The Never-ending Story: Multigene Approaches to the Phylogeny of Amaryllidaceae

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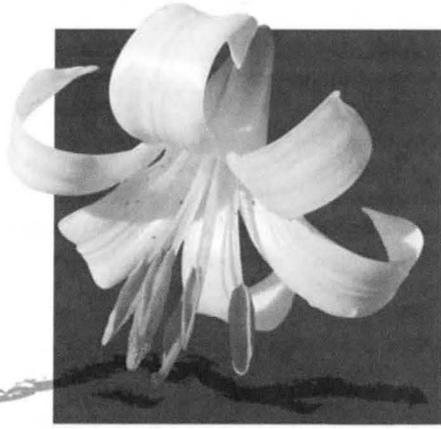### Excluding Poales

Asparagales
Alliaceae/Amaryllidaceae

THE NEVER-ENDING STORY: MULTIGENE APPROACHES TO THE PHYLOGENY OF AMARYLLIDACEAE

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ABSTRACT

Results to date with various plastid genes confirmed the monophyly of the Amaryllidaceae s.s. as a whole, strongly supported the mostly African tribe Amaryllideae as sister to the rest of the family, and resolved geographically-based monophyletic groups, but failed to resolve the relationships among several basal lineages in the family (the African Haemantheae and Cyrtantheae, the Australasian Calostemmateae, and the American and Eurasian sister clades). We present analysis of plastid *ndh*F sequences that fully resolved the major clades of the family. The baccate-fruited Haemantheae and Calostemmateae are sister tribes, and the African endemic Cyrtantheae is sister to them both. This clade is sister to an American/Eurasian clade. We also present preliminary nuclear ribosomal ITS sequence analysis of the Eurasian clade. Lycorideae are basal in the group and begin a grade that continues with *Hannonia*, then *Pancratium*, then *Lapiedra*. The genera *Galanthus*, *Narcissus*, and *Sternbergia* are resolved as monophyletic with strong support. *Leucojum* is paraphyletic and recognition of *Acis* for the mostly autumn-flowering Mediterranean species is supported. Recent phylogenetic analyses of various tribes and genera of the family are reviewed. Above the family level, Agapanthaceae, Alliaceae, and Amaryllidaceae form a well-supported monophyletic group, but exact resolution of the relationships among the three subclades varies depending on the sequence matrix utilized. The Angiosperm Phylogeny Group II has advocated combining all three into a single family, Alliaceae. We discuss this decision, which has historical precedent, but recommend that Amaryllidaceae be conserved as the name for the family in such a treatment.

Key words: Amaryllidaceae, Asparagales, cladistics, DNA, monocotyledons, phylogeny.

INTRODUCTION

Amaryllidaceae are one of the few families of the higher Asparagales well defined by other than molecular characters, namely the combination of umbellate cymes, inferior ovaries, and unique alkaloid chemistry (Meerow and Snijman 1998), but morphological characters alone fail to adequately resolve phylogenetic relationships within the family (Meerow et al. 2000a). Some of these characters, such as inflorescence type, are likely synapomorphies for a deeper grouping of families (Agapanthaceae, Alliaceae) that could be subsumed in Amaryllidaceae (though this name would need to be conserved over Alliaceae). The four most recent infrafamilial classifications of Amaryllidaceae are those of Traub (1963), Dahlgren et al. (1985), Müller-Doblies and Müller-Doblies (1996), and Meerow and Snijman (1998). Traub's scheme included Alliaceae, Hemerocallidaceae, and Ixoliriaceae as subfamilies, in part following Hutchinson (1934, 1959). Within his subfamily Amaryllidoideae, he erected two informal taxa, "infrafamilies" Amarylloidinae and Pancratioidinae, both of which were polyphyletic (Meerow 1995). Dahlgren et al. (1985) dispensed with any subfamilial classification above the level of tribe, recognizing eight, and treated as Amaryllidaceae only those genera in Traub's Amaryllidoideae. Stenomesseae and Eustephieae were combined. Meerow (1995) resurrected Eustephieae from Stenomesseae and suggested that two new tribes might need to be recognized, Calostemmateae and Hymenocallideae. Müller-Doblies and Müller-Doblies (1996) recognized ten tribes

(among them Calostemmateae) and 19 subtribes, many of them monogeneric; Meerow and Snijman (1998) recognized 13 tribes, with two subtribes only in one of them. Discussion of character evolution within the family can be found in Meerow (1995), Meerow and Snijman (1998), and Meerow et al. (1999).

The precise relationship of Amaryllidaceae to other Asparagales remained elusive until Fay and Chase (1996) used molecular data to argue that Agapanthaceae, Alliaceae, and Amaryllidaceae form a monophyletic group (also evident in Chase et al. 1995) and that together they are related most closely to Hyacinthaceae s.s. and the resurrected family Themidaceae (the former tribe Brodiaeeae of Alliaceae). They recircumscribed Amaryllidaceae to include *Agapanthus* L'Hér., previously included in Alliaceae, as subfamily Agapanthoideae. This recircumscription was based on phylogenetic analysis of plastid *rbc*L sequence data, with only four genera of Amaryllidaceae s.s. included in the analysis. All the epigynous genera were treated as Amaryllidoideae. Subsequent analyses of multiple DNA sequences from both the chloroplast and nuclear genomes have shown quite strongly that *Agapanthus*, Amaryllidaceae, and Alliaceae represent a distinct lineage within the monocot order Asparagales (Meerow et al. 1999; Fay et al. 2000), but the exact relationships among the three groups have been difficult to resolve with finality (Graham et al. 2006).

Meerow et al. (1999) presented cladistic analyses of plastid DNA sequences *rbc*L and *trn*L–F alone and in combi-

nation for 51 genera of Amaryllidaceae and 31 genera of related asparagalean families. The combined analysis was the most highly resolved of the three and provided good support for the monophyly of Amaryllidaceae and indicated Agapanthaceae as its sister family (though bootstrap support for this relationship was still weak at 60%). Alliaceae were in turn sister to the Amaryllidaceae/Agapathaceae clade. In Fay et al.'s (2000) analysis of Asparagales using four chloroplast DNA regions, Alliaceae were resolved as sister to Amaryllidaceae, with *Agapanthus* sister to them both. Again, bootstrap support was weak at best. Based on these data, it would be possible to argue for recognizing Amaryllidaceae in a modified Hutchinsonian (1934) sense, i.e., with three subfamilies, Agapanthoideae, Allioideae, and Amaryllidoideae. Meerow et al. (1999) opted to recognize a monotypic Agapanthaceae. The latest Angiosperm Phylogeny Group (AGP II 2003) recommends treating all three as a single family, Alliaceae (which currently has nomenclatural priority), but also optionally suggest recognition of "Amaryllidaceae s.s." using their "bracketing" system.

Based on the cladistic relationships of chloroplast DNA sequences (Meerow et al. 1999) the family originated in Africa and infrafamilial relationships are resolved along biogeographic lines. Tribe Amaryllideae, entirely southern African with the exception of pantropical *Crinum* L., were sister to the rest of Amaryllidaceae with very high bootstrap support. The remaining two African tribes of the family, Haemantheae (including Gethyllideae) and Cyrtantheae, were well supported, but their position relative to the Australasian Calostemmateae and a large clade comprising the Eurasian and American genera, was not clear. Most surprising, the Eurasian and American elements of the family were each monophyletic sister clades. Ito et al. (1999) resolved a very similar topology for a more limited sampling of Amaryllidaceae and related asparagoids using plastid *mat*K sequences, but *Agapanthus* was sister to a diverse clade of Agavaceae, Anthericaceae, Funkiaceae, and Hyacinthaceae in their trees, the former three families represented by a single species each. There was no bootstrap support for this position of *Agapanthus* in their analyses.

The relationships of the endemic American genera were well resolved using the spacer regions of nuclear ribosomal DNA (Meerow et al. 2000*b*). Seventy-seven species of the monophyletic American Amaryllidaceae were analyzed using *Pancratium* L. as the outgroup. The American genera of the family form two major clades. The first, or "hippeastroid" clade, are diploid (*n* = 11), primarily the extra-Andean element of the family (though several of the genera do have Andean representatives), comprising the genera treated as the tribe Hippeastreae in most recent classifications (Dahlgren et al. 1985; Müller-Doblies and Müller-Doblies 1996; Meerow and Snijman 1998). The second clade constitutes the tetraploid-derived (*n* = 23) Andean-centered tribes. In addition, the Andean clade is characterized by three consistent deletions, two in the ITS1 and one in the ITS2 regions. Several genera within the hippeastroid clade resolve as polyphyletic (*Rhodophiala* C. Presl., *Zephyranthes* Herb.) and the possibility of reticulate evolution (i.e., early hybridization) in these lineages was hypothesized (Meerow et al. 2000*b*). A petiolate-leafed Andean subclade, containing elements of both Eucharideae and Stenomesseae, was resolved

with a bootstrap = 93%. In both of the major American clades, there is a small tribe that is sister to the rest of the clade, Eustephieae in the Andean group, and Griffineae in the hippeastroid clade. These two small tribes may represent either ancestral or merely very isolated elements of their respective clades.

To date, the relationships of the remaining endemic African tribal clades of the family after Amaryllideae branches remain unresolved. In the intervening years since Monocots II in Sydney (Wilson and Morrison 2000), we have been working on several fronts. A number of generic and tribal phylogenetic analyses have been completed (Meerow and Snijman 2001; Meerow et al. 2002, 2003; Meerow and Clayton 2004). In this paper we review the progress made on amaryllid phylogeny since Monocots II and present the first results of phylogenetic analyses across the entire family using plastid *ndh*F sequences, as well as preliminary analyses of the Eurasian clade of the family using ITS.

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*b*). GenBank accession numbers are listed in Table 1.

DNA Extraction, Amplification, and Sequencing Protocols

Amplification of the ribosomal DNA ITS1/5.8S/ITS2 region was accomplished using flanking primers (in the 18S and 26S loci) AB101 and AB102 (Douzery et al. 1999) and the original White et al. (1990) internal primers ITS2 and ITS3 were used to amplify the spacers along with the intervening 5.8S gene as described by Meerow et al. (2000*b*). The plastid *ndh*F gene was amplified and sequenced using the eight primers of Olmstead and Sweere (1994). All polymerase chain reaction (PCR) amplifications were performed on a Gene Amp® PCR System 9700 (Perkin-Elmer Applied Biosystems, Foster City, California, USA).

Amplified products were purified using QIAquick (QIAGEN, Inc., Valencia, California, USA) columns, following manufacturer's 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 an ABI 3100 automated sequencer (according to the manufacturer's protocols; Applied Biosystems).

Sequence Alignment

The ITS sequences of the Eurasian clade were aligned using CLUSTAL_X, applying various combinations of gap opening and extension penalties. The resulting alignments were then imported into Sequencher vers. 4.1 (Gene Codes Corp., Ann Arbor, Michigan, USA) for further manual editing. As there is a significant amount of sequence divergence among the major lineages in the Eurasian clade, the alignment used for the analysis presented here should be considered very preliminary. The *ndh*F sequences across the entire family were readily aligned using Sequencher alone.

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

Taxon	Voucher specimen or accession no.	GenBank accession	
		*ndh*F	ITS
Acis autumnalis (L.) Herb.	*Meerow 2604*	AY434489	—
A. nicaeensis (Ardoino) Lledó, A. P. Davis & M. B. Crespo	*Meerow 2613*	—	AY751419
A. tingitana (Baker) Lledó, A. P. Davis & M. B. Crespo	*Meerow 2614*	—	AY751418
A. tricophylla (Schousb.) Sweet	*Meerow 2601*	—	AY7751417
Agapanthus africanus (L.) Hoffmanns.	UCBG 45.0288 (UC)	AF508405	—
Boophone disticha (L. f.) Herb.	*Malan 121* (NBG)	AY434486	—
Brunsvigia comptonii W. F. Barker	*Chase 612* (K)	AY434495	—
Cryptostephanus vansonii Verdoorn	*Meerow 2310*	AY434490	—
Cyrtanthus herrei (F. M. Leight.) R. A. Dyer	*van Zyl 104* (NBG)	AY434484	AY751428
Eustephia darwinii Vargas	*Meerow 2436*	AY434479	—
Galanthus nivalis L.	*Meerow 2608*	AY747081	AY943930
G. peshmenii A. P. Davis & C. D. Brickell	*Meerow 2609*	AY434490	AY751424
G. plicatus M. Bieb. subsp. *plicatus*	*Meerow 2610*	—	AY751422
subsp. *byzantinus* (Baker) D. A. Webb	*Meerow 2600*	—	AY751421
G. reginae-olgae Orph.	*Meerow 2611*	—	AY751423
G. woronowii Losinsk.	*Meerow 2612*	—	AY751433
Gethyllis ciliaris L. F.	*Duncan 1123* (NBG)	AY434491	—
Griffinia parviflora Ker Gawl.	*Meerow 2603*	AY434478	—
Hannonia hesperidum Braun-Blanqu. & Maire	*Meerow 2615*	—	AY751427
Hippeastrum papilio (Ravenna) J. Van Scheepen	*Meerow 2406*	AY434475	—
H. reticulatum Herb.	*Meerow 2407*	AY434481	—
Hymenocallis tubiflora Salisb.	*Meerow 2440*	AY434482	—
Ismene vargasii (Velarde) Gereau & Meerow	*Meerow 2308*	AY434493	—
Lapiedra martinezii Laf.	*Meerow 2607*	AY434488	AY751425
Leucojum aestivum L.	*Meerow 2612*	—	AY751420
Lycoris radiata Herb.	*Meerow 606*	—	AY751430
Narcissus alcaracensis S. Ríos Ruiz, D. Rivera Nuñez, F. Alcaraz Ariza & C. Obón de Castro	*Meerow 2616*	—	AY751413
N. calcicola Mendonca	*Meerow 2617*	—	AY751414
N. nanus Steud.	*Meerow 2618*	—	AY751415
N. viridiflorus Schousb.	*Meerow 2619*	—	AY751416
Pancratium canariense Ker Gawl.	*Meerow 1142*	—	AF223531
P. tenuifolium Hochst. ex A. Rich.	*Meerow 2427*	—	AF223537
P. zeylanicum L.	*Preuss s. n.* (no voucher)	—	AY751431
Paramongaia weberbaueri Velarde	*Meerow 2303*	AY434480	—
Proiphys cunninghamii (Lindl.) Mabb.	*Meerow 1118* (FLAS)	AY434487	—
Scadoxus membranaceus (Baker) Friis & Nordal	NBG 708/88	AY434485	—
Sprekelia formosissima Herb.	*Meerow 1151*	AY434476	—
Sternbergia colchicifolia Waldst. & Kit.	*Meerow 2620*	—	AY751408
S. greuteriana Kamari & R. Artelari	*Meerow 2605*	—	AY751409
S. lutea Ker Gawl. ex Schult. f.	*Meerow 2621*	—	AY751411
S. lutea 'Angustifolia'	*Meerow 2622*	—	AY751410
S. sicula Tineo ex Guss.	*Meerow 2602*	—	AY751412
Ungernia flava Boiss. & Haussk. ex Boiss.	*Chase 3640* (K)	AY434483	AY751429
Vagaria ollivieri Maire	*Archibald et al. 4484* (RSA)	—	AY751426
Worsleya rayneri (Hook. f.) Traub & Moldenke	*Meerow 2411*	AY434477	AF223475

Analyses

Aligned matrices were analyzed using the parsimony algorithm of PAUP* vers. 40b10 for Macintosh (Swofford 2001), 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. For the *ndh*F matrix, a branch-and-bound (Hendy and Penny 1982) search was

conducted under the Fitch (equal) weights (Fitch 1971) criterion with a simple addition sequence. A heuristic search with 5000 random addition replications was conducted for the Eurasian clade ITS analysis, with tree-bisection-reconnection (TBR) branch swapping (saving no more than 100 trees from each replication). Node confidence was tested with 5000 replications of bootstrap analysis (Felsenstein 1985). *Agapanthus praecox* Willd. was used as the outgroup
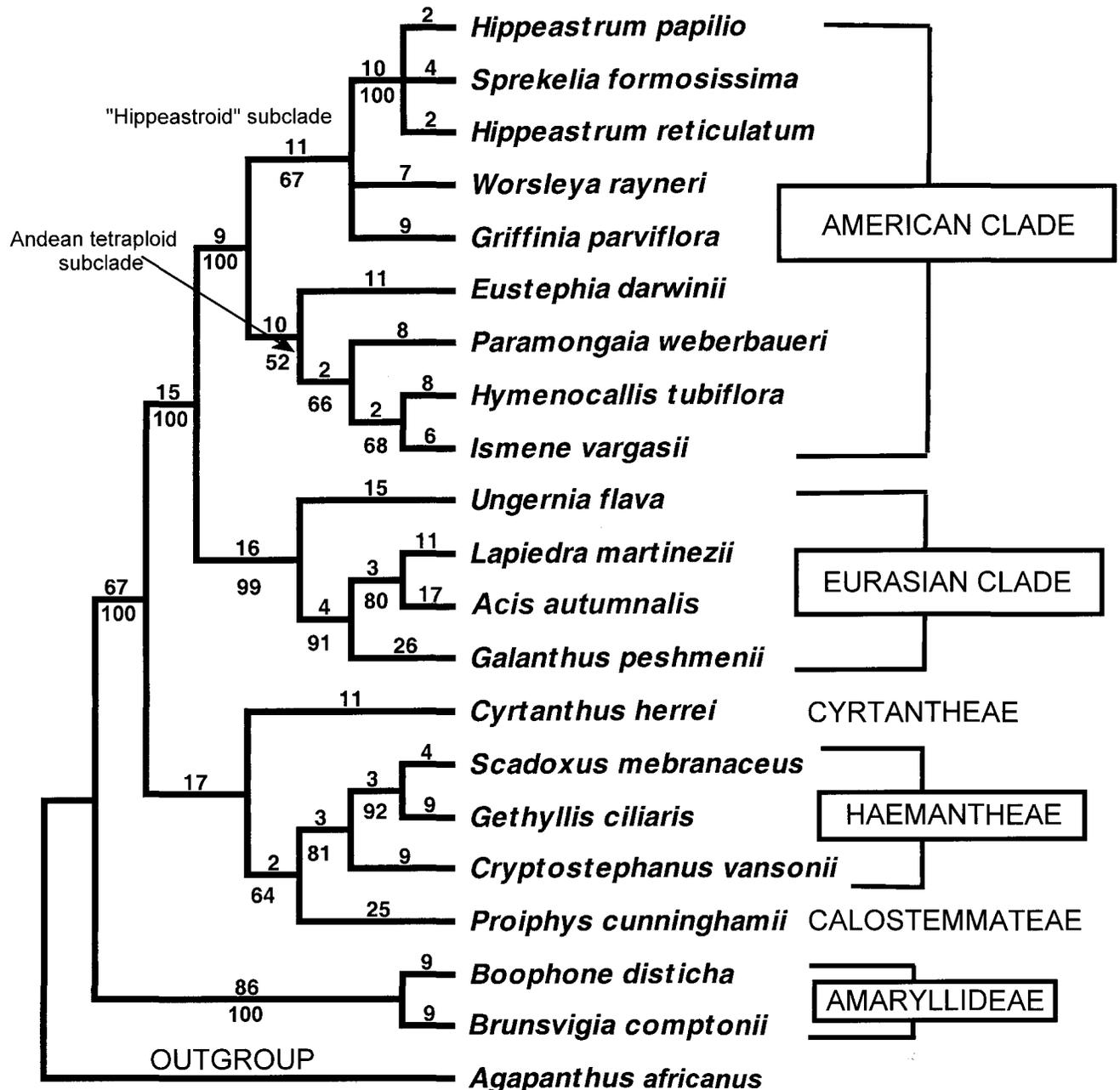
Fig. 1.—Single most-parsimonious tree found by branch-and-bound search of plastid *ndh*F sequence matrix for representative genera of Amaryllidaceae with *Agapanthus praecox* used as the outgroup. Numbers below branches are bootstrap support percentages.

for the *ndh*F analyses across the family, while *Cyrtanthus herrei* and *Worsleya rayneri* were used as outgroups in the ITS analyses of the Eurasian clade. Alignments and trees shown here are to be considered preliminary and part of larger works in progress, thus they have not been deposited in TreeBASE at this time.

RESULTS

Plastid ndh*F Sequence Phylogeny of Amaryllidaceae*

Of the 2098 total characters in our alignment, 103 were potentially parsimony informative. A branch-and-bound search found a single tree (Fig. 1) of length = 361 steps,

consistency index (CI) = 0.86, and retention index (RI) = 0.82. The tribe Amaryllideae is the first terminally resolved branch of the tree with bootstrap support of 100%. Cyrtantheae are resolved as sister to a subclade of Haemantheae (the baccate-fruited African Amaryllidaceae) and Calostemmateae (the Austalasian endemic clade of the family). There is <50% bootstrap support for the resolution of *Cyrtanthus* W. Aiton. The Haemantheae/Calostemmateae sister relationship have weak support (64%). This clade is in turn sister to the Eurasian/American clade, the latter with 100% bootstrap support. The American clade and the Eurasian clades have bootstrap support values of 100% and 99%, respectively. Lycorideae (from central and eastern Asia), repre-
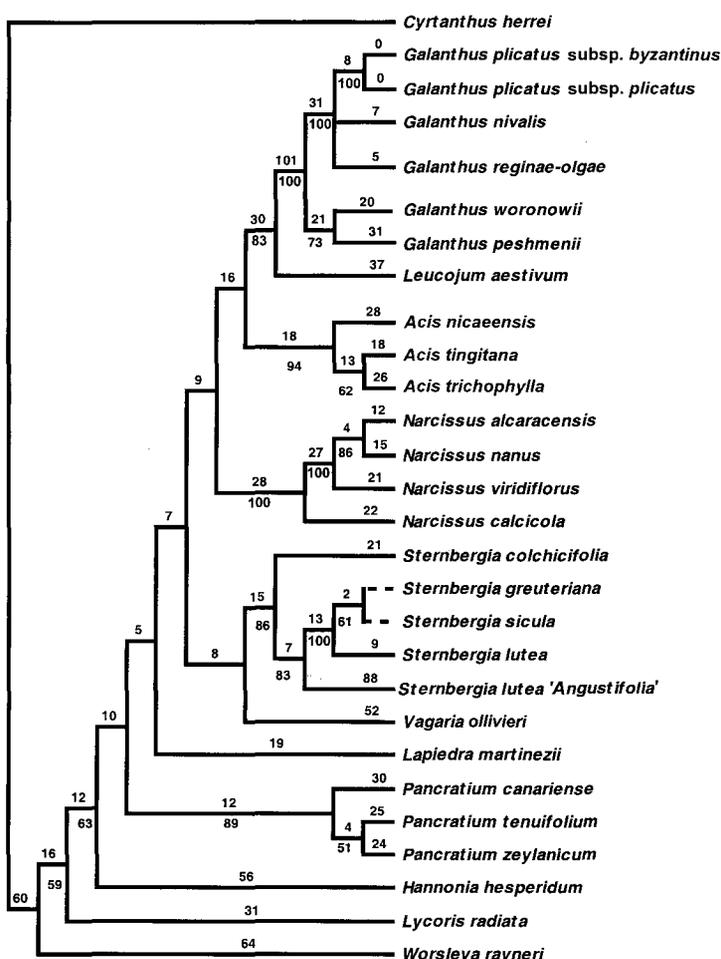
Fig. 2.—Single most-parsimonious tree found by heuristic search of nuclear ribosomal DNA spacer sequence matrix for representative species of the Eurasian clade of Amaryllidaceae with *Cyrtanthus herrei* and *Worsleya rayneri* used as outgroups. Numbers below branches are bootstrap support percentages.

sented here by *Lycoris radiata,* are sister to the predominately Mediterranean remainder of the Eurasian group. In the American clade, the hippeastroid and Andean tetraploid subclades (see Meerow et al. 2000*b*) are resolved with bootstrap support of 67% and 52%, respectively.

ITS Sequence Phylogeny of the Eurasian Clade

Of the 698 total characters in our current alignment, 339 were potentially parsimony informative. A single tree of length = 1140 was found (Fig. 2), with a CI of 0.60 and a RI of 0.73. While several genera are resolved as monophyletic with excellent bootstrap support (*Galanthus* L.: 100%, *Narcissus* L.: 100%, *Pancratium*: 89%, and *Sternbergia* L.: 86%), albeit with limited sampling. One species of *Leucojum* L. (*L. aestivum*) resolves as sister to *Galanthus* (83%) rather than as part of an otherwise monophyletic *Leucojum* (94% bootstrap).

The relationships among the terminal clades, however, mostly lack bootstrap support, except for the position of *Lycoris* Herb., which begins a basal grade in the Eurasian clade (bootstrap = 59%), followed by *Hannonia* Braun-Blanqu. & Maire (bootstrap = 63%), then *Pancratium,* and next *Lapiedra* Lag. The remainder of the tree forms two sister clades.

Vagaria Herb. is sister to *Sternbergia* in one of them, and *Narcissus* is sister to *Galanthus/Leucojum* in the other.

DISCUSSION

Overall Family Phylogeny

The intrafamilial relationships of Amaryllidaceae resolved by *ndh*F sequences are completely congruent with the relationships supported by plastid *rbc*L and *trn*L–F sequences (Meerow et al. 1999), with additional benefit of increased bootstrap support for most of the clades, and the satisfying resolution of the basal polytomy that plagued the previous analyses. The African baccate-fruited Haemantheae and the Australasian Calostemmateae are sister tribes, and Cyrtantheae are in turn sister to them both (though this latter relationship has <50% bootstrap support). *Cyrtanthus* retains the loculicidally dehiscent capsule and phytomelanous seed so common in the Eurasian/American clade. In so far as the globose, indehiscent capsule of Calostemmateae resembles the unripe berry of many Haemantheae, this resolution has at least some morphological support. To this we might add the convergent evolution of a petiolate leaf with reticulate-like venation (*Scadoxus* Raf. in Haemantheae and *Proiphys*

Herb. in Calostemmateae). This resolution would suggest that the common ancestor of the two tribes was extant when Australia and Africa were closer together, or a long-distance dispersal event took place early in the diversification of the clade. Either hypothesis awaits biogeographic analysis. The bulbiform pseudoseed of Calostemmateae is actually an adventitious bulbil formed by the precocious germination of the developing seed (Rendle 1901). The mature, indehiscent capsule of the tribe resembles the unripe berry of many Haemantheae. It is tempting to speculate if the fruit morphology of Calostemmateae, with its unusual contents, might have been derived through neotonous evolution from a berry-fruited ancestor.

Amaryllideae

This tribe, with much of its generic diversity confined to South Africa, is sister to the rest of Amaryllidaceae and has high support in all molecular phylogenies of the family published to date (Ito et al. 1999; Meerow et al. 1999). Compared to other tribes in Amaryllidaceae, Amaryllideae are marked by a large number of morphological synapomorphies (Snijman and Linder 1996): extensible fibers in the leaf tissue, bisulculate pollen with spinulose exines, 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 adapted for oceanic dispersal (Koshimizu 1930), ranges through Africa, Madagascar, Asia, Australia, and America.

Recognition of 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 synapomorphic characters for the tribe. Previous treatments of the tribe included elements of Haemantheae (Pax 1888; Pax and Hoffmann 1930; Hutchinson 1934, 1959). Traub's (1957, 1963) concept was largely adopted by Dahlgren et al. (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 based on morphological, seed anatomical, and cytological data suggested that two monophyletic groups could be recognized in the tribe. Subtribe Crininae are defined by indehiscent, rostellate capsules, corky testa, and the partially chlorophyllous endosperm of their seeds. Subtribe Amaryllidinae are characterized by a staminal tube (although rudimentary in Amaryllis L. and lost in Strumaria Jacq. ex Willd. and Carpolyza Salisb.) and stomatose seeds with an enlarged, green integument (except Amaryllis). Snijman and Linder (1996) also recognized the polyphyly of Boophone Herb. (sensu Arnold and De Wet 1993), though the formal reestablishment of the segregate genus Crossyne Salisb. 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 Herb., Cybistetes Milne-Redh. & Schweick.), Boophoninae (Boophone, Brunsvigia Heist., Crossyne), Amaryllidinae (Amaryllis, Namaquanula D. Müll.-Doblies & U. Müll.-Doblies, Nerine Herb.), and Strumariinae, the latter containing several segregate genera from Hessea Herb. 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, Nerine, and Strumaria) nested within an Amaryllis–Boophone–Crinum grade. The plastid matK sequence analysis of Ito et al. (1999), who studied only five taxa (Amaryllis, Brunsvigia, Crinum, Nerine, and Strumaria), also supported the basal position of Amaryllis.

Meerow and Snijman (2001) used a combination of nuclear ribosomal DNA spacer sequences and morphology to further explore the phylogenetic relationships of the tribe. Amaryllis and Boophone formed a grade at the base of their tree (Fig. 3) and were recognized as the monotypic subtribes Amaryllidinae and Boophonidinae. Two other subtribes were recognized: Crininae (which incorporates Crinum, Ammocharis, and Cybistetes), and Strumariinae (which includes Strumaria, Brunsvigia, Crossyne, Hessea, Namaquanula, and Nerine). Carpolyza was placed into synonymy with Strumaria.

Crinum, which is most species-rich in southern Africa, is also the only pantropical genus of Amaryllidaceae. Meerow et al. (2003) presented phylogenetic and biogeographic analyses of both plastid and nuclear ribosomal DNA for all continental groups of the genus and related African genera, with Amaryllis used as the outgroup (Fig. 4). They reported that C. baumii Harms is more closely related to Ammocharis and Cybistetes than to Crinum s.s. Three clades were resolved in Crinum s.s. One unites a monophyletic American group with tropical and North African species. The second includes only southern African species plus the Australian endemic C. flaccidum Herb. The third includes monophyletic Madagascar, Australasian, and Sino-Himalayan clades, with southern African species. The salverform, radially symmetrical perianths of subgen. Crinum evolved several times in the genus from ancestors with zygomorphic perianths (subgen. Codonocrinum Baker); thus, neither subgenus is monophyletic (Fig. 4). Biogeographic analyses place the origin of Crinum in southern Africa (Meerow et al. 2003). The genus underwent three major waves of radiation corresponding to the three main clades resolved in their gene trees (Fig. 4). Two entries into Australia for the genus were indicated, as were separate Sino-Himalayan and Australasian dispersal events.

Haemantheae

This baccate-fruited tribe is another morphologically well-marked group with strong molecular support (Fig. 1). Fleshy fruits have evolved only once in 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) and Clivia Lindl. in the tribe Amaryllidiformes, while Gethyllis was classified with Sternbergia in Oporanthiformes. Salisbury (1866) recognized the distinct tribes Haemantheae and Geth-
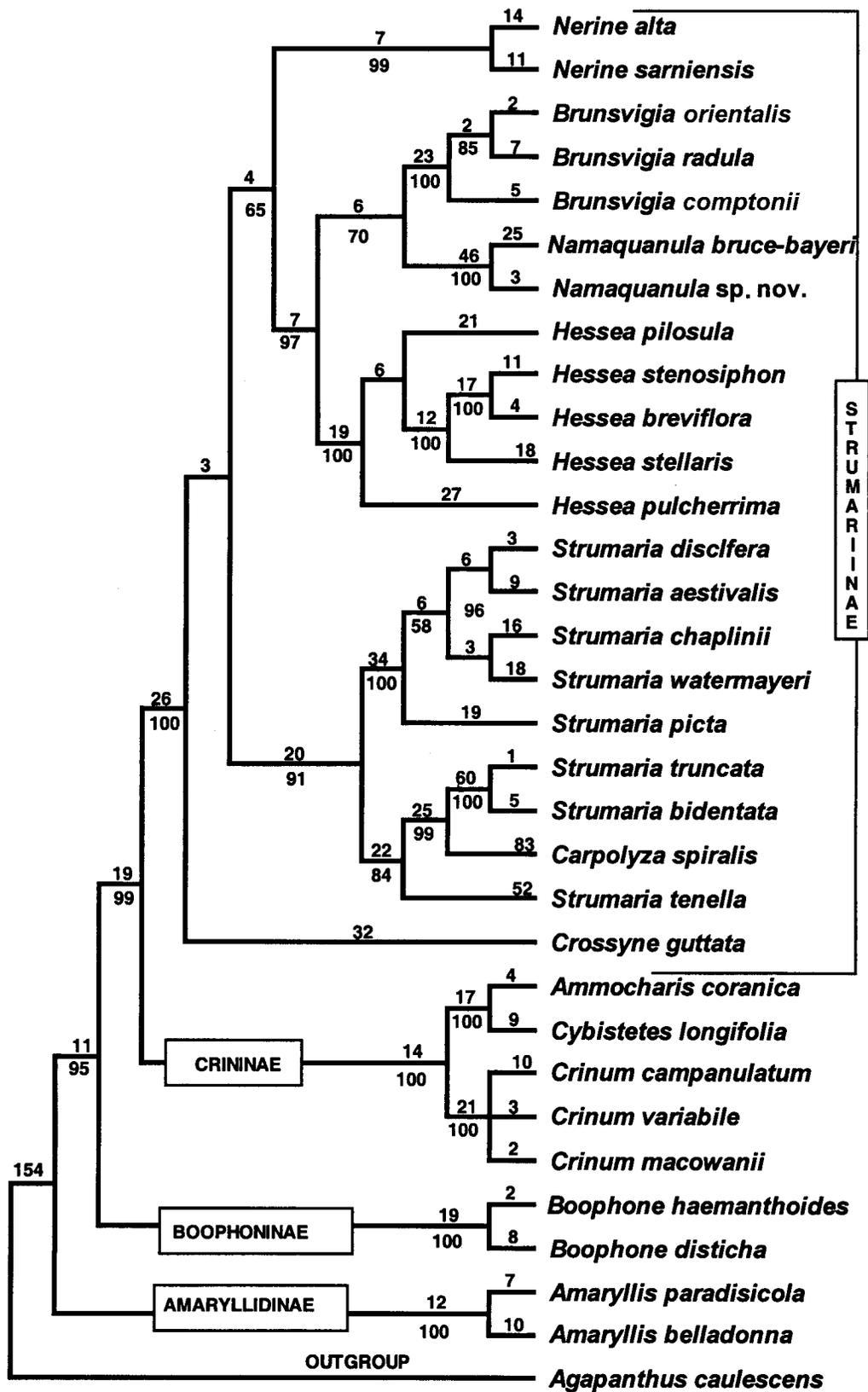
Fig. 3.—Phylogenetic tree derived from cladistic analyses of nuclear ribosomal DNA spacer sequences combined with morphological character matrix across the Amaryllidaceae tribe Amaryllideae (Meerow and Snijman 2001). Numbers above the lines are branch lengths. Numbers below the lines are bootstrap support percentages.
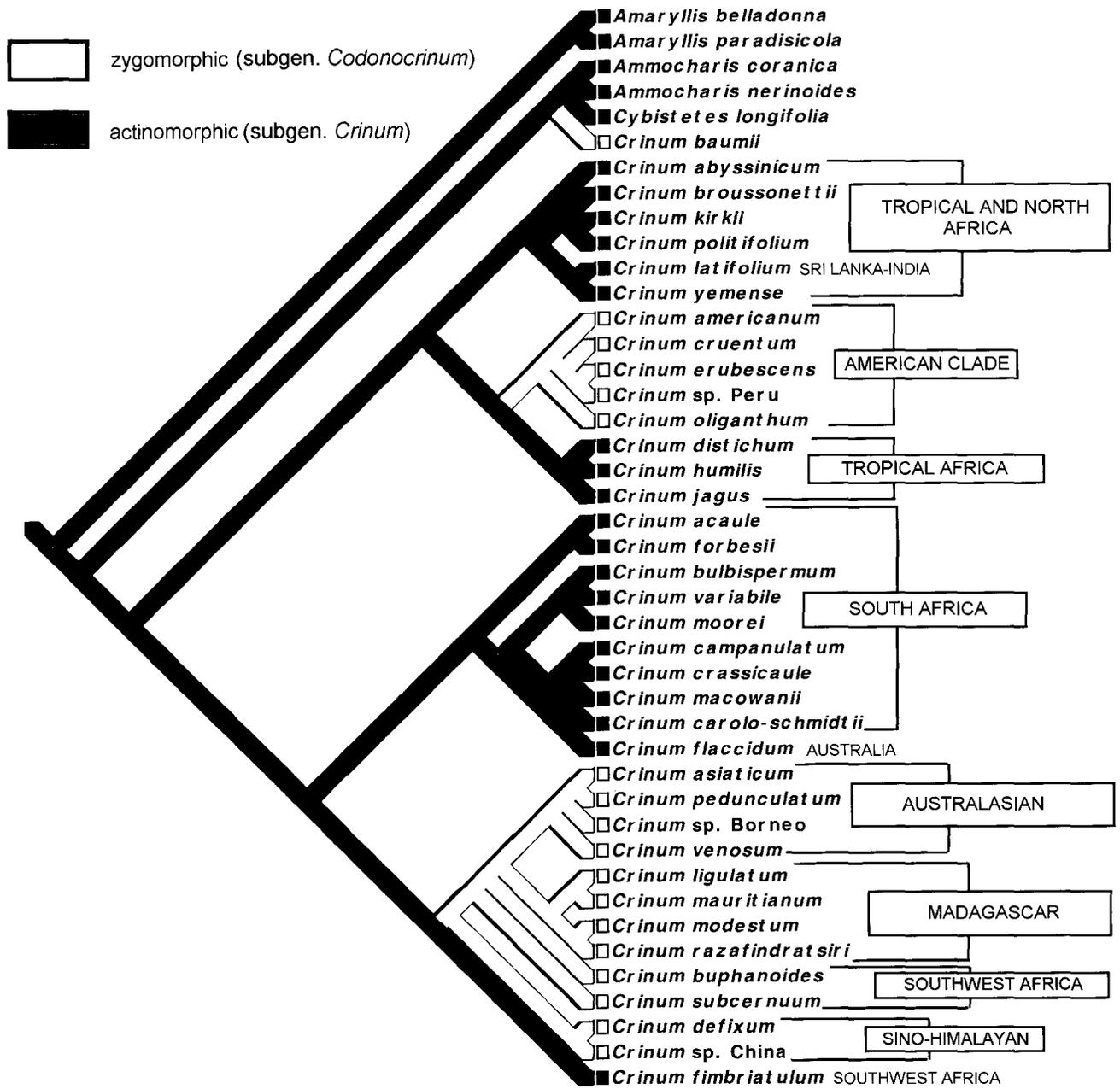
Fig. 4.—Phylogenetic tree derived from cladistic analyses of combined chloroplast *trn*L–F and nuclear ribosomal DNA spacer sequences across 43 species of *Crinum* and related genera (Meerow et al. 2003). Distribution of floral morphology and geographic position of the clades are shown.

yllideae Bentham and Hooker (1883) united *Cryptostephanus* Baker with *Narcissus* in their subtribe Coronatae, while maintaining *Haemanthus*, *Clivia*, and *Apodolirion* Baker in subtribe Genuinae. *Cryptostephanus* has perianthal appendages at the throat of the flower that Bentham and Hooker (1883) considered comparable to the corona of *Narcissus*. Pax (1888) situated *Haemanthus* and *Clivia* in his subtribe Haemanthinae, 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 (1888) 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 should be combined. Melchior (1964) placed both *Clivia* and *Cryptostephanus* in Haemantheae. *Scadoxus* was segregated from *Haemanthus* by Friis and Nordal (1976). Dahlgren et al. (1985) largely adopted Traub's (1963) classification, though Gethyllideae and Clivieae were subsumed in Haemantheae.

Both Müller-Doblies and Müller-Doblies (1996) and Meerow and Snijman (1998) recognized two tribes for the baccate-fruited genera: Haemantheae (*Haemanthus, Clivia, Cryptostephanus,* and *Scadoxus*) and Gethyllideae (*Gethyllis* and *Apodolirion*). Müller-Doblies and Müller-Doblies (1996) further recognized two fleshy-fruited subtribes in Haemantheae, Haemanthinae (*Haemanthus* and *Scadoxus*), Cliviinae (*Clivia* and *Cryptostephanus*).

Using three plastid DNA sequences, Meerow et al. (1999) 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 DNA 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. Using plastid *mat*K sequences, Ito et al. (1999), also resolved a monophyletic Haemantheae, though only three genera were sampled. *Haemanthus* and *Scadoxus* were sister taxa in their study, with 98% bootstrap support.

Using a combination of chloroplast and nuclear DNA sequences, Meerow and Clayton (2004) investigated the phylogeny of the Haemantheae across 19 species representing all genera of the tribe (Fig. 5). Two main clades were 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. The second clade contains all of the genera that form true bulbs, 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 certain species. Biogeographic analysis rooted the tribe in eastern South Africa, with several subsequent dispersals to the winter rainfall western Cape region and tropical Africa. 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. Gouws (1949) noted the striking similarities between the karyotype of *Clivia* and *Cryptostephanus. Cryptostephanus* is the only member of the tribe with the ancestral state of phytomelan in the seed coat.

The sister relationship of *Haemanthus* and *Scadoxus* is well supported by the morphological synapomorphy of the brush-like inflorescence, 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 were resolved that corresponded to the eastern Cape (*H. albiflos* Jacq., *H. humilis* Jacq.) vs. the western Cape endemics (*H. grantiticus* Snijman, *H. pumilio* Jacq.) (Snijman 1984). *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 ho-

mologous to one large, metacentric chromosome in the complement of *Haemanthus* and considered this to be an incidence of disploid reduction.

One of four included *Gethyllis* species, *G. lanuginosa* Marloth, resolved as sister to *Apodolirion* in Meerow and Clayton's (2004) analyses. Wilsenach (1965) found little variation among the karyotypes of representatives of both genera, all species of which so far investigated have $2n = 12$ chromosomes (Wilsenach 1965; Vosa 1986). Traub (1963) expressed doubt about maintaining *Apodolirion* and *Gethyllis* as distinct genera, an argument also taken up to some extent by Hilliard and Burtt (1973). They are differentiated by the capitate stigma in *Gethyllis* (vs. trilobed 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, but there are species of each in both climatic regions of the Cape. Clearly this question requires further investigation.

Eurasian Clade

Our tree is congruent with that of Lledo et al. (2004), in that the polyphyly of *Leucojum* is resolved with strong support. Lledo et al. (2004) resurrected the genus *Acis* Salisb. for the erstwhile Mediterranean *Leucojum* that resolve as sister to a *Galanthus/Leucojum* clade in their work as in ours (Fig. 2). However, their study focused on *Galanthus* and *Leucojum* with only a few other members of the Eurasian clade used as outgroups. This being the case, a straightforward comparison of our trees outside of the resolution of these two genera is not tenable. However, they also included a family-wide analysis combining the *mat*K sequences of Ito et al. (1999) and the *rbc*L and *trn*L–F sequence data of Meerow et al. (1999). In those trees, *Lycoris* also resolves as sister to the rest of the Eurasian genera (bootstrap = 70%). *Lapiedra* and *Vagaria* are sister genera, however (bootstrap = 70%), in turn sister to Galantheae (*Galanthus, Acis,* and *Leucojum*) in that combined plastid gene tree. ITS sequences for this group are plagued by both paralogy and significant divergence among the major groups, thus there is substantial room for error in constructing a larger alignment among the more easily aligned terminal (generic) clades. At present, the more limited sampling of this clade for *ndh*F (Fig. 1) is not congruent with the ITS phylogeny. We are developing a more extensive *ndh*F sequence matrix for the Eurasian group that we believe will provide well-supported resolution of these internal nodes.

The Future of Amaryllidaceae

It now appears that a well-resolved phylogeny of Amaryllidaceae is within our grasp. Similarly, the intimate relationship among Amaryllidaceae, Alliaceae, and *Agapanthus* appears unassailable at this point in time (see Graham et al. 2006). We are continuing our sampling with *ndh*F and ultimately will combine the preexisting plastid matrices with it, along with the 60-character matrix developed previously (Meerow et al. 2000a). As best as can be attempted without a fossil record, we would also like to try and date the significant divergence events in the evolutionary history of the
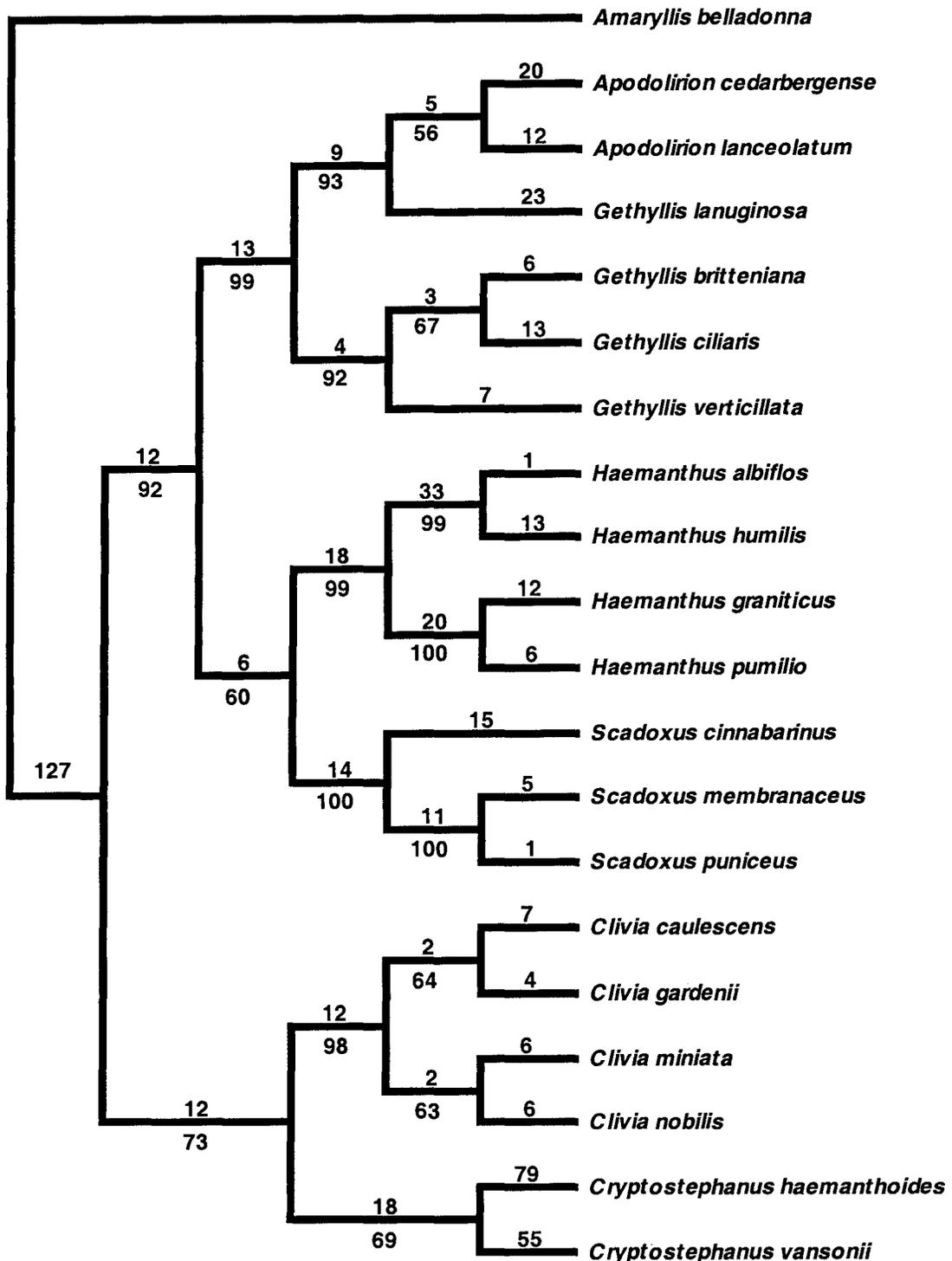
Fig. 5.—Phylogenetic tree derived from cladistic analyses of combined chloroplast *trn*L–F and nuclear ribosomal DNA spacer sequences across 19 species of Amaryllidaceae tribe Haemantheae, with *Amaryllis belladonna* L. used as the outgroup (Meerow and Clayton 2004). Numbers above branches are branch lengths. Numbers below branches are bootstrap support.

group. We are also still attempting to understand the curious pattern of polyphyly resolved by ITS within certain genera of the American hippeastroids (Meerow et al. 2000*b*) and are currently testing various plastid spacer regions in the hopes that one or more will allow us to corroborate or challenge these results.

AGP II (2003) advocates recognizing all Agapanthaceae,

Alliaceae, and Amaryllidaceae as three subtaxa of a single family, citing Alliaceae Batsch (1786) as the name of earliest priority. They do, however, leave open the option for recognizing three families. The former would essentially reestablish the family concept of Hutchinson (1934, 1959) with a few modifications. However, we believe that nomenclatural stability would be better served by conserving the name

Amaryllidaceae for the family and are preparing a proposal for this action.

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