American Journal of Botany 88(12): 2321–2330. 2001.

PHYLOGENY OF AMARYLLIDACEAE TRIBE AMARYLLIDEAE BASED ON NRDNA ITS SEQUENCES AND MORPHOLOGY[1]

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We present the results of cladistic analyses of morphology, nrDNA ITS sequences, and a combination of the two for tribe Amaryllideae of the Amaryllidaceae. The morphologically based analysis supports the recognition of *Amaryllis* as sister to two major clades, equivalent to Snijman and Linder's (1996, *Annals of the Missouri Botanical Garden* 83: 362–386) Crininae and Amaryllidinae (less *Amaryllis*). A single tree is found with a successively weighted ITS sequence matrix. *Amaryllis* and *Boophone* form a grade at the base of the tree. All the other genera are included in two clades conforming to Snijman and Linder's (1996) subtribes Amaryllidinae (less *Amaryllis*, thus now Strumariinae) and Crininae (less *Boophone*). Within Strumariinae, *Strumaria* sensu lato is resolved as polyphyletic. *Strumaria* subg. *Gemmaria* is sister to the rest of the subtribe. *Hessea* is monophyletic only if *Namaquanula* is excluded. The monotypic *Carpolyza* is embedded within *Strumaria* sensu stricto. The consensus of the combined analysis is highly resolved, and most similar to the sequence topology. Based on the results of the combined analyses, the major clades are recognized as subtribes, and *Carpolyza* is placed into synonymy under *Strumaria*.

Key words: Amaryllidaceae; cladistic analysis; ITS; molecular systematics; monocotyledons; phylogeny; ribosomal DNA; South Africa.

Amaryllidaceae tribe Amaryllideae is endemic to Africa, with the exception of the pantropical genus *Crinum*. Much of the tribe's generic diversity is confined to South Africa (Snijman and Linder, 1996). In plastid DNA-based phylogenies of the family, the Amaryllideae is sister to the rest of the Amaryllidaceae with high bootstrap and jackknife support (Ito et al., 1999; Meerow et al., 1999). Compared to other tribes in Amaryllidaceae, Amaryllideae is marked by a large number of synapomorphies (Snijman and Linder, 1996; Meerow and Snijman, 1998): extensible fibers in the bulb tunics, bisulculate pollen with spinulose exine, scapes with a sclerenchymatous sheath, unitegmic or ategmic ovules, and nondormant, water-rich, nonphytomelanous seeds with chlorophyllous embryos. A few of the genera extend outside of South Africa proper, but only *Crinum*, with seeds well suited to oceanic dispersal (Koshimizu, 1930), ranges through Asia, Australia, and America.

Snijman and Linder's (1996) phylogenetic analysis of the tribe based on morphological, floral and seed anatomical, and cytological data resulted in recognition of two monophyletic subtribes: Crininae (*Boophone*, *Crinum*, *Ammocharis*, and *Cybistetes*) and Amaryllidinae (*Amaryllis*, *Nerine*, *Brunsvigia*, *Crossyne*, *Hessea*, *Strumaria*, and *Carpolyza*). Meerow et al.'s (1999) incomplete sampling of this tribe for three plastid sequences resolved *Amaryllis* as sister to the rest of the tribe. Most recently, Weichhardt-Kulessa et al. (2000) presented an analysis of internal transcribed spacer (ITS) sequences for a part of the tribe (subtribe Strumariinae sensu D. & U. Müller-Doblies [1985, 1996]). Recent taxonomic history of the tribe is summarized in Table 1.

In this paper, we present the results of cladistic analysis of nrDNA internal transcribed spacer (ITS) sequences and a morphological data matrix for the tribe, separately and in combination, and compare the results with those of Snijman and Linder (1996) and Weichhardt-Kulessa et al. (2000).

MATERIALS AND METHODS

Plant materials—Species used in the sequence analyses, voucher specimens, and GenBank accession numbers (AF373068-99) are listed in a table archived at the following World Wide Web site: http://ajbsupp.botany.org/. Thirty-one species of Amaryllideae were sampled, representing all genera of the tribe, with *Agapanthus caulescens* used as outgroup. *Agapanthus* resolved as sister to Amaryllidaceae with plastid sequence data (Meerow et al., 1999).

Morphological characters—Forty morphological characters were coded for the 32 species utilized in the molecular analyses (Tables 2 and 3). All characters were unordered in the analyses. Because species are the terminal units in the molecular analyses, rather than genera and subgenera, as in Snijman and Linder's (1996) morphological analysis of the tribe, an additional seven characters were added to their morphological data set. Phyllotaxis was found to vary among species of particular genera (characters 6 and 7); as was the presence or absence of a cataphyll (character 4); a midrib on the leaf (character 8); perigone symmetry (character 13); perigone tube (character 14); the degree to which the stamens are adnate to the style (character 19); and the position of filament appendages (character 20). Another modification of the previously published matrix (Snijman and Linder, 1996) was the use of *Agapanthus* instead of Haemantheae sensu Dahlgren, Clifford, and Yeo (1985) (scored for states observed in *Cyrtanthus*) as the outgroup.

DNA extraction, amplification, and sequencing protocols—Genomic DNA was extracted from silica gel dried leaf tissue as described by Meerow et al. (2000b). Amplification of the ribosomal DNA ITS1/5.8S/ITS2 region was 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 sequence. Amplified products were purified using QIAquick (Qiagen, Valencia, California, USA) columns, following manufacturer's protocols. Polymerase chain reaction (PCR) amplifications were performed on an ABI 9700 (Perkin-Elmer Applied

[1] Manuscript received 28 November 2000; revision accepted 31 July 2001.
Some of the sequences were generated at the DNA Sequencing Core of the Interdisciplinary Center for Biotechnology Research at the University of Florida. Portions of this work were supported by NSF grant DEB-968787.
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TABLE 1. Four most recent classifications of Amaryllidaceae tribe Amaryllideae. The lines indicate subsequent segregation or inclusion of genera.

Traub (1963)	Dahlgren, Clifford, and Yeo (1985)[a]	Müller-Doblies and Müller-Doblies (1996)[b]	Snijman (1992), Meerow and Snijman (1988)
Crineae	**Amaryllideae**	**Amaryllideae**	**Amaryllideae**
Crinum L.	*Crinum* L.	subtr. Amaryllidinae	subtr. Amaryllidinae
Ammocharis Herb.	*Ammocharis* Herb.	*Amaryllis* L.	*Amaryllis*
		[1]*Namaquanula* D. and U. M-D.	
		Nerine	
Nerine Herb.	*Nerine*	subtr. Boophoninae	*Nerine*
Boophone Herb.	*Boophone*	*Boophone*	
	Brunsvigia	*Brunsvigia*	
Cybistetes Milne-Redh. and Scweick.	*Cybistetes*	[2]*Crossyne* Salisb.	*Crossyne*
	Amaryllis		
Brunsvigia Heist.	*Brunsvigia*		
Strumarieae		subtr. Strumariinae	
Strumaria Jacq.	*Strumaria*	*Strumaria*	*Strumaria*
Hessea Herb.	*Hessea*	*Hessea*	*Hessea*
Carpolyza Salisb.	*Carpolyza*	*Carpolyza*	*Carpolyza*
		[3]*Bokkeveldia* D. and U. M-D.	
		[3]*Gemmaria* D. and U. M-D.	
		[3]*Tedingea* D. and U. M-D.	
		[1]*Dewinterella* D. and U. M-D.	
		subtr. Crininae	subtr. Crininae
		Crinum	*Crinum*
		Ammocharis	*Ammocharis*
		Cybistetes	*Cybistetes*
			Boophone

[a] As Dahlgren, Clifford, and Yeo (1985) did not consistently list the component genera in their tribal concepts, their exact generic composition is inferred. Most of their delimitations are presumed to have followed Traub (1963).

[b] Superscript 1 = segregate from *Messea*; superscript 2 = segregate from *Boophone*; superscript 3 = segregate from *Strumaria*.

Biosystems, Foster City, California, USA), running 28 cycles of the following program: 4 min at 94°C, 1 min at 52°C, and 3 min at 72°C.

Cycle sequencing reactions were performed directly on purified PCR products on the ABI 9700, using standard dideoxy cycle protocols for sequencing with dye terminators on either an ABI 377 or ABI 310 automated sequencer (according to the manufacturer's protocols; Applied Biosystems).

Sequence alignment—The ITS sequences were aligned using the program CLUSTALX (Higgins and Sharp, 1988; Thompson et al., 1997) with a gap opening penalty of 15 and a gap extension penalty of 0.666, with subsequent manual editing using the sequence editing program Sequencher (Gene Codes, Ann Arbor, Michigan, USA). The aligned matrix is archived in a table that can be accessed online at the following World Wide Web site: http://ajbsupp.botany.org/.

Analyses—Aligned matrices were analyzed using the parsimony algorithm of PAUP* for Macintosh (version 4.0b8; Swofford, 1998), with the MULPARS option invoked. Tree branches were retained only if unambiguous support was available (i.e., branches were collapsed only if the minimum length = 0). Gaps were coded as missing characters. A successive weighting (SW) strategy (Farris, 1969) was implemented. The SW strategy is a useful tool employed to reduce the global effect of highly homoplasious base positions on the resulting topologies (Wenzel, 1997; Lledó et al., 1998; Meerow et al., 1999). Whole category weights (codon or transversion) exhibit broad and overlapping ranges of consistency (Olmstead, Reeves, and Yen, 1998), whereas successive weighting independently assesses each base position of the multiple alignment based on their consistency in the initial analysis.

The initial tree search was conducted under the Fitch (equal) weights (Fitch, 1971) criterion with 1000 random sequence additions (Maddison, 1991) and tree bisection and reconnection (TRB) branch swapping. We permitted only ten trees to be held at each step to reduce the time spent searching trees at suboptimal levels. All trees collected in the 1000 replicates were swapped onto completion or an upper limit of 5000 trees. The characters were then reweighted by the rescaled consistency index (a base weight of 1000 was used to maintain integer values), and a further 500 replications of random sequence additions were conducted with the weighted matrix saving 20 trees per replication. These trees were then swapped on to completion or an upper limit of 5000 trees. The resulting trees were then used to reweight the matrix a second time by the rescaled consistency index, and another 500 replicates of random sequence addition were conducted, saving 20 trees per replication, with subsequent swapping on those trees. This cycle was repeated until two successive rounds found trees of the same length.

Internal support was determined by bootstrapping (Felsenstein, 1985; 5000 replicates) and calculation of Bremer (1988) decay indices (DI) using the program TreeRot (Sorenson, 1996). The cut-off bootstrap percentage is 50. A bootstrap value >75% is considered good support, 65–75% is designated moderate support, and <65% is weak. One hundred replicate heuristic searches were implemented for each constraint statement postulated by TreeRot, saving 10 trees per replicate. TreeRot was run with equal weights imposed on the data.

RESULTS

Morphological data—Of the 40 characters coded for the species, 36 were parsimony informative. With equal weights imposed on the data, 84 equally most parsimonious trees were found with a length of 97 steps, a consistency index (CI) = 0.52 and a retention index (RI) = 0.81. The strict consensus of these trees is not well resolved, and support is low for many of the clades (Fig. 1). After two rounds of SW, one tree was found with weighted length = 41 079 (Fitch length = 98) and a weighted CI = 0.72 (Fitch CI = 51) and RI = 0.90 (Fitch RI = 81). The weighted tree (Fig. 2) was much more resolved than the consensus of the Fitch trees (Fig. 1), and support percentages increased markedly. The additional step of the SW tree is essentially the ''cost'' of optimizing consistent characters over highly homoplastic base positions (Lledó et al., 1998; Meerow et al., 1999). Even with successive weighting, few clades are defined by more than one or two synapomorphies.

Amaryllis is the first branch in both the unweighted and weighted analyses (bootstrap = 87% in both). The apomor-

TABLE 2. Morphological characters for cladistic analysis of Amaryllideae using exemplars and *Agapanthus* as the outgroup. (*indicates an autapomorphy in a multistate character.)

Character no.	Character states
Bulb and leaves	
1	Bulb tunics when torn: 0 = without threads; 1 = producing threads.
2	Outer bulb tunics: 0 = fibrous; 1 = brittle.
3	Leaf habit: 0 = annual; 1 = lasting beyond a year.
4	Cataphyll: 0 = absent; 1 = present.
5	Foliage leaf number: 0 = at least four; 1 = 2–4; 2 = always 2.
6	Leaf arrangement: 0 = not overlapping; 1 = overlapping.
7	Leaf position: 0 = held above the ground; 1 = adpressed to the ground.
8	Leaf midrib: 0 = prominent; 1 = absent.
9	Leaf surface: 0 = glabrous; 1 = pubescent (at least in juveniles); *2 = bristly.
10	Leaf margin: 0 = unthickened; 1 = heavily thickened.
11	Leaf margin: 0 = untoothed; 1 = with branched short teeth; *2 = with branched long bristles.
Inflorescence and flowers	
12	Pedicel length at anthesis: 0 = less or equaling the perigone; 1 = at least twice the perigone.
13	Perigone (perianth) symmetry: 0 = actinomorphic; 1 = zygomorphic.
14	Perigone (perianth) tube: 0 = shorter than tepals; 1 = longer than tepals; 2 = absent.
15	Flower color at senescence: 0 = dark pink; 1 = brown; *2 = dark blue.
16	Stamen relative length: 0 = about equal; 1 = biseriate.
17	Stamen position: 0 evenly arranged; 1 = declinate
18	Stamen tube: 0 = absent; 1 = rudimentary; 2 = conspicuous.
19	Stamen adnation to style: 0 = free; 1 = adnate equally at base; 2 = adnate unequally beyond the base.
20	Filaments appendages: 0 = absent; 1 = ventral
21	Filament trichomes: 0 = absent; 1 = present proximally.
22	Anther shape: 0 = straight; 1 = curved.
23	Anther insertion: 0 = dorsifixed; 1 = subcentrifixed; 2 = centrifixed.
24	Pollen aperture: 0 = monosulate; 1 = bisulculate.
25	Pollen sculpturing: 0 = reticulate; 1 = spinulose.
26	Nectar wells: 0 = around style; 1 = in axils between inner filaments and style; 2 = in deep wells between inner filaments and style.
27	Style form: 0 = slender throughout; 1 = lower half thickened; 2 = broad-based.
28	Style position: 0 = central; 1 = ecentric.
29	Ovule: 0 = bitegmic; 1 = unitegmic.
Infructescence and seed	
30	Scape habit at seed shed: 0 = persisting to bulb; 1 = abscising at ground level.
31	Fruiting head: 0 = remaining attached to scape; 1 = detaching from scape.
32	Fruit: 0 = dehiscent; 1 = indehiscent.
33	Fruit: 0 = nonrostellate; 1 = rostellate.
34	Fruit shape: 0 = regular; 1 = irregular.
35	Testa: 0 = without stomata; 1 = stomatose.
36	Integument at seed shed: 0 = undifferentiated; 1 = enlarging.
37	Integument at seed shed: 0 = nonchlorophyllous; 1 = chlorophyllous.
38	Endosperm at seed shed: 0 = noncorky; 1 = corky externally.
39	Endosperm: 0 = nonchlorophyllous; 1 = partially chlorophyllous.
Chromosomes	
40	Basic number: 0 = 11; 1 = 10; *2 = 7.

phies for *Amaryllis* are the following two homoplasious characters: absence of a perigone tube and a rudimentary stamen tube. The two subtribes of Snijman and Linder (1996) are supported with the exclusion of *Amaryllis* from the Amaryllidinae (which according to the International Code for Botanical Nomenclature [ICBN] must now be referred to as the Strumariinae), Crininae with a weighted bootstrap of 74% (60% Fitch), and Strumariinae with 90% (78% Fitch). However, the Strumariinae clade has an unweighted DI of 2 and Crininae has a DI of only 1. Crininae is defined by four nonhomoplasious synapomorphies: indehiscent, irregularly shaped fruit, and cork-covered seeds, with partially chlorophyllous endosperm (but see position of *Boophone* in the sequence phylogeny below). The monophyly of *Boophone* and a sister relationship for *Ammocharis* and *Cybistetes* receive moderate to strong support in both the Fitch and weighted analyses, but *Crinum* is very poorly resolved in both analyses. *Boophone* is weakly supported as part of Crininae in the Fitch (60%) and only slightly better in the SW (66%) analyses. Within Strumariinae, *Hessea* sensu lato (s.l., i.e., including *Namaquanula*) is resolved as monophyletic in the SW trees with moderate (80%) support (Fig. 2), but not in the Fitch consensus (Fig. 1). Flowers turning brown at senescence is unique to the clade but presence of cataphyll, two leaves, and scape abscising at ground level are homoplasious synapomorphies. *Hessea pulcherrima* is sister to *Namaquanula* with a bootstrap = 62%. With equal weights imposed, the clade is unresolved (Fig. 1). *Strumaria* s.l. is resolved as monophyletic in both trees but without bootstrap support and a DI = 1. The sister relationship of *Carpolyza* to *Strumaria* receives strong support in the SW (bootstrap = 94%) and moderate in the Fitch trees (79%). Five nonhomoplasious characters uphold the *Carpolyza-Strumaria*

TABLE 3. Morphological character state matrix used in the cladistic analyses of Amaryllideae. ? = missing or inapplicable data.

Taxon	Character state matrix
Crinum variabile	100?000100101001100001011001100111000110
Crinum macowanii	100?00010010 1?0110000101100110011100 0110
Crinum campanulatum	10??000100001101100001011001100101?????0
Namaquanula sp. nov.	110?100100010010020010011000 1???????????
Namaquanula bruce-bayeri	11001001000100100201100110001100000111000
Strumaria tenella	10000001000102000010000111201000000111001
Strumaria bidentata	10011001000?020000110001111010000011100 1
Boophone haemanthoides	10000101000000000000000011000101110000 11?
Hessea pulcherrima	10012001000102100101101110001100000111000
Strumaria aestivalis	10012001100102000020001112101100000111001
Agapanthus caulescens	??0?000000000021100000000001000000000002
Amaryllis paradisicola	10000000100012011000101100110000000000?
Amaryllis belladonna	100?000000001201110001011001100000000000
Carpolyza spiralis	10000001000100000020001111101000001110 01
Hessea stenosiphon	10012001000101110200002110001100001110 00
Strumaria chaplinii	10012011001020000100011112011000011100 1
Hessea breviflora	10012001000100100200002110001100001110 00
Strumaria watermeyeri	10012011?00?020000200011111010000011100 1
Brunsvigia radula	11002011211110011200000110011100001110 0?
Nerine alta	10000001000010011200000110011000001110 00
Strumaria discifera	10012001100102000010001111101?0000111001
Cybistetes longifolia	11100001001000001000010110011101100001 10
Strumaria truncata	10011001000002000020000112101000001110 01
Crossyne guttata	11000011012100000200000110011100000111000
Brunsvigia orientalis	1100001101111001120?000110011100001110 0?
Boophone disticha	10000101000000000000000011000101110000 110
Hessea stellaris	10012001000100100200002110001100001110 00
Ammocharis coranica	11100001001000000000010110011001110000 110
Hessea pilosula	10012001100100100200002110001100001110 00
Nerine sarniensis	10000001000000010200000110001000001110 00
Strumaria picta	10001001100?020000100011111010000011100 1
Brunsvigia comptonii	110?101101001001120000011001110000 11100?

clade: stamen tube absent, stamens adnate to the style; nectar in axils between style and inner stamens; style thickened in lower half; and basic chromosome number = 10. *Nerine* is poorly resolved in both analyses, and *Brunsvigia* and *Crossyne* form a well-supported (SW bootstrap = 87%), but internally poorly resolved clade. Synapomorphies for the latter are the brittle bulb tunics, leaves adpressed to the ground, thickened leaf margins, and scapes abscising at ground level (but see position of *Crossyne* in the combined analysis below). However, all states except thickened leaf margins are found elsewhere in the tribe.

Sequence data—Of the 759 base positions included in the matrix, 271 were parsimony informative. Eight equally parsimonious trees were found with the unweighted (Fitch) data matrix. The strict consensus tree is shown in Fig. 3. The trees were 949 steps long, with a consistency index (CI) = 0.65 and a retention index (RI) = 0.71. After two rounds of successive weighting (base weight = 1000), a single tree was found of length = 466 019 (Fitch length = 949), with CI = 0.83 (Fitch = 0.65) and RI = 0.84 (Fitch = 0.71). The topology of the single weighted tree (Fig. 4) was more resolved than the consensus of the Fitch trees (Fig. 3) and is one of the eight topologies resolved without weights imposed.

Amaryllis, followed by *Boophone*, form a basal grade in the trees (Figs. 3–4). The position of *Boophone* receives weak support in the Fitch trees (Fig. 3; bootstrap = 51%) but strong support (85%) in the SW tree (Fig. 4). In trees one step longer, its position is unresolved relative to the rest of the tribe. Two major clades are then resolved with high support in both the weighted (Fig. 4) and unweighted trees (Fig. 3), conforming respectively to Snijman and Linder's (1996) Crininae (less *Boophone*) and Amaryllidinae (less *Amaryllis*), which is now called Strumariinae s.l. The Crininae clade consists of two well-supported sister subclades, one representing the genus *Crinum* and the other containing *Ammocharis* and *Cybistetes*. The Strumariinae clade resolves into a collection of three smaller basal clades in the SW tree with varying support. Five species of *Strumaria* that represent subgenus *Gemmaria* sensu Snijman (1994) form a well-supported clade that is sister to the rest of the Strumariinae. Next, a clade containing *Carpolyza*, *Crossyne*, and three additional species of *Strumaria* is resolved with weak support at a node above the basal node. Support for *Crossyne* as part of this clade is weak in the SW tree (Fig. 4; 59%). In the Fitch trees, the position of *Crossyne* is unresolved (Fig. 3). The sister relationship between *S. bidentata* and *S. truncata* is well supported in both weighted and unweighted analyses (Figs. 3–4); while the positions of *Carpolyza* and *S. tenella* are moderately supported in the Fitch trees and strongly supported in the SW tree. The third, moderately supported clade within Strumariinae s.l. consists of two sister groups in the SW tree (Fig. 4). The first is a strongly supported *Hessea* sensu stricto (s.s.) clade that is equally well supported in the Fitch trees (Fig. 3). The weakly supported sister clade unites *Brunsvigia* with two *Namaquanula* species with moderate support only in the SW tree and places this group as sister to *Nerine*.

Combined data—Combining independent character matrices, whether both molecular or molecular and morphological,

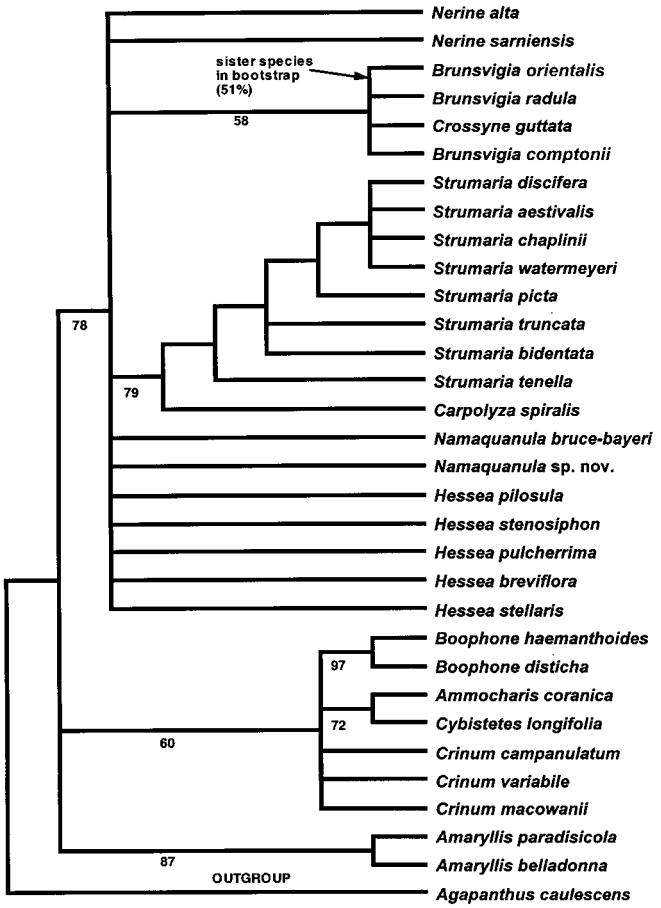

Fig. 1. Strict consensus of 84 trees generated by Fitch analysis of 40 morphological characters across the Amaryllideae. Numbers below the lines are bootstrap support values.

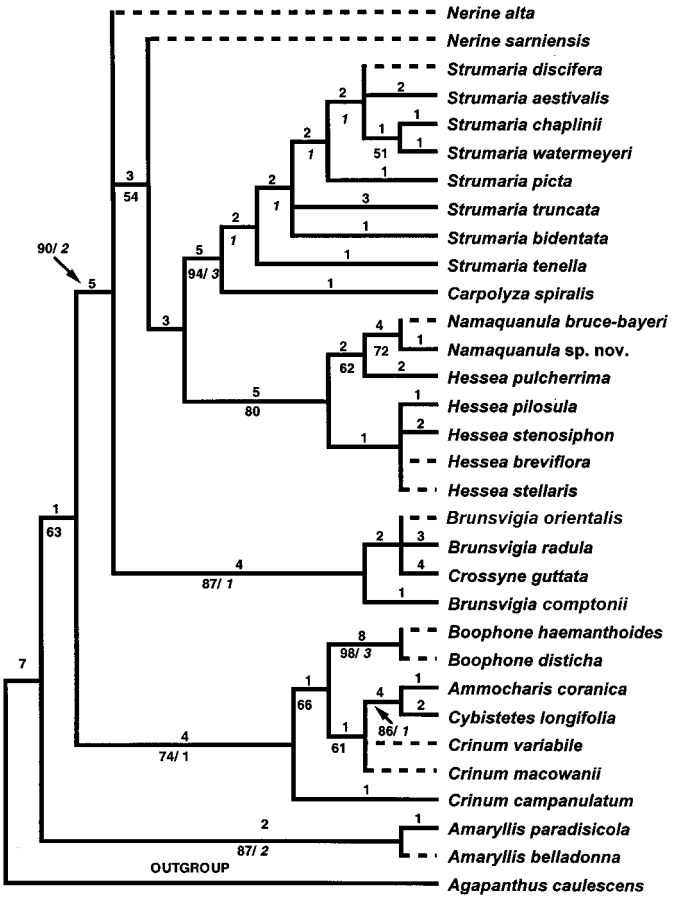

Fig. 2. Single most parsimonious tree found by successive weighting of 40 morphological characters across the Amaryllideae. Numbers above the line are branch lengths; below the lines are bootstrap and Bremer index (in italics) values, respectively. Dashed lines represent zero-length branches.

very often increases the resolution of the ingroup and the bootstrap support of the internal nodes of the phylogenetic trees (Olmstead and Sweere, 1994; Chase et al., 1995; Yukawa et al., 1996; Rudall et al., 1998; Soltis et al., 1998; Meerow et al., 1999). Nonetheless, there is controversy about whether different data sets should be analyzed separately or together (de Queiroz, Donoghue, and Kim, 1995; Huelsenbeck, Bull, and Cunningham, 1996). Congruence of the independent matrices has generally been demonstrated before they are combined, but it has also been argued that incongruence should not be a predetermined factor against doing so (Seelanan, Schnabel, and Wendel, 1997; Dubuisson, Hebant-Mauri, and Galtier, 1998). Miyamoto and Fitch (1995) argue that data sets should always be analyzed independently, as underlying assumptions, constraints, or weighting strategies will vary from data set to data set. Kluge (1989) and Nixon and Carpenter (1996) argue that simultaneous analysis of multiple data sets better maximizes parsimony and allows secondary signals to appear from the combined data. Bull et al. (1993), Rodrigo et al. (1993), and de Queiroz (1993) advocated combining data only after a statistical test of congruence, what Huelsenbeck, Bull, and Cunningham (1996) call ''conditional combination.'' Before combining the morphological and ITS data sets, we performed a partition homogeneity test on the matrices (Farris, Kluge, and Bult, 1994; Farris et al., 1995). One thousand heuristic searches were conducted, each with ten random addition replications, saving ten trees from each for TBR branch-swapping. The P value = 0.002, indicating substantial incongruence between the morphological and ITS matrices. Much of the apparent incongruence can be attributed to the weak resolution of the morphologically based topologies, and we felt that it would still be informative to combine the two matrices in a single analysis. This seems especially useful given the degree of difficulty that has been encountered with cladistic analysis of purely morphological data in Amaryllidaceae (Meerow et al., 2000a).

Of the 799 characters in the combined matrix, 307 were parsimony informative. With equal weights, five trees were found of length = 1063, CI = 0.62, and RI = 0.71. The strict consensus is shown in Fig. 5. After two rounds of SW, one tree (Fig. 6) was found of length = 504 155 (Fitch = 1063) with CI = 81 (Fitch = 62) and RI = 84 (Fitch = 71). The single SW tree is the same as one of the five found with equal weights imposed. The combined trees differ from the ITS trees only in the resolution of a monophyletic *Strumaria* (including *Carpolyza*) with high support (bootstrap = 91%, DI = 3) in the SW tree and moderate (83%, DI = 3) in the Fitch trees. Morphological apomorphies for the clade are loss of the perigone tube, loss of the staminal tube, stamens equally adnate at the base to the style, nectar wells between the inner filaments and the style, the style strumose in the lower half, and

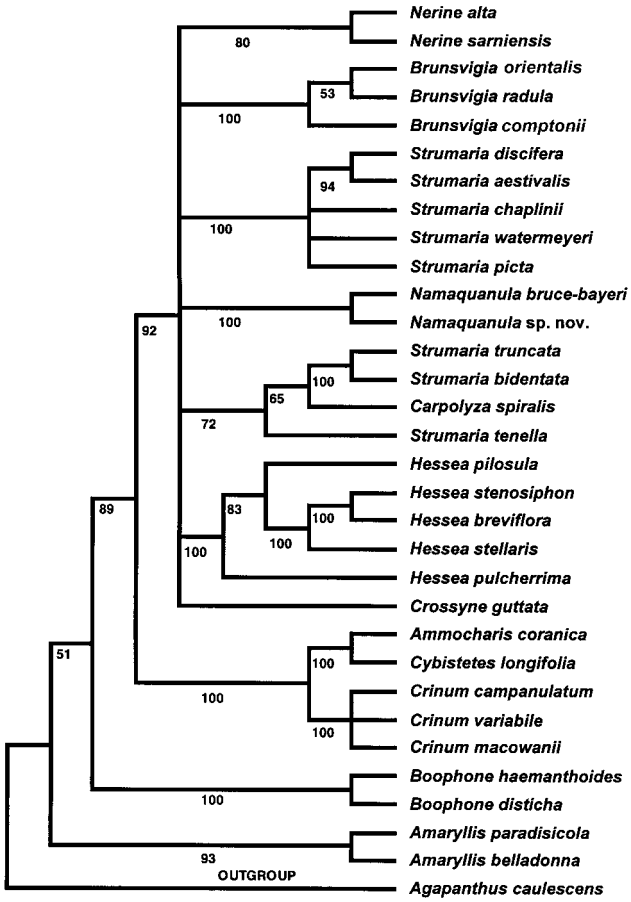

Fig. 3. Strict consensus of eight most parsimonious trees found by cladistic analysis of aligned ITS sequences across the Amaryllideae with equal weights imposed. Numbers below the lines are bootstrap support values.

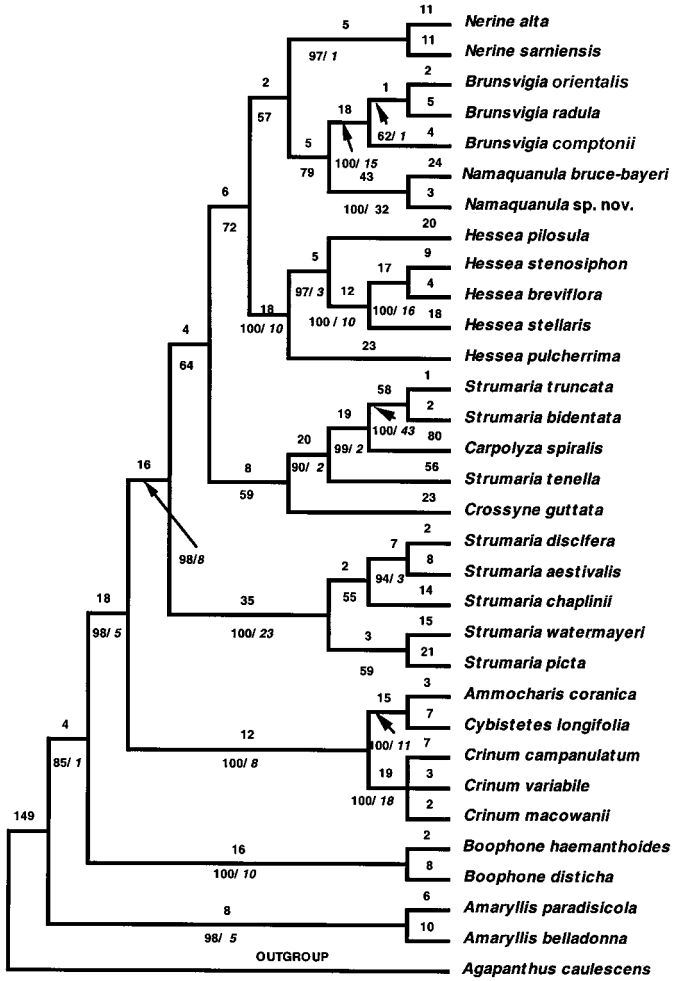

Fig. 4. Single most parsimonious trees found with successively weighted ITS sequence matrix of Amaryllidaceae tribe Amaryllideae. Numbers above the lines are branch lengths. Numbers below the lines or directed by arrows are bootstrap and Bremer index (in italics) values, respectively.

a base chromosome number of $x = 10$. *Crossyne* is resolved as sister to the rest of Strumariinae with 100% bootstrap support in both analyses and a DI of 16. The nine morphological apomorphies that support this clade in the combined analysis are pedicels at least twice the perigone length at anthesis, a conspicuous stamen tube, a dehiscent capsule, a nonrostellate fruit, a stomatose testa, an enlarged and chlorophyllous integument in the mature seed, and noncorky endosperm. *Brunsvigia* and *Namaquanula* form a sister clade to *Hessea* in the SW tree (Fig. 6), with *Nerine* sister to both. The sister relationship of *Namaquanula* to *Brunsvigia* receives moderate support in the SW tree (bootstrap = 70%), as does the relationship of *Nerine* to that clade (65%). The only morphological synapomorphy that joins *Brunsvigia* and *Namaquanula* is a brittle outer bulb tunic. Morphological synapomorphies linking these two genera to *Hessea* are 2–4 leaves, flowers brown at senescence, and infructescence abscising at ground level, all of which are homoplasious. Although the rest of the tree is identical to the resolution obtained from ITS alone, bootstrap and DI values are higher for certain clades (e.g., resolution of *Boophone* as sister to both subtribes Crininae s.s. and Strumariinae s.l.).

DISCUSSION

Recognition of the Amaryllideae as a natural group was first advanced by Traub (1957, albeit as Crineae), on the basis of the bulb tunic fibers that appear when this tissue is torn. Unitegmic ovules and bisulculate pollen (Huber, 1969; Schulze, 1984), as well as scapes with a sclerenchymatous sheath (Arroyo and Cutler, 1984), are additional autapomorphic characters for the tribe. Previous treatments of the tribe included elements of Haemantheae (Pax, 1887; Pax and Hoffmann, 1930; Hutchinson, 1934, 1959). Traub's (1957, 1963) concept was largely adopted by Dahlgren, Clifford, and Yeo (1985). Traub (1957) originally recognized two subtribes, Crininae and Strumariinae, which he elevated to tribal rank (Traub, 1963) and then later (Traub, 1965, 1970) combined again. Müller-Doblies and Müller-Doblies (1985) formally reinstated Strumariinae at the subtribal level.

Snijman and Linder's (1996) cladistic analysis of the tribe suggested that two monophyletic groups could be recognized in the tribe. Subtribe Crininae was defined by indehiscent, rostellate capsules, the corky testa, and the partially chlorophyllous endosperm of their seeds. Subtribe Amaryllidinae was characterized by a staminal tube (although rudimentary in *Amaryllis* and lost in *Strumaria* and *Carpolyza*) and stomatose seeds with an enlarged, green integument (except *Amaryllis*). Snijman and Linder (1996) also recognized the polyphyly of

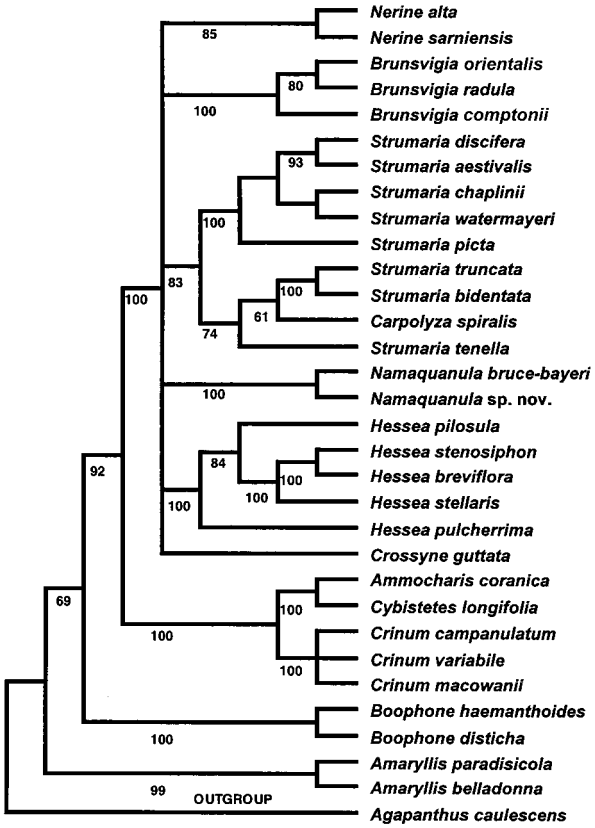

Fig. 5. Strict consensus of five equally most parsimonious trees found by cladistic analysis of the combined ITS and morphological matrix for the Amaryllideae. Numbers below the lines are bootstrap values.

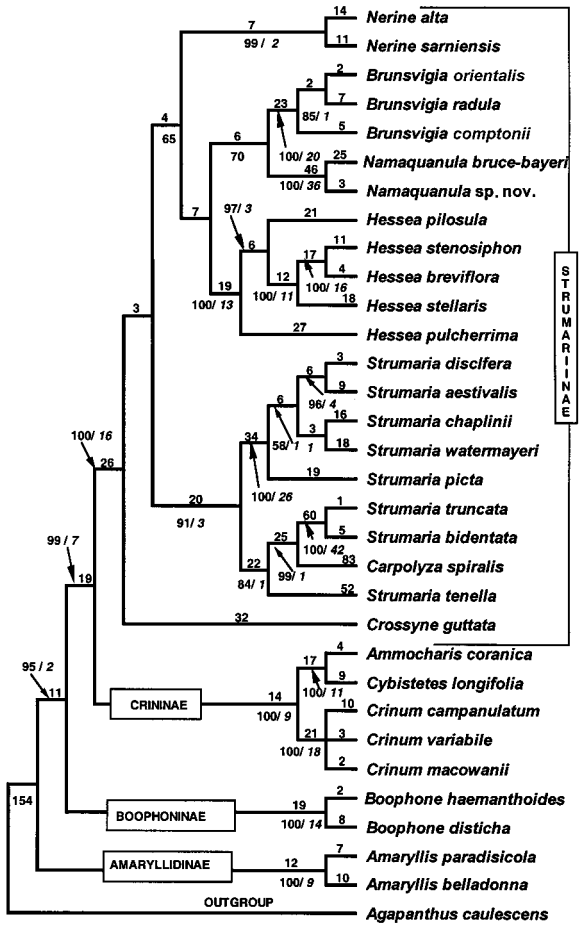

Fig. 6. Single most parsimonious trees found with successively weighted combined ITS sequence and morphological character matrix across tribe Amaryllideae. Numbers above the lines are branch lengths. Numbers below the lines or directed by arrows are bootstrap and Bremer index (in italics) values, respectively.

Boophone (sensu Arnold and De Wet, 1993), though the formal reestablishment of the segregate genus *Crossyne* was accomplished by Müller-Doblies and Müller-Doblies (1994). Müller-Doblies and Müller-Doblies (1996) recognized four subtribes with little discussion and no phylogenetic analysis: Crininae (*Crinum*, *Ammocharis*, *Cybistetes*), Boophoninae (*Boophone*, *Brunsvigia*, *Crossyne*), Amaryllidinae (*Amaryllis*, *Nerine*, *Namaquanula*), and Strumariinae, the latter containing several segregate genera from *Hessea* and *Strumaria*. Meerow et al.'s (1999) analysis of plastid DNA sequences resolved *Amaryllis* as sister to the rest of the tribe, with a monophyletic ''Amaryllidinae'' (*Brunsvigia*, *Hessea*, *Strumaria*, *Nerine*) nested within an *Amaryllis-Boophone-Crinum* grade. The plastid *matK* sequence analysis of Ito et al. (1999), who studied only five taxa (*Amaryllis*, *Nerine*, *Brunsvigia*, *Strumaria*, *Crinum*), also supports the basal position of *Amaryllis*.

The results from the nuclear ITS sequences are more congruent with the plastid DNA phylogeny (Meerow et al., 1999) than the morphologically based analyses presented here and by Snijman and Linder (1996). Both *Boophone* and *Amaryllis* form a grade at the base of the tree (Figs. 3–4). Our sequence-based (Figs. 3–4) and combined trees (Figs. 5–6) suggest that placing *Amaryllis* together with *Nerine*, *Crossyne*, *Brunsvigia*, *Namaquanula*, and *Strumaria* and placing *Boophone* within Crininae renders both subtribes polyphyletic. However, with the exception of *Amaryllis* and *Boophone*, Snijman and Linder's (1996) subtribes are strongly supported monophyletic groups. Within Crininae, *Crinum* is monophyletic. The relationships within Strumariinae, as recognized here, are more complex, and the basal resolution of the subtribe is poorly supported in many places. Snijman and Linder (1996) were able to resolve both a monophyletic *Hessea* and *Strumaria*, as did Snijman (1994), and justified submerging Müller-Doblies and Müller-Doblies' (1985) segregate genera (*Namaquanula* and *Dewinterella* into *Hessea*; *Bokkeveldia*, *Gemmaria*, and *Tedingea* into *Strumaria*), as well as Snijman's (1991) own *Kamiesbergia* into *Hessea*. Our results (Figs. 3–4) suggest that both *Hessea* (*Namaquanula* included) and *Strumaria* sensu Snijman (1994) may be polyphyletic. But after the ITS sequence data were combined with the morphological data (Figs. 5–6) only *Namaquanula* (*N. bruce-bayeri* and an undescribed species) appears justified as a segregate. Snijman (1994) also included *H. pulcherrima* in *Hessea* subg. *Namaquanula*, a resolution that appears in the SW morphological tree (Fig. 2) but in our gene tree, as in that of Weichhardt-Kulessa et al. (2000), this species does not resolve with the other *Namaquanula* species. Moreover, the only recognized member of subg. *Kamiesbergia*, *H. stenosiphon*, resolves as sister to a member of subg. *Hessea* with strong support. Weichhardt-Kulessa et al. (2000) resolved *Namaquanula* as sister to *Hessea*, but no species of *Brunsvigia* was included in their analysis.

In the case of *Strumaria*, two independent origins are inferred from our ITS sequence trees (Figs. 3–4), though their basal relationships are not well supported. One group is sister to the rest of the Strumariinae and is entirely composed of species from Snijman's (1994) subgenus *Gemmaria*. The second clade of *Strumaria* species, within which the monotypic *Carpolyza* is nested, combines species from subg. *Tedingea* and subg. *Strumaria* sensu Snijman (1994). The resolution of *Crossyne* as sister to *Strumaria* subg. *Strumaria* has weak support only in the weighted analysis of the molecular data (Fig. 4). In contrast to the molecular analyses, the combined data analyses, however, support the monophyly of *Strumaria* with inclusion of *Carpolyza* (Figs. 5–6) as a genus with two well-supported, divergent lineages.

None of the subgenera or sections that Snijman (1994) recognized in *Strumaria*, and which Müller-Doblies and Müller-Doblies (1985, 1994) recognized as genera, were characterized by more than two nonhomoplasious synapomorphies. Some of the subgeneric taxa in *Hessea* were slightly better supported. Many of the characters used are floral characters that are demonstrably homoplastic within the family (Meerow and Snijman, 1998; Meerow et al., 1999). If our sequence-based phylogeny is accurate, it suggests that morphological homoplasy is more rampant within the Strumariinae than might have been suspected.

Subtribes Crininae and Boophoninae (as emended below) are fairly widespread throughout sub-Saharan Africa (Snijman and Linder, 1996), with a decided bias towards summer rainfall areas. Only the monotypic *Cybistetes* is confined to the western Cape and southern Namibia. On the other hand, subtribes Amaryllidinae and Strumariinae (as recircumscribed here) are restricted entirely to southern Africa. Among the zygomorphic flowered genera of these two subtribes (*Amaryllis*, *Brunsvigia*, *Crossyne*, and *Nerine*), *Nerine* is predominantly a summer rainfall genus with a few outlying species in the winter rainfall region of the western Cape. *Brunsvigia* is equally distributed in the winter and summer rainfall areas of the west and east, respectively, while *Amaryllis* and *Crossyne* are restricted to southern Africa's winter rainfall region. The actinomorphic genera of subtribe Strumariinae (*Hessea*, *Namaquanula*, and *Strumaria* [including *Carpolyza*]) are endemic to southwestern Africa, from southern Namibia to the southern Cape with two species in the semiarid summer rainfall region. Prior to the Pliocene, Africa's southwestern region enjoyed a moist environment (Coetzee, 1978, 1983, 1986; Hendey, 1983; Scholtz, 1985), with the earliest evidence of modern semiarid, winter rainfall pattern dating to the Late Pliocene, but not fully established until the Early Pleistocene (Hendey, 1983; Tankard and Rogers, 1978). Moreover, the winter rainfall region of southern Africa experienced a more recent pattern of expansion and contraction with concurrent wetter and drier conditions during glacial and interglacial periods of the Quaternary (Tankard, 1976; van Zinderen Bakker, 1976; Tyson, 1986; Cockcroft, Wilkinson, and Tyson, 1987). These recent climatic changes have undoubtedly played an important role in the evolution of the Amaryllideae, especially the Strumariinae, and chiefly the actinomorphic genera (Snijman, 1992), but the detailed history of these late Pleistocene and Quaternary events is still to emerge for the Cape region (Cowling et al., 1999). Snijman (1992) generated area cladograms for *Namaquanula*, *Hessea*, and *Strumaria*, all of which were incongruent. These suggested that the southwestern Cape might have comprised a series of transient biotas, each generating a different speciation pattern and phytogeographic history. She hypothesised that lineage divergence in *Hessea* was caused predominantly by vicariance, whereas allopatric speciation by peripheral isolation was most frequent in *Strumaria* (Snijman, 1992).

In conclusion, ITS sequences resolve a phylogeny of Amaryllideae that is only partially congruent with the present and previous morphologically based topologies (Snijman and Linder, 1996). *Amaryllis*, which Snijman and Linder (1996) treated within Strumariinae (as Amaryllidinae), resolves as sister to the remainder of the tribe in all analyses. In contrast to the morphological topologies (Figs. 1–2), *Boophone* is not allied within subtribe Crininae but forms the second most basal branch of the phylogeny after *Amaryllis*. Two major lineages are subsequently resolved in all the analyses. The most diverse taxonomically is the southern African lineage that encompasses *Crossyne*, *Strumaria*, *Nerine*, *Hessea*, *Namaquanula*, and *Brunsvigia*. The other is the predominantly sub-Saharan African group that includes *Crinum*, *Cybistetes*, and *Ammocharis*. Furthermore, apart from their Crininae, Müller-Doblies' and Müller-Doblies' (1996) subtribes Amaryllidinae, Strumariinae, and Boophoninae are not supported by the ITS sequence data and combined data. Their Amaryllidinae and Boophoninae are polyphyletic and their Strumariinae is paraphyletic. Both Snijman's (1994) *Strumaria* and *Hessea* appear polyphyletic. The manner by which Snijman's (1994) concepts of these genera are polyphyletic is consistent with only two of the previously recognized segregate genera, *Namaquanula* and *Gemmaria* (Müller-Doblies and Müller-Doblies, 1985). In the combined analyses, *Gemmaria* nevertheless resolves within a strongly supported *Strumaria* s.l., which is diagnosed by several morphological synapomorphies (filaments adnate to the style; style base swollen; basic chromosome number = 10). *Carpolyza*, however, appears to be embedded in *Strumaria* s.l. Given the weak support at some of the internal nodes within the Strumariinae clade (Figs. 4 and 6), no taxonomic changes are proposed at this time, except the submergence of *Carpolyza* into *Strumaria* and the reinstatement of *Namaquanula* sensu Müller-Doblies and Müller-Doblies (1985). Because valid names for the major monophyletic groups generated in the molecular and combined analyses have already been used at subtribal level in earlier classifications (Müller-Doblies and Müller-Doblies, 1996), we propose the recircumscription of four subtribes for the Amaryllideae. These are the monotypic Amaryllidinae and Boophoninae, Crininae (which incorporates *Crinum*, *Ammocharis*, and *Cybistetes*), and Strumariinae (which includes *Crossyne*, *Strumaria*, *Nerine*, *Hessea*, *Namaquanula*, and *Brunsvigia*). We justify this proposal on the grounds that the monophyly of each of these subtribal clades is well-supported in our combined analyses and that the revised classification conveys the current understanding of the interrelationships of groups of genera within the Amaryllideae more succinctly than by choosing to leave them unrecognized. Clearly, the phylogenetic relationships within the subtribe Strumariinae are complex, and further sampling, as well as a complementary plastid sequence matrix, are desirable to more clearly resolve the generic relationships within the group.

TAXONOMIC CHANGES

We provide a brief synopsis of the emended subtribes in the Amaryllideae followed by the full synonymy of *Carpolyza* that is embedded in *Strumaria*.

Subtribe **Amaryllidinae** Pax in Engler & Prantl, Nat. Pflanzenfam. 2, 5: 105. 1887 emend.

Leaf with a prominent midrib; flowers zygomorphic, without a perigone tube; stamens declinate, proximally connate into a rudimentary filament tube; scape not detaching from bulb during seed dispersal; fruit dehiscent; seeds large, pink or colorless, only the embryo green. Endemic to the winter rainfall region of southern Africa. *Amaryllis* L. (2 species).

Subtribe **Boophoninae** D. & U. Müll.-Doblies, Feddes Repertorium 107: S. c. 3. 1996 emend.

Leaves spreading into an erect fan. Inflorescence of numerous helicoid cymes; pedicels elongating and radiating after anthesis; flowers actinomorphic, with a perigone tube; stamens free; fruit indehiscent, trigonal, 3-ribbed; fruiting head detaching from top of scape; seeds endosperm-rich, partially chlorophyllous, cork-covered. Widespread in sub-Saharan Africa. *Boophone* Herb. (2 species).

Subtribe **Crininae** Pax in Engler & Prantl, Nat. Pflanzenfam. 2, 5: 108. 1887; Müller-Doblies & Müller-Doblies, Feddes Repertorium 107: S. c. 3. 1996.

Leaves often with an intercalary meristem, usually fringed with cartilaginous teeth, apex often truncate. Flowers actinomorphic to zygomorphic, with a perigone tube; stamens free; fruit indehiscent, irregular, often rostellate; scape not abscising during seed dispersal except in *Cybistetes* where it detaches at ground level; seeds lacking an integument, endosperm-rich, partially chlorophyllous, cork-covered. Widespread in the tropics and sub-Saharan Africa. *Crinum* L. (~65 species), *Ammocharis* Herb. (5 species), *Cybistetes* Milne-Redh. & Schweick. (1 species).

Subtribe **Strumariinae** Traub ex Müller-Doblies & Müller-Doblies, Bot. Jahrb. 107: 18. 1985 emend.

Leaves often prostrate. Flowers zygomorphic or actinomorphic, with or without a perigone tube; stamens connate into a tube proximally (except in *Strumaria* where one whorl of stamens is fused to the style); fruit dehiscent; seeds with a well-developed chlorophyllous integument and stomatose testa. Southern Africa. *Crossyne* Salisb. (2 species), *Strumaria* Jacq. (24 species), *Nerine* Herb. (~23 species), *Hessea* Herb. (13 species), *Namaquanula* D. & U. Müll.-Doblies (2 species), and *Brunsvigia* Heist. (~23 species).

Strumaria Jacq., Collecteana 5: 49 (1797). Type: *Strumaria truncata* Jacq. *Carpolyza* Salisb., The paradisus londinensis 1: t. 63 (1807). Type: *Carpolyza spiralis* (L' Hérit.) Salisb.

Strumaria spiralis (L' Hérit.) Aiton, Hortus Kewensis 2: 213 (1811). Type: figure in L' Hérit., Sertum anglicum 3 t. 13 (1792). *Amaryllis spiralis* L' Hérit., Sertum anglicum 1: 10 (1789); *Crinum spirale* (L' Hérit.) Andr., The botanists repository 2: t. 92 (1800); *Carpolyza spiralis* (L' Hérit.) Salisb., The paradisus londinensis 1: t. 63 (1807); *Hessea spiralis* (L' Hérit.) Bergius ex Schlechtend., Linnaea 1: 252 (1826).

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