Available online at www.sciencedirect.com

ScienceDirect

South African Journal of Botany 76 (2010) 217–238

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

Floral and macroecological evolution within *Cyrtanthus* (Amaryllidaceae): Inferences from combined analyses of plastid *ndhF* and nrDNA ITS sequences

D.A. Snijman [a,*], A.W. Meerow [b]

[a] *Compton Herbarium, South African National Biodiversity Institute, Private Bag X7, Claremont 7735, South Africa*
[b] *USDA-ARS-SHRS, National Germplasm Repository, 13601 Old Cutler Road, Miami, Florida 33158, USA*

Received 2 June 2009; received in revised form 14 September 2009; accepted 26 October 2009

Abstract

One of the most diverse members of Amaryllidaceae is *Cyrtanthus* Aiton, a large, sub-Saharan Africa genus of approximately 55 species found mostly in South Africa. To investigate phylogenetic and biogeographic relationships within *Cyrtanthus*, sequence data from the plastid *ndhF* gene and the ITS nrDNA region for 41 species were analyzed with parsimony, maximum likelihood, and Bayesian-inference approaches. Various recombination detection algorithms were used to test for interspecific hybridization in the ITS alignment. The genus resolved as monophyletic, comprising three poorly to well-supported major lineages: a predominantly Afrotemperate lineage, largely restricted to seasonally moist sites in summer rainfall southern Africa, a subtropical lineage found mostly in nonseasonal rainfall regions, often in dry habitats, and a Cape Floristic Region-centered lineage in which most species are concentrated in the summer-dry to nonseasonal rainfall southwest. The ITS sequence alignment shows no evidence for reticulation between any of the species. Relationships inferred by the molecular data disagree with those derived from morphological data, but agree with previously published groupings based on karyotype morphology. Fitch optimization of selected floral characters on the combined gene tree reveals recurrent patterns of convergence. Ornithophilous floral forms occur in parallel among the three primary clades, putatively sphingophilous species are concentrated in the Afrotemperate lineage in seasonally moist upland grasslands; the brush-type *Aeropetes tulbaghia* butterfly and inferred long-proboscid fly pollination syndromes are unique in the Cape lineage. Macroecological factors inferred to have influenced the evolution of *Cyrtanthus* are changes in rainfall seasonality, the advent of fire, and the availability of new habitats at high and low altitudes and in rock-free soils or rock crevices. This study gives greater clarity on relationships within the genus and enables its division into three informal infrageneric groups.

Keywords: Africa; Amaryllidaceae; *Cyrtanthus*; Floral morphology; Molecular systematics; Monocotyledons

1. Introduction

The genus *Cyrtanthus* Aiton is endemic to sub-Saharan Africa, with well over 90% of its species concentrated in South Africa (Dyer, 1939; Reid and Dyer, 1984). With about 55 species it is the largest genus of southern Africa's Amaryllidaceae (Snijman and Archer, 2003) and one of the largest in the family overall. Within this ecologically diverse region *Cyrtanthus* extends from the summer-dry southwest to the summer rainfall northeast. A remarkable response of several species within fire-prone ecosystems is their dependence on fire to flower (Gordon-Gray and Wright, 1969; Le Maitre and Midgley, 1992; Keeley, 1993).

Traub (1963) placed *Cyrtanthus* in its own tribe, Cyrtantheae, a treatment maintained by Meerow and Snijman (1998), whereas Müller-Doblies and Müller-Doblies (1996) placed *Cyrtanthus* in the tribe Haemantheae, albeit as a monotypic subtribe, Cyrtanthinae, based on bulb morphology and chromosome number, a classification accepted by Dahlgren et al. (1985). Combined *rbcL* and plastid *trnL-F* sequences (Meerow et al., 1999) indicated a position for *Cyrtanthus* as sister to the remainder of Amaryllidaceae after the branching of the tribe Amaryllideae. Plastid *ndhF* sequences (Meerow and

* Corresponding author. Fax: +27 21 7614151.
E-mail address: d.snijman@sanbi.org.za (D.A. Snijman).

0254-6299/$ - see front matter © 2009 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2009.10.010

Snijman, 2006), however, resolved *Cyrtanthus* as sister to a clade comprising Calostemmateae and Haemantheae. An ITS alignment across the entire family (Meerow, unpubl. data) places *Cyrtanthus* as sister to Haemantheae, but until additional data are available the position of the genus relative to the rest of the family remains ambiguous.

The genus exhibits a high level of floral morphological diversity which is unparalleled in any other genus of the family. Conversely, the genus shows great consistency in chromosome number, with $2n=16$ characteristic of most, if not all, of the species (Wilsenach, 1963; Ising, 1970; Strydom et al., 2007). It is also the only African genus with the flattened, winged, phytomelanous seed, so common in the American clade of the family (Meerow and Snijman, 1998). Following Baker's (1888, 1896) treatments of *Cyrtanthus*, Dyer (1939) provided a synoptic review of the genus and Nordal (1979) revised the two East African species. The most recent account is that of Reid and Dyer (1984); six new species have since been described (Hilliard and Burtt, 1986; Snijman and Van Jaarsveld, 1995; Snijman, 1999, 2001, 2003, 2007).

The showiness of the flowers in *Cyrtanthus* is comparable to those of Orchidaceae and Iridaceae in southern Africa, families for which extensive data on pollination systems are available (Goldblatt et al., 1995, 1998; Johnson et al., 1998; Linder and Kurzweil 1999; Goldblatt and Manning, 1999, 2000). These studies indicate that shifts in pollination systems occur frequently within groups of closely related species and have necessitated the revision of several genera formerly classified on floral similarities (Goldblatt and Manning, 1998, 2007).

Using plastid *ndhF* and nrDNA ITS sequences, this study explores the phylogeny of *Cyrtanthus*. We use the phylogeny to examine ancestral habitats and distributions within the lineage and we test whether floral morphology is congruent with the phylogeny generated by the molecular data. By means of floral types (Vogel, 1954; Faegri and Van der Pijl, 1979; Johnson and Bond, 1994; Goldblatt and Manning, 2006) we infer the pollinators of species, trace the evolution of selected floral characters and assess the taxonomic implications for the genus.

2. Material and methods

2.1. Sampling

Plastid *ndhF* sequences and nrITS were obtained for 42 taxa of *Cyrtanthus* (Table 1). Many of the unsampled species belong to two groups of closely allied species (the *C. macowanii* group and *C. loddigesianus* group) that are often difficult to distinguish from each other, but representatives from each of these groups, (*C. epiphyticus*, *C. macowanii*, *C. suaveolens*) and (*C. helictus*, *C. loddigesianus*, *C. smithiae*), are included in our sample. *Amaryllis belladonna* (Amaryllideae) was designated as functional outgroup for both gene regions, but *Calostemma luteum* (Calostemmateae) and *Clivia nobilis* (Haemantheae) were included in the matrix to allow the generation of bootstrap support percentages for a monophyletic *Cyrtanthus*. Leaf samples were collected from living collections at the Kirstenbosch National Botanical Garden, South Africa, and from populations in the field and they were preserved in silica gel for later extraction.

2.2. DNA extraction and amplification and sequence alignment

Genomic DNA was extracted from 30 mg of silica gel dried leaf tissue using the FastDNA Kit (BIO 101 Inc., Carlsbad, CA) according to manufacturer's protocols with a FP 120 FastPrep cell disrupter (Savant Instruments Inc., Holbrook, NY). Samples were quantified with a GeneQuant pro RNA/DNA calculator (Amersham Pharmacia Biotech Inc., Piscataway, CA, USA).

2.2.1. ndhF

The plastid *ndhF* gene was amplified using the primers of Olmstead and Sweere (1994) and Graham et al. (1998). The gene was amplified and sequenced as described by Pires and Sytsma (2002), but with 4% DMSO added to the 50 µl reaction mix.

2.2.2. ITS

Amplification and sequencing of the ribosomal DNA ITS1/5.8S/ITS2 region were accomplished using flanking primers (18S, 26S) AB101 and AB102 (Douzery et al., 1999), and the original White et al. (1990) internal primers ITS2 and 3 to amplify the spacers along with the intervening 5.8S gene as described by Meerow et al. (2000).

Amplified products were purified with an Exonuclease I and Shrimp Alkaline Phosphatase treatment. Cycle sequencing reactions were performed directly on purified PCR products using standard dideoxy cycle protocols for sequencing with dye terminators on either an ABI 3100 or ABI 3730 automated sequencer (according to the manufacturer's protocols; Applied Biosystems, Foster City, California, USA).

Both the ITS and *ndhF* sequences were readily aligned manually and unambiguously using Sequencher™ 4.8 (Gene Codes, Ann Arbor, MI, USA).

2.3. Phylogenetic analyses

The *ndhF* and ITS matrices were analyzed separately and in combination using parsimony with PAUP∗ v. 4.0b10 (Swofford, 2002), and with two model-based approaches, maximum likelihood (ML), utilizing TreeFinder (Jobb, 2008) and, for the combined analysis only, Bayesian analysis (BA), with MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Best fit nucleotide substitution model was determined for each gene region with KAKUSAN v.3 (Tanabe, 2007), which also generates input files for these two programs. Best fit models were evaluated using the corrected Akaike Information Criterion (AICc; Akaike, 1973; Shono, 2000) for ML and the Bayesian Information Criterion (BIC) with significance determined by Chi-square analysis.

Parsimony tree searches were heuristic, conducted under the Fitch (equal) weights (Fitch, 1971) criterion with 2000 rounds of random addition sequence, saving no more than 20 minimum length trees per search for swapping using tree branch reconnection (TBR). Tree branches were collapsed if the

Table 1
Taxa, vouchers and sequence GenBank accession numbers for samples used in the phylogenetic analyses of *ndhF* and ITS sequence alignments, and origin of the plant material.

Taxon [a]	Voucher [b] or Garden accession number (n/n) [c]	Origin [d]	GenBank accession number	
			ndhF	ITS
Amaryllis belladonna L.	Chase 612 (K)	Ex Hort.	AY225029	AF373084
Calostemma luteum Sims	Meerow 3114 (FTG)	Ex Hort.	FJ264206	GQ160818
Clivia nobilis Lindl.	Chase 3080 (K)	Ex Hort.	FJ264209	AY280349
Cyrtanthus angustifolius (L.f.) Aiton	Snijman 1647 (NBG)	CPP-WC, Farm Buffelshoek	GQ160860	GQ160819
C. attenuatus R.A.Dyer	NBG 368/69	OFS, Ficksberg	GQ160861	GQ160820
C. aureolinus Snijman	Snijman 1980 (NBG)	CPP-WC, Farm Wilgermond	GQ160862	GQ160821
C. brachyscyphus Baker	Nichols 321 (NBG)	CPP-EC, Gonubi River	GQ160863	GQ160822
C. breviflorus Harv.	Nichols s.n. (NBG)	NAT, Eshowe	GQ160864	GQ160823
C. carneus Lindl.	NBG 175/94	CPP-WC, Farm Springfield	GQ160865	GQ160824
C. collinus Ker Gawl.	Bayer 6923 (NBG)	CPP-EC, Zuurberg	GQ160865	GQ160825
C. contractus N.E.Br.	Meerow s.n. (photo)	CPP-EC, Old Cathcart Road	GQ160867	GQ160826
C. debilis Snijman	Snijman 1717 (NBG)	CPP-WC, Outeniqua Mountains	GQ160868	GQ160827
C. elatus (Jacq.) Traub	NBG 915/82	Ex Hort.	FJ264214	GQ160828
C. epiphyticus J.M.Wood	Roux 1462 (PRE)	LES, Sehlabathebe	GQ160869	GQ160829
C. eucallus R.A.Dyer	McNeil s.n. (NBG)	TVL-MP, Aurora Mine	GQ160870	GQ160830
C. falcatus R.A.Dyer	Unknown (NBG 182510)	NAT, Underberg	GQ160871	GQ160831
C. fergusoniae L.Bolus	Bayer 2668 (NBG)	CPP-WC, Farm Botterkloof	GQ160872	GQ160832
C. flammosus Snijman & Van Jaarsv.	Van Jaarsveld 14721 (NBG)	CPP-EC, Kouga Dam	GQ160873	GQ160833
C. flanaganii Baker	Snijman 2230 (NBG)	CPP-EC, Tiffendel	GQ160874	GQ160834
C. galpinii Baker	Nichols 357 (NBG)	NAT, Hluhluwe	GQ160875	GQ160835
C. guthrieae L.Bolus	Olivier s.n. (NBG)	CPP-WC, Bredasdorp	GQ160876	GQ160836
C. helictus Lehm.	Snijman 1632 (NBG)	CPP-EC, Cathcart	GQ160877	GQ160837
C. herrei (F.M.Leight.) R.A.Dyer	Hall s.n. = Van Zijl 104 (NBG)	CPP-NC, Richtersveld	AY434484	AY751428
C. huttonii Baker	Meerow s.n. (photo)	CPP-EC, Amathole Mountains	GQ160878	GQ160838
C. labiatus R.A.Dyer	McMurtry s.n. (NBG)	CPP-EC, Baviaanskloof	GQ160879	GQ160839
C. leptosiphon Snijman	Goldblatt 10621 (NBG)	CPP-WC, Buffelsjags River	GQ160880	GQ160840
C. leucanthus Schltr.	Snijman 1267 (NBG)	CPP-WC, Palmiet River	GQ160881	GQ160841
C. loddigesianus (Herb.) R.A.Dyer	Unknown (NBG 190511)	CPP-EC, Buffelsfontein	GQ160882	GQ160842
C. mackenii subsp. *cooperi* (Baker) Snijman	R.McMaster 30 (NBG)	CPP-EC, Stutterheim	GQ160883	GQ160843
C. mackenii Hook.f. subsp. *mackenii*	J.C.McMaster s.n. (NBG)	CPP-EC, Haga Haga	GQ160884	GQ160844
C. macmasteri Snijman	J.C.McMaster s.n. (NBG)	CPP-EC, Farm Keibolo	GQ160885	GQ160845
C. macowanii Baker	Zsabo 286 (NBG)	CPP-EC, Maclear District	GQ160886	GQ160846
C. montanus R.A.Dyer	Skinner s.n. (NBG)	CPP-EC, Baviaanskloof	GQ160887	GQ160847
C. obliquus (L.f.) Aiton	Meerow s.n. (Photo)	CPP-EC, Longkloof	GQ160888	GQ160848
C. odorus Ker Gawl.	Snijman 1721 (NBG)	CPP-WC, Tradouw Pass	GQ160889	GQ160849
C. parviflorus Baker	Snijman & Manning 1875 (NBG)	CPP-EC, NE of Grahamstown	GQ160890	GQ160850
C. sanguineus (Lindl.) Walp. subsp. *sanguineus*	Unknown (NBG 182888)	Ex Hort.	GQ160891	GQ160851
C. sp.	Tait s.n. (NBG)	CPP-EC, Springs	GQ160892	GQ160854
C. smithiae Watt ex Harv.	Snijman 1636 (NBG)	CPP-EC, Fort Brown	GQ160893	GQ160852
C. spiralis Burch. ex Ker Gawl.	Unknown (NBG 176820)	CPP-EC, Settlers Park	GQ160894	GQ160853
C. stenanthus Baker var. *stenanthus*	Manning 2679 (NBG)	TVL-MP, E of Wakkerstroom	GQ160895	GQ160855
C. suaveolens Schönland	Snijman 1637 (NBG)	CPP-EC, Amathole Mountains	GQ160896	GQ160856
C. tuckii Baker var. *transvaalensis* I.Verd.	Manning 2678 (NBG)	TVL-MP, E of Wakkerstroom	GQ160897	GQ160857
C. ventricosus Willd.	Snijman 1661 (NBG)	CPP-WC, Table Mountain	GQ160898	GQ160858
C. wellandii Snijman	Snijman 1575 (NBG)	CPP-EC, Kabeljouws River	GQ160899	GQ160859

[a] Author abbreviations follow Brummitt and Powell (1992).
[b] Herbarium acronyms are according to Holmgren et al. (1990).
[c] Accession number for Kirstenbosch Botanical Garden.
[d] Abbreviations for geographical regions follow Brummitt (2001).

minimum length = 0. Gaps were coded as missing characters in all of the analyses, as there were no informative indels in either matrix. Before combining the ITS and *ndhF* data sets, we performed an incongruence length difference test (ILD = partition homogeneity test in PAUP) on the matrices (Farris et al., 1994, 1995) to assess the degree of congruence between them. One thousand heuristic searches were conducted for the ILD, each with 10 random addition replications, saving no more than 20 trees from each for TBR branch swapping. Internal support was determined by bootstrapping (BP; Felsenstein, 1985; 5000 heuristic replicates with simple addition, TBR branch swapping, saving 20 trees per replicate). For the combined analysis, partitioned Bremer (decay) indices (Bremer, 1988) using TreeRot v. 3.0 (Sorenson and Franzosa, 2007) were also calculated. The cut-off BP value was 50%. A BP value greater than 75% was considered good support, 65–75% was designated moderate support, and less than 65% as weak (Meerow and Snijman, 2001; Meerow et al., 2002; Meerow and Clayton, 2004). Five hundred

heuristic searches with random addition sequence were implemented for each constraint statement postulated by TreeRot, saving no more than 10 trees per search. A minimum DI=2 was considered to represent good support for a clade (Meerow and Snijman, 2001; Meerow et al., 2002; Meerow and Clayton, 2004).

Two parallel runs were performed in MrBayes, each consisting of four chains, one "cold" and three incrementally heated. Five million Markov chain Monte Carlo (MCMC) generations were run, with convergence diagnostics calculated every 1000th generation for monitoring the stabilization of log likelihood scores. Trees in each chain were sampled every 100th generation. A 50% majority rule consensus tree was generated from the sampled trees after discarding the first 25% (12,500 trees).

2.4. Morphological characters and macroecological parameters

We scored a set of 23 morphological characters (Appendix A) and four ecological parameters (altitude, rainfall seasonality, groundwater availability, and soil rockiness) (Appendix B) for parsimony analyses (morphological only) as described above and/or optimization on gene trees. Derived from live plants, dried specimens at NBG and current literature (Dyer, 1939; Gordon-Gray and Wright, 1969; Nordal, 1979; Reid and Dyer, 1984; Snijman and Van Jaarsveld, 1995; Snijman, 1999, 2001, 2003, 2007), the morphological data were treated as 13 binary state characters and ten unordered multi-state characters. Fire-dependence (character 23) was ascribed to species tightly keyed to fire events and that flower over a relatively wide period (several months) following a fire (Keeley, 1993). It was determined from herbarium records (BOL, NBG, and PRE), and assigned to species which showed short discontinuous flowering times scattered over a period of several months and very rarely in successive years at any particular locality. These were not confused with species occupying dry, fire-free areas with erratic rainfall that also show long gaps between flowering periods. Chromosome morphology was omitted from the analysis due to an absence of data for many species.

Species' distributions were obtained from locality data on herbarium specimens (in BOL, NBG, PRE, and SAM) and these were plotted onto the vegetation map of southern Africa (Mucina and Rutherford, 2006) from which the associated data on altitude and rainfall seasonality were derived. Data on soil rockiness and groundwater availability were derived from field studies and herbarium specimens. Although the macroecological estimates are rough, similar parameters have given valuable insights in other studies (Hardy and Linder, 2005; Linder and Hardy, 2005; Linder et al., 2006). To accommodate ancestral polymorphisms, the ecological parameters (Appendix B) were coded as binary states: presence or absence (Hardy and Linder, 2005; Linder and Hardy, 2005). Both DELTRAN (delayed transformation) and ACCTRAN (accelerated transformation) optimizations, as implemented in MacClade 4.08 (Maddison and Maddison, 2001), were used on one fully resolved tree, following the method of Linder et al. (2006).

Floral morphology was used to hypothesize pollinators by matching sets of floral features with those constituting flower types as recognized by Faegri and Van der Pijl (1979), Johnson and Bond (1994), and Goldblatt and Manning (2006). Although important when inferring pollination syndromes, nectar data are still absent for the genus. We identified four flower types within *Cyrtanthus* and listed their inferred pollinators alongside the species in the cladogram which traces the evolution of macroecological habitats. Where the floral morphology of a species could not be matched with any one of the classical flower types the pollinators were regarded as equivocal. The following sets of characters were sought: *classic butterfly-flowers*: radially symmetrical, vividly coloured, including pure red, nectar guides (if present) simple, rim generally flat, often narrow (not allowing access of a bird's bill) and not much divided, odour weak, nectar well hidden in a tube; *brush-type butterfly-flowers*: as above but stamens long, projecting from a funnel-shaped perianth; *moth-flowers*: pendent or held horizontally, long-tubed with rim absent or bent back, mostly white or drably coloured, strongly perfumed at night, landing surface present in noctuid-type, but absent in sphingid-type; *bird-flowers*: usually hanging, vividly coloured, odourless, deep-tubed, with a hard wall, lip or margin curved back, filaments stiff; *long-proboscid fly-flowers*: perianth white to pink with dark markings towards throat, odourless, with a long cylindrical tube (1.0–1.5 mm diameter).

2.5. Testing for interspecific hybridization

Tests of recombinant signal in the ITS alignment of *Cyrtanthus* were conducted with the program RDP3 (Martin et al., 2005a). RDP3 (Recombination Detection Program version 3) is a Windows 95/98/NT/XP program for detecting and analyzing recombination signals in a set of aligned DNA sequences. In addition to its own RDP method (Martin and Rybicki, 2000), RDP3 implements a Bootscanning method (Salminen et al., 1995; Martin et al., 2005b), the GENECONV method (Padidam et al., 1999), the Maximum Chi-Square method (MaxChi; Maynard Smith, 1992; Posada and Crandall, 2001), the Chimaera method (Posada and Crandall, 2001), the Sister Scanning Method (SiScan; Gibbs et al., 2000), the 3SEQ method (Boni et al., 2007), the Reticulate compatibility matrix method (Jakobsen and Easteal, 1996) and the TOPAL DSS method (McGuire and Wright, 1998, 2000). RDP3 also functions as a Windows interface for other recombination detection and analysis programs including LARD (Likelihood Assisted Recombination Detection; Holmes et al., 1999) and LDHAT (McVean et al., 2002, 2004). Breakpoint polishing and checking for misalignment were options that were invoked, and the matrix was analyzed both with and without the requirement for phylogenetic evidence for recombination signals. All tests were permuted by simulation 1000 times, using SEQ-GEN (Rambaut and Grassly, 1997) parametric simulations in order to generate simulated alignments with approximately the same distribution of polymorphic sites as the actual data matrix. Highest acceptable P value was set to 0.05 with Bonferroni correction applied. The full gamut of tests was run five times.

3. Results and discussion

3.1. Phylogenetic analyses of molecular data

3.1.1. ndhF

The *ndhF* matrix consisted of 2040 characters, 129 of which were variable and 36 were parsimony informative. The heuristic search found 153 equally parsimonious trees that condensed to one (Fig. 1) when branches of minimum length=0 were collapsed. The tree was 147 steps in length, with consistency index (CI)=0.898 (CI with informative characters only=0.712) and retention index (RI)=0.913. Branch lengths are short in the tree, with nine being the largest number of apomorphies (ancestral node of *C. labiatus* and *C. montanus*). The monophyly of *Cyrtanthus* is supported with BP=99%, after which three distinct clades are resolved (Fig. 1), with weak BP. In Clade A (BP<50%), the best supported group (BP=84%) unites a large group of the narrow-leafed species, after a grade of three species is formed with little or no parsimony support. Surprisingly, the two subspecies of *C. mackenii* do not resolve as sister taxa, probably an artefact of the overall short branch lengths (the two subspecies differ by only two base substitutions across the entire *ndhF* alignment). The second clade, B (BP=70%), was largely unresolved internally except for strong to moderately supported sister relationships between *C. carneus–C. herrei* (BP=81%), *C. collinus–C. ventricosus* (BP=83%), and *C. labiatus–C. montanus* (BP=96%). The third weakly supported (BP=53%) clade C contains two well-supported groups that are weakly united (BP=57%), then form a polytomy with *C. eucallus*, *C. galpinii* and *C. obliquus*.

The transversional model with gamma distributed rate heterogeneity (TVM+G) was the best fit for the *ndhF* alignment. The ML tree, with a likelihood score=−3747.7, was very similar to the parsimony topology with a few exceptions (Fig. 1). Clade B was resolved as sister to Clade A with weak (59%) BP. There was an additional sister species relationships resolved in Clade A: *C. brachyscyphus* with *C. mackenii* subsp. *cooperi* (63% BP). In Clade B, *C. leptosiphon* resolved as sister

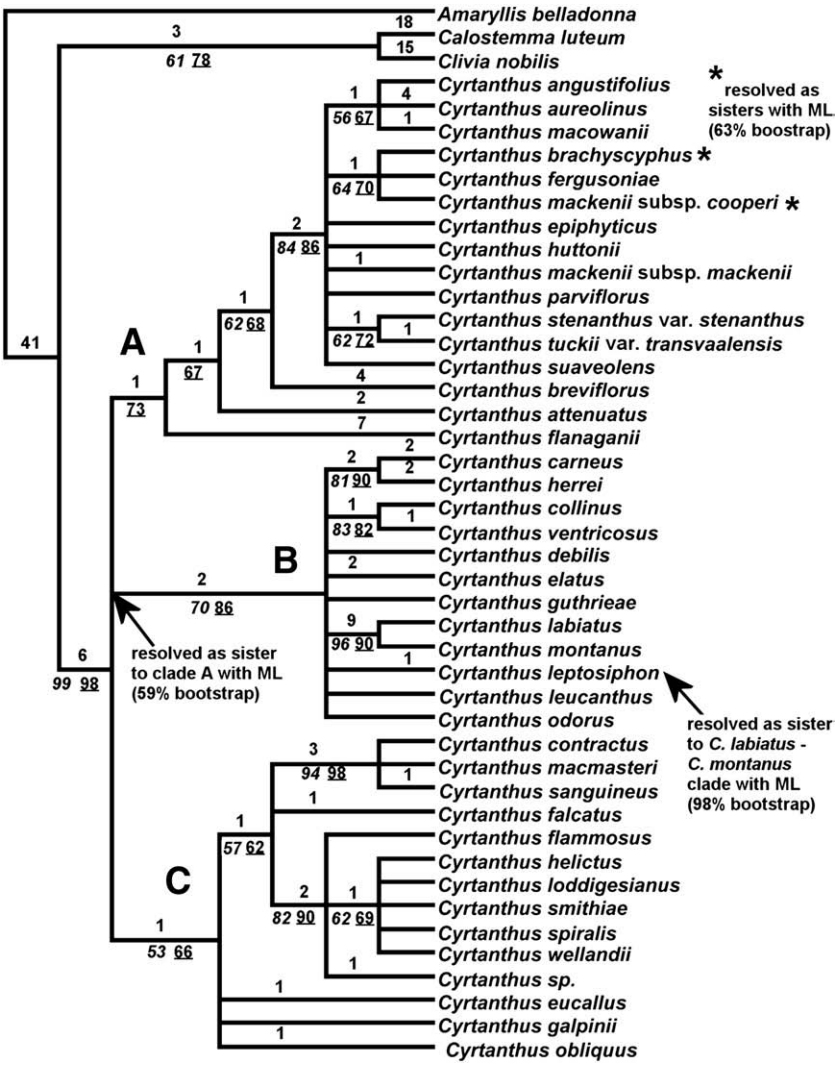

Fig. 1. Single tree found by parsimony analysis of the *ndhF* sequence alignment across 42 *Cyrtanthus* taxa. Numbers above branches are branch lengths. Numbers below branches are parsimony (italic) and ML (underline) bootstrap percentages.

to the *C. labiatus–C. montanus* clade (98% BP). Overall, bootstrap support tended to be higher with ML than parsimony, and several branches received BP > 50% with ML that did not in the parsimony analysis (Fig. 1).

3.1.2. ITS

The ITS matrix had a total of 661 characters, of which 121 were parsimony informative. Fifty-three equally most parsimonious trees were found, which condensed to four when branches of minimum length = 0 were collapsed. The trees were 379 steps long with consistency index (CI) = 0.784 (CI with informative characters only = 0.650) and retention index (RI) = 0.804. *Cyrtanthus* resolves as monophyletic with 100% BP (Fig. 2). Three clades in the genus are resolved, labelled A, B and C (Fig. 2). Clade A has 64% BP, and contains exactly the same taxa as Clade A with *ndhF* (Fig. 1), albeit with greater resolution and some differences in the terminal relationships of several taxa. The subspecies of *C. mackenii* are sisters with 81% BP, while *C. tuckii* var. *transvaalensis* forms a well-supported clade with *C. angustifolius* and *C. aureolinus*, but its sister in the *ndhF* tree (Fig. 1), *C. stenanthus*, is part of a different subclade that is sister to *C. macowanii* (BP = 93%). Clade B with ITS also contains the same species as its equivalent with *ndhF* with one exception: *C. ventricosus* is unresolved between Clades B and C. Clade B has 50% BP with ITS, even though it resolved in all four trees. Clade C with ITS is also similar to that resolved with *ndhF* except *C. falcatus* joins *C. obliquus* and *C. ventricosus* in a polytomy with both Clades B and C in the ITS trees (Fig. 2). Bootstrap support for Clade C is 68%. Two spiral-leafed species, *C. helictus* and *C. smithiae*, are sisters with 97% BP support, and, as in the *ndhF* tree, are included in a subclade with *C. flammosus*, *C. loddigesianus*, and *C. wellandii*. Absent from this grouping, however, is *C. spiralis*, which with *ndhF* formed a weakly supported subclade with the other two spiral-leafed species, *C. loddigesianus* and *C. wellandii* (Fig. 1), and *C.* sp., which instead is sister to *C. sanguineus* with 72% BP (Fig. 2). With *ndhF*, *C. sanguineus* formed a well-supported (94% BP) polytomy with *C. contractus* and *C. macmasteri* (Fig. 1).

Of the available models of molecular evolution, the best model fit to the ITS alignment was the general time reversible with gamma distributed rate heterogeneity (GTR + G). The ML tree log likelihood score was −2944.738. Major differences with the parsimony trees were few. Clade A received 95% BP with ML versus 64% with parsimony. In Clade B, *C. collinus* was sister to *C. debilis–C. elatus* with BP = 70%. *Cyrtanthus ventricosus*, which with parsimony was unresolved between Clades B and C, was sister to Clade B (BP = 67%) with ML. In

Fig. 2. Strict consensus tree of four most equally parsimonious trees found by cladistic analysis of the ITS sequence alignment across 42 *Cyrtanthus* taxa. Numbers below branches are parsimony (italic) and ML (underline) bootstrap percentages.

Clade C, *C. contractus* was sister to the rest of the clade (BP=69%) in the ML topology, and *C. eucallus* and *C. galpinii* were sister at BP=60%.

The null hypothesis that interspecific hybridization had not figured in the evolution of *Cyrtanthus* species was accepted. None of the recombination detection algorithms used by RDP found any sequence evidence for reticulation between any *Cyrtanthus* species.

3.1.3. Combined loci

The ILD resulted in the rejection of the null hypothesis that the *ndhF* and ITS alignments were congruent ($P=0.01$), but in fact, the same main clades are resolved by both topologies (Figs. 1 and 2), with most differences concentrated in the terminal resolutions within each of the three. Partitioned decay indices indicate congruent support for Clades A and B (Fig. 3). The accuracy of the ILD as an arbiter of combinability has declined steadily since Farris et al. (1994, 1995) first recommended a *P* value of 0.05 as the threshold for determining non-combinability. Numerous studies have concluded that *P* values<0.05, and even as low as 0.001, should not preclude data set combination (Sullivan, 1996; Cunningham, 1997a,b; DeSalle and Brower, 1997; Sidall, 1997; Davis et al., 1998; Flynn and Nedbal, 1998; Messenger and McGuire, 1998; Yoder et al., 2001). This along with the main clade congruence between the two trees justified a combined analysis. For ML and Bayesian analyses, a mixed model, retaining each partition's best fit nucleotide substitution model, was applied.

Fifty-three equally most parsimonious trees were found (Fig. 3), which condensed to 20 when branches of minimum length=0 were collapsed. The trees were 544 steps long with consistency index (CI)=0.789 (CI with informative characters only=0.622) and retention index (RI)=0.806. Clades A, B and C were resolved with 84, 75 and 72% BP (DI=3, 2, 1, respectively). *Cyrtanthus falcatus* and *C. obliquus* formed a polytomy with Clade C at 59% BP (DI=1). Clades B and C were sister groups with 65% BP (DI=1). In Clade C, a sister relationship between *C. contractus* and *C. sanguineus* received weak BP=53%; as did a sister relationship of *C. galpinii* to the single well-resolved subclade (BP=53%), unresolved in the strict consensus (Fig. 3).

The ML tree had a log likelihood=−2944.7. Clades A and B were almost identical to their resolution with parsimony. In Clade A, a few subclades received weak bootstrap support with

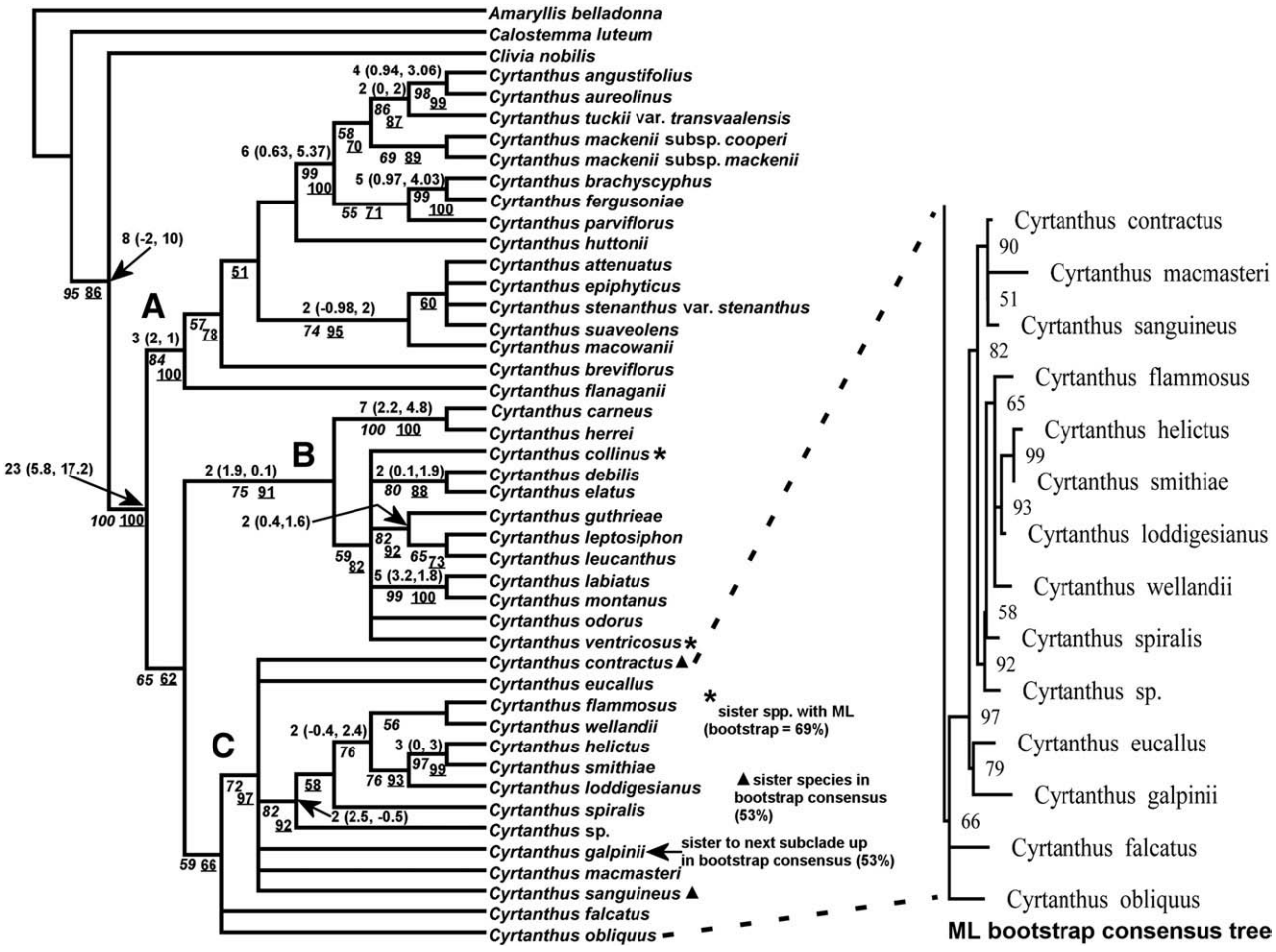

Fig. 3. Strict consensus of 20 most equally parsimonious trees found by cladistic analysis of combined *ndhF* and ITS sequence alignments across 42 *Cyrtanthus* taxa. Numbers above branches are decay indices (if>1); in parentheses are partitioned decay indices (*ndhF*, ITS). Incongruence between the two partitions at a given node is indicated by a negative value for one of the partitioned scores. Numbers below branches are parsimony (italic) and ML (underline) bootstrap percentages.

ML, vs. <50% with parsimony (Fig. 3). In Clade B, *C. collinus* and *C. ventricosus* were sister species (BP=69%). Clade C has the most disparate internal resolution between the two methods of analysis. In the ML tree, *C. galpinii* and *C. eucallus* were sisters at 79% BP. *Cyrtanthus contractus*, *C. macmasteri*, and *C. sanguineus* formed a clade with 90% BP. All of these species were unresolved within Clade C with parsimony.

All stabilization diagnostics indicated that the log likelihood scores in the Bayesian analysis converged before 500,000 iterations, thus our 50% majority consensus (Fig. 4) of trees sampled from the latter 3.75 million generations is a conservative estimate of clade credibility (CC). Clades A, B and C had CC=1, 1, and 0.99, respectively, and the same species resolution as the parsimony and ML trees for A and B. Sister relationship of *C. collinus* and *C. ventricosus*, resolved by ML but not parsimony, received a CC=0.85. There is weak and polytomous subclade resolution (CC=0.55) in Clade B for these two species with *C. debilis–C. elatus*, *C. labiatus–C. montanus* and *C. odorus*. The level of support for Clade C in the Bayesian tree (Fig. 4) is significantly more robust than with either parsimony or ML (Fig. 3). The well-supported ML clade (BP=90%) of *C. contractus*, *C. macmasteri* and *C. sanguineus* in Clade C was also resolved by Bayesian analysis (CC=1.00), as was the sister relationship of *C. eucallus* and *C. galpinii* (CC=0.92). As with the parsimony and ML analyses, *C. falcatus* and *C. obliquus* occupy unresolved positions in Clade C in the Bayesian topology.

3.2. Phylogenetic analyses of morphological data

Heuristic search found 2276 equally most parsimonious trees with the morphological data set. Trees were 126 steps long, with CI=0.278, and RI=0.664. The homoplasy index was a high 0.838. The trees were very poorly resolved (not shown). In the strict consensus of all trees, ten of the 14 species placed in Clade C of the combined gene tree formed a monophyletic group, but with <50% BP. The six morphological synapomorphies at the ancestral node were leaves distinctly narrowed towards the base, perigone tube more-or-less abruptly inflated from near the base into a bell-shaped tube, tube less than twice the length of the segments, flowers with dark stripes leading into the throat, anthers just exserted from the perigone throat, and stigma minutely 3-lobed. Within this clade, *C. helictus*, *C. smithiae* and *C. loddigesianus* formed a subclade with 63% BP, a monophyletic group that was also resolved in the ITS and combined analyses (Figs. 2 and 3). The four morphological

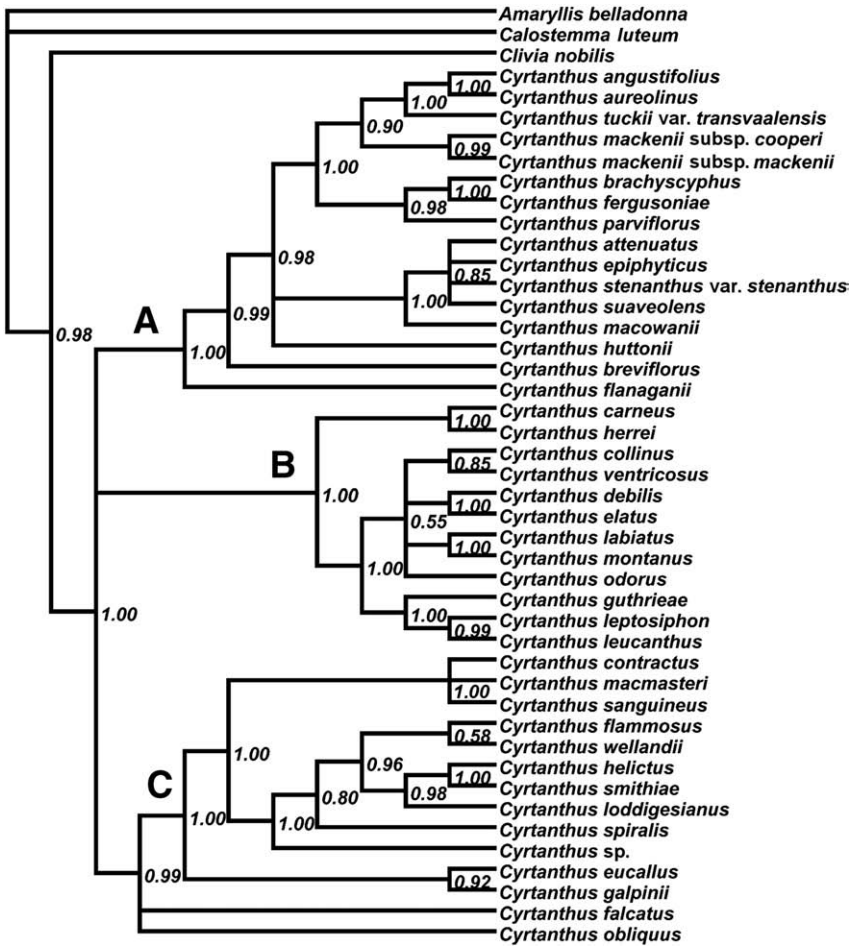

Fig. 4. Fifty percent majority rule consensus tree of 37,500 trees sampled after burn-in from Bayesian analysis of the combined *ndhF* and ITS sequence matrices across 42 *Cyrtanthus* taxa. Numbers at nodes represent clade credibility scores (as proportion of trees in which that clade was resolved).

synapomorphies for this small clade were solitary bulbs; cream-coloured, pale lemon or white flower colour; red, pink or green stripes on the backs of segments and outside of tube; and strongly biseriate stamens.

Combining the morphological data with the sequence matrices yielded 45 trees of length = 775 steps, CI = 0.662 and RI = 0.695 (not shown). Clade A, and most of Clades B and C were resolved as in the combined gene tree, but a fourth clade was formed consisting of *C. carneus* and *C. herrei* (Clade B in Fig. 3) and *C. falcatus* and *C. obliquus* (Clade C in Fig. 3). The two morphological synapomorphies supporting this subclade were clump-forming bulbs, as large as 65–100 mm in diameter at maturity.

We concluded that the morphological data matrix was not very informative from the perspective of phylogenetic analysis, and then traced the evolution of selected floral characters through the optimization of their states on the combined gene tree using MacClade v4.08 (Maddison and Maddison, 2001).

3.3. Biogeography and macroecology

The three major lineages generated in our analyses show different biogeographic affinities although their distributions overlap to some extent for a few extant species.

Clade A encompasses taxa concentrated in southern Africa's Grassland Biome together with a few outlying species in the Savanna Biome to the east and north, the Indian Ocean Coastal Belt Biome in the extreme east and the Fynbos Biome in the south (Fig. 5a). As such, it falls mostly in the Afrotemperate Phytogeographical Region (Galley et al., 2007), made up of the Afromontane phytochorion (White, 1983) in the north and the Cape Floristic Region in the south. Apart from *C. tuckii* var. *transvaalensis*, which is found in grassland of the Highveld in the northern parts of South Africa, most other extanct species in this 'Afrotemperate' lineage (*C. attenuatus*, *C. epiphyticus*, *C. huttonii*, *C. mackenii* subsp. *cooperi*, *C. macmasteri*, *C. macowanii*, *C. stenanthus* var. *stenanthus*, *C. suaveolens*), including the earliest diverging species (*C. flanaganii*), are endemic to or occur in the present-day southeastern African temperate grasslands. The few species that presently occur outside this grassland area may therefore best be interpreted as instances of secondary range expansion. These are: *C. angustifolius*, *C. fergusoniae* and *C. aureolinus* in the Cape Region and *C. mackenii* subsp. *mackenii* and *C. brachyscyphus* that occupy drainage lines on the subtropical Indian Ocean Coastal Belt, the youngest biome in South Africa (Mucina and Rutherford, 2006). Likewise, southern Africa appears to be the source area for the widespread *C. breviflorus* which extends northwards in a series of disjunct populations along mountain corridors to East Africa and Angola.

The extant species of Clade B are almost entirely restricted to the Fynbos and Succulent Karoo Biomes which constitute the Greater Cape Region (Jürgens, 1991; Born et al., 2007), hereafter referred to as 'the Cape' (Fig. 5b, d). Two species, *C. labiatus* and *C. montanus* from the Baviaanskloof Mountains, Eastern Cape, are found at the interface of the Fynbos and Albany Thicket Biomes. One species, the Richtersveld endemic *C. herrei*, occurs in the semi-arid Succulent Karoo. *Cyrtanthus herrei* is one of several other known outliers found outside the core Cape Region (sensu Goldblatt and Manning, 2002) in the Richtersveld: *Amaryllis paradisicola* is sister to the Western Cape *A. belladonna* (Snijman and Williamson, 1998), *Moraea gariepensis*, is sister to the Cape-centered *M. ramosissima* (Goldblatt et al., 2002), and *Trachyandra adamsonii* (Manning, 1990) and *Walleria gracilis* have disjunct populations in the Richtersveld and Western Cape (Manning et al., 2001). The disjunction between *C. herrei* and its sister species, the southwestern Cape *C. carneus*, therefore suggests vicariance, probably corresponding with phases of range expansion and contraction during climatic oscillations of the Pleistocene (Midgley et al., 2001, 2005).

Most species in the 'Cape' lineage are presently concentrated on the summer-dry, southwest coast forelands (west of 21°E), with only half this number in the Fynbos of the nonseasonal rainfall Eastern Cape (Fig. 5b). This pattern corresponds with species richness in the Cape flora as a whole (Cowling and Lombard, 2002; Linder, 2003). Most of the species (*C. carneus*, *C. elatus*, *C. guthrieae*, *C. labiatus*, *C. leptosiphon*, *C. leucanthus*, *C. montanus*, and *C. odorus*) are range-restricted habitat specialists confined to specific vegetation types and/or soils. Only two species (*C. collinus* and *C. ventricosus*) are widespread, occupying the same soils and aspect in habitats on the continuous Cape Fold mountain ranges. *Cyrtanthus collinus* is found on the coastal and inland mountains of the southern Cape and *C. ventricosus* extends from the Cape Peninsula into the Eastern Cape.

Most species of Clade C are located in the eastern lowlands and midlands of southern Africa (Fig. 5c, d), where they are concentrated in the subtropical biomes: Albany Thicket and Savanna (Cowling et al., 2005; Mucina and Rutherford, 2006). Nested in this lineage are *C. flammosus* and *C. spiralis* which are narrowly endemic to the Albany Thicket Biome and confined to the Savanna Biome are *C. eucallus* and *C. galpinii* in the Lowveld. Other species straddle the Albany Thicket and Savanna Biomes: the Eastern Cape *C. helictus* and, extending northwards from the Albany region through South Africa, Zimbabwe, western Mozambique and East Africa into Sudan, is *C. sanguineus* (Nordal, 1979). Only one species, *C. contractus*, extends beyond the Savanna Biome into the Sub-Escarpment and Highveld grasslands (Fig. 5d), which suggests that this is a later radiation into the temperate grasslands of the high-lying interior. Two representatives extend beyond the Albany Thicket Biome into the eastern Fynbos Biome: *C. obliquus*, adapted to nutrient-poor soils, occupies rocky habitats in east–west tending valleys, whereas the narrowly distributed *C. loddigesianus* extends south westwards along fixed coastal dunes that were probably exposed after the retreat of the sea level to its current position, an event dated at two to three million years ago (Siesser and Dingle, 1981). Only *C. wellandii*, in the Humansdorp district, is strictly endemic to the Fynbos Biome. Despite several species belonging to the semi-arid Albany Thicket Biome being arid-adapted, only *C. smithiae* extends beyond this Biome along valleys into the southern parts of the Nama Karoo. Essentially, the transitional nature of the distribution ranges evident in this lineage reflects the complexity

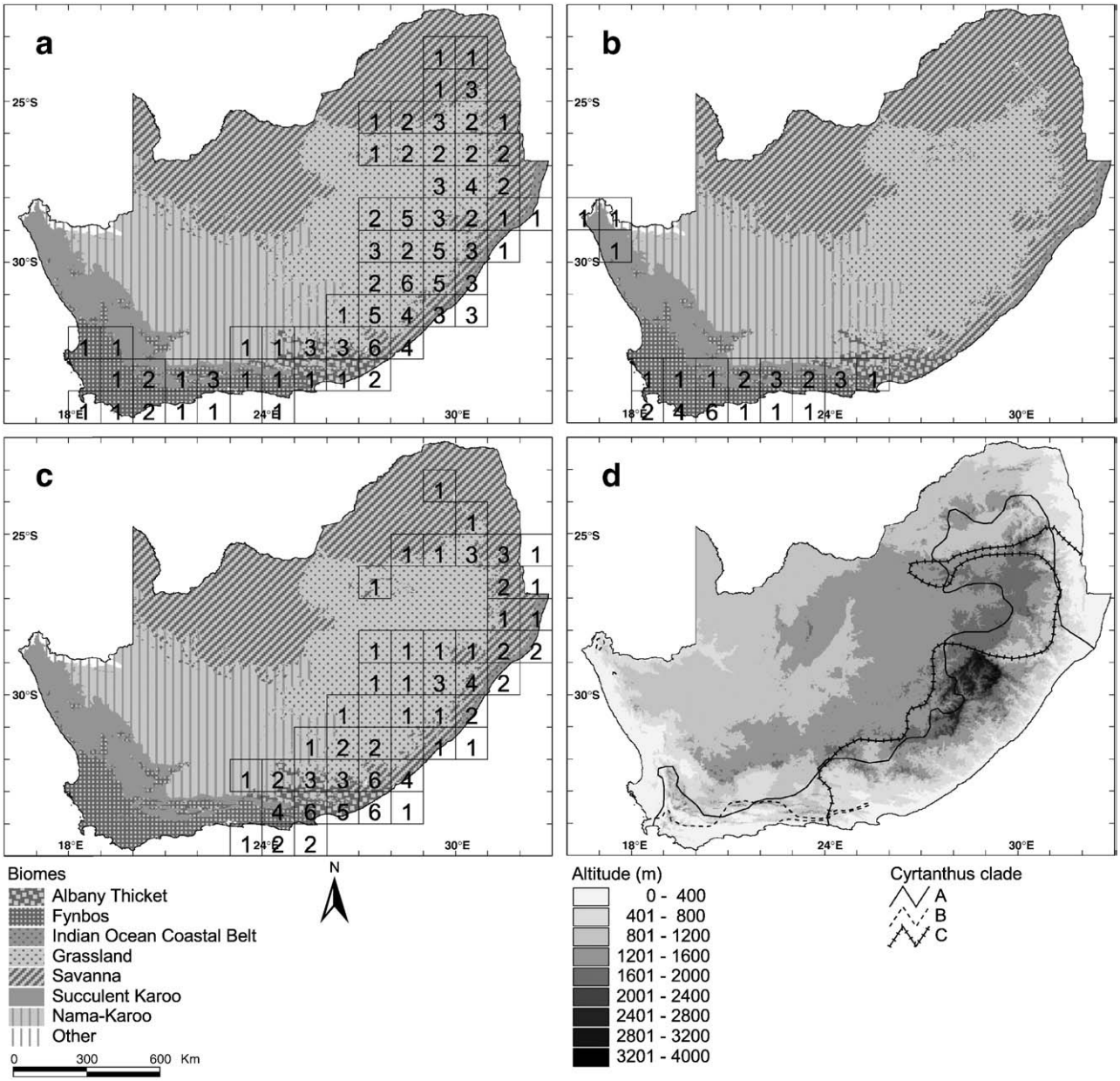

Fig. 5. *Cyrtanthus* in southern Africa: (a–c) species richness per one geographical degree square for Clade A, Clade B and Clade C respectively within the major biomes; (d) distribution outlines for Clades A, B and C relative to altitude above sea level.

of the Albany Thicket Biome itself which is hypothesized to have incorporated many lineages from adjacent vegetation formations while it was contracting during climatic deterioration in the past (Cowling et al., 2005).

Habitats in the Albany Thicket Biome are fire-free, but most in the Fynbos, Grassland and Savanna Biomes are fire-prone (Mucina and Rutherford, 2006) and the latter are home to the fire-specialists (*C. angustifolius*, *C. breviflorus*, *C. contractus*, *C. tuckii* var. *transvaalensis* and *C. ventricosus*), the most widespread extant species of the genus.

Although the 'Cape' lineage of *Cyrtanthus* displays geographical patterns similar to many of the taxa regarded as 'core' Cape groups as defined by Linder (2003), it is nevertheless nested within extra-Cape clades that are essentially Afrotemperate and subtropical in nature, suggesting that the evolutionary history of *Cyrtanthus* is strongly tied to the subcontinent's southeastern region. Incomplete sampling at the base of a tree is known to affect ancestral analyses (Bremer, 1992), but our sample of species from the Cape lacks only *C. ochroleucus* (Herb.) Burch. ex Steud., previously treated as a close ally of *C. mackenii* and *C. aureolinus* (Snijman, 2007) but considered here to be a close relative of *C. odorus*, *C. staadensis* Schönland which is believed to be closely allied to *C. contractus* but often confused with *C. collinus* (Snijman, 1999), and the poorly known *C. inaequalis* O'Brien. So their omission is unlikely to alter the tree topology.

To date, few attempts have been made to reconstruct the evolution of lineages with broad distributions in southern Africa

(Linder et al., 2006). Major ecological factors that have been implicated in the lineage diversification of *Zaluzianskya*, Brassicaceae, *Melianthus* and Arctotidinae within southern Africa are past shifts in annual precipitation and rainfall seasonality, and changes in altitude (Archibald et al., 2005; Mummenhoff et al., 2005; Linder et al., 2006; McKenzie and Barker, 2008). Our reconstruction of the ecological evolution of *Cyrtanthus* (Fig. 6) suggests that stony, well-drained uplands (300–1500 m) were the ancestral niche. The earliest rainfall seasonality of the lineage remains ambiguous but subsequent divergence is clearly evident among the major lineages. The occupation of summer-wet conditions and seasonally moist sites is concentrated in the 'Afrotemperate' clade (Clade A) and appears to have been accompanied by a contemporaneous shift (with ACCTRAN) or later shift (with DELTRAN) to highlands (1500–3000 m), followed, however, by the exploitation by several species of upland and lowland habitats and nonseasonal rainfall conditions. The only instance of adaptation to strictly summer-dry conditions is in the 'Cape' clade (Clade B). ACCTRAN infers that adaptation to a summer-dry climate is the most recent state. DELTRAN, however, resolved summer-dry habitats at the base of the clade with a reversal to non-seasonal habitats. This suggests that lineages which receive not just winter rainfall but summer moisture from clouds formed on the south coast forelands off the warm Agulhas current as well, are recently derived, as has been suggested for *Thamnochortus* (Hardy and Linder, 2005). Our use of summer-dry conditions encompasses climates on mountains in the northwest as well as on coastal lowlands in the southwest, hence summer drought may be a compound ecological attribute. The former habitat may represent long-term, relatively stable inland refugia, whereas the southwestern Cape lowlands may have provided

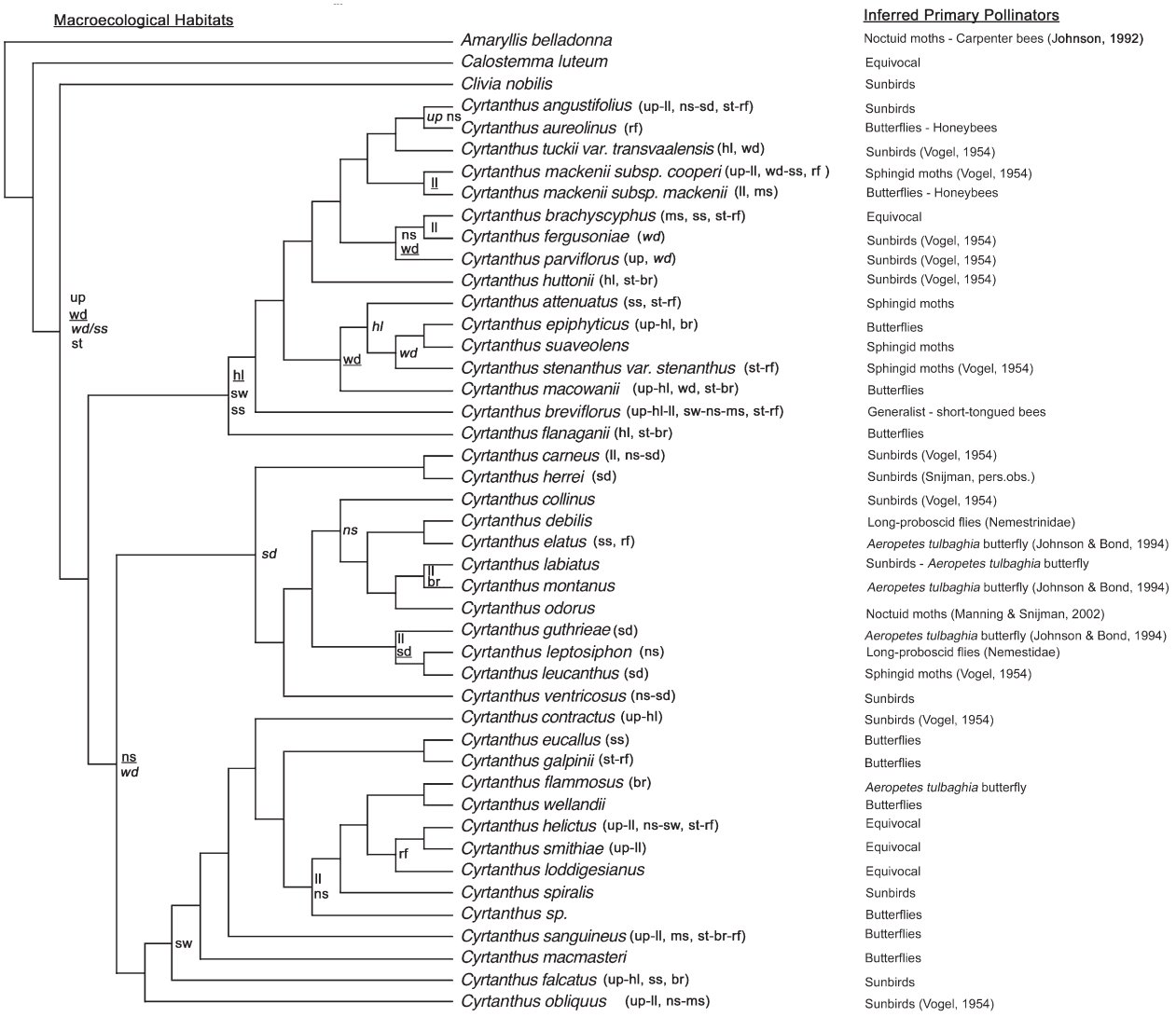

Fig. 6. Macroecological attributes mapped onto a fully resolved consensus tree of *Cyrtanthus* and a list of inferred primary pollinators. Abbreviations: ACCTRAN underlined, DELTRAN in italics, non-italics and not underlined reflect agreement between ACCTRAN and DELTRAN, altitude (up = uplands, hl = highlands, and ll = lowlands), rainfall (ns = nonseasonal, ms = moderately seasonal, sw = summer-wet/winter-dry, and sd = summer-dry/winter-wet), groundwater (wd = well-drained and ss = streams or seepages), soil rockiness (st = stony, bd = bedrock, and rf = rock-free).

less stable environments in which the winter-rainfall patterns were more transient during sea-level changes of the Quaternary (Siesser and Dingle, 1981; Deacon et al., 1992), particularly when the seashore on the Agulhas Bank was almost 200 km distant from the present one (Dingle and Rogers, 1972). Apart from this ambiguity, the late occupation of lowland coastal sites is inferred from all three lineages. This is thought to be a general feature of the Cape flora (Linder and Hardy, 2005) and our reconstruction for *Cyrtanthus* suggests this is true of extra-Cape areas as well, and may correspond with the extensive fluvial action and dissection of the forelands below the eastern escarpment from the end of the Pliocene (Partridge and Maud, 1987). Most recently, species evolved through adaptation to rock-free sandy soils on the coast or to newly exposed, steep rock-faces, mostly in river valleys (notably the vegetatively reproducing *C. labiatus*, *C. montanus* and *C. flammosus* in the Baviaanskloof, Eastern Cape). Given the absence of reliable external calibration data with which to estimate the age of the lineages, we cannot as yet place these events in a temporal framework and cannot link lineage diversification within *Cyrtanthus* with important historical factors in the region, particularly the upwelling of the cold Benguela Current in the middle Miocene and the erosion cycles of the Miocene and Pliocene following the uplift in southern Africa's eastern region (Cowling et al., 2009).

We conclude, nevertheless, that of the external selective forces that have influenced the evolution of *Cyrtanthus*, the most obvious are geomorphic developments which led to habitats being partitioned according to differences in altitudes and levels of rockiness, and climate changes which effected shifts in rainfall seasonality and influenced the likelihood of fire. As such, *Cyrtanthus*, primed by its geophytic habit, became increasingly adapted to a disturbance regime which facilitated the fragmentation of populations and encouraged speciation in newly available habitat types.

3.4. Floral morphology, convergence and inferred pollinators

The optimization of several floral characters on the strict consensus tree shows that the deflection of the style from the central position is independently derived in all three major lineages (Fig. 7a). A downwardly deflected style has evolved at least once in both the 'Cape' and 'subtropical' clades, and its placement against the upper tepals has only arisen in the 'Cape' and 'Afrotemperate' clades (Fig. 7a). This latter state is associated with the most strongly bilabiate flower type in the genus and is limited to two distantly related Cape species: *C. fergusoniae* (in the 'Afrotemperate' clade) and *C. labiatus* (in the 'Cape' clade). In these, the lower tepals are distinctly reflexed and the upper three tepals form a hood over the stamens and style. Stamens that are included in the perigone tube are concentrated in the 'Afrotemperate' clade, with only one occurrence (*C. wellandii*) in the 'subtropical' clade (Fig. 7b). Floral scent, which is known in only seven species in our study, has evolved at least six times and is represented in each of the primary clades, most often in the 'Afrotemperate' lineage (Fig. 7c). Moreover, a shift from red to some other floral colour has occurred in all the major lineages and is most pronounced in the 'Afrotemperate' lineage (Fig. 7d), as has a shift in the shape of the mature floral tube from straight to curved (Fig. 8a), and in the presentation of the flower from spreading to pendulous. The erect floral habit (Fig. 8b) is confined to the 'Cape' clade and is shared by at least seven species.

In effect, *Cyrtanthus* shows greater evolutionary lability in floral symmetry and colour than any other genus of Amaryllidaceae. Changes in floral symmetry are known in the Amaryllidaceae as a whole, which led Meerow et al. (1999) to suggest that it is likely under simple genetic control. Zygomorphy (with one plane of symmetry both vertically and face on) in *Cyrtanthus*, however, is associated with both sternotribic and nototribic flowers. Interestingly, nototribic flowers are unknown in any other African genus of Amaryllidaceae and both states have not yet been noted in any other single genus of the family. Zygomorphy in *Cyrtanthus* is associated with the flowers being directed sideways through the curvature of the floral tube. This predisposes the stamens and style to further bending which changes their orientation in the mouth. From the cladogram we infer that the evolution of pronounced zygomorphy in *Cyrtanthus* progressed after a slight widening and marked lengthening of the perigone tube in the ancestral lineage (Fig. 8c, d). At first zygomorphy appears to have been weak but, probably through co-evolution with increasingly specialized insect visitors, sternotribic or nototribic flowers evolved. Increased zygomorphy in *Cyrtanthus* also appears to have been associated with the evolution of enhanced visual patterns, such as dark bands and stripes in the floral throat that are particularly characteristic of the 'subtropical' lineage and to a lesser extent the 'Cape' lineage. Fundamental to the elaboration of all these characteristics, however, was the increase in the length of the floral tube which shows recurrent development of increased floral robustness, sufficient to accommodate visits by birds.

The cladogram also points to the shift from weak zygomorphy to floral actinomorphy which is considered to be the derived state in Amaryllidaceae (Snijman, 1992). In the Cape, the large showy red flowers of *C. guthrieae*, *C. elatus* and *C. montanus* retain several plesiomorphic characters: a straight floral tube, no floral markings, a truncate stigma, and equally long stamens exserted from the throat, but importantly the position of the flower switched from the plesiomorphic horizontal state to an apomorphic suberect one (Fig. 8b). The erect floral position is associated with increased size and showiness, leading to perfect actinomorphy in *C. guthrieae* and *C. montanus*. The only other change towards near perfect radial floral symmetry is evident in species from the moist upland grasslands of southern Africa: in the funnel-form flowers of *C. breviflorus* and in the secondarily narrowed and much elongated perigone tube of *C. attenuatus* and *C. stenanthus*. Unlike the Cape species, the stamens of *C. attenuatus* and *C. stenanthus* form two distinct whorls, the derived state for *Cyrtanthus* (Fig. 7b).

Published observations on pollinators and inferred pollination systems based on flower types reveal that the tubular floral form in *Cyrtanthus* favours sunbirds and insects with long mouthparts, mainly butterflies and moths, and less often long-proboscid flies (Fig. 6). Moreover, with similar floral forms found repeatedly among the primary clades, many distantly related species appear to share the same class of pollinators,

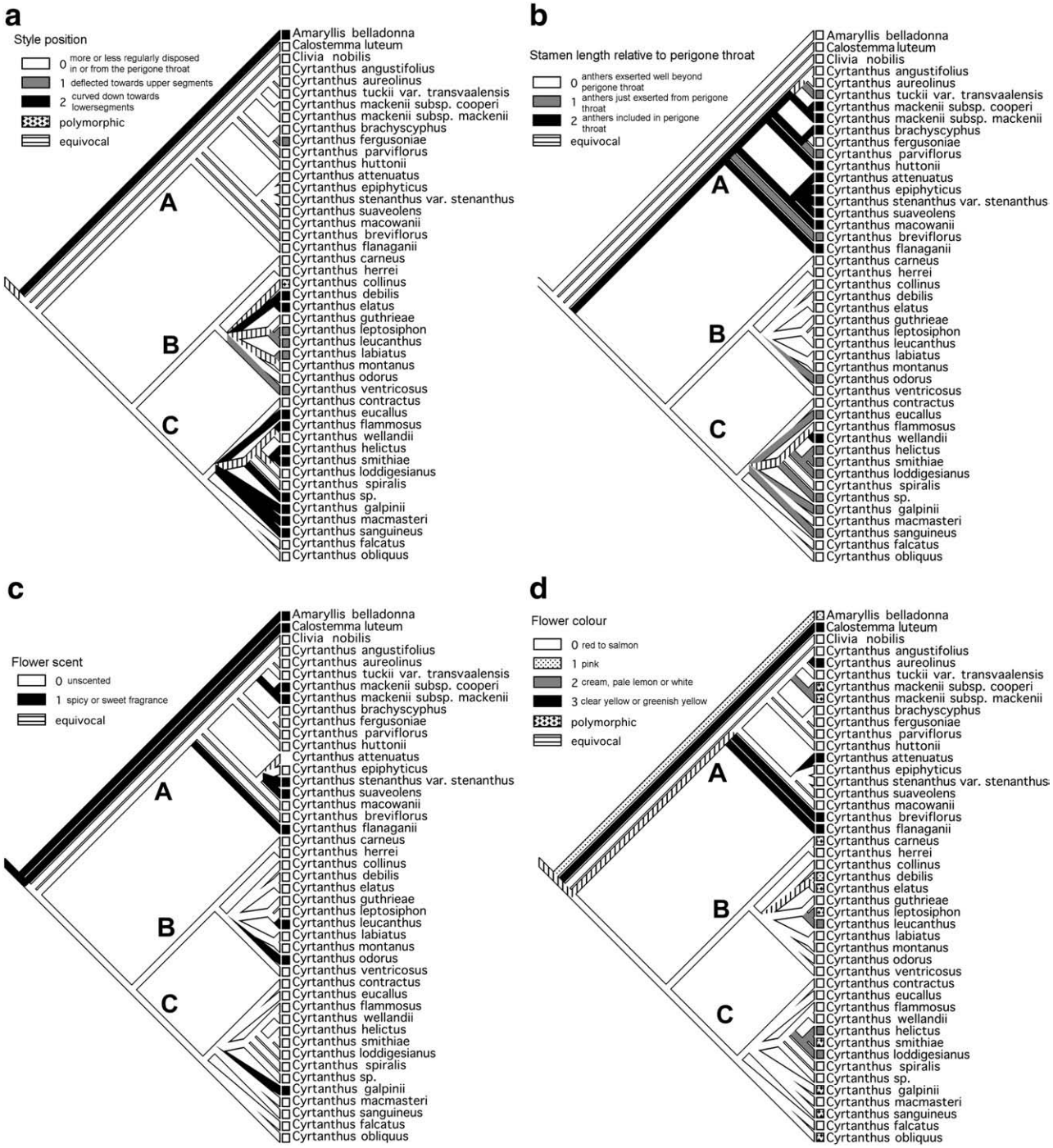

Fig. 7. Fitch optimization of floral characters onto the strict consensus tree of 20 equally most parsimonious trees found by analysis of combined *ndhF* and ITS sequence matrices of *Cyrtanthus*. Clades A, B and C represent the 'Afrotemperate', 'Cape' and 'subtropical' clades respectively. (a) style more or less regularly disposed in or from perigone tube (0), style deflected towards the upper segments (1), style curved down towards the lower segments (2); (b) anthers well-exserted beyond perigone throat (0), just exserted from throat (1), included in throat (2); (c) flowers not scented (0), spicy or sweetly scented (1); (d) flowers red to salmon (0), pink (1), cream-coloured, pale lemon or white (2), clear yellow or greenish yellow (3).

particularly of sunbirds and butterflies. A few floral forms are, nevertheless, unique to a particular clade which possibly indicates that the selective environment for each lineage is slightly different.

Ornithophily involving sunbirds (family Nectariniidae) is inferred to have evolved one or more times in each major lineage: in the tubular, red-flowered *C. angustifolius*, *C. collinus* (Vogel, 1954), *C. contractus* (Vogel, 1954), *C. huttonii* (Vogel, 1954), *C. parviflorus* (Vogel, 1954), *C. spiralis* and *C. tuckii* (Vogel, 1954); in the tubular, red- to dusky pink-flowered *C. carneus* (Vogel, 1954) and *C. ventricosus*; in the bilabiate, red-flowered *C. fergusoniae*

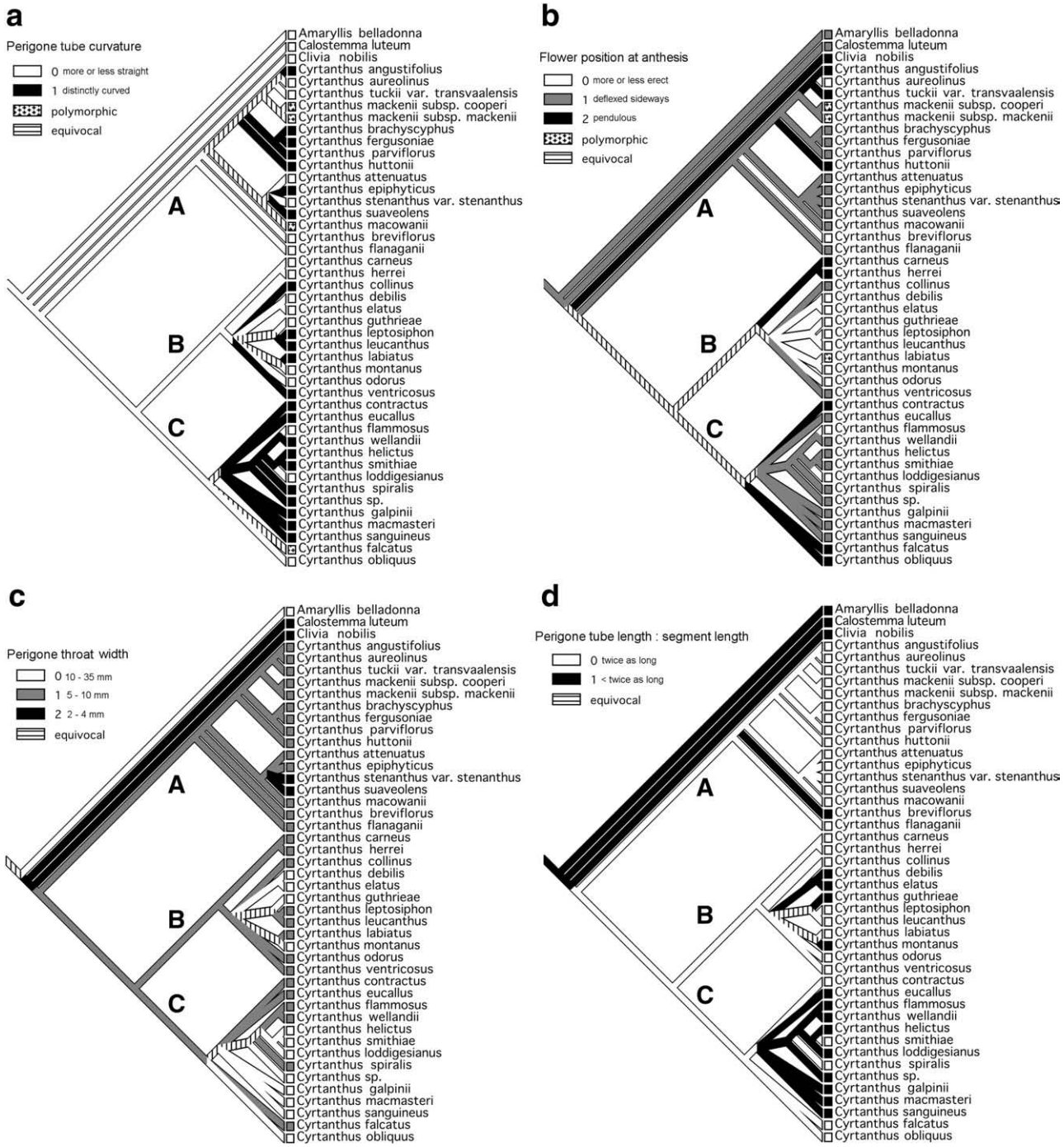

Fig. 8. Fitch optimization of floral characters onto the strict consensus tree of 20 equally most parsimonious trees found by analysis of combined *ndhF* and ITS sequence matrices of *Cyrtanthus*. Clades A, B and C represent the 'Afrotemperate', 'Cape' and 'subtropical' clades respectively. (a) perigone tube more or less straight (0), distinctly curved (1); (b) flowers more or less erect at anthesis (0), deflexed sideways (1), pendulous (2); (c) perigone throat 10–35 mm wide (0), 5–10 mm wide (1), 2–4 mm wide (2); (d) perigone tube at least twice as long as segments (0), less than twice the length of segments (1).

(Vogel, 1954) and *C. labiatus*; as well as in the orange and red-flowered *C. herrei* (Snijman, pers obs.) and *C. obliquus* (Vogel, 1954). *C. herrei* with its specialized laterally winged filaments has the firmest flowers of the putative ornithophilous species, and *C. falcatus*, which is also considered to be bird-pollinated, has a unique sharply recurved scape topped with pendulous, reddish flowers.

The high-altitude grassland species (*C. attenuatus*, *C. stenanthus* var. *stenanthus* and *C. suaveolens*) in the 'Afrotemperate' clade have the longest and most slender floral tubes, the shortest tepals, and the most concealed stamens in the entire genus, which together with scent and pale or dull colours (sweet in *C. stenanthus* var. *stenanthus* and clove-scented in *C. suaveolens*), is typical of sphingid moth pollination (Vogel, 1954). The concentration of

putative sphingophilous *Cyrtanthus* species in the grasslands of southern Africa, particularly in seasonally moist habitats, follows the pattern for all southern African Amaryllidaceae (Manning and Snijman, 2002) as well as for *Satyrium* (Van der Niet, 2006). Floral scent and narrow perigone tubes have also evolved independently in two species of the 'Cape' clade. The cream-flowered *C. leucanthus* is inferred to be sphingophilous (Vogel, 1954), whereas the dull red-flowered *C. odorus* is regarded as noctuid moth-pollinated (Manning and Snijman, 2002).

Many other tubular-flowered species belonging to the 'Afrotemperate' clade are inferred to be butterfly-pollinated. They have a medium-sized floral throat (5–10 mm wide) with flowers arranged in a somewhat compact, one-sided cluster on which butterflies can settle and manoeuvre. Exclusive to four species of this primary clade is clear yellow floral colouring (Fig. 7d) that has evolved in parallel four times. Other specializations associated with this colour shift are: more or less upright flowers in both *C. aureolinus* and *C. breviflorus*, minute quantities or the absence of nectar in *C. aureolinus*, and a short, funnel-shaped floral tube, with the lower narrow part never exceeding 5 mm in length, in *C. breviflorus*. Native honey bees (*Apis mellifera*) have been seen collecting pollen from the exserted anthers of *C. aureolinus* (Snijman, 2007), thus in general they may act as secondary pollinators of the long-tubed species having anthers and stigma located at the entrance to the floral tube. *Cyrtanthus breviflorus* in particular, which provides easiest access to the base of the floral tube, appears to be most suited to a variety of generalist insects, including short-tongued bees.

Pollination by the large satyrid butterfly *A. tulbaghia* has been reported for several red-flowered members of the 'Cape' clade (*C. guthrieae*, *C. elatus* and *C. montanus*) that have features which match the brush-type syndrome (Johnson and Bond, 1994). Also in the 'Cape' clade are *C. leptosiphon* and *C. debilis* which have distinctive long, slender tubes and cream to pale pink flowers respectively with dark pink stripes or spots as nectar guides that constitute the syndrome for long-proboscid fly pollination (family Nemestrinidae) (Snijman, 1999). As yet these are the only *Cyrtanthus* species believed to belong to the guild of late summer- and autumn-flowering species pollinated by *Prosoeca ganglbauri* and *P. longipennis* (Goldblatt and Manning, 2000, 2006).

Compared with the other two primary clades, the 'subtropical' lineage (Clade C) reflects relatively low levels of variation in floral form and the absence of floral scent (except in *C. galpinii*). Similar, low levels of floral variation, as reflected in different pollination syndromes, have been reported for *Satyrium* in the coastal bush and woodlands found mostly at low altitudes in eastern southern Africa (Van der Niet, 2006), which in general may reflect reduced levels of pollination syndromes within this area. But apart from the ornithophilous *C. contractus*, little has been published on the pollination of this group of species. The *A. tulbaghia* butterfly is probably the primary pollinator of *C. flammosus* from the Baviaanskloof Mountains, Eastern Cape, but swallowtail butterflies (family Papilionidae) may be implicated in the pollination of the large, red-flowered species from the more northerly subtropical areas. As yet nothing is known about visitors to the unscented, pale flowers of *C. helictus*, *C. smithiae* and *C. loddigesianus* which occupy habitats with erratic rainfall in the southeastern Cape.

A comparison of sister species relationships in our study group shows that the 'Cape' clade reflects the highest level of diversification in inferred pollination syndromes (Fig. 6). The '*Aeropetes*' syndrome is known to combine elements of both classical bird and butterfly syndromes (Johnson and Bond, 1994) and this is reflected in the close association between the sunbird- and *A. tulbaghia* butterfly-pollination syndromes in sister species of the Cape *Cyrtanthus*. In addition, the pollination syndromes of the *A. tulbaghia* butterfly, other Lepidoptera and long-proboscid flies (*Prosoeca* spp.) appear to be closely linked. There is no evidence from any of the sister species pairs, however, to suggest that taxa with bird-pollinated flowers share a most recent common ancestor with those characterized by long-proboscid fly-flowers, thus morphological changes of this kind may be phylogenetically constrained in *Cyrtanthus*.

4. Taxonomic conclusion

The difficulty of determining the species relationships in *Cyrtanthus* (Dyer, 1939; Reid and Dyer, 1984) is clearly a result of the evolutionary nature of the genus: one in which different lineages are inferred to have repeatedly converged on various suites of morphological characteristics, apparently as an adaptation to shared classes of pollinators. In particular, the similarity in the floral characters of the putatively bird-pollinated species that are segregated between different clades is actually greater than that among the members within each of the main lineages. Not surprisingly, there is strong incongruence between the evolutionary relationships indicated in our study and the current taxonomic treatment of *Cyrtanthus* (Dyer, 1939; Reid and Dyer, 1984), in which the species with bird-adapted flowers are arranged together, as are the large, red-flowered, butterfly-pollinated species. Despite greater clarity on the phylogeny of *Cyrtanthus*, the repeated reversals in floral morphology, however, complicate the morphological characterization of the primary clades.

Despite the lack of support by Strydom et al. (2007) for the value of chromosome morphology in identifying groups of species in *Cyrtanthus*, Ising's (1970) groupings based on karyotypes correspond well with those generated by plastid *ndhF* and nrDNA ITS sequences. Ising's groups one and two ((*C. brachyscyphus*, *C. parviflorus*, *C. tuckii*, *C. mackenii*, *C. breviflorus*, *C. huttonii*) and (*C. epiphyticus* and *C. stenanthus*)), along with other unsampled species (*C. erubescens* Killick, *C. rotundilobus* N.E.Br., *C. obrienii* Baker) correspond with members of the 'Afrotemperate' clade. Group three (*C. contractus*, *C. eucallus*, *C. sanguineus* and *C. nutans* R.A.Dyer), which was erroneously called a form of *C. breviflorus*, falls within the 'subtropical' clade. Group four (*C. obliquus*, *C. herrei*, *C. falcatus* and *C. flanaganii*), however, comprises elements from all three primary clades. Sharing a large habit and somewhat similar vegetative morphology, these four species resolve as sisters to the rest of the taxa in each of the primary clades to which they belong. *Cyrtanthus elatus* and

C. staadensis, the only Cape species in Ising's study, were not allied to any of the above groups.

Further analysis of additional gene sequence data may strengthen support for the major clades resolved in this study, but since we consider the recognition of formal subgenera based on the present results to be premature, we propose the division of *Cyrtanthus* into three informal groupings, mainly as a framework for future taxonomic studies in the genus (Appendix C). Many of the species not sampled are doubtfully distinct from those included in this study, but some are undoubtedly distinct and their placement must be regarded as tentative.

Lastly, the phylogeny presented here should facilitate further critical studies on the range of pollination systems that appear to characterize the genus and thereby contribute to an understanding of the underlying processes involved in speciation.

Acknowledgements

The authors acknowledge Graham Duncan, John Manning, and Cameron and Rhoda McMaster for providing the study material, Les Powrie and Michelle Smith for the assistance with the figures, and Colin Paterson-Jones for the help in the field. This research was partially supported by the National Science Foundation (USA) grant DEB 0129179 to AWM.

Appendix A

Morphological data matrix for *Cyrtanthus*. Characters and character states are: 1—Plants evergreen (0), deciduous (1). 2—Bulbs solitary (0), clumped (1). 3—Bulb 65–100 mm across (0), less than 60 mm across at maturity (1). 4—Leaves strap-shaped and parallel-sided (0), distinctly narrowed towards base (1), linear (2). 5—Leaf straight (0), twisted (1), spiralled (2). 6—Spathe valves 2 (0), 3 or 4 (1). 7—Flowers more or less erect at anthesis (0), deflexed sideways (1), pendulous (2). 8—Perigone tube not abruptly inflated from near the base, varying from tubular to somewhat trumpet-shaped (0), more or less abruptly inflated from near the base into a bell-shaped tube (1). 9—Perigone tube more or less straight (0), distinctly curved (1). 10—Perigone tube at least twice as long as segments (0), less than twice the length of segments (1). 11—Perigone throat 10–35 mm wide (0), 5–10 mm wide (1), 2–4 mm wide (2). 12—Perigone segments all connivent and more or less straight (0), all spreading more or less evenly (1); upper connivent and more or less hooded with lower spreading, thus somewhat bilabiate (2), all recurved and rolled back when fully mature (3). 13—Perigone segments longer than broad (0), approximately as long as broad (1). 14—Flowers red to salmon (0), pink (1), cream-coloured, pale lemon or white (2), clear yellow or greenish yellow (3). 15—Flowers not striped (0), with dark stripes leading into the throat (1), red-, pink- or green-striped on backs of segments and outside of the tube (2). 16—Flowers not scented (0), spicy or sweetly scented (1). 17—Anthers well exserted beyond perigone throat (0), just exserted from throat (1), included in throat (2). 18—Stamens weakly biseriate to more or less uniseriate, i.e. outer anthers reaching above the base of inner anthers to both whorls almost perfectly aligned (0), strongly biseriate, i.e. outer anthers falling short of inner anthers (1). 19—Filaments filiform (0), distinctly laterally winged towards base (1). 20—Filaments inserted in the upper half of tube (0), near the base of tube (1). 21—Style more or less regularly disposed in or from perigone tube (0), style deflected towards the upper segments (1), style curved down towards the lower segments (2). 22—Stigma minutely 3-lobed, at most 0.25 mm long (0), tricuspidate with short narrow branches, 0.5–2.0 mm long (1), trifid with long slender branches, 2.5–5.0 mm long, rarely up to 10.0 mm (2). 23–Flowering not keyed to fire (0), following fire at any time during a five-month fire season and in the absence of leaves or with leaves just emerging (1).

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Amaryllis belladonna	1	0	0	1	0	0	1	0	0	1	0	1	0	1	0	1	0	1	0	0	2	0	0
Calostemma luteum	1	0	1	0	0	1	1	0	0	1	2	1	0	3	0	1	0	0	na	0	0	0	0
Clivia nobilis	0	na	na	0	0	1	2	0	0	1	2	0	0	0	0	0	0	0	0	0	0	1	0
Cyrtanthus angustifolius	0/1	1	1	0	0	0	2	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1	1
C. attenuatus	1	0	1	1	0	0	1	0	0	0	1	1	1	3	0	?	2	1	0	0	0	1	0
C. aureolinus	0/1	0	1	0	1	0	0	0	0	0	1	1	0	3	0	0	0	0	0	0	0	1	1
C. brachyscyphus	0	1	1	1	0	0	1	0/1	1	0	1	0	0	0	0	0	2	1	0	0	0	1	0
C. breviflorus	0/1	0	1	0/2	0	0	0	0	0	1	1	1	0	3	0	0	1	0	0	0	0	1	1
C. carneus	0	1	0	0	1	1	2	0	0	0	1	1	0	0/1	0	0	0	0	1	0	0	0	0
C. collinus	0/1	0	1	1	0	0	1	0/1	1	0	1	1	0	0	0	0	0	0	0	0	0/1	0	0
C. contractus	1	0	1	1	0	0	2	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1
C. debilis	1	0	1	2	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	1	2	1	1
C. elatus	0	1	1	0	0	0/1	0	0	0	1	0	1	0	0/1	0	0	0	0	0	0	2	0	0
C. epiphyticus	0	0	1	1	0	0	1	0	1	0	1	1	0	0	0	0	2	1	0	0	0	1	0
C. eucallus	0/1	1	1	1	0	0	1	1	1	1	1	1	0	0	1	0	1	0	0	0	2	1	0
C. falcatus	1	1	0	0	0	1	2	0	0/1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
C. fergusoniae	1	0	1	2	0	0	1	0	1	0	1	2	0	0	0	0	0	0	0	0	1	0	0
C. flammosus	0	1	1	1	0	0	0	1	0	1	1	1	0	0	1	0	0	0	0	0	2	2	0
C. flanaganii	1	1	1	0	0	0	1	0	0	0	1	1	0	3	0	1	2	1	0	0	0	1	0
C. galpinii	1	0	1	1	0	0	1	1	1	1	0	1	0	0/1	1	1	1	0	0	0	2	2	0
C. guthrieae	1	0	1	2	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0

Appendix A (*continued*)

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
C. helictus	1	0	1	2	2	0	1	1	1	1	0	1	0	2	2	0	1	1	0	0	2	2	0
C. herrei	0	1	0	0	1	1	2	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0
C. huttonii	1	1	1	0	0	0	2	0	1	0	1	1	0	0	0	0	2	0	0	0	0	1	0
C. labiatus	0	1	1	0	0	0	0/1	0	1	0	1	2	0	0	0	0	0	0	0	0	1	0	0
C. leptosiphon	1	1	1		0	0	0	0	1	0	1	2	0	1/2	2	0	0	1	0	0	1	0	0
C. leucanthus	1	0	1	2	0	0	0	0	1	0	1	1	0	2	0	1	0	1	0	0	1	0	1
C. loddigesianus	1	0	1	1	0	0	0	1	0	1	0	1	0	2	2	0	1	1	0	0	0	2	0
C. mackenii subsp. *cooperi*	1	0	1	0	0	0	0/1	0	0/1	0	1	3	1	1/2	0	1	2	1	0	0	0	1	0
C. mackenii subsp. *mackenii*	0	1	1	0	0	0	1/2	0	0/1	0	1	3	1	2/3	0	1	2	1	0	0	0	1	0
C. macmasteri	1	0	1	1	0	0	1	1	1	1	0	1	0	0	1	0	0	0	0	0	2	1	0
C. macowanii	1	0	1	1	0	0	1	0	0/1	0	1	1/3	1	0	0	0	2	1	0	0	0	1	0
C. montanus	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
C. obliquus	0	1	0	0	1	1	2	0	0	0	0	0	0	0/3	0	0	0	0	0	1	0	0	0
C. odorus	1	0	1	2	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	1	1
C. parviflorus	1	0	1	2	0	0	1	0	1	0	1	1	0	0	0	0	1	1	0	0	0	1	0
C. sanguineus subsp. *sanguineus*	0	1	1	1	0	0	1	1	1	1	0	1	0	0/1	1	0	1	0	0	0	2	2	0
C. sp. (Tait s.n.)	1	1	0	1	0	0	1	1	1	1	0	1	0	0	1	0	1	0	0	0	2	2	0
C. smithiae	1	0	1	1	2	0	1	1	1	0	0	1	0	1/2	2	0	1	1	0	0	2	2	0
C. spiralis	0/1	1	1	0	2	0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	0
C. stenanthus var. *stenanthus*	1	0	1	2	0	0	1	0	0	0	2	1	0	0	0	1	2	1	0	0	0	1	0
C. suaveolens	1	0	1	1	0	0	1	0	1	0	2	3	1	0	0	1	2	1	0	0	0	1	0
C. tuckii var. *transvaalensis*	1	0	1	1	0	0	2	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	1
C. ventricosus	1	0	1	1	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	1	1	0	1
C. wellandii	1	0	1	1	0	0	1	1	1	1	1	1	0	0	1	0	2	1	0	0	0	0	0

Appendix B

Macroecological data matrix for *Cyrtanthus* optimized on combined *ndhF* and ITS gene trees. Characters and character states are: 1—Lowlands, 0–300 m: absent (0), present (1). 2—Uplands, 300–1500 m: absent (0), present (1). 3—Highlands, 1500–3000 m: absent (0), present (1). 4—Nonseasonal, wet in any month of year: absent (0), present (1). 5—Moderately seasonal, summer-wet/winter-moist: absent (0), present (1). 6—Strongly seasonal, summer-wet/winter-dry: absent (0), present (1). 7—Seasonal, summer-dry/winter-wet: absent (0), present (1). 8—Well-drained: absent (0), present (1). 9—Seepages and stream-sides: absent (0), present (1). 10—Rock-free: absent (0), present (1). 11—Stony: absent (0), present in (1). 12—Bedrock: absent (0), present in (1).

Taxon	1	2	3	4	5	6	7	8	9	10	11	12
Amaryllis belladonna	1	1	0	0	0	0	1	1	1	0	1	0
Calostemma luteum	1	0	0	0	1	0	0	0	1	1	0	0
Clivia nobilis	1	1	0	0	1	0	0	1	0	1	1	0
Cyrtanthus angustifolius	1	1	0	1	0	0	1	0	1	1	1	0
C. attenuatus	0	0	1	0	0	1	0	0	1	1	1	0
C. aureolinus	0	1	0	1	0	0	0	0	1	1	0	0
C. brachyscyphus	1	0	0	0	1	0	0	0	1	1	1	0
C. breviflorus	1	1	1	0	1	1	0	0	1	1	1	0
C. carneus	1	0	0	1	0	0	1	1	0	0	1	0
C. collinus	0	1	0	1	0	0	0	1	0	0	1	0
C. contractus	0	1	1	0	0	1	0	1	0	0	1	0
C. debilis	0	1	0	1	0	0	0	1	0	0	1	0
C. elatus	0	1	0	1	0	0	0	0	1	1	0	0
C. epiphyticus	0	1	1	0	0	1	0	1	0	0	0	1
C. eucallus	0	1	0	0	0	1	0	0	1	0	1	0
C. falcatus	0	1	1	0	0	1	0	0	1	0	0	1
C. fergusoniae	1	0	0	1	0	0	0	1	0	0	1	0
C. flammosus	1	0	0	1	0	0	0	1	0	0	0	1
C. flanaganii	0	0	1	0	0	1	0	0	1	0	1	1
C. galpinii	0	1	0	0	0	1	0	1	0	1	1	0
C. guthrieae	1	0	0	0	0	0	1	1	0	0	1	0
C. helictus	1	1	0	1	0	1	0	1	0	1	1	0
C. herrei	0	1	0	0	0	0	1	1	0	0	0	1
C. huttonii	0	0	1	0	0	1	0	0	1	0	1	1
C. labiatus	1	0	0	1	0	0	0	1	0	0	0	1
C. leptosiphon	1	0	0	1	0	0	0	1	0	0	1	0
C. leucanthus	1	0	0	0	0	0	1	1	0	0	1	0

(continued on next page)

Appendix B (*continued*)

Taxon	1	2	3	4	5	6	7	8	9	10	11	12
C. loddigesianus	1	0	0	1	0	0	0	1	0	1	0	0
C. mackenii subsp *cooperi*	1	1	0	0	0	1	0	1	1	1	0	0
C. mackenii subsp. *mackenii*	1	0	0	0	1	0	0	0	1	0	1	0
C. macmasteri	0	1	0	0	0	1	0	1	0	0	1	0
C. macowanii	0	1	1	0	0	1	0	1	0	0	1	1
C. montanus	1	0	0	1	0	0	0	1	0	0	0	1
C. obliquus	1	1	0	1	1	0	0	1	0	0	1	0
C. odorus	0	1	0	1	0	0	0	1	0	0	1	0
C. parviflorus	0	1	0	1	0	0	0	1	0	0	1	0
C. sanguineus subsp. *sanguineus*	1	1	0	0	1	0	0	1	0	1	1	1
C. smithiae	1	1	0	1	0	0	0	1	0	1	0	0
C. sp (Tait s.n.)	1	0	0	1	0	0	0	1	0	0	1	0
C. spiralis	1	0	0	1	0	0	0	1	0	0	1	0
C. stenanthus var. *stenanthus*	0	0	1	0	0	1	0	1	0	1	1	0
C. suaveolens	0	0	1	0	0	1	0	1	0	0	1	0
C. tuckii var. *transvaalensis*	0	0	1	0	0	1	0	1	0	0	1	0
C. ventricosus	0	1	0	1	0	0	1	1	0	0	1	0
C. wellandii	1	0	0	1	0	0	0	1	0	0	1	0

Appendix C

Proposed informal groups within the genus *Cyrtanthus* based on the results of the analysis of combined *ndhF* and ITS sequence alignments shown in Figs. 3 and 4. An asterisk indicates taxa not included in the phylogenetic analyses. In the absence of known synapomorphies or strong morphological markers for the groups the placement of some unsampled taxa remains tentative.

A.1. Gastronema group

Leaves straight or spiralled, rarely twisted. *Spathe valves* 2, rarely 3 or 4. *Flowers* deflexed sideways, rarely pendulous or suberect, red, pink, cream-coloured, pale lemon or white, mostly with dark stripes leading into throat or running along backs of segments; tube more or less abruptly inflated from near base into a bell-shaped tube above, less to more than twice as long as segments; throat mostly broad (up to 35 mm); segments more or less evenly spreading, longer than broad. *Stamens* weakly to strongly biseriate; anthers mostly exserted, rarely included in the tube. *Style* more or less evenly disposed within tube or curved downwards to lower segments; stigma minutely 3-lobed to deeply divided into 3 branches up to 5 mm long.

17 species, mostly in southern Africa, with one species extending northwards into Sudan. Found in Albany Thicket, Savanna, Fynbos, Indian Ocean Coastal Belt and Grassland Biomes, in a variety of habitats.

C. obliquus (L.f.) Aiton
C. falcatus R.A.Dyer
C. contractus N.E.Br.
∗*C. nutans* R.A.Dyer
C. eucallus R.A.Dyer
C. galpinii Baker
∗*C. thorncroftii* C.H.Wright
C. macmasteri Snijman
C. sanguineus (Lindl.) Walp. subsp. *sanguineus*
∗*C. sanguineus* subsp. *ballyi* Nordal
∗*C. sanguineus* subsp. *minor* Nordal
∗*C. sanguineus* subsp. *salmonoides* (Bally & Carter) Nordal
∗*C. sanguineus* subsp. *wakefieldii* (Sealy) Nordal
∗*C. staadensis* Schönland
C. spiralis Burch. ex Ker Gawl.
C. flammosus Snijman & Van Jaarsv.
C. wellandii Snijman
C. loddigesianus (Herb.) R.A.Dyer
C. helictus Lehm.
∗*C. clavatus* (L'Hér.) R.A.Dyer
C. smithiae Watt ex Harv.

A.2. Monella group

Leaves straight, infrequently twisted. *Spathe valves* 2, rarely 3 or 4. *Flowers* deflexed sideways or erect, rarely pendulous, red, pink, cream-coloured or pale lemon, rarely with dark stripes in throat or on backs of segments; tube narrow or widening gradually to throat, at least twice as long as segments or seldom as long; throat approximately 5–10 mm wide; segments evenly spreading or upper three connivent and more or less hooded, longer than broad. *Stamens* weakly to strongly biseriate; anthers well exserted from throat. *Style* more or less evenly disposed within tube or deflected towards upper segments; stigma minutely tricuspidate or with 3 short branches up to 2 mm long.

14 species, nearly all endemic to the Greater Cape Floristic Region, southern Africa. Most common in the Fynbos Biome, with one species in the Succulent Karoo Biome, and two species located between the Fynbos and Albany Thicket Biomes. Often in stony or rocky habitats.

C. carneus Lindl.
C. herrei (F.M.Leight.) R.A.Dyer
C. collinus Ker Gawl.
C. odorus Ker Gawl.
∗*C. ochroleucus* (Herb.) Burch. ex Steud.
C. ventricosus Willd.
C. guthrieae L.Bolus
C. leptosiphon Snijman
C. leucanthus Schltr.
C. debilis Snijman
C. elatus (Jacq.) Traub
C. labiatus R.A.Dyer
C. montanus R.A.Dyer
∗*C. inaequalis* O'Brien

A.3. Cyrtanthus group

Leaves straight, rarely twisted. *Spathe valves* 2. *Flowers* deflexed sideways, rarely pendulous or suberect, red, pink, cream-coloured or pale lemon, white or clear yellow, without contrasting stripes; tube narrow or widening gradually to throat, less to more than twice as long as segments; throat 2–10 mm wide; segments evenly spreading to rolled back, rarely all connivent, as long as or longer than broad. *Stamens* weakly to strongly biseriate; anthers included in tube to shortly or rarely well exserted from throat. *Style* regularly disposed in tube, only rarely deflexed towards upper segments; stigma with 3 branches 0.5–2.0 mm long, rarely shorter.

24 species, mostly southern African with a few reaching East Africa and Angola. Commonly found in the Grassland Biome, less so in Savanna, Indian Ocean Coastal Belt and Fynbos Biomes, often favouring seasonally moist habitats.

C. flanaganii Baker
C. breviflorus Harv.
∗*C. bicolor* R.A.Dyer
∗*C. brachysiphon* Hilliard & B.L.Burtt
∗*C. erubescens* Killick
C. macowanii Baker
∗*C. rotundilobus* N.E.Br.
∗*C. obrienii* Baker
C. suaveolens Schönland
C. stenanthus Baker var. *stenanthus*
∗*C. stenanthus* var. *major* R.A.Dyer
C. epiphyticus J.M.Wood
C. attenuatus R.A.Dyer
C. huttonii Baker
C. parviflorus Baker
C. fergusoniae L.Bolus
C. brachyscyphus Baker
∗*C. flavus* P.E.Barnes
C. mackenii Hook.f. subsp. *mackenii*
C. mackenii subsp. *cooperi* (Baker) Snijman

∗*C. tuckii* Baker var. *tuckii*
C. tuckii var. *transvaalensis* I.Verd.
∗*C. tuckii* var. *viridilobus* I.Verd.
∗*C. junodii* P.Beauv.
C. aureolinus Snijman
C. angustifolius (L.f.) Aiton
∗*C. rhodesianus* Rendle
∗*C. welwitschii* Hiern ex Baker

A.4. Insufficiently known taxa

∗*C. rhododactylus* Stapf (no authentic specimens are known)
∗*C. striatus* Herb. (probably conspecific with *C. angustifolius*)

References

Akaike, H., 1973. Information Theory and an Extension of the Maximum Likelihood Principle. In: Petrov, B.N., Csaki, F. (Eds.), Second International Symposium on Information Theory. Akademia Kiado, Budapest, pp. 267–281.

Archibald, J.K., Mort, M.E., Wolfe, A.D., 2005. Phylogenetic relationships within *Zaluzianskya* (Scrophulariaceae s.s., tribe Manuleeae): classification based on DNA sequences from multiple genomes and implications for character evolution and biogeography. Systematic Botany 30, 196–215.

Baker, J.G., 1888. Handbook of the Amaryllideae. George Bell, London.

Baker, J.G., 1896. Flora Capensis 6, 2. L. Reeve and Co., Ashford, Kent.

Boni, M.F., Posada, D., Feldman, M.W., 2007. An exact nonparametric method for inferring mosaic structure in sequence triplets. Genetics 176, 1035–1047.

Born, J., Linder, H.P., Desmet, P., 2007. The Greater Cape Floristic Region. Journal of Biogeography 34, 147–162.

Bremer, K., 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42, 198–213.

Bremer, K., 1992. Ancestral area: a cladistic reinterpretation of the center of origin concept. Systematic Biology 41, 436–445.

Brummitt, R.K., 2001. World Geographical Scheme for Recording Plant Distributions, International Working Group on Taxonomic Databases for Plant Sciences (TDWC) Edition 2. Hunt Institute for Botanical Documentation, Pittsburgh.

Brummitt, R.K., Powell, C.E., 1992. Authors of Plant Names. Royal Botanic Gardens, Kew.

Cowling, R.M., Lombard, A.T., 2002. Heterogeneity, speciation/extinction history and climate: explaining regional plant diversity patterns in the Cape Floristic Region. Diversity and Distributions 8, 163–179.

Cowling, R.M., Procheş, Ş., Vlok, J.H.J., 2005. On the origin of southern African subtropical thicket vegetation. South African Journal of Botany 71, 1–23.

Cowling, R.M., Procheş, Ş., Partridge, T.C., 2009. Explaining the uniqueness of the Cape flora: incorporating geomorphic evolution as a factor for explaining its diversification. Molecular Phylogenetics and Evolution 51, 64–74.

Cunningham, C.W., 1997a. Is congruence between data partitions a reliable predictor of phylogenetic accuracy? Empirically testing an iterative procedure for choosing among phylogenetic methods. Systematic Biology 46, 464–478.

Cunningham, C.W., 1997b. Can three incongruence tests predict when data should be combined? Molecular Biology and Evolution 14, 733–740.

Dahlgren, R.M.T., Clifford, H.T., Yeo, P.F., 1985. The Families of the Monocotyledons. Springer, Berlin.

Davis, J.I., Simmons, M.P., Stevenson, D.W., Wendel, J.F., 1998. Data decisiveness, data quality, and incongruence in phylogenetic analysis: an example from the monocotyledons using mitochondrial *atp* A sequences. Systematic Biology 47, 282–310.

Deacon, H.J., Jury, M.R., Ellis, F., 1992. Selective Regime and Time. In: Cowling, R.M. (Ed.), The Ecology of Fynbos: Nutrients, Fire and Diversity. Oxford University Press, Cape Town, p. 6-2.

Desalle, R., Brower, V.Z., 1997. Process partitions, congruence, and the independence of characters: inferring relationships among closely related Hawaiian *Drosophila* from multiple gene regions. Systematic Biology 46, 751–764.

Dingle, R.V., Rogers, J., 1972. Pleistocene palaeogeography of the Agulhas Bank. Transactions of the Royal Society of South Africa 40, 155–165.

Douzery, J.P., Pridgeon, A.M., Kores, P., Kurzweil, H., Linder, H.P., Chase, M.W., 1999. Molecular phylogenetics of Diseae (Orchidaceae): a contribution from nuclear ribosomal ITS sequences. American Journal of Botany 86, 887–899.

Dyer, R.A., 1939. Description, Classification and Phylogeny. A Review of the Genus *Cyrtanthus*. Herbertia 6, 65–103 (published 1940).

Faegri, K., Van der Pijl, L., 1979. The Principles of Pollination Ecology. Pergamon Press, Oxford.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1995. Constructing a significance test for incongruence. Systematic Biology 44, 570–572.

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

Fitch, W.M., 1971. Toward defining the course of evolution: minimum change for a specific tree topology. Systematic Zoology 20, 406–416.

Flynn, J.J., Nedbal, M.A., 1998. Phylogeny of the Carnivora (Mammalia): congruence vs. incompatibility among multiple data sets. Molecular Phylogenetics and Evolution 9, 414–426.

Galley, C., Bytebier, B., Bellstedt, D.U., Linder, H.P., 2007. The Cape element in the Afrotemperate Flora: from Cape to Cairo? Proceedings of the Royal Society, London. B, vol. 274, pp. 535–543.

Gibbs, M.J., Armstrong, J.S., Gibbs, A.J., 2000. Sister-Scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. Bioinformatics 16, 573–582.

Goldblatt, P., Manning, J.C., 1998. Gladiolus in southern Africa. Fernwood Press, Cape Town.

Goldblatt, P., Manning, J.C., 1999. The long-proboscid fly pollination system in *Gladiolus* (Iridaceae). Annals of the Missouri Botanical Garden 86, 758–774.

Goldblatt, P., Manning, J.C., 2000. The long-proboscid fly pollination system in southern Africa. Annals of the Missouri Botanical Garden 87, 146–170.

Goldblatt, P., Manning, J.C., 2002. Plant diversity in the Cape Region of southern Africa. Annals of the Missouri Botanical Garden 89, 281–302.

Goldblatt, P., Manning, J.C., 2006. Radiation of pollination systems in the Iridaceae of sub-Saharan Africa. Annals of Botany 97, 317–344.

Goldblatt, P., Manning, J.C., 2007. A revision of the southern African genus *Babiana*, Iridaceae: Crocoideae. Strelitzia 18, 1–98.

Goldblatt, P., Manning, J.C., Bernhardt, P., 1995. Pollination biology of *Lapeirousia* (Iridaceae) in southern Africa; floral divergence and adaptation for long-tongued fly pollination. Annals of the Missouri Botanical Garden 82, 517–534.

Goldblatt, P., Manning, J.C., Bernhardt, P., 1998. Adaptive radiation of bee-pollinated *Gladiolus* species (Iridaceae) in southern Africa. Annals of the Missouri Botanical Garden 85, 492–517.

Goldblatt, P., Savolainen, V., Porteous, O., Sostaric, I., Powell, M., Reeves, G., Manning, J.C., Barraclough, T.G., Chase, M.W., 2002. Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. Molecular Phylogenetics and Evolution 25, 341–360.

Gordon-Gray, K.D., Wright, F.B., 1969. *Cyrtanthus breviflorus* and *Cyrtanthus luteus* (Amaryllidaceae): observations with particular reference to Natal populations. Journal of South African Botany 35, 35–62.

Graham, S.W., Kohn, J.R., Morton, B.R., Eckenwalder, J.E., Barrett, S.C.H., 1998. Phylogenetic congruence and discordance among one morphological and three molecular data sets from Pontederiaceae. Systematic Biology 47, 545–567.

Hardy, C.R., Linder, H.P., 2005. Intraspecific variability and timing in ancestral ecology reconstruction: a test case from the Cape flora. Systematic Biology 54, 299–316.

Hilliard, O.M., Burtt, B.L., 1986. Notes on some plants of southern Africa chiefly from Natal: XII. Notes from the Royal Botanic Garden Edinburgh 43, 189–228.

Holmes, E.C., Worobey, M., Rambaut, A., 1999. Phylogenetic evidence for recombination in Dengue virus. Molecular Biology and Evolution 16, 405.

Holmgren, P.K., Holmgren, N.H., Barnett, L.C., 1990. Index Herbariorum. Part I: The Herbaria of the World, Eighth Edition. Regnum Vegetabile, vol. 120. New York Botanical Garden, New York.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Ising, G., 1970. Evolution of karyotypes in *Cyrtanthus*. Hereditas 65, 1–28.

Jakobsen, I.B., Easteal, S., 1996. A program for calculating and displaying compatibility matrices as an aid in determining reticulate evolution in molecular sequences. CABIOS 12, 291–295.

Jobb, G., 2008. TREEFINDER version of October 2008. Munich, Germany. Distributed by the author at www.treefinder.de.

Johnson, S.D., Bond, W.J., 1994. Red Flowers and Butterfly Pollination in the Fynbos of South Africa. In: Arianoutsou, M., Groves, R.H. (Eds.), Plant–Animal Interactions in Mediterranean-type Ecosystems. Kluwer Academic Publishers, Netherlands, pp. 137–148.

Johnson, S.D., Linder, H.P., Steiner, K.E., 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). American Journal of Botany 85, 402–411.

Jürgens, N., 1991. A new approach to the Namib Region I: phytogeographic subdivision. Vegetatio 97, 21–38.

Keeley, J.E., 1993. Smoke-induced flowering in the fire-lily *Cyrtanthus ventricosus*. South African Journal of Botany 59, 638.

Le Maitre, D.C., Midgley, J.J., 1992. Plant Reproductive Ecology. In: Cowling, R.M. (Ed.), The Ecology of Fynbos: Nutrients, Fire and Diversity. Oxford University Press, Cape Town, pp. 135–174.

Linder, H.P., 2003. The radiation of the Cape flora, southern Africa. Biological Reviews of the Cambridge Philosophical Society 78, 597–638.

Linder, H.P., Kurzweil, H., 1999. Orchids of Southern Africa. A.A. Balkema, Rotterdam.

Linder, H.P., Hardy, C.R., 2005. Species Richness in the Cape Flora: A Macroevolutionary and Macroecological Perspective. Plant Species-level Systematics: New Perspectives on Pattern and Process. In: Bakker, F.T., Chatrou, L.W., Gravendeel, B., Pelser, P.B. (Eds.), Regnum Vegetabile, vol. 143. A.R.G. Ganter Verlag, Ruygel, Lichtenstein, pp. 47–73.

Linder, H.P., Dlamini, T., Henning, J., Verboom, G.A., 2006. The evolutionary history of *Melianthus* (Melianthaceae). American Journal of Botany 93, 1052–1064.

Maddison, D.R., Maddison, W.P., 2001. MacClade 4: Analysis of Phylogeny and Character Evolution. Version 4.04. Sinauer Associates, Sunderland, Massachusetts.

Manning, J.C., 1990. A new species of *Trachyandra* section Liriothamnus (Asphodelaceae) from the Richtersveld. South African Journal of Botany 56, 1–5.

Manning, J., Snijman, D., 2002. Hawkmoth pollination in *Crinum variabile* (Amaryllidaceae) and the biogeography of sphingophily in southern African Amaryllidaceae. South African Journal of Botany 68, 212–216.

Manning, J.C., Goldblatt, P., Batten, A., 2001. *Walleria gracilis*. Flowering Plants of Africa 57, 44–47.

Martin, D., Rybicki, E., 2000. RDP: detection of recombination amongst aligned sequences. Bioinformatics 16, 562–563.

Martin, D.P., Williamson, C., Posada, D., 2005a. RDP2: recombination detection and analysis from sequence alignments. Bioinformatics 21, 260–262.

Martin, D.P., Posada, D., Crandall, K.A., Williamson, C., 2005b. A modified bootscan algorithm for automated identification of recombinant sequences and recombination breakpoints. AIDS Research and Human Retroviruses 21, 98–102.

Maynard Smith, J., 1992. Analyzing the mosaic structure of genes. Journal of Molecular Evolution 34, 126129.

McGuire, G., Wright, F., 1998. TOPAL: recombination detection in DNA and protein sequences. Bioinformatics 14, 219–220.

McGuire, G., Wright, F., 2000. TOPAL 2.0: improved detection of mosaic sequences within multiple alignments. Bioinformatics 16, 130–134.

McKenzie, R.J., Barker, N.P., 2008. Radiation of southern African daisies: biogeographic inferences for subtribe Arctotidae (Asteraceae, Arctotideae). Molecular Phylogenetics and Evolution 49, 1–16.

McVean, G., Awadalla, P., Fearnhead, P., 2002. A coalescent-based method for detecting and estimating recombination from gene sequences. Genetics 160, 1231–1241.

McVean, G.A.T., Myers, S.R., Hunt, S., Deloukas, P., Bentley, D.R., Donnelly, P., 2004. The fine-scale structure of recombination rate variation in the human genome. Science 304, 581–584.

Meerow, A., Clayton, J.R., 2004. Generic relationships among the baccate-fruited Amaryllidaceae (tribe Haemantheae) inferred from plastid and nuclear non-coding DNA sequences. Plant Systematics and Evolution 244, 141–155.

Meerow, A., Snijman, D.A., 1998. Amaryllidaceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants, vol. 3. Springer, Berlin, pp. 83–110.

Meerow, A., Snijman, D.A., 2001. Phylogeny of Amaryllidaceae tribe Amaryllideae based on nrDNA ITS sequences and morphology. American Journal of Botany 88, 2321–2330.

Meerow, A., Snijman, D.A., 2006. The Never-ending Story: Multigene Approaches to the Phylogeny of Amaryllidaceae. In: Columbus, J.T., Friar, E.A., Porter, J.M., Prince, L.M., Simpson, M.G. (Eds.), Monocots: Comparative Biology and Evolution (excluding Poales). Aliso, vol. 22. Rancho Santa Ana Botanic Garden, pp. 355–366.

Meerow, A.W., Fay, M.F., Guy, C.L., Li, Q.-B., Zaman, F.Q., Chase, M.W., 1999. Systematics of Amaryllidaceae based on cladistic analysis of plastid *rbc*L and *trn*L-F sequence data. American Journal of Botany 86, 1325–1345.

Meerow, A.W., Guy, C.L., Li, Q.-B., Yang, S.-Y., 2000. Phylogeny of the American Amaryllidaceae based on nrDNA ITS sequences. Systematic Botany 25, 708–726.

Meerow, A.W., Guy, C.L., Li, Q.-B., Clayton, J.R., 2002. Phylogeny of the tribe Hymenocallideae (Amaryllidaceae) based on morphology and molecular characters. Annals of the Missouri Botanical Garden 89, 400–413.

Messenger, S.L., Mcguire, J.A., 1998. Morphology, molecules, and the phylogenetics of cetaceans. Systematic Biology 47, 90–124.

Midgley, G.F., Hannah, L., Roberts, R., MacDonald, D.J., Allsopp, J., 2001. Have Pleistocene climatic cycles influenced species richness patterns in the Greater Cape Mediterranean Region? Journal of Mediterranean Ecology 2, 137–144.

Midgley, G.F., Reeves, G., Klak, C., 2005. Late Tertiary and Quaternary Climate Change and Centres of Endemism in the Southern African Flora. Phylogeny and Conservation. In: Purvis, A., Gittleman, J.L., Brooks, T. (Eds.), Conservation Biology, vol. 8. Cambridge University Press, Cambridge, pp. 230–242.

Mucina, L., Rutherford, M.C. (Eds.), 2006. The vegetation of South Africa, Lesotho and Swaziland. Strelitzia, vol. 19. South African National Biodiversity Institute, Pretoria.

Müller-Doblies, D., Müller-Doblies, U., 1996. Tribes and subtribes and some species combinations in Amaryllidaceae J. St.-Hil. emend R. Dahlgren & al. 1985. Feddes Repertorium S.c., 1-9.

Mummenhoff, K., Al-Shehbaz, I.A., Bakker, F.T., Linder, H.P., Mühlhausen, A., 2005. Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape flora of southern Africa. Annals of the Missouri Botanical Garden 92, 400–424.

Nordal, I., 1979. Revision of the genus *Cyrtanthus* (Amaryllidaceae) in East Africa. Norwegian Journal of Botany 26, 183–192.

Olmstead, R.G., Sweere, J.A., 1994. Combining data in phylogenetic systematics—an empirical approach using 3 molecular data sets in the Solanaceae. Systematic Biology 43, 467–481.

Padidam, M., Sawyer, S., Fauquet, C.M., 1999. Possible emergence of new geminiviruses by frequent recombination. Virology 265, 218–225.

Partridge, T.C., Maud, R.R., 1987. Geomorphic evolution of southern Africa since the Mesozoic. South African Journal of Geology 90, 179–208.

Pires, J.C., Sytsma, K.J., 2002. A phylogenetic evaluation of a biosystematic framework: *Brodiaea* and related petaloid monocots (Themidaceae). American Journal of Botany 89, 1342–1359.

Posada, D., Crandall, K.A., 2001. Evaluation of methods for detecting recombination from DNA sequences: computer simulations. Proceedings of the National Academy of Sciences of the United States of America 98, 13757–13762.

Rambaut, A., Grassly, N.C., 1997. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. Computational Applied Bioscience 13, 235–238.

Reid, C., Dyer, R.A., 1984. A Review of the Southern African Species of *Cyrtanthus*. Botanical Research Institute, Pretoria and American Plant Life Society La Jolla, California.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Salminen, M.O., Carr, J.K., Burke, D.S., McCutchan, F.E., 1995. Identification of breakpoints in intergenotypic recombinants of HIV type 1 by Bootscanning. AIDS Research and Human Retroviruses 11, 1423–1425.

Shono, H., 2000. Efficiency of the finite correction of Akaike's Information Criteria. Fisheries Science 66, 608–610.

Sidall, M.E., 1997. Prior agreement: arbitration or arbitrary? Systematic Biology 46, 765–769.

Siesser, W.G., Dingle, R.V., 1981. Tertiary sea-level movements around southern Africa. Journal of Geology 89, 83–96.

Snijman, D.A., 1992. Systematic studies in the tribe Amaryllideae (Amaryllidaceae). PhD Dissertation, University of Cape Town, South Africa.

Snijman, D.A., 1999. New species and notes on *Cyrtanthus* in the southern Cape, South Africa. Bothalia 29, 258–263.

Snijman, D.A., 2001. A new species of *Cyrtanthus* (Cyrtantheae) from the southern Cape, South Africa. Bothalia 31, 31–34.

Snijman, D.A., 2003. A new *Cyrtanthus* species (Amaryllidaceae: Cyrtantheae) endemic to the Albany Centre, Eastern Cape, South Africa. Bothalia 33, 145–147.

Snijman, D.A., 2007. Notes on new and misunderstood taxa of *Cyrtanthus* (Amaryllidaceae: Cyrtantheae) from the Western Cape, Eastern Cape and KwaZulu-Natal, South Africa. Bothalia 37, 1–8.

Snijman, D.A., Van Jaarsveld, E.J., 1995. *Cyrtanthus flammosus*. Flowering plants of Africa 54, 100–103.

Snijman, D.A., Williamson, G., 1998. A new species of *Amaryllis* from the Richtersveld, South Africa. Bothalia 28, 192–196.

Snijman, D.A., Archer, R.H., 2003. Amaryllidaceae. Plants of Southern Africa: An Annotated Checklist. In: Germishuizen, G., Meyer, N.L. (Eds.), Strelitzia, vol. 14. National Botanical Institute, Pretoria, pp. 957–967.

Sorenson, M.D., Franzosa, E.A., 2007. TreeRot, Version 3. Boston University, Boston, MA.

Swofford, D.L., 2002. PAUP∗ Phylogenetic Analysis Using Parsimony (∗and Other Methods), v. 4.0 beta 10. Sinauer Associates, Sunderland.

Strydom, A., Kleynhans, R., Spies, J.J., 2007. Chromosome studies on African plants. 20. Karyotypes of some *Cyrtanthus* species. Bothalia 37, 103–108.

Sullivan, J., 1996. Combining data with different distributions of among-site variation. Systematic Biology 45, 375–380.

Tanabe, A.S., 2007. Kakusan: a computer program to automate the selection of a nucleotide substitution model and the configuration of a mixed model on multilocus data. Molecular Ecology Notes 7, 962–964.

Traub, H.P., 1963. Amaryllid notes, 1963. Plant Life 19, 57–62.

Van der Niet, T., 2006. Systematics and radiation patterns of *Satyrium* Sw. (Orchidaceae). Dissertation zur Erlangung der naturwissenschaftlichen Doktorwürde, Universität Zürich.

Vogel, S., 1954. Blütenbiologische Typen als Elemente der Sippengliederung. Botanische Studien, vol. 1. Gustav Fischer, Jena, pp. 1–337.

White, F., 1983. The Vegetation of Africa. Unesco, Paris, France.

White, T.J., Bruns, Lee, S., Taylor, J., 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J., White, T. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, Orlando, pp. 315–322.

Wilsenach, R., 1963. A cytotaxonomic study of the genus *Cyrtanthus*. Cytologia 28, 170–180.

Yoder, A.D., Irwin, J.A., Payseur, B.A., 2001. Failure of the ILD to determine data combinability for Slow Loris phylogeny. Systematic Biology 50, 408–424.

Edited by JC Manning