

Generic relationships among the baccate-fruited Amaryllidaceae (tribe Haemantheae) inferred from plastid and nuclear non-coding DNA sequences

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Abstract. Using sequences from the plastid *trnL-F* region and nrDNA ITS, we investigated the phylogeny of the fleshy-fruited African tribe Haemantheae of the Amaryllidaceae across 19 species representing all genera of the tribe. ITS and a combined matrix produce the most resolute and well-supported tree with parsimony analysis. Two main clades are resolved, one comprising the monophyletic rhizomatous genera *Clivia* and *Cryptostephanus*, and a larger clade that unites *Haemanthus* and *Scadoxus* as sister genera to an *Apodolirion/Gethyllis* subclade. One of four included *Gethyllis* species, *G. lanuginosa*, resolves as sister to *Apodolirion* with ITS. Relationships among the *Clivia* species are not in agreement with a previous published phylogeny. Biogeographic analysis using the divergence/vicariance method roots the tribe in Eastern South Africa, with several subsequent dispersals to the winter rainfall Western Cape region. Chromosomal change from an ancestral $2n=22$ (characteristic of *Clivia*) is associated with each main clade. Reduction in number has occurred in all but *Cryptostephanus*, which has $2n=24$ chromosomes. Increasing the sampling across all of the species in the tribe will allow a more detailed understanding of the biogeographic patterns inherent in the parsimony topology, which undoubtedly reflect Quaternary climatic changes in Southern Africa.

Key words: Amaryllidaceae, Haemantheae, geophytes, South Africa, monocotyledons, DNA, phylogenetics, systematics.

Baccate fruits have evolved only once in the Amaryllidaceae (Meerow et al. 1999), and solely in Africa, but the genera possessing them have not always been recognized as a monophyletic group. *Haemanthus* L. and *Gethyllis* L. were the first two genera of the group to be described (Linnaeus 1753). Herbert (1837) placed *Haemanthus* (including *Scadoxus* Raf.) and *Clivia* Lindl. in the tribe Amaryllidiformes, while *Gethyllis* was classified with *Sternbergia* L. in Oporanthiformes. Salisbury (1866) recognized the distinct tribes Haemantheae Salisb. and Gethyllideae Salisb. Bentham and Hooker (1883) united *Cryptostephanus* Baker with *Narcissus* L. in their subtribe Coronatae, while maintaining *Haemanthus*, *Clivia* Lindl. and *Apodolirion* Baker in subtribe Genuinae. *Cryptostephanus* has perianthal appendages at the throat of the flower that Bentham and Hooker (1883) considered homologous to the corona of *Narcissus*. Pax (Pax 1887) situated *Haemanthus* and *Clivia* in his subtribe Haemanthinae

Pax, placed *Gethyllis* and *Apodolirion* in Zephyranthinae (on the basis of their fused spathe bracts and single-flowered inflorescences), and *Cryptostephanus* within Narcissinae, a treatment largely followed by Hutchinson (1934), though Pax's (1887) subtribes were elevated to the rank of tribe. All of these groups were polyphyletic, uniting genera from disparate lineages within the family (see discussion by Nordal and Duncan 1984).

Traub (1963) was the first to recognize the relationship between *Clivia* and *Cryptostephanus*, but placed both as the sole genera in tribe Clivieae Traub. *Haemanthus* was relegated to the monotypic Haemantheae, while *Gethyllis* and *Apodolirion* were placed alone in Gethyllideae, with the suggestion that the two genera were likely indistinct. Melchior (1964) placed both *Clivia* and *Cryptostephanus* in Haemantheae. Dahlgren et al. (1985) largely adopted Traub's (1963) classification, though Gethyllideae and Clivieae were subsumed in Haemantheae.

The two most recent formal classifications of the Amaryllidaceae are those of Müller-Doblies and Müller-Doblies (1996), and Meerow and Snijman (1998). Both recognized two tribes for the baccate-fruited genera: Haemantheae (*Haemanthus*, *Scadoxus*, *Clivia* and *Cryptostephanus*) and Gethyllideae (*Gethyllis* and *Apodolirion*). Müller-Doblies and Müller-Doblies (1996) further recognized two subtribes in Haemantheae, Haemanthinae D. & U.M.-D. (*Haemanthus* and *Scadoxus*) and Cliviinae D. & U.M.-D. (*Clivia* and *Cryptostephanus*). *Scadoxus* was segregated from *Haemanthus* by Friis and Nordal (1976). All of the baccate-fruited genera are endemic to Africa.

Meerow et al. (1999), using three plastid DNA sequences, confirmed the monophyly of Haemantheae, but indicated that Gethyllideae was embedded within the former tribe, and thus could not be recognized without rendering Haemantheae paraphyletic. The level of sampling and the number of phylogenetically informative base substitutions were insufficient to resolve the relationships within the tribe in

that study beyond the well-supported sister relationship of *Apodolirion* and *Gethyllis* which together terminated a successive grade beginning with *Clivia*, followed by *Cryptostephanus*, *Scadoxus* and *Haemanthus*. However, bootstrap support for each branch in the grade was moderate to strong. Ito et al. (1999), using plastid *matK* sequences also resolved a monophyletic Haemantheae, though only three genera were sampled. *Haemanthus* and *Scadoxus* were sister taxa in their study with 98% bootstrap support.

As treated here, Haemantheae consists of six genera. *Cryptostephanus* (2 spp.) and *Clivia* (5 spp.) are bulbless, rhizomatous perennials. With the exception of the newly described *Clivia mirabilis* Rourke, both genera are found in summer rainfall regions. *Clivia* is adapted to butterfly and sunbird pollination, and has showy orange and yellow flowers. The species are chiefly understory herbs of coastal and Afro-montane forest. The two species of *Cryptostephanus* are either savanna or forest herbs. The small flowers have a paraperigone, and it is the only genus in the tribe whose seeds have a phytomelanous testa. *Scadoxus* (9–12 spp.) and *Haemanthus* (21 spp.) have long been recognized intuitively as sister taxa (in the past treated as a single genus; e.g. Björnstad and Friis 1972). Both genera have brush-like inflorescence morphology, in which the bracts often form part of the pollinator attraction system. *Scadoxus* are forest understory herbs, some species of which do not form true bulbs. The genus is most common in the African tropics. *Haemanthus*, all species forming bulbs, is strictly southern African, with species in both the summer and winter rainfall regions of the Cape (Snijman 1984). Finally, *Gethyllis* (ca. 35 species, Müller-Doblies 1986) and *Apodolirion* (ca. 6 species, Müller-Doblies 1986) are two closely related uni-flowered Cape endemics that both retain the ovary inside the bulbs until the large, fleshy, aromatic fruit matures. They are differentiated by the capitate stigma in *Gethyllis* (vs. tri-lobed in *Apodolirion*) and the often numerous stamens in *Gethyllis* (vs. six in *Apodolirion*). *Gethyllis* is most common

in the winter rainfall region of South Africa, *Apodolirion* in the summer rainfall zone.

The purpose of this present study was to establish baseline generic relationships of the genera of the Haemantheae by increasing the sampling for the plastid *trnL-F* region, and adding sequences from the internally transcribed spacer (ITS) of nuclear ribosomal DNA.

Materials and methods

Sampling. Genomic DNA was extracted from silica gel dried leaf tissue of the taxa listed in Table 1 as described by Meerow et al. (2000).

DNA extraction, amplification and sequencing protocols. The *trnL-trnF* region was amplified and sequenced using the primers of Taberlet et al. (1991) as described by Meerow et al. (1999). 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 intron as described by Meerow et al. (2000). All polymerase chain reaction (PCR) amplifications were performed on an ABI 9700 (Perkin-Elmer Applied Biosystems, Foster City, California, USA).

Amplified products were purified using QIAquick (Qiagen, Valencia, California, USA) columns, following manufacturers' protocols. 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 310 or ABI 3100 automated sequencer (according to the manufacturer's protocols; Applied Biosystems, Foster City, California, USA).

Sequence alignment. Both the ITS and *trnL-F* matrices were readily aligned manually using Sequencher 4.1 (Gene Codes, Ann Arbor, Michigan, USA). The alignment is accessible through GenBank or from the first author (miaam@ars-grin.gov).

Analyses. The ITS matrix consisted of 19 taxa (two *Apodolirion* spp., four *Clivia* spp., two *Cryptostephanus* spp., four *Gethyllis* spp., three *Haemanthus* spp., three *Scadoxus* spp., and one species of *Amaryllis*, the latter designated as outgroup).

The plastid *trnL-F* matrix consisted of the same 19 taxa, plus the addition of *Haemanthus humilis*. *Amaryllis* is the basal most genus within the tribe Amaryllideae (Meerow and Snijman 2001) that in turn is sister to the rest of the Amaryllidaceae. The sister group relationships of Haemantheae are so far unresolved (Meerow et al. 1999). We experimented with a species of *Cyrtanthus* Herb. (Cyrtantheae) and *Calostemma* R. Brown (Calostemmatae) as outgroups, but found that *Amaryllis* presented the least number of alignment ambiguities and generated the shortest trees. Resolution of the sister relationships of Haemantheae remain unclear (Meerow et al. 1999); however the tribe Amaryllideae is sister to all other genera in the family with high bootstrap support, even with as highly conserved a gene as *rbcL* (Meerow et al. 1999). At present we are working to successfully align ITS sequences across the entire Amaryllidaceae in order to resolve the basal polytomy that resolves after the branching of tribe Amaryllideae with all plastid sequences that have been applied to the problem to date (Ito et al. 1999; Meerow et al. 1999, 2000). We feel it is better to use as outgroup the most basal genus in a tribe that is indisputably outside of the ingroup of interest.

Aligned matrices were analyzed using the parsimony algorithm of PAUP* for Macintosh (version 4.0b10; Swofford 1998), with the MULPARS option invoked. Tree branches were retained only if unambiguous support was available (i.e. branches were collapsed if the minimum length = 0). Gaps were coded as missing characters in the initial analyses, but a gap matrix was also constructed from each alignment using the program PAUPGAP (Anthony Cox, RBG Kew), which applies a strict interpretation of gaps (i.e. no partial homology). This binary matrix was added to the sequence alignment and analyzed in combination. For all matrices, a branch and bound (Hendy and Penny 1982) search was conducted under the Fitch (equal) weights (Fitch 1971) criterion with furthest addition sequence.

We also combined the two data matrices, opting for the "total evidence" approach (Dubuisson et al. 1998, Seelanan et al. 1997). However, before combining the ITS and *trnL-F* data sets (and the gap matrices with the sequence alignments), we performed partition homogeneity tests on the matrices (Farris et al. 1994, 1995) to assess the degree of congruence between them. Five hundred

Table 1. Species, voucher specimens and GenBank sequence accession numbers used in the phylogenetic analyses of Haemantheae. Vouchers are deposited at NBI unless otherwise stated

Taxon	Voucher Specimen or Accession	GenBank Accession No. or Literature Citation		Area Code ¹
		ITS	<i>trnL-F</i> (<i>trnL</i> , <i>trnL-F</i> spacer)	
<i>Amaryllis belladonna</i> L.	M. W. Chase 612 (K)	Meerow and Snijman (2001)	Meerow et al. (1999)	A
<i>Apodolirion cedarbergense</i> D. M.-D.	Dulse s. n.	AY280344	AY278957, AY278971	A
<i>A. lanceolatum</i> (Thunb.) Baker	NBG 714/88	AY280345	Meerow et al. (1999)	A
<i>Clivia caulescens</i> R. A. Dyer	Rourke 2167	AY280346	AY278958, AY278973	B
<i>C. gardenii</i> Hook.	Rourke 2160	AY280357	AY278960, AY278974	B
<i>C. miniata</i> Regel	Rourke 2143	AY280348	AY278961, AY278975	B
<i>C. nobilis</i> Lindl.	M. W. Chase 3080 (K)	AY280349	Meerow et al. (1999)	B
<i>Cryptostephanus haemanthoides</i> Pax	Koopowitz 11040	AY280350	AY278962, AY278976	D
<i>C. vansonii</i> Verdoorn	Meerow 2310 (FTG)	AY280351	Meerow et al. (1999)	C
<i>Gethyllis briteniana</i> Baker	Van Jaarsveld 5618	AY280352	AY278963, AY278977	A
<i>G. ciliaris</i> (Thunb.) Thunb.	Duncan 1123	AY280353	Meerow et al., (1999)	A
<i>G. lanuginosa</i> Marl.	Van Jaarsveld 4377	AY280354	AY278964, AY278978	A
<i>G. verticillata</i> R. Br. ex Herb.	Meerow 2350 (FTG)	AY280355	AY278965, AY278979	A
<i>Haemanthus albiflos</i> Jacq.	Meerow 2351 (FTG)	AY280356	AY278966, AY278980	B
<i>H. graniticus</i> Snijman	Snijman 308	AY280357	AY278967, AY278981	AB
<i>H. humilis</i> Jacq.	M. W. Chase 2025 (K)	–	Meerow et al. (1999)	B
<i>H. pumilio</i> Jacq.	Snijman 668	AY280358	AY278968, AY278982	A
<i>Scadoxus cinnabarinus</i> (Decne.) Friis & Nordal	M. W. Chase 549 (K)	AY280360	Meerow et al. (1999)	E
<i>S. membranaceus</i> (Bak.) Friis & Nordal	NBG 708/88	AY280360	AY278969, AY278983	B
<i>S. puniceus</i> (L.) Friis & Nordal	NBG 43/72	AY280361	AY278970, AY278984	BE

¹A = Western South Africa, B = Eastern South Africa, C = Zimbabwe, D = East Africa, E = Tropical Africa

heuristic searches were conducted for each test, each with 10 random addition replications, saving no more than 20 trees from each for TBR branch swapping.

Internal support was determined by bootstrapping (Felsenstein 1985; 5000 replicates with simple addition) and calculation of Bremer (1988) decay indices (DI) using the program TreeRot v. 2.1 (Sorenson 1996). The cut-off bootstrap percentage is 50. A bootstrap 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). Five hundred replicate heuristic searches were implemented for each constraint statement postulated by TreeRot, saving 10 trees per replicate. A minimum DI = 2 was considered to represent good support for a clade.

The biogeographic patterns inferred from our gene trees were assessed using both Fitch optimization (Maddison et al. 1992) with MacClade version 4.03 (Maddison and Maddison 2001) and the dispersal-vicariance method of analysis (Ronquist 1997) using the program DIVA version 1.1 (Ronquist 1996). The program uses vicariance (i.e. allopatric speciation) in its optimization of ancestral distributions but takes into consideration dispersal and extinction events and indicates their direction (Ronquist 1996, 1997). The most parsimonious reconstructions minimize such events. Unlike Fitch optimization, DIVA does not restrict widespread distributions to terminals or limit ancestral distributions to single areas (Ronquist 1996). The single tree from the combined sequence analysis was used for optimization of five coded geographic areas (Table 1). Fitch optimization of area data was performed on the same tree using a single multistate character (Table 1). An exact optimization (versus heuristic) was invoked in the analysis by allowing the maximum number of alternative reconstructions to be held at any node. The maximum areas allowed at ancestral nodes was set to the minimum (2) in order to reduce ambiguities at the more basal nodes of the tree (DIVA tends to optimize all possible areas at the lower nodes of the tree if the maximum value is used). Five biogeographic areas were coded for the analysis (Table 1), based on the distributions of the taxa in our sequence matrix. Western South Africa is equivalent to the winter-rainfall region; Eastern South Africa, to the summer-rainfall zone.

Results

Plastid *trnL-F*. Of the 975 characters included in the analysis, 23 were parsimony informative. Three equally parsimonious trees were found of length = 96 steps, consistency index (CI) = 0.94, and retention index (RI) = 0.86 (Fig. 1A). The tree is not well-resolved, and only two genera form monophyletic groups, *Cryptostephanus* (bootstrap = 95%, DI = 3) and *Scadoxus* (no bootstrap support, DI = 1). *Apodolirion* and *Gethyllis* form a clade (bootstrap = 81%, DI = 2), but neither genus is resolved as monophyletic. When a binary gap matrix is added to the sequence alignment, the number of parsimony informative characters increases to 57, out of a total of 1039. The gap matrix is mostly incongruent with the sequence alignment ($P = 0.136$ in the partition homogeneity test). Two trees were found (partially shown in Fig. 1B), of length = 199, CI = 0.77 and RI = 0.65. Support for a monophyletic *Cryptostephanus* is increased (bootstrap = 99%, DI = 5), *Clivia* is resolved in one of the two trees as monophyletic without support (Fig. 1B), and *Apodolirion* and *Gethyllis* are each resolved as monophyletic sister clades (individually without support), but with a lower bootstrap (65%). The monophyly of *Scadoxus* is lost with the addition of the gap matrix.

ITS. Of the 749 characters (ITS1, 5.8S gene, ITS2) included in the analyses, 153 were parsimony informative. The search found 12 equally most parsimonious trees of length = 513, CI = 0.76 and RI = 0.73 (Fig. 2A). The larger number of characters results in a much more resolved tree topology than that from *trnL-F*. Two main clades are resolved. One unites *Cryptostephanus* and *Clivia* with a bootstrap of 53% and DI = 1. *Clivia* is monophyletic with strong support (bootstrap = 96%, DI = 6), but a monophyletic *Cryptostephanus* has no support and is not resolved in all twelve trees.

The second clade, well supported with a bootstrap = 96% and DI = 6, consists of two subclades. One unites *Apodolirion* and *Gethyllis*

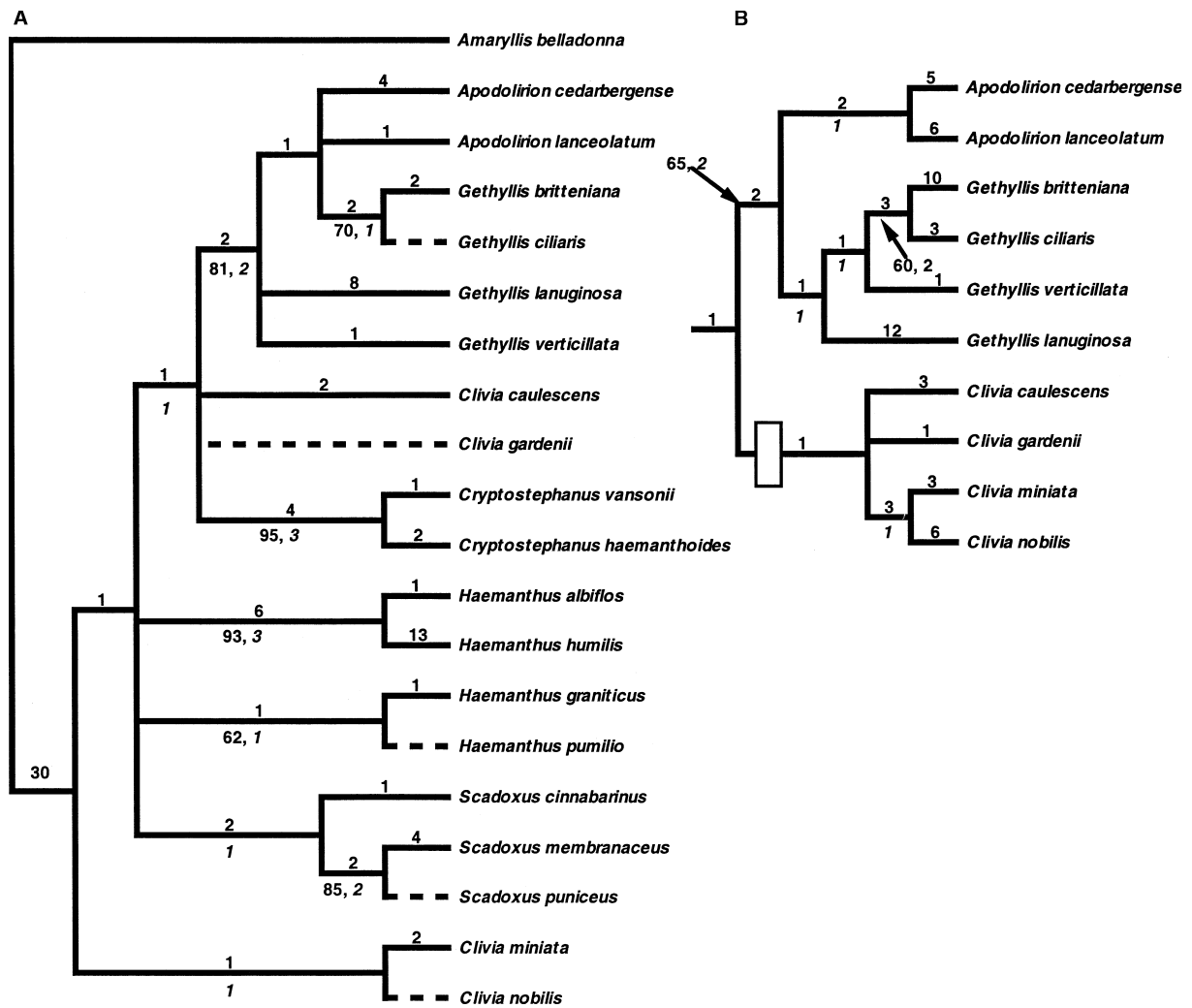


Fig. 1. Trees found by phylogenetic analysis of plastid *trnL-F* DNA sequences across 19 species of Haemantheae, with *Amaryllis belladonna* used as outgroup. **A** One of three most parsimonious trees found with gaps coded as missing data. **B** Increased resolution gained by adding a binary strict gap matrix to the sequence alignment (two trees found). Numbers above branches are branch lengths. Numbers below branches are bootstrap percentages and decay indices (*italic*), respectively. Dashed lines are zero-length branches. A white bar across a branch signifies a collapsed node in the strict consensus of all trees

with a bootstrap of 97% and DI = 6, though one species of *Gethyllis* (*G. lanuginosa*) is embedded within *Apodolirion* (bootstrap = 93%, DI = 4). The remaining three *Gethyllis* species are united with a bootstrap = 93% and DI = 3. The second subclade is weakly supported (bootstrap = 56%, DI = 1), and unites *Haemanthus* and *Scadoxus* as sister groups, each strongly supported (Fig. 2).

The ITS gap matrix and sequence alignment are largely incongruent ($P = 0.038$), and

addition of the matrix resulted in 191 parsimony informative characters of a total of 844. Three equally parsimonious trees were found of length = 642, CI = 0.77 and RI = 0.73 (partially shown in Fig. 2B). The only topological changes from the trees generated by sequence matrix alone are 1) the breakup of *Cryptostephanus* such that *C. vansonii* is resolved as sister to the rest of the tribe (with no support), 2) *Gethyllis ciliaris* and *G. verticillata* switch positions, and 3) higher resolution within

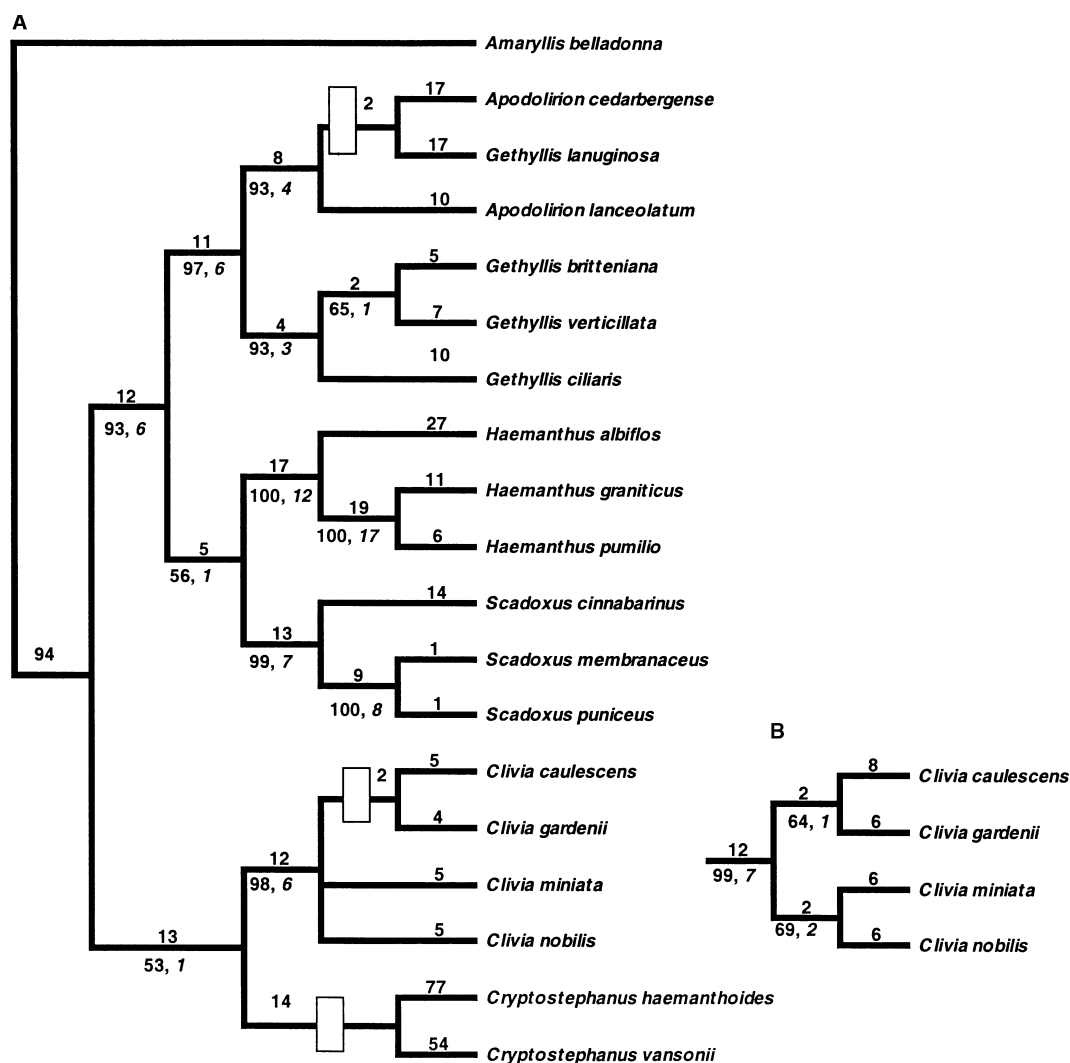


Fig. 2. Trees found by phylogenetic analysis of nrDNA ITS sequences across 18 species of Haemantheae, with *Amaryllis belladonna* used as outgroup. **A** One of twelve most parsimonious trees found with gaps coded as missing data. **B** Increased resolution gained by adding a binary strict gap matrix to the sequence alignment (three trees found). Numbers above branches are branch lengths. Numbers below branches are bootstrap percentages and decay indices (*italic*), respectively. A white bar across a branch signifies a collapsed node in the strict consensus of all trees

Clivia (Fig. 2B). *C. caulescens* and *C. gardenii* become sister species (bootstrap = 64%, DI = 1), and *C. miniata* is united with *C. nobilis* (bootstrap = 69, DI = 2).

Combined analysis. The P value = 0.46 indicates a moderate level of congruence between the ITS and *trnL-F* sequence matrices. A single most parsimonious tree was found of length = 612, CI = 0.80, RI = 0.73 (Fig. 3). The tree is fully resolved, with the same two main

clades resolved by ITS alone (Fig. 2). *Cryptostephanus* is resolved as monophyletic with moderate support (bootstrap = 69, DI = 4), and the support for its sister relationship to *Clivia* also increases (bootstrap = 73%, DI = 5). *Apodolirion* receives weak bootstrap support, but *Gethyllis* is still resolved as paraphyletic, due to the resolution of *G. lanuginosa* as sister to *Apodolirion*. Support for the sister relationship of *Scadoxus* and

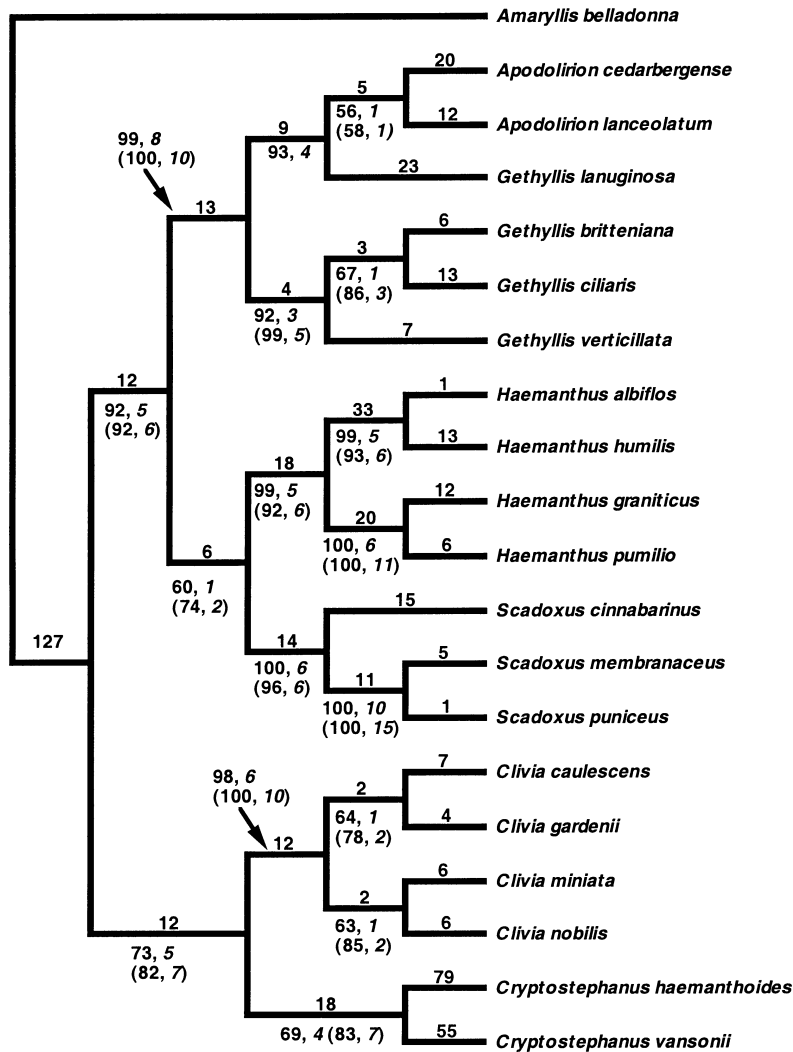


Fig. 3. Single most parsimonious tree found by phylogenetic analysis of combined nrDNA ITS and plastid *trnL-F* sequences across 19 species of Haemantheae with *Amaryllis belladonna* used as outgroup, and gaps coded as missing characters. Numbers above branches are branch lengths. Numbers below branches are bootstrap percentages and decay indices (italic), respectively. If these changed when a strict gap matrix was added to the sequence alignment, the revised bootstrap and DI are shown between parentheses

Haemanthus remains weak, however. If both gap matrices are added to the combined sequence data set, there is increased support for all of the clades (Fig. 3), but no change in tree topology.

Biogeographic analysis. DIVA hypothesizes six vicariance events to account for the optimal reconstruction of area on the combined sequence topology (Fig. 4), and roots the ancestral node of Haemantheae in Eastern South Africa (equivocal with Fitch optimization). Dispersal to the Western Cape occurred twice, once for the ancestor of *Apodolirion* and *Gethyllis*, and again within *Haemanthus*. The Western Cape *Clivia mirabilis*, not included in our analysis, would ostensibly represent a third

vicariance event. Moreover, the remaining four *Apodolirion* species not included in our analysis are from the summer rainfall areas of the Eastern Cape, as are two of the 32 described species of *Gethyllis*, thus vicariance events between the winter and summer rainfall regions of South Africa are undoubtedly greater than three.

Discussion

Our combined *trnL-F* and ITS analysis (Fig. 3), the most completely resolved and best supported tree for Haemantheae, divides the tribe into two main clades. The smaller clade, uniting *Clivia* and *Cryptostephanus*, represents

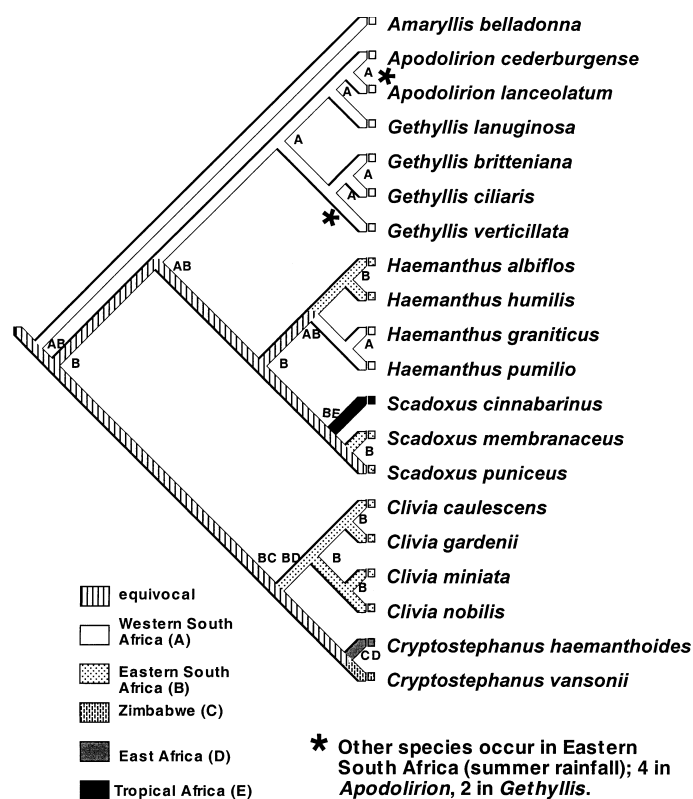


Fig. 4. Single most parsimonious tree found by phylogenetic analysis of combined nrDNA ITS and plastid *trnL-F* sequences across 19 species of Haemantheae with *Amaryllis belladonna* used as outgroup showing optimization of biogeographic data. Fitch optimization is indicated by pattern; dispersal-vicariance optimization is coded by small letters at ancestral nodes

entirely rhizomatous genera that never form bulbs (Fig. 5A). Meerow et al. (1999) consider the bulbless state plesiomorphic for the family. *Cryptostephanus* is also the only genus of the tribe that retains the plesiomorphic character of a phytomelanous testa (Fig. 5B; Meerow and Snijman 1998; loss of phytomelan in the tribe Amaryllideae is considered an independent event). The second clade contains all of the genera that form true bulbs (Fig. 5A), though *Scadoxus* is polymorphic for this character and has been misdiagnosed as being entirely rhizomatous (Friis and Nordal 1976). It is unclear whether bulbs form in *Scadoxus* only under certain environmental conditions or if bulb formation is limited to just certain species.

The second clade contains two subclades that can be characterized morphologically as well (Fig. 5). The sister relationship of *Haemanthus* and *Scadoxus* is only resolved by ITS and by the combined analysis, but is well supported by the morphological synapomor-

phy of the brush-like inflorescence (Fig. 5C), facilitated by the reduction in perianth size (all species) and the dominance of the spathe bracts during anthesis [this occurs in at least some of the species of each genus (Friis and Nordal 1976, Nordal and Duncan 1984)]. Within *Haemanthus*, well-supported sister clades are resolved that correspond to Eastern Cape (*H. albiflos*, *H. humilis*) vs. Western Cape (*H. graniticus*, *H. pumilio*) endemics (Snijman 1984). The gethyllid subclade is characterized by a suite of morphological characters: uniflory, obsolete scape (Fig. 5D), and the long, aromatic, cylindrical, many-seeded fruit of both recognized genera, in contrast to the one or few seeded berry of the other genera in the tribe.

Chromosomal change appears to have figured importantly in cladogenesis within Haemantheae (Fig. 5E). *Clivia* has $2n = 22$ chromosomes, the plesiomorphic number for the family (Meerow 1995), while *Cryptostephanus* has $2n = 24$, which may have been derived

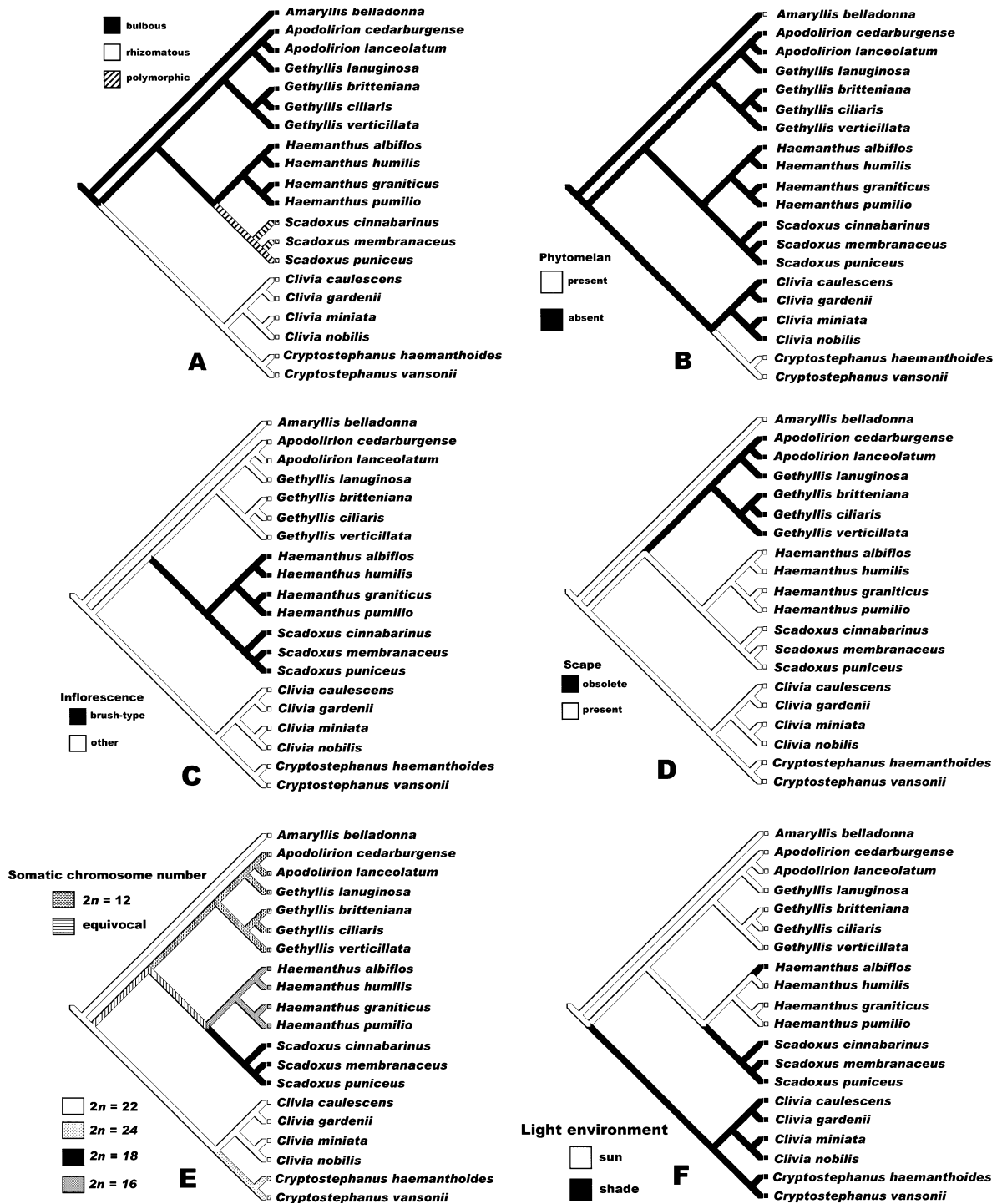


Fig. 5. Fitch optimization of selected characters on the single most parsimonious tree found by phylogenetic analysis of combined nrDNA ITS and plastid *trnL-F* sequences across 19 species of Haemantheae with *Amaryllis belladonna* used as outgroup. A Presence of bulb. B Presence of phytomelan in the testa. C Inflorescence morphology. D Scape development. E Somatic chromosome number. F Light environment

from the ancestral $x=11$ (Gouws 1949). The inferred trend is reduction in number from the ancestral $2n=22$. Only $2n=12$ chromosomes has been found in either genus of the erstwhile Gethyllideae (Wilsenach 1965, Vosa 1986). *Scadoxus* and *Haemanthus* have $2n=18$ and 16 chromosomes, respectively (Vosa and Marchi 1980). Vosa and Marchi (1980) demonstrated that two small teleocentric chromosomes in the karyotype of *Scadoxus* are homologous to one large, metacentric chromosome in the complement of *Haemanthus*. Vosa and Marchi (1980) considered this to be an incidence of dispoloid reduction, and identified the two short chromosomes of *Scadoxus* that were likely homologous to a single long chromosome in *Haemanthus*. Vosa and Snijman (1984) documented further recombination events in the evolutionary history of *Haemanthus* that could be correlated with speciation patterns. Translocations appeared to be frequent occurrence in the genus.

Gouws (1949) noted the striking similarities between the karyotype of *Clivia* and *Cryptostephanus*. The latter genus has more acrocentric/sub-telocentric chromosomes than *Clivia*. The “extra” chromosome in the haploid complement of *Cryptostephanus* does not appear typical of a supernumerary (“B”) chromosome (Jones and Rees 1982). None of the chromosomes in the haploid karyotype of *C. vansonii* are telocentric, and the three shortest, sub-telocentric chromosomes all have apparent homologs in the diploid complement (Gouws 1949). An alternative origin for $2n=24$ in *Cryptostephanus* is a tetraploid derivation from the $2n=12$ that demarcates the gethyllids. However, our tree topology (Fig. 3) would suggest the origin of this number in *Apodolirion* and *Gethyllis* occurred after the divergence of *Clivia* and *Cryptostephanus*.

As was suggested by previous plastid analyses (Meerow et al. 1999), recognition of a distinct tribe Gethyllideae for *Apodolirion* and *Gethyllis* would render the Haemantheae paraphyletic. The two genera do, however, form a monophyletic subclade that is sister to *Haemanthus/Scadoxus* (Fig. 3). Unlike all of

the other genera in the tribe which have a few-seeded berry fruit, the fleshy fruit of both *Apodolirion* and *Gethyllis* is a long, aromatic, cylindrical, many-seeded structure (Meerow and Snijman 1998). The seeds of these genera are small and hard, in contrast to the larger, water-rich, more or less fleshy seeds of the rest of the genera in Haemantheae. The scape remains inside the bulbs of *Gethyllis* and *Apodolirion*, and both are uni-flowered with fused spathe bracts. At least some species of *Gethyllis* have 18 or more stamens. Traub (1963) expressed doubt about maintaining *Apodolirion* and *Gethyllis* as distinct genera, an argument also taken up to some extent by Hilliard and Burt (1973). Wilsenach (1965) found little variation among in the karyotypes of representatives of both genera. While our sampling is hardly complete, there are two well supported clades resolved within the gethyllids (Fig. 3); however *G. lanuginosa* is sister to *Apodolirion* in the ITS and combined phylogeny. In the *trnL-F* sequence with gap matrix (Fig. 1B), the two genera are resolved as distinct sisters. This is clearly a question that will benefit from a full sampling of all known species of both genera, and ultimately, recognition of a single genus may be warranted.

Our combined analysis resolves two subclades within *Clivia* with moderate to strong support (Fig. 3). These sister species relationships are not in agreement with the maximum likelihood topologies postulated by Ran et al. (2001) in their study of *Clivia* using ITS and 5.8S nrDNA sequences, in which sister relationships are the reverse of those in Fig. 3. While our *C. caulescens* sequence appears congruent with that of Ran et al. (2001), downloaded from GenBank, our sequence of *C. nobilis* is most congruent with Ran et al.’s (2001) *C. gardenii*. Our *C. miniata* and *C. gardenii* sequences are also considerably divergent from those of Ran et al.’s (2001). Koopowitz (2002) points out that *C. miniata* overlaps with *C. nobilis* in the southern part of the former’s range, and with *C. gardenii* at its more northerly limits. Though the three species are somewhat ecologically specialized,

mixed populations of *C. miniata* and either of the other two species have been observed (Koopowitz 2002). Though Koopowitz (2002) concludes that natural hybridization is rare among *Clivia* species, some degree of historical introgression of genes of one species into another can not be ruled out. Conrad and Reeves (2002), using four non-coding plastid regions (a total of 17 phylogenetically informative base substitutions), resolved yet a third topology for *Clivia*, in which *C. miniata* and *C. caulescens* are sister species, with *C. gardenii*, *C. nobilis*, and finally *C. mirabilis* Rourke [recently described from the Western Cape (Rourke 2002)] forming a successive grade. Understanding the genetic relationships among *Clivia* species would clearly benefit from a population genetic marker approach such as microsatellite DNA.

A detailed biogeographic analysis of Haemantheae is premature given that *Apodolirion*, *Gethyllis*, *Haemanthus* and *Scadoxus* were not fully sampled, especially in regard to species of both *Apodolirion* and *Gethyllis* from Eastern South Africa. However, both Fitch optimization of biogeographic data onto our combined tree and divergence/vicariance analysis (Fig. 4) indicates that the probable origins of the tribe are in eastern South Africa. Only two genera occur outside of South Africa. *Scadoxus* is found from the Arabian peninsula west to Senegal and south to the East Cape (Friis and Nordal 1976, Nordal and Duncan 1984), but only three of the nine recognized species occur in South Africa (*S. membranaceus*, *S. multiflorus* (Martyn) Raf. and *S. puniceus*). *Cryptostephanus* is absent from South Africa completely, and is distributed from South Central to East Africa (Koopowitz 2002).

Prior to the Pliocene, Africa's southwestern region was a more mesic, subtropical environment (Coetzee 1978, 1983, 1986; Hendey 1983; Scholtz 1985). The earliest evidence of modern semi-arid, winter-rainfall pattern dates to the Late Pliocene [though Coetzee (1993) hypothesizes an earlier establishment of the Benguella current, as well as replacement of forest by

savanna and grassland in the mid-Tertiary], but it may not have been 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, Crockcroft et al. 1987). Divergence of the three main clades within Haemantheae may thus have occurred during the Pliocene, while speciation within their component genera might have been engendered by more recent paleoclimatic events. However, a detailed history of these late Pleistocene and Quaternary events in the Cape region is elusive (Cowling et al. 1999). In all of the African tribes of Amaryllidaceae, most genera have species in both the winter and summer rainfall regions; only in the tribe Amaryllideae does Western Cape endemism occur at the generic level (Snijman and Linder 1996).

Patterson and Givnish (2002) discussed the correlation of rhizomatous growth habit, baccate fruits and net-veined leaves with colonization of low light habitats in Liliales. In Haemantheae we can see partial support for a similar scenario (Fig. 5F), insofar as three genera of the tribe (*Clivia*, *Cryptostephanus* and *Scadoxus*) are predominately plants of low-light habitats and lack bulbs (completely or in part; Fig. 5A). However, net venation only occurs in *Scadoxus*, and at least one species of *Haemanthus* (*H. albiflos*) has secondarily colonized shady habitats (Fig. 5F). Given the position of *Clivia* and *Cryptostephanus* as sister to the rest of the tribe, there is at least a reasonable possibility that the bulbless condition and the evolution of berry fruits, in conjunction with a forest understory habitat, was a basal event in the divergence of the Haemantheae from the rest of the family.

Taxonomically, our sequence phylogeny would support the recognition of three subtribes in Haemantheae: Cliviinae D. & U. M.-D., Haemanthinae Pax and Gethyllidinae. Only the latter has yet to be formally named.

Amaryllidaceae J. St.-Hil., tribe Haemantheae, subtribe Gethyllidinae (Dumort.) Meerow, stat. nov. Tribe Gethyllideae Dumort., Anal. Fam., Pl. 58, 1829. Type: *Gethyllis* L., 1753.

In conclusion, our combined analysis results in a well-resolved, well-supported phylogenetic framework for the Haemantheae that can be augmented further by increasing the depth of sequence sampling for *Apodolirion*, *Gethyllis*, *Haemanthus* and *Scadoxus*. Such a project is underway in South Africa (G. Reeves, personal communication), and will hopefully clarify some the questions remaining about generic and species relationships within the tribe, as well as illuminate the phytogeographic history of the baccate-fruited amaryllids.

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