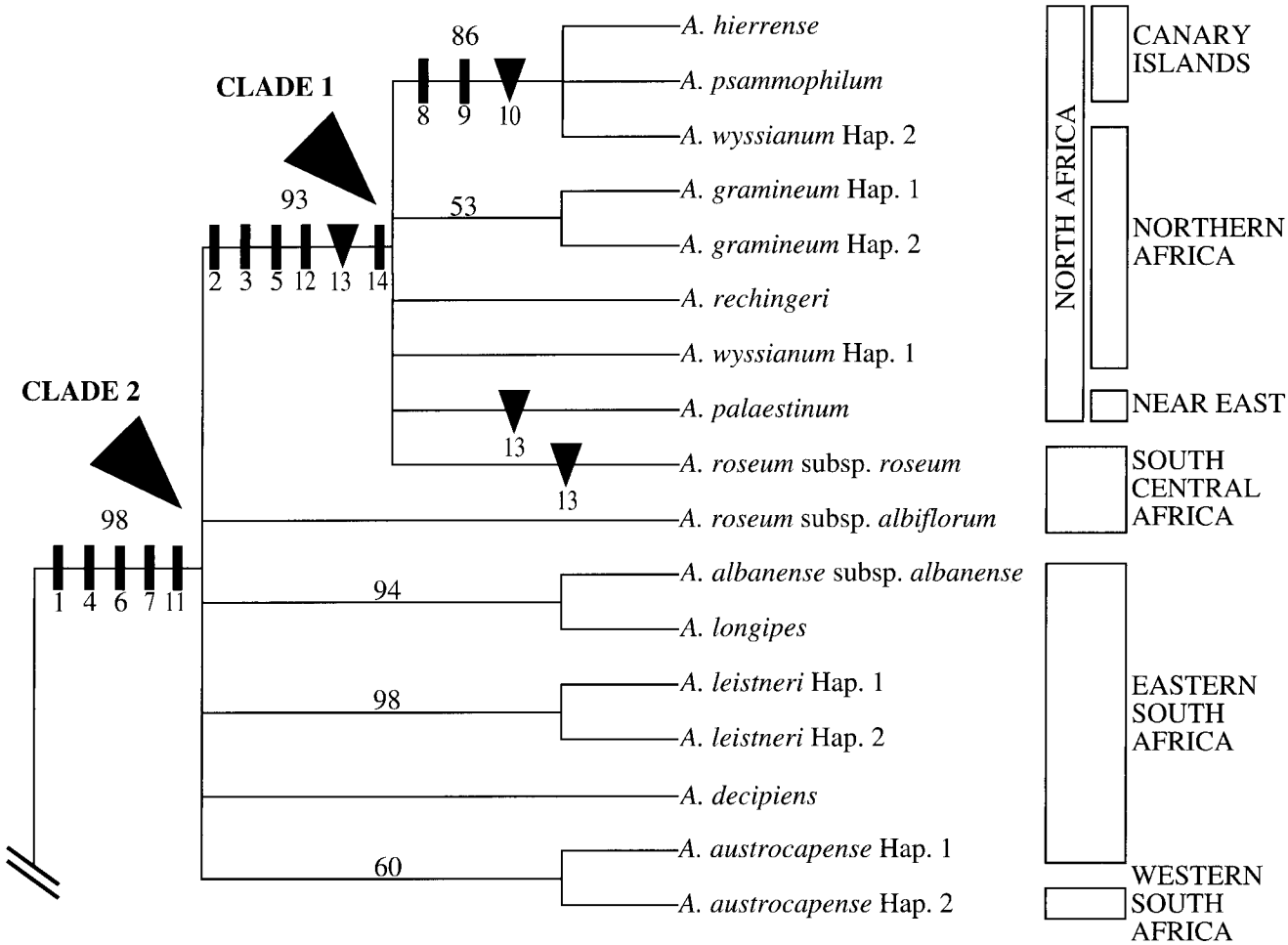

Fig. 3.—Distribution of the synapomorphic changes at cpDNA sequence level in Clade 1 and 2 of the bootstrap strict consensus tree.

Eckl. & Zeyh. (Linder et al. 1992), and *Moraea* Mill. (Goldblatt et al. 2002). In all of these cases it can be observed that greater morphological diversity occurs in the western species than in the eastern ones.

This biogeographic pattern, with a west to east direction, could serve as an evolutionary model for many other species with the same disjunct distribution, given that this disjunction is very common in many South African xerophytic genera.

South–North Disjunction

The geographical south-north disjunction of *Androcymbium* has 50 taxa in the southern African region (South Africa and south-central Africa), and 6 taxa in North Africa. This pattern is similar to many other genera with high species diversity in South Africa and that also have some species in the north, i.e., *Erica* with 644 taxa in the southern African region, but only 25 in North Africa and Europe (Brown and Lomolino 1998), or *Moraea* with nearly 200 taxa in South Africa and only one in the Mediterranean basin (Goldblatt et al. 2002). Other examples are *Aloe* L., *Dracaena* Vand., *Echium* L., *Lobostemon* Lehm., *Olea* L., and *Pelargonium* L'Hér. These disjunctions could have originated by dispersal or vicariance. The dispersalist hypothesis explains the disjunct patterns of distribution by dispersion, due to the disappearance of pre-existing barriers; whereas, vicariance explains the disjunctions as the result of the appearance of barriers that fragmented the distribution of ancestral taxa. From the sequencing of three cpDNA noncoding regions and Bremer's analysis, we found that the North African species are derived from the South African ones. This suggests that this disjunction originated by dispersal, with western South Africa as the center of origin. The preexisting barrier was a tropical zone that developed into an arid corridor—the arid track. This corridor connected the south with the north of Africa in the Upper Miocene (Balinsky 1962).

The North African species that form Clade 1 (BS 93%), show a set of synapomorphies at sequence level that also occur in *A. roseum* subsp. *roseum* (Fig. 3; Table 4). This species appears to provide evidence for the connection between South Africa and North Africa and could be the most probable ancestor of this latter species group. *Androcymbium roseum* is currently widespread in south-central Africa, in zones with arid conditions, occurring specifically in ravines and riverside habitats. Given its apparent inability to establish populations far from sites that experience periodic flooding, and the need for arid conditions for their development, it is possible to suggest that either this species or its ancestor could have arrived in the Mediterranean basin by following

Table 4. Synapomorphic changes associated with the major clades, using the three noncoding regions.

Synapomorphy	Base	Canarian species + *A. wyssianum* Hap. 2 (BS 86%)	Clade 1[a] (BS 93%)	Clade 2[b] (BS 98%)	Rest of *Androcymbium*	Type[c]	cpDNA region
1	32	T	T	T	A	Tv.	*trn*L–*trn*F IGS
2	282	T	T	C	C	Ts.	
3	286	A	A	G	G	Ts.	
4	423	A	A	A	T	Tv.	
5	448	G	G	A	A	Ts.	
6	671	C	C	C	T	Ts.	*trn*Y–*trn*D IGS
7	753	A	A	A	T	Tv.	
8	764–769	INSERTION	—	—	—	6 bp.	
9	801	A	G	G	G	Ts.	
10	931	C	A	A	A	Tv.	
11	1080	A	A	A	G	Ts.	*trn*L INTRON
12	1096	T	T	C	C	Ts.	
13	1179–1192	INSERTION	INSERTION[d]	—	—	14 bp.	
14	1128	A	A	T	T	Tv.	

[a] Northern African species + *A. roseum* subsp. *roseum.*
[b] Northern African species + Eastern South African species + *A. austrocapense* Hap. 2.
[c] Abbreviations: bp, base pairs; Ts, transitions; Tv, transversions.
[d] The species *A. roseum* and *A. palaestinum* have the insertion duplicated.

Table 5. Ancestral area analysis, following Bremer (1992); a higher gain-to-loss ratio indicates a higher probability of being the ancestral area.

	Gains	Losses	G/L[a]	AA[b]
Western South Africa	5	7	0.71	1.0
Eastern South Africa	4	8	0.50	0.7
Northern Africa	4	14	0.29	0.4
South-Central Africa	3	13	0.23	0.3
Canary Islands	2	16	0.13	0.2
Near East	1	15	0.07	0.1

[a] Gains/Losses.
[b] Rescaled quotient.

the major river courses. Indeed, there is geological evidence indicating the existence of an Upper Miocene watercourse (i.e., Eonile Canyon) connecting the eastern part of central Africa with the northern portion of the continent (Said 1981, 1993), coinciding with the formation of the Miocene arid track. In previous work with cpDNA RFLPs (Caujapé-Castells et al. 1999), an ancestral species is dated from North Africa at 12.1 ± 2.8 million years ago (mya) (Upper Miocene). The relationships among *A. roseum* and the northern species is also supported by an enormous similarity in plant macromorphology (Membrives 2000), microfeatures of pollen (Martín et al. 1993), and seed coat (Pedrola-Monfort 1993), providing additional indirect evidence of the ancestral nature. Unlike *A. roseum* subsp. *roseum, A. roseum* subsp. *albiflorum* is included in the clade containing all the species of eastern South Africa, in addition to species of the Canary Islands, North Africa and the Near East (Fig. 3; Table 4). Therefore, those groups made up of the species of North Africa and the species of eastern South Africa (Clade 2) are more closely related to each other than to the species from western South Africa.

The center of origin of *Androcymbium* appears to be in western South Africa and the phylogenetic tree (Fig. 2) supports a southwestern to southeastern to northern Africa directionality of dispersal. This biological and geological evidence explains the pattern of distribution via dispersion. Collectively, this supports the *Androcymbium* dispersalist hypothesis starting from a center of origin situated in western South Africa with distribution to North Africa and the important role of the Miocene arid track and Eonile Canyon.

The disjunctions formed via dispersion may originate in two ways: by a single long-range event, or several progressive, short-range events. If long-range dispersal was a factor in the distribution of *Androcymbium* before desertification of Africa, then we would expect that some of the species in eastern South Africa must be more recent than their western and northern congeners. In our phylogenetic tree it is observed that the most recent species are the North African ones. Our study seems more consistent with the dispersion hypothesis of *Androcymbium* by multiple, progressive, and short-range events.

Northern Africa Disjunction

Within northern Africa, we can find another disjunction between the Atlantic coast of Morocco and the Canary Islands. Pedrola-Monfort and Caujapé-Castells (1998) pro-

posed three hypotheses that could account for the origin of the Canarian species. The first is that their origins lie in two different mainland taxa. The second possibility is that a single mainland taxon could have colonized both groups of islands at different times. The third alternative assumes the existence of a mainland taxon from which one of the Canarian taxa originated (probably *A. hierrense,* given the geological history of these islands), which in turn would have been the ancestor of the other one. Caujapé-Castells et al. (2001) indicated that the origin of the Canary Islands species *A. psammophilum* and *A. hierrense* could be explained by a single colonization event from an ancestor related to the mainland *A. wyssianum,* agreeing with the third hypothesis.

In our phylogenetic tree, the Canarian species form a clade with *A. wyssianum* Hap. 2 (Essaouria population, Morocco) (BS 86%). This population of *A. wyssianum* possesses a set of changes at DNA sequence level that occur only in the Canarian species, *A. psammophilum and A. hierrense.* Crossability among individuals of the Canarian species and *A. wyssianum* Hap. 2 indicate that there is reproductive incompatibility, discounting the likelihood of introgression.

The hypothesis supported by all these data is that the Canarian species originated from a related ancestor with *A. wyssianum* Hap. 2, the population of Essaouria (Morocco). The inclusion of this new population in our analysis has become an important key to the understanding of the relationship between the Canarian and mainland species, and agrees with the hypothesis proposed by Caujapé-Castells et al. (2001).

Taxonomic Implications

Because of the appearance of *Bulbocodium, Colchicum,* and *Merendera* in the ingroup with *Androcymbium,* we discard a monophyletic origin of the genus. Nevertheless it is obvious that tribe Colchiceae (sensu Dahlgren et al. 1985) is monophyletic and to make *Androcymbium* monophyletic requires only four more steps. We propose the reunification of these four genera. Following the International Code of Botanical Nomenclature (Greuter et al. 1994; sect. 3, art. 11.3), the correct name is the earliest legitimate name, in this case *Colchicum.*

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Appendix 1. GenBank accession numbers. ID: idem. Hap: haplotype.

Taxon	Haplotype	GenBank accession number		
		*trn*L intron	*trn*L–*trn*F IGS	*trn*Y–*trn*D IGS
North Africa				
A. gramineum	1	AY608517	AY608520	AY608528
A. gramineum	2	AY608516	AY608521	AY608529
A. hierrense		AY608514	AY608523	AY608531
A. palaestinum		AY136755	AY608527	AY608534
A. psammophilum		AY136756	AY608524	AY608532
A. rechingeri		AY608518	AY608525	AY608535
A. wyssianum	1	AY608519	AY608526	AY608533
A. wyssianum	2	AY608515	AY608522	AY608530
West South Africa				
A. albanense subsp. *clanwilliamense*		AY622747	AY622708	AY611748
A. austrocapense	2	AY136757	AY622696	AY611741
A. bellum		AY622738	AY622700	AY611742
A. burchellii subsp. *burchellii*		AY622739	AY622701	AY611743
A. burchellii subsp. *pulchrum*	1	AY622740	AY622702	AY611744
A. burchellii subsp. *pulchrum*	2	AY622741	ID Hap. 1	ID Hap. 1
A. capense		AY622742	AY622703	AY611745
A. ciliolatum		AY622743	AY622704	AY611746
A. circinatum	1	AY622744	AY622705	AY611747
A. circinatum	2	AY622745	AY622706	ID Hap. 1
A. cuspidatum		AY622746	AY622707	AY611749
A. dregei		AY622748	AY622709	AY611750
A. eghimocymbion		AY622749	AY622710	AY611751
A. hantamense		AY622750	AY622711	AY611752
A. henssenianum		AY622751	AY622712	AY611753
A. huntleyi		AY622752	AY622713	AY611754
A. irroratum	1	AY622753	AY622714	AY611755
A. irroratum	2	AY622754	AY622715	AY611756
A. irroratum	3	AY622755	AY622716	ID Hap. 2
A. poeltianum	1	AY622756	AY622717	AY611757
A. poeltianum	2	AY622757	AY622718	AY611758
A. villosum		AY622758	AY622719	AY611759
A. walteri		AY622759	AY622720	AY611760
East South Africa				
A. albanense subsp. *albanense*		AY622733	AY622695	AY611765
A. austrocapense	1	ID Hap. 2	ID Hap. 2	AY611766
A. decipiens		AY622734	AY622697	AY611767
A. leistneri	1	AY622735	AY622698	AY611768
A. leistneri	2	AY622736	ID Hap. 1	AY611769
A. longipes		AY622737	AY622699	AY611770
Namibia				
A. melanthioides	1	AY622732	AY622694	AY611763
A. melanthioides	2	ID Hap. 1	ID Hap. 1	AY611764
A. roseum subsp. *albiflorum*		AY622731	AY622693	AY611762
A. roseum subsp. *roseum*		AY622730	AY622692	AY611761
Outgroups				
Alstroemeria aurantiaca		AY622764	AY622728	AY622773
Baeometra uniflora		AY155494	AY622729	AY622769
Bulbocodium vernum		AY622763	AY622727	AY622767
Colchicum lusitanum		AY154475	AY622722	AY622768
Gloriosa superba		AY154476	AY622721	AY622766
Merendera montana		AY154477	AY622724	AY622770
Onixotis triquetra		AY622762	AY622723	AY622765