

SYSTEMATICS OF AMARYLLIDACEAE BASED ON CLADISTIC ANALYSIS OF PLASTID *RBCL* AND *TRNL-F* SEQUENCE DATA¹

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Cladistic analyses of plastid DNA sequences *rbcL* and *trnL-F* are presented separately and combined for 48 genera of Amaryllidaceae and 29 genera of related asparagalean families. The combined analysis is the most highly resolved of the three and provides good support for the monophyly of Amaryllidaceae and indicates Agapanthaceae as its sister family. Alliaceae are in turn sister to the Amaryllidaceae/Agapanthaceae clade. The origins of the family appear to be western Gondwanaland (Africa), and infrafamilial relationships are resolved along biogeographic lines. Tribe Amaryllideae, primarily South African, is sister to the rest of Amaryllidaceae; this tribe is supported by numerous morphological synapomorphies as well. The remaining two African tribes of the family, Haemantheae and Cyrtantheae, are well supported, but their position relative to the Australasian Calostemmateae and a large clade comprising the Eurasian and American genera, is not yet clear. The Eurasian and American elements of the family are each monophyletic sister clades. Internal resolution of the Eurasian clade only partially supports currently accepted tribal concepts, and few conclusions can be drawn on the relationships of the genera based on these data. A monophyletic Lycorideae (Central and East Asian) is weakly supported. *Galanthus* and *Leucojum* (Galantheae pro parte) are supported as sister genera by the bootstrap. The American clade shows a higher degree of internal resolution. Hippeastreae (minus *Griffinia* and *Worsleya*) are well supported, and Zephyranthinae are resolved as a distinct subtribe. An Andean clade marked by a chromosome number of $2n = 46$ (and derivatives thereof) is resolved with weak support. The plastid DNA phylogenies are discussed in the context of biogeography and character evolution in the family.

Key words: Amaryllidaceae; cladistic analysis; molecular systematics; monocotyledons; phylogeny; plastid DNA.

The Amaryllidaceae J. St.-Hil., a cosmopolitan (predominantly pantropical) family of petaloid monocots, represent one of the elements of the Linnaean *Hexandria monogynia* (Linnaeus, 1753), the 51 genera of which have been variously classified since as liliaceous or amaryllidaceous. This basic dichotomy represents the generally uncertain phylogenetic placement of many petaloid monocots until the past two decades. Seven of the 51 genera that Linnaeus placed in *Hexandria monogynia* have since been included within a common taxonomic unit, as section Narcissi (Adanson, 1763; de Jussieu, 1789), family Amaryllideae (Jaume-St.-Hilaire, 1805; Brown, 1810), order Amaryllidaceae (Lindley, 1836; Herbert, 1837), tribe Amarylleae (Bentham and Hooker, 1883), suborder Amarylleae (Baker, 1888), subfamily Amaryllidoideae (Pax, 1888); family Amaryllidaceae

(Hutchinson, 1934, 1959), and subfamily Amarylloideae (Traub, 1963). Brown (1810) was the first to propose that the genera with superior ovaries be excluded from Amaryllidaceae, a restriction followed faithfully until Hutchinson (1934). Herbert (1837) recognized that the Taccaceae were not allied to Amaryllidaceae, and Pax (1888) formally removed Velloziaceae as part of the family (Herbert's suborder Xerophyteae). Hutchinson's (1934, 1959) classification was the first radical recircumscription of Amaryllidaceae since Brown (1810). In defining the unifying character of the family to be "an umbellate inflorescence subtended by an involucre of one or more spatheaceous bracts," he segregated Agavaceae, Hypoxidaceae, and Alstroemeriaceae and added tribes Agapantheae, Allieae, and Gilliesieae (Alliaceae). Takhtajan (1969) recognized Amaryllidaceae in the narrow sense, and maintained a distinct Alliaceae. Cronquist (1988) and Thorne (1976) included Amaryllidaceae within broad concepts of Liliaceae.

Concepts of familial and ordinal limits of the monocotyledons were radically challenged by Huber (1969), who emphasized less conspicuous characters, particularly embryological characters, over gross floral or vegetative morphology. Huber's work highlighted the heterogeneity present in many traditional monocot families, especially Liliaceae. Much of this work was refined and placed into phylogenetic context by Dahlgren and coworkers (Dahlgren and Clifford, 1982; Dahlgren and Rasmussen, 1983;

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Dahlgren, Clifford, and Yeo, 1985). In Dahlgren, Clifford, and Yeo's (1985) synthesis, Amaryllidaceae and Alliaceae are both recognized as members of the order Asparagales, an order of 31 families that have evolved many traits in parallel with Liliales. One of the most important and consistent characters separating these two orders is the presence of phytomelan in the seed coat of Asparagales (Huber, 1969). To date, phylogenetic analyses of the monocotyledons, based on both morphological and gene sequence matrices, have supported this classification with some amendment (Duvall et al., 1993; Stevenson and Loconte, 1995; Chase et al., 1995a, b), but the precise relationship of Amaryllidaceae to other Asparagales remained elusive until Fay and Chase (1996) used molecular data to argue that Amaryllidaceae, Agapanthaceae, and Alliaceae form a monophyletic group and that together they are related most closely to Hyacinthaceae s.s. and the resurrected family Themidaceae (the former tribe Brodiaeae of Alliaceae).

Despite a lack of consensus on generic limits and tribal delimitations within the Amaryllidaceae, cladistic analysis has only rarely been applied to problems in the family, such as by Nordal and Duncan (1984) for *Haemanthus* and *Scadoxus*, two closely related, baccate-fruited African genera, Meerow (1987a, 1989) for *Eucrosia* and *Eucharis* and *Caliphruria*, respectively, and Snijman (1994) and Snijman and Linder (1996) for various taxa of tribe Amaryllideae. Applying phylogenetic studies for the entire family is difficult due to homoplasy for many conspicuous characters within this highly canalized group (Meerow, 1987a, 1989, 1995). This led Meerow (1995) to conclude that "future reconstruction attempts will greatly benefit from the inclusion of molecular data."

The four most recent infrafamilial classifications (Table 1) of Amaryllidaceae are those of Traub (1963), Dahlgren, Clifford, and Yeo (1985), Müller-Doblies and Müller-Doblies (1996), and Meerow and Snijman (1998). Traub's scheme included Alliaceae, Hemerocallidaceae, and Ixioliriaceae as subfamilies, following Hutchinson (1934, 1959) in part. Within his subfamily Amarylloideae, he erected two informal taxa, "infrafamilies" Amarylloideinae and Pancratioidinae, both of which were polyphyletic (Meerow, 1995). Dahlgren, Clifford, and Yeo (1985) dispensed with any subfamilial classification above the level of tribe, recognizing eight, and treated as Amaryllidaceae only those genera in Traub's Amarylloideae. Stenomessaeae and Eustephieae were combined. Meerow (1995) resurrected Eustephieae from Stenomessaeae and suggested that two new tribes may need to be recognized, Calostemmatae and Hymenocallideae. Müller-Doblies and Müller-Doblies (1996) recognized ten tribes (among them Calostemmatae) and 19 subtribes, many of them monogeneric; Meerow and Snijman (1998) recognize 14 tribes, with two subtribes only in one of them (Table 1).

Fay and Chase (1996) recircumscribed Amaryllidaceae to include *Agapanthus*, previously included in Alliaceae, as subfamily Agapanthoideae. This recircumscription was based on phylogenetic analysis of *rbcL* sequence data, with only four genera of Amaryllidaceae s.s. included in the analysis. All the epigynous genera were treated as Amaryllidoideae. Bootstrap support for this treatment was weak (63%). The sampling within Amaryllidaceae

s.s. in Fay and Chase (1996) did not allow sufficient resolution of the generic relationships within the family, and we present here phylogenetic analyses of three plastid DNA sequence data sets for a much wider range of taxa.

The phylogenetic application of sequences of *rbcL* is well documented (e.g., Chase et al., 1993; Olmstead and Palmer, 1994) and has been used to clarify relationships between and within a number of asparagoid families, including Themidaceae (Fay and Chase, 1996), Asphodelaceae (de Bruijn et al., unpublished data), Alliaceae (Fay et al., unpublished data), and Orchidaceae (Cameron et al., 1999). Within Amaryllidaceae, however, levels of resolution obtained within some major clades, particularly those from the Neotropics, were not sufficient to elucidate tribal relationships fully (Fay et al., 1995). For this reason, we chose to combine our *rbcL* matrix with two for the *trnL* intron/*trnL-F* spacer region of noncoding plastid DNA, for which Taberlet et al. (1991) had developed "universal" primers for amplification. Sequences of this region have been used in phylogenetic studies of Crasulaceae (Kim, t'Hart, and Mes, 1996; Mes, Van Bredereode and t'Hart, 1996; Mes, Wijers, and t'Hart, 1997), Gentianaceae (Gielly and Taberlet, 1996; Gielly et al., 1996), Paeoniaceae (Sang, Crawford and Stuessy, 1997), Proteaceae (Maguire et al., 1997), Ranunculaceae (Kita, Ueda, and Kadota, 1995), among others, either alone or in combination with other loci. This region of the plastid genome evolves more than three times faster, on average, than *rbcL* (Gielly and Taberlet, 1994) and can therefore potentially add increased resolution to a phylogeny generated by *rbcL* sequences.

Combining independent character matrices, whether both molecular or molecular and morphological, very often increases the resolution of the ingroup and the bootstrap support of the internal nodes of the phylogenetic trees (Chase et al., 1995b; Olmstead and Sweere, 1994; Rudall et al., 1998; Soltis et al., 1998). In this paper we present the first family-wide phylogenetic analysis of Amaryllidaceae using three plastid DNA sequences, alone and in combination, and comment on the evolutionary and biogeographic implications of the results.

MATERIALS AND METHODS

Plant materials—The sources of plant material and vouchers/accessions used in this analysis are listed in Table 2, along with GenBank or EMBL accession numbers for the sequences.

DNA extraction, gene amplification, and sequencing—Sequences for *rbcL* were generated at both RBG Kew and the University of Florida (Table 2); all *trnL-F* sequences were obtained at Kew.

RBG Kew—DNA was extracted from 1.0 g fresh, 0.2–0.25 g silica gel-dried leaves, or ~0.1 g material from herbarium sheets using the 2X CTAB method of Doyle and Doyle (1987). All samples were then purified on cesium chloride/ethidium bromide gradients (1.55 g/mL density). Gene amplification of the *rbcL* gene was carried out using forward primers that match the first 20 or 26 base pairs (bp) of the coding region and reverse primers that correspond to 20-bp sequences that begin at position 1352 or 1367 in the coding region (Table 3; Chase and al., 1995a). The *trnL-trnF* region was amplified using the c and f primers of Taberlet et al. (1991). Amplified products were purified using Magic mini columns (Promega, Madison, Wisconsin) or QIAquick (Qiagen, Valencia, California) columns, following manufacturers protocols. Stan-

TABLE 2. Taxa, voucher specimens, and GenBank accession numbers used in the plastid DNA sequence phylogeny analyses of Amaryllidaceae.

Taxon	Voucher	<i>rbcL</i>	<i>trnL</i> gene	<i>trnL-F</i> spacer
Alliaceae				
<i>Allium sicutum</i> var. <i>bulgaricum</i>	M. W. Chase 835 (K)	GBAN-Z69200 ^a	GBAN-AF117001	GBAN-AF117057
<i>A. subhirsutum</i> L.	M. W. Chase 439 (K)	GBAN-Z69205	GBAN-AF117000	GBAN-AF117058
<i>Gilliesia graminea</i> Lindl.	M. W. Chase 450 (K)	GBAN-Z69208	GBAN-AF117018	GBAN-AF117045
<i>Iphelon uniflorum</i> (Graham) Raf.	M. W. Chase 627 (K)	GBAN-AF116992	GBAN-AF117021	GBAN-AF117049
<i>Leucocoryne pauciflora</i> R. Phil.	UC Irvine Arboretum 8182	GBAN-AF116998	GBAN-AF117025	GBAN-AF117053
<i>Mitula spicata</i> Prain	Grey-Wilson & Phillips 752 (K)	GBAN-AF116991	GBAN-AF117002	GBAN-AF117056
<i>Nothoscordum bivalve</i> Britton	M. W. Chase 247 (NCU)	GBAN-Z69202	GBAN-AF117024	GBAN-AF117052
<i>Pabellonia incrassata</i> (Phil.) Quezada and Martic.	UCI Arboretum 8247	GBAN-Z69209	GBAN-AF117026	GBAN-AF117054
<i>Solaria atropurpurea</i> (Phil.) Rav.	M. W. Chase 693 (K)	GBAN-Z69207	GBAN-AF117022	GBAN-AF117050
<i>Stemmatium narcissoides</i> Phil.	Beckett, Cheese & Watson 4688 (NY)	N/A	GBAN-AF117020	GBAN-AF117048
<i>Tristagma bivalve</i> (Lindl.) Traub	M. W. Chase 692 (K)	GBAN-Z69206	GBAN-AF117023	GBAN-AF117051
<i>Tulbaghia violacea</i> Harv.	M. W. Chase 248 (NCU)	GBAN-Z69203	GBAN-AF116999	GBAN-AF117030
Agapanthaceae				
<i>Agapanthus africanus</i> Hoffm.	M. W. Chase 627 (K)	GBAN-Z69221	GBAN-AF117028	GBAN-AF117060
<i>Agapanthus campanulatus</i> F. M. Leighton	M. W. Chase 1008 (K)	GBAN-Z69220	GBAN-AF117029	GBAN-AF117059
Amaryllidaceae				
<i>Amaryllis belladonna</i> L.	M. W. Chase 612 (K)	GBAN-Z69219	GBAN-AF10479	GBAN-AF104744
<i>Apodolirion lanceolatum</i> Benth. and Hook.	Kirstenbosch, NBG 714/88	GBAN-AF116944	GBAN-AF104789	GBAN-AF104767
<i>Boophone disticha</i> (L. f.) Herb.	M. W. Chase 2246 (K)	GBAN-AF116945	GBAN-AF104801	GBAN-AF104726
<i>Brunsvigia comptonii</i> W. F. Barker	M. W. Chase 2240 (K)	GBAN-AF116946	GBAN-AF104813	GBAN-AF104722
<i>Calphurria korsakoffii</i> (Traub) Meerow	M. W. Chase 962 (K)	GBAN-AF116947	GBAN-AF104810	GBAN-AF104731
<i>Calostemma lutea</i> Sims	M. W. Chase 1505 (K)	GBAN-AF116948	GBAN-AF104790	GBAN-AF104740
<i>Chlidanthus fragrans</i> Herb.	Meerow 2312 (FLAS)	GBAN-AF116949	GBAN-AF104770	GBAN-AF104723
<i>Clivia nobilis</i> Lindl.	M. W. Chase 3080 (K)	GBAN-AF116950	GBAN-AF104776	GBAN-AF104763
<i>Crinum yemense</i> Delfers	M. W. Chase 1595 (K)	GBAN-AF116951	GBAN-AF104784	GBAN-AF104756
<i>Cryptostephanus vansonii</i> Verdoorn	Meerow 2310 (FLAS)	GBAN-AF116952	GBAN-AF104804	GBAN-AF104743
<i>Cyrtanthus elatus</i> (Jacq.) Traub	M. W. Chase 1572 (K)	GBAN-AF116953	GBAN-AF104818	GBAN-AF104753
<i>Eucharis castelnaeana</i> (Baill.) Macbr.	Schunke 14156 (FLAS)	GBAN-AF116954	GBAN-AF104798	GBAN-AF104766
<i>Eucrosia eucrosioides</i> (Pax) Traub	Meerow 1117 (FLAS)	GBAN-AF116955	GBAN-AF104788	GBAN-AF104742
<i>Eustephia darwinii</i> Vargas	M. W. Chase 559 (K)	GBAN-AF116956	GBAN-AF104794	GBAN-AF104727
<i>Galanthus plicatus</i> M. Bieb.	M. W. Chase 741 (K)	GBAN-Z69218	GBAN-AF104799	GBAN-AF104730
<i>Gethyllis ciliaris</i> (Thunb.) Thunb.	Duncan 1123 (NBG)	GBAN-AF116957	GBAN-AF104816	GBAN-AF104745
<i>Griffithia hyacinthina</i> Ker Gawler	Meerow 2106 (FLAS)	GBAN-AF116958	GBAN-AF104771	GBAN-AF104736
<i>Habranthus martiniezii</i> Ravenna	M. W. Chase 1023 (K)	GBAN-AF116959	GBAN-AF104772	GBAN-AF104738
<i>Haemanthus humilis</i> Jacq.	M. W. Chase 2025 (K)	GBAN-AF116960	GBAN-AF104781	GBAN-AF104721
<i>Haemodia hesperidium</i> Braun-Blanq. and Maire	M. W. Chase 2023 (K)	GBAN-AF116961	GBAN-AF104812	GBAN-AF104734
<i>Hesperomilla marginata</i> (Pax) A. T. Hunz.	M. W. Chase 2238 (K)	GBAN-AF116962	GBAN-AF104813	GBAN-AF104741
<i>Hippeastrum papilio</i> (Rav.) Van Scheepen	M. W. Chase 1901 (K)	GBAN-AF116963	GBAN-AF104807	GBAN-AF104757
<i>Hymenocallis marginata</i> (Pax.) A. T. Hunz.	Meerow 2307 (FLAS)	GBAN-AF116964	GBAN-AF104775	N/A
<i>Ismene longipetala</i> (Lindl.) Meerow	M. W. Chase 3583 (K)	GBAN-AF116965	GBAN-AF104796	N/A
<i>Ismene narcissiflora</i> Jacq.	Meerow 2306 (FLAS)	GBAN-AF116966	GBAN-AF104796	GBAN-AF104719
<i>Ismene vargasii</i> (Velarde) Gereau and Meerow	Meerow 2308 (FLAS)	GBAN-AF116967	GBAN-AF104787	GBAN-AF104768
<i>Lapidia martiniezii</i> Lag.	M. W. Chase 1528 (K)	GBAN-AF116968	GBAN-AF104802	GBAN-AF104732
<i>Leptochiton quitensis</i> (Herb.) Sealy	M. W. Chase 1116 (FLAS)	GBAN-AF116969	GBAN-AF104806	GBAN-AF104750
<i>Leucocjum autumnale</i> L.	M. W. Chase 607 (K)	GBAN-AF116970	GBAN-AF104779	GBAN-AF104755
<i>Lycoris squamigera</i> Maxim.	M. W. Chase 2014 (K)	GBAN-AF116971	GBAN-AF104780	GBAN-AF104733
<i>Narcissus elegans</i> (Haw.) Spach	M. W. Chase 617 (K)	GBAN-AF116972	GBAN-AF104791	GBAN-AF104746
<i>Nerine bowdenii</i> Will. Wats.	M. W. Chase 616 (K)	GBAN-AF116973	GBAN-AF104769	GBAN-AF104751

TABLE 2. Continued.

Taxon	Voucher	<i>rbcL</i>	<i>trnL</i> gene	<i>trnL-F</i> spacer
<i>Pamitanthe peruviana</i> [Stapf]	Meerow 2304 (FLAS)	GBAN-AFI16974	GBAN-AFI04814	GBAN-AFI04759
<i>Pancratium canariensis</i> L.	Meerow 1142 (FLAS)	GBAN-AFI16975	GBAN-AFI04778	GBAN-AFI04718
<i>Paramongaia weberbaueri</i> Valarde	Meerow 2303 (FLAS)	GBAN-AFI16976	GBAN-AFI04777	GBAN-AFI04764
<i>Phaedranassa dubia</i> (HBK) Macbr.	M. W. Chase 1834 (K)	GBAN-AFI16977	GBAN-AFI04809	GBAN-AFI04729
<i>Proiphys cunninghamii</i> (Ait. ex Lindl.) Mabb.	Meerow 1118 (FLAS)	GBAN-AFI16978	GBAN-AFI04785	GBAN-AFI04762
<i>Rauhia decora</i> Ravenna	M. W. Chase 1573 (K)	GBAN-AFI16979	GBAN-AFI04805	GBAN-AFI04735
<i>Rhodophiala moelleri</i> (R. Phil.) Traub	M. W. Chase 1908 (K)	GBAN-AFI16980	GBAN-AFI04782	GBAN-AFI04720
<i>Scadoxus cinnabarinus</i> (Decne.) I. Friis and I. Nordal	M. W. Chase 549 (K)	GBAN-AFI16981	GBAN-AFI04783	GBAN-AFI04754
<i>Sprekelia formosissima</i> (L.) Herb.	M. W. Chase 577 (K)	GBAN-AFI16982	GBAN-AFI04808	GBAN-AFI04728
<i>Stenomesson pearcei</i> Bak.	M. W. Chase 1591 (K)	GBAN-Z69217	GBAN-AFI04800	GBAN-AFI04739
<i>Stenomesson variegatum</i> (R. and P.) Bak.	Meerow 1159 (FLAS)	GBAN-AFI16983	GBAN-AFI04811	GBAN-AFI04724
<i>Sternbergia lutea</i> (L.) Spreng.	M. W. Chase 615 (K)	GBAN-AFI16984	GBAN-AFI04793	GBAN-AFI04747
<i>Strumaria truncata</i> Jacq.	Snijman 281 (NBG)	GBAN-AFI16985	GBAN-AFI04819	GBAN-AFI04765
<i>Traubia modesta</i> (R. A. Phil.) Ravenna	Meerow 2301 (FLAS)	GBAN-AFI16986	GBAN-AFI04792	GBAN-AFI04748
<i>Ungernia flava</i> Boiss. ex Haussk. ex Boiss.	M. W. Chase 3640 (K)	GBAN-AFI16987	GBAN-AFI04797	GBAN-AFI04749
<i>Vagarina parviflora</i> Herb.	M. W. Chase 1066 (K)	GBAN-AFI16988	GBAN-AFI04786	GBAN-AFI04760
<i>Worsleya rayneri</i> (Hook.) Traub and Moldenke	Meerow 2302 (FLAS)	GBAN-AFI16989	GBAN-AFI04774	GBAN-AFI04761
<i>Zephyranthes filifolia</i> Herb. ex Baker	M. W. Chase 1836 (K)	GBAN-AFI16990	GBAN-AFI04815	GBAN-AFI04737
Anthericaceae				
<i>Anthericum liliago</i>	M. W. Chase 515 (K)	GBAN-Z69225	GBAN-AFI17005	GBAN-AFI17033
<i>Echeandia</i> sp.	M. W. Chase 602 (K)	GBAN-Z69225	GBAN-AFI17014	GBAN-AFI17039
<i>Leucocrinum montanum</i> Nutt. ex A. Gray	M. W. Chase 795 (K)	GBAN-Z77252	GBAN-AFI17003	GBAN-AFI17031
Behniaceae				
<i>Behnia reticulata</i> Didr.	Goldblatt 9273 (MO)	GBAN-Z69226	GBAN-AFI17007	GBAN-AFI17035
Convallariaceae				
<i>Aspidistra elatior</i> Blume	M. W. Chase 833 (K)	GBAN-Z77269	GBAN-AFI17016	GBAN-AFI17044
<i>Liriope platyphylla</i> F. T. Wang & T. Tang	M. W. Chase 1102 (K)	GBAN-Z77271	GBAN-AFI17009	GBAN-AFI17038
<i>Peltosanthus</i> sp.	M. W. Chase 497 (K)	GBAN-Z77272	GBAN-AFI17006	GBAN-AFI17034
<i>Polygonatum hookeri</i> Baker	M. W. Chase 847 (K)	GBAN-Z73695	GBAN-AFI17010	GBAN-AFI17036
Hemerocallidaceae				
<i>Geitonoplesium cymosum</i>	Adelaide B. G. 880709	GBAN-AFI16997	GBAN-AFI17027	GBAN-AFI17055
Hyacinthaceae				
<i>Albuca shawii</i> Baker	M. W. Chase 1012 (K)	GBAN-Z69223	GBAN-AFI17012	GBAN-AFI17042
<i>Hyacinthus orientalis</i> L.	M. W. Chase 1503 (K)	GBAN-AFI16995	GBAN-AFI17013	GBAN-AFI17043
<i>Ornithogalum longibracteatum</i> Jacq.	M. W. Chase 1507 (K)	GBAN-Z69224	GBAN-AFI17008	GBAN-AFI17037
Laxmanniaceae				
<i>Eustrephus latifolius</i> R. Br.	Adelaide B. G. 880587	GBAN-AFI16996	GBAN-AFI17004	GBAN-AFI17032
Themidaceae				
<i>Bessera elegans</i> Schult.	M. W. Chase 626 (K)	GBAN-Z69215	GBAN-AFI17015	GBAN-AFI17040
<i>Brodiaea jolonensis</i> Eastw.	M. W. Chase 1831 (K)	GBAN-AFI16993	GBAN-AFI17017	GBAN-AFI17046
<i>Milla magnifica</i> E. Moore	Meerow 2309 (FLAS)	GBAN-AFI16994	GBAN-AFI17011	GBAN-AFI17041
<i>Mulla maritima</i> S. Wats.	M. W. Chase 779 (K)	GBAN-Z69213	GBAN-AFI17019	GBAN-AFI17047

^a The prefix GBAN has been added for linking the online version of *American Journal of Botany* to GenBank and is not part of the actual GenBank accession number.

TABLE 3. PCR and sequencing primers for *rbcL* and *trnL-F* used in this study.

Sequence	Primer name
<i>rbcL</i>	
Royal Botanic Gardens, Kew	
5' ATGTCACCACAAACAGAAAC ^{3'}	1F
5' GCGTTGGAGAGAGATCGTTTTCT ^{3'}	636F
5' TCGCATGTACCYGCAGTTGC ^{3'}	724R
	(monocots)
5' CTTTCCAAAATTTCCACAAGCAGCA ^{3'}	1368R
University of Florida	
5' ATGTCACCACAAACAGAAACTAAAGCAAGT ^{3'}	Zurawski's Z-1
5' AATTTGATCTCCTTCCATATTTGCGA ^{3'}	Zurawski's Z-1375R
5' AAACCTTTCCAAGGCCCGC ^{3'}	ALM1 (=Z-427)
5' GCGACTTCGGTCTTTTTC ^{3'}	ALM2
5' GGTAAGTGAAGGGGAA ^{3'}	ALM3
5' GCGGCCTTGAAAGTTT ^{3'}	ALM4
<i>trnL-F</i> (Taberlet et al., 1991)	
5' CGAAATCGGTAGACGCTACG ^{3'}	<i>trnL-c</i>
5' ATTTGAAGTGGTGACACGAG ^{3'}	<i>trnL-f</i>
5' GGGGATAGAGGGACTTGAAC ^{3'}	<i>trnL-d</i>
5' GTTCAAGTCCCTCTATCCC ^{3'}	<i>trnL-e</i>

25 ng of pGEM-T[®] vector (Promega, Madison, Wisconsin), 50–100 ng PCR products, 1 μ L of 10 \times ligase buffer [300 mmol/L Tris-HCl, pH 7.8, 100 mmol/L MgCl₂, 100 mmol/L dithiothreitol, 5 mmol/L ATP, and 1.5 U T4 DNA ligase (Promega, Madison, Wisconsin)]. The ligations were incubated at 16°C overnight. One hundred microlitres of competent *E. coli* XL-1 Blue cells were transformed with 2.5 μ L of each ligation mixture, and spread on a Luria-Bertani (LB) agar plate (100 \times 15 mm) containing 50 μ g/mL of ampicillin and 12.5 μ L tetracycline. The plate was spread with 50 μ L of 2% X-gal and 50 μ L of 100 mmol/L isopropyl-beta-D-thiogalactopyranoside before using. The plate was incubated at 37°C overnight. Individual colonies were counted, and white colonies were selected to grow overnight at 37°C in LB media containing 50 μ g/mL ampicillin. Plasmid DNA was digested with Pst I and Sst II, and the restricted DNAs were fractionated on a 0.8% agarose gel to verify the presence of the cloned insert. Plasmid DNA containing the cloned, amplified insert was purified, and DNA sequencing was accomplished using the Taq DyeDeoxy[®] Terminator Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, California) on an automated sequencer (Applied Biosystems Inc., Foster City, California) by the DNA sequencing Core of the Interdisciplinary Center for Biotechnology Research at the University of Florida. Sequencing was accomplished using vector T₇ and Sp6 primers along with specific primers for the *rbcL* gene received from G. Zurawski (Table 3). The complete sequence of both strands was determined using sets of synthetic primers (Table 3).

Sequence alignment—Sequences of *rbcL* were easily aligned manually because no length variation was detected. For *trnL-F*, two methods were employed. Sequences of several taxa representing the range of probable variation in the matrix were aligned using the Clustal option in Sequence Navigator (Applied Biosystems, Inc.), followed by manual optimisation and alignment of subsequent sequences. Alternatively, the program Sequencher (Gene Codes, Inc., Ann Arbor, Michigan) was used to align sequences of closely related taxa with subsequent builds of these smaller alignments performed manually. Copies of the aligned matrices are available from the senior author.

Analysis—Aligned matrices were analyzed using the parsimony al-

gorithm of the software package PAUP* for Macintosh (v4.0 d59-64, Swofford, 1998) with a successive weighting (SW; Farris, 1969) strategy. SW was employed to globally reduce the effect of highly homoplasious base positions on the resulting topologies (Lledó et al., 1998; Wenzel, 1997). Whole category weights (codon or tranversion) exhibit broad and overlapping ranges of consistency (Olmstead, 1997), whereas SW 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 and SPR (subtree pruning-regrafting) branch swapping but permitting 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 on to either completion or an upper limit of 5000 trees. The characters were then reweighted by the rescaled consistency index, and a further 50 replications of random sequence additions were conducted with the weighted matrix saving 15 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 50 replications of random sequence addition conducted, saving 15 trees per replication, with subsequent swapping on those trees. This cycle was repeated until two successive rounds found trees of the same length. All analyses were run with the MULPARS option and ACCTRAN optimization. Branches with zero length were collapsed if the maximum value = 0 ("amb +"). Internal support was determined by bootstrapping (5000 replicates) with the final reweighted character matrix and with the jackknife program (5000 replicates) of Farris et al. (1996) without SW weights applied. The cut-off bootstrap percentage is 50; minimum jackknife support percentage is 63 (Farris et al., 1996). The *rbcL* matrix consisted of 81 taxa, 51 Amaryllidaceae s.s. representing 48 genera, and 30 additional taxa representing 28 genera of Agapanthaceae, Alliaceae, Anthericaceae, Behniaceae, Convallariaceae, Hyacinthaceae, Laxmanniaceae, and Themidaceae, with *Geitonoplesium* sp. (Hemerocallidaceae) used as outgroup. The *trnL-F* matrix includes these same with the addition of *Stemmatium narcissoides* (Alliaceae).

The *trnL-F* region consists of an intron, a short exon, and an intergene spacer (Taberlet et al., 1991). We combined the components of *trnL-F* because they are nearly all noncoding, but each of the two larger regions was analyzed separately to determine whether they were congruent. Because they were congruent (results not shown), we lumped them together as the "noncoding matrix" to compare directly with *rbcL* before we combined all of them.

RESULTS

The *rbcL* matrix alone—Of 1340 included base positions in the analyses, 226 were parsimony informative. More than 5000 equally most parsimonious Fitch trees were found (tree length = 974) with a consistency index (CI) of 0.62 and a retention index (RI) of 0.71. SW produced at least 5000 equally parsimonious trees with a length of 450819 (Fitch length = 975), a CI = 0.88 (Fitch = 0.62), and RI = 0.89 (Fitch = 0.70). The large number of equally parsimonious trees is largely the result of the short branch lengths that occur within most of the internal clades (Figs. 1–2) and the imposed constraints against collapsing zero-length branches. However, the strict consensus of the weighted trees is more resolved than that of the Fitch trees. The additional step of the SW trees is essentially the "cost" of optimizing consistent characters over highly homoplastic base positions (Lledó et al., 1998). The Amaryllidaceae are not resolved as monophyletic in the strict consensus of all 5000 SW trees; in these *Agapanthus* and Amaryllidaceae tribe Amaryllideae

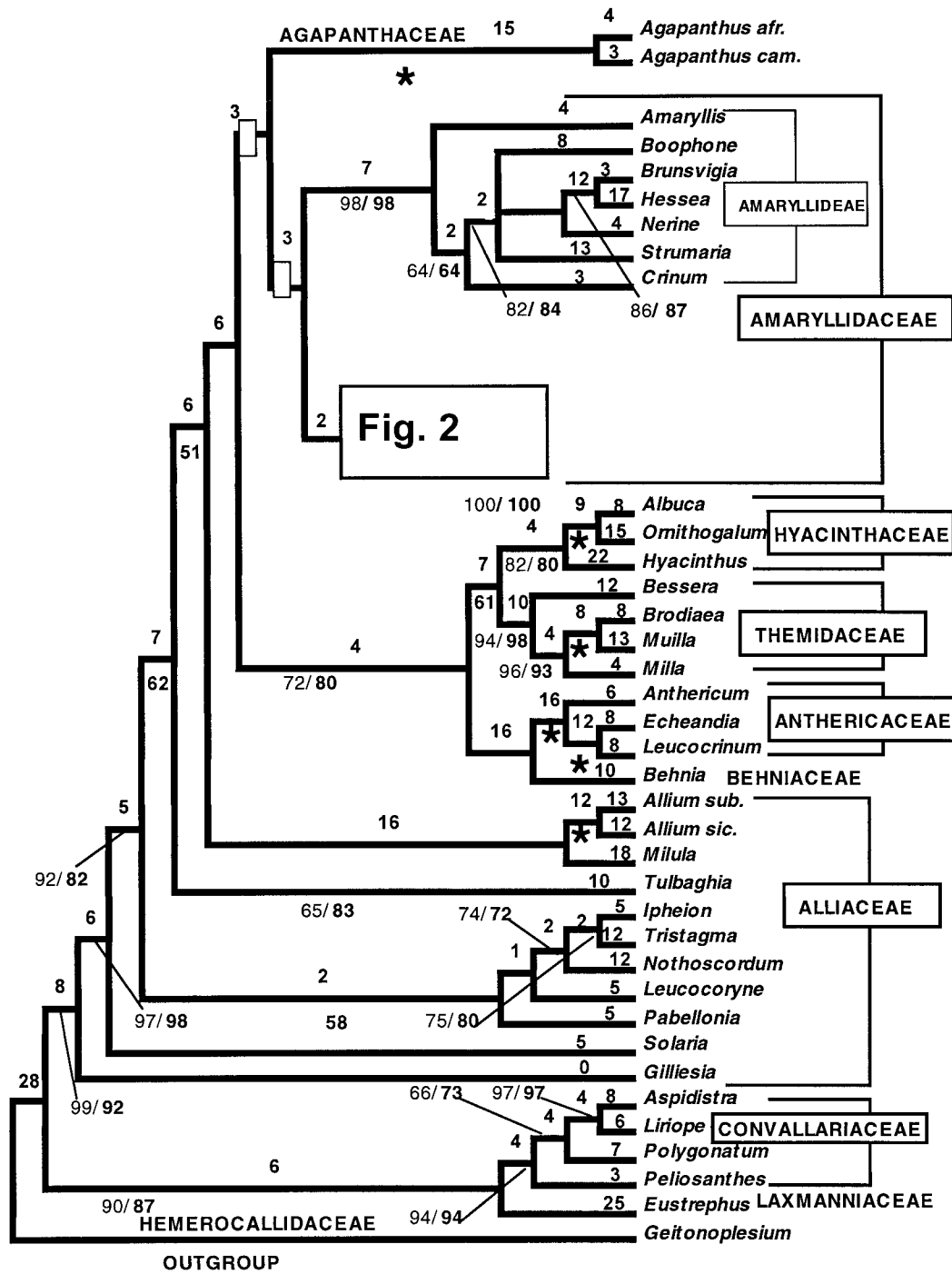


Fig. 1. One of 5000 equally parsimonious trees generated by cladistic analysis of the successively weighted *rbcL* sequence matrix for Amaryllidaceae and other Asparagalean genera. Numbers above branches are branch lengths. Bootstrap (plain) and jackknife (boldface) percentages are below branches supported by one or both. An asterisk below a branch signifies that both bootstrap and jackknife = 100%. A white bar across a branch signifies lack of resolution in the strict consensus tree of the 5000 trees. “*Agapanthus afr.*” = *A. africanus*, “*Agapanthus cam.*” = *A. campanulatus*, “*Allium sub.*” = *A. subhirsutum*, “*Allium sic.*” = *A. siculum* var. *bulgaricum*. The tree is continued in Fig. 2.

form a polytomy with the rest of Amaryllidaceae sensu stricto (s.s.). Moreover, these clades have no bootstrap or jackknife support.

The *rbcL* matrix (Fig. 1) resolves Hyacinthaceae/Themidaceae as sister to Anthericaceae/Behniaceae with moderate bootstrap and jackknife support and positions

this clade as sister to *Agapanthus*/Amaryllidaceae but with no jackknife or bootstrap support. The Alliaceae are resolved as an unsupported paraphyletic grade.

In many of the trees (Fig. 1), the African tribe Amaryllideae is sister to the rest of Amaryllidaceae s.s. This monophyletic group has high bootstrap and jackknife

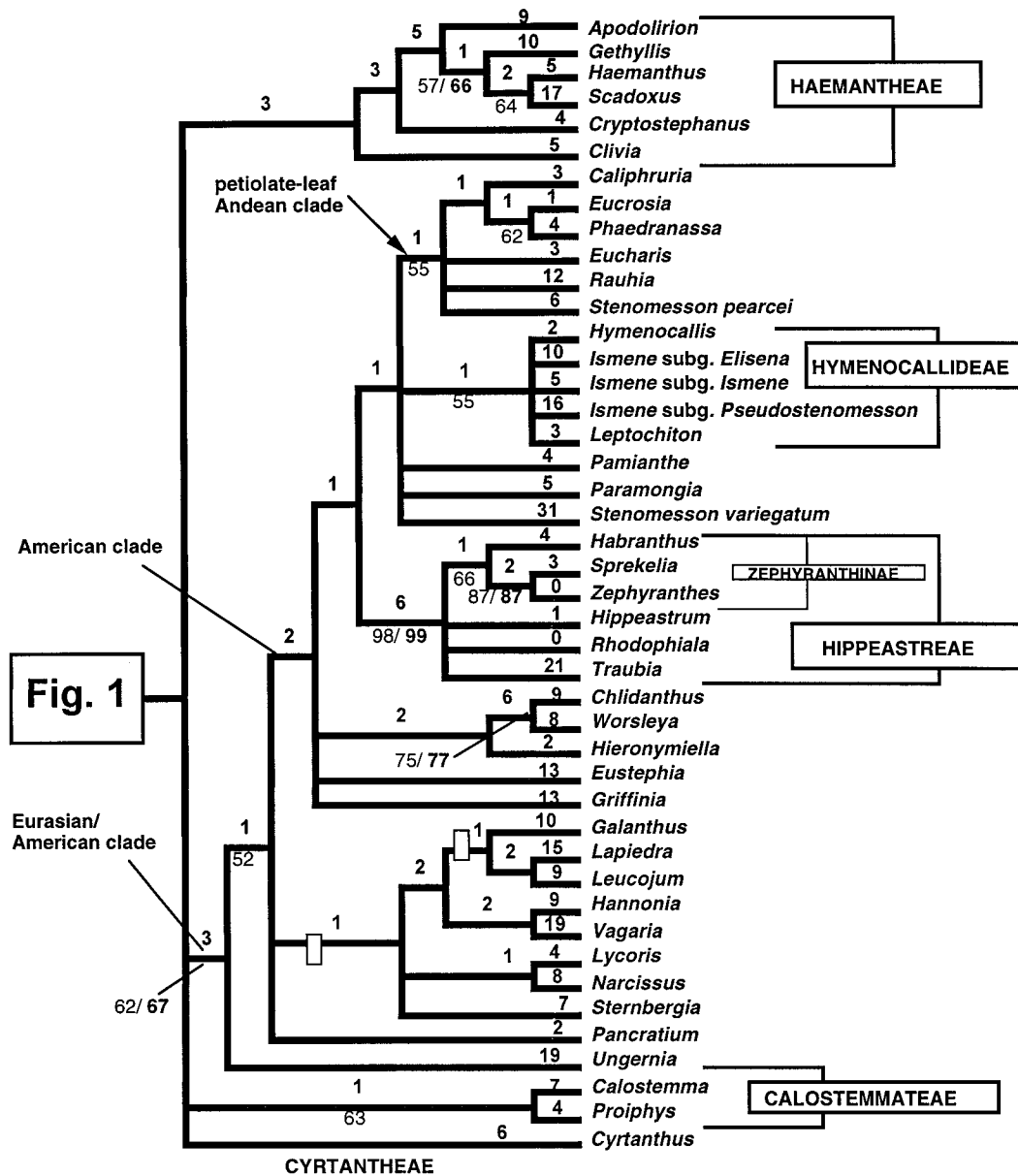


Fig. 2. One of 5000 equally parsimonious trees generated by cladistic analysis of the successively weighted *rbcL* sequence matrix for Amaryllidaceae and other Asparagalean genera. Numbers above branches are branch lengths. Bootstrap (plain) and jackknife (boldface) percentages are below branches supported by one or both. A white bar across a branch signifies lack of resolution in the strict consensus tree of the 5000 trees. The tree is continued in Fig. 1.

support (Fig. 1). The rest of the family forms a polytomy (Fig. 2) that includes a baccate-fruited clade (Haemantheae, including Gethyllideae), the Cyrtantheae (confined to Africa), Calostemmatae (Australasia), and a monophyletic Eurasian/American group. Of these latter, only the Eurasian/American clade has any bootstrap (62) and jackknife support (67). Calostemmatae have a bootstrap percentage of 63 but no jackknife support. Within the Haemantheae, *Apodolirion* and *Gethyllis* (Gethyllideae) are resolved as sister taxa in the Fitch topologies, but not in the SW trees (Fig. 2).

Within the Eurasian/American clade, the American genera are monophyletic in all trees (Fig. 2) but lack bootstrap and jackknife support. The Eurasian genera

form a polytomous grade within this clade. These sequences resolve the tribe Hippeastreae (excluding *Griffinia* and *Worsleya* = *Griffineae* Ravenna emend.) and Hymenocallideae. Tribe Hippeastreae are the only clade in Amaryllidaceae s.s. other than tribe Amaryllideae that is supported by bootstrap and jackknife percentage greater than 90% (Fig. 2). *Worsleya* appears as sister to *Chlidanthus* (Eustephieae), and *Griffinia* is unresolved (Fig. 2). A subclade representing subtribe Zephyranthinae is supported by low bootstrap percentage, but the remaining relationships within this clade are unresolved. The rest of the American tribes (Eucharideae, Eustephieae, and Stenomesseae) are not resolved by *rbcL*, but one unexpected clade with weak bootstrap support (55) encompasses all

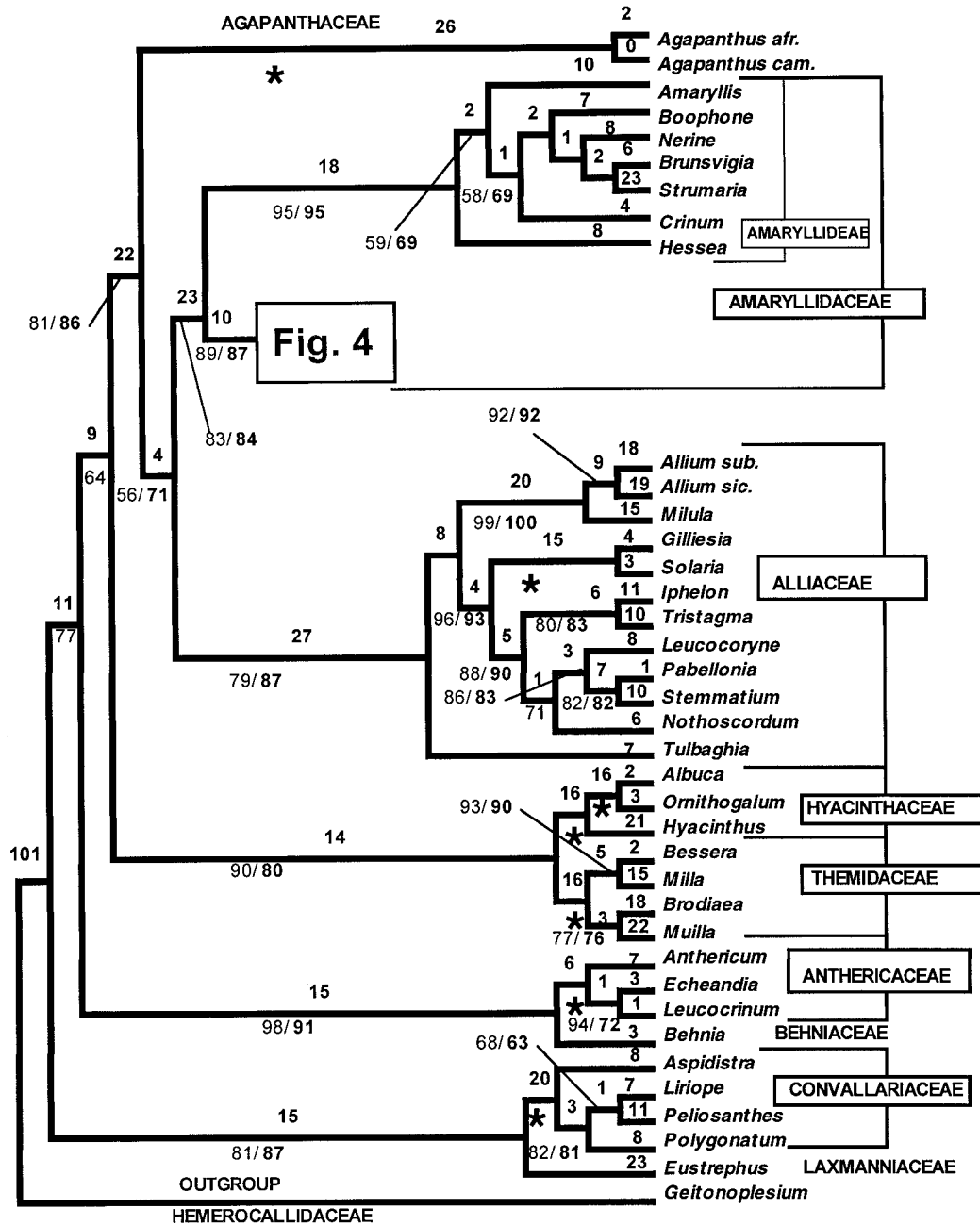


Fig. 3. One of 5000 equally parsimonious trees generated by cladistic analysis of successively weighted *trnL-F* sequence matrix for Amaryllidaceae and other Asparagalean genera. Numbers above branches are branch lengths. Bootstrap (plain) and jackknife (boldface) percentages are below branches supported by one or both. An asterisk below a branch signifies that both bootstrap and jackknife = 100%. “*Agapanthus afr.*” = *A. africanus*, “*Agapanthus cam.*” = *A. campanulatus*, “*Allium sub.*” = *A. subhirsutum*, “*Allium sic.*” = *A. siculum* var. *bulgaricum*. The tree is continued in Fig. 4.

the included petiolate-leaved Andean taxa with $2n = 46$ chromosomes.

The *trnL-F* matrix alone—Of the 1389 base positions (including gaps) included in the analysis, 378 were parsimony informative. More than 5000 equally most parsimonious trees were found of length = 1540 with CI = 0.66 and RI = 0.73. SW found more than 5000 equally parsimonious trees of length = 747723 (Fitch = 1541) with CI = 0.89 (Fitch = 0.66) and RI = 0.91 (Fitch 0.73),

the strict consensus of which is more resolved than the initial Fitch consensus. The *trnL-F* matrix (Fig. 3) resolves a monophyletic Amaryllidaceae s.s. (bootstrap and jackknife support > 80%) as sister to Alliaceae with low bootstrap (56%) and somewhat higher jackknife support (71%). *Agapanthus* is sister to the Amaryllidaceae/Alliaceae clade with supporting bootstrap and jackknife percentages of 81 and 87%, respectively.

In all *trnL-F* topologies the well-supported Amaryllidaceae is sister to the rest of Amaryllidaceae, with high

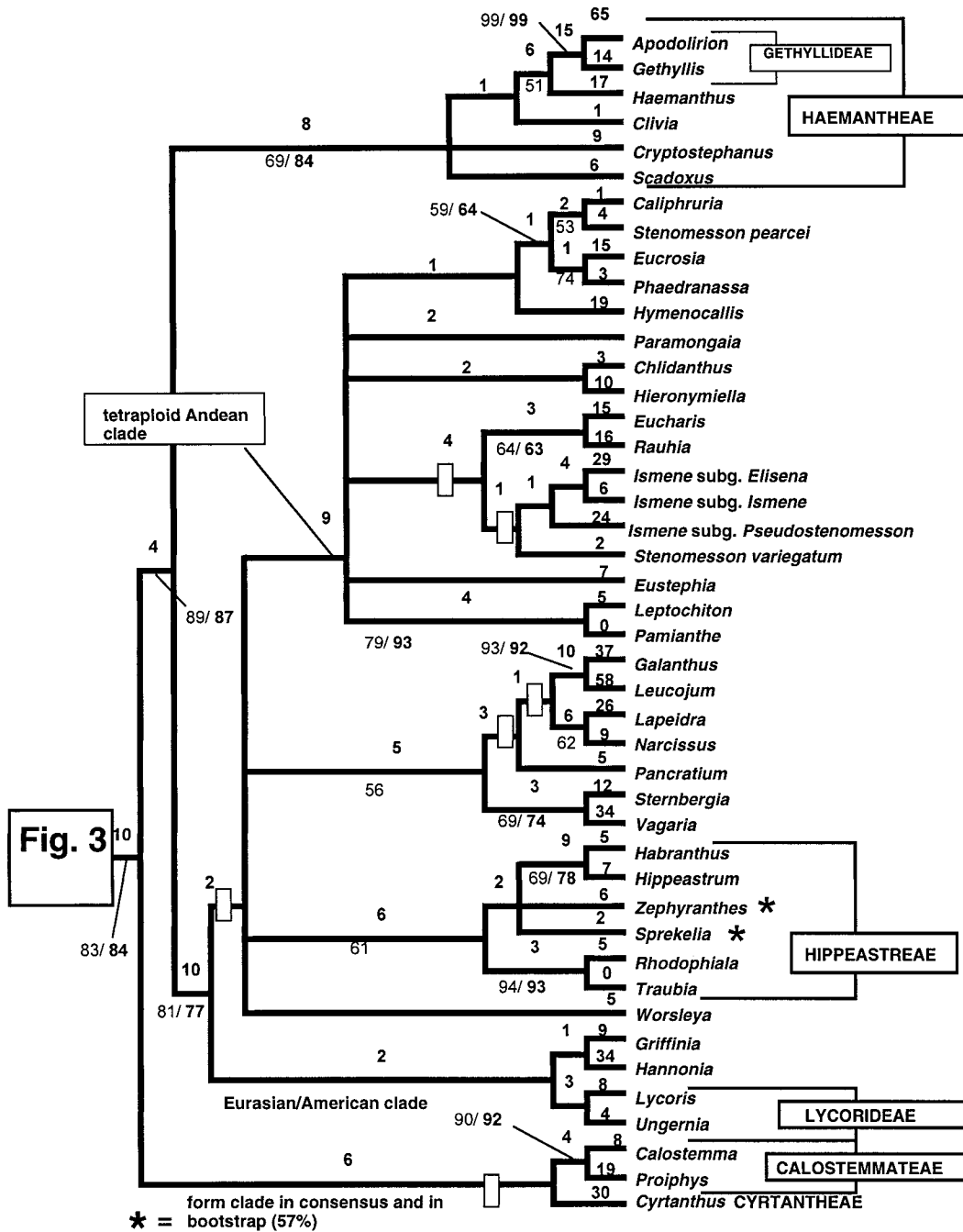


Fig. 4. One of 5000 equally parsimonious trees generated by cladistic analysis of successively weighted *trnL-F* sequence matrix for Amaryllidaceae and other Asparagalean genera. Numbers above branches are branch lengths. Bootstrap (plain) and jackknife (boldface) percentages are below branches supported by one or both. A white bar across a branch signifies lack of resolution in the strict consensus tree of the 5000 trees. The tree is continued in Fig. 3.

bootstrap and jackknife support. As with *rbcL*, the remaining African tribes (Haemantheae, Gethyllideae, Cyrtantheae) and Australasian Calostemmatae (itself, a well-supported clade) form an unresolved polytomy with the American/Eurasian taxa in the strict consensus (Fig 4). Unlike the *rbcL* topology, Gethyllideae resolves as a well-supported monophyletic subclade of Haemantheae that is sister to *Haemanthus* (Fig. 4).

Hannonia and Lycorideae (*Lycoris* and *Ungernia*) are

outside of an otherwise monophyletic Eurasian clade in which Galantheae (*Galanthus* and *Leucojum*) are resolved with high bootstrap (93%) and jackknife (92%) percentages (Fig. 4). The monophyletic Lycorideae form a weak clade with *Griffinia* and *Hannonia* as sister genera.

Compared to the *rbcL* topology, the American genera are less resolved by *trnL-F*; *Griffinia* and *Worsleya* appear outside the clade comprising all other American taxa

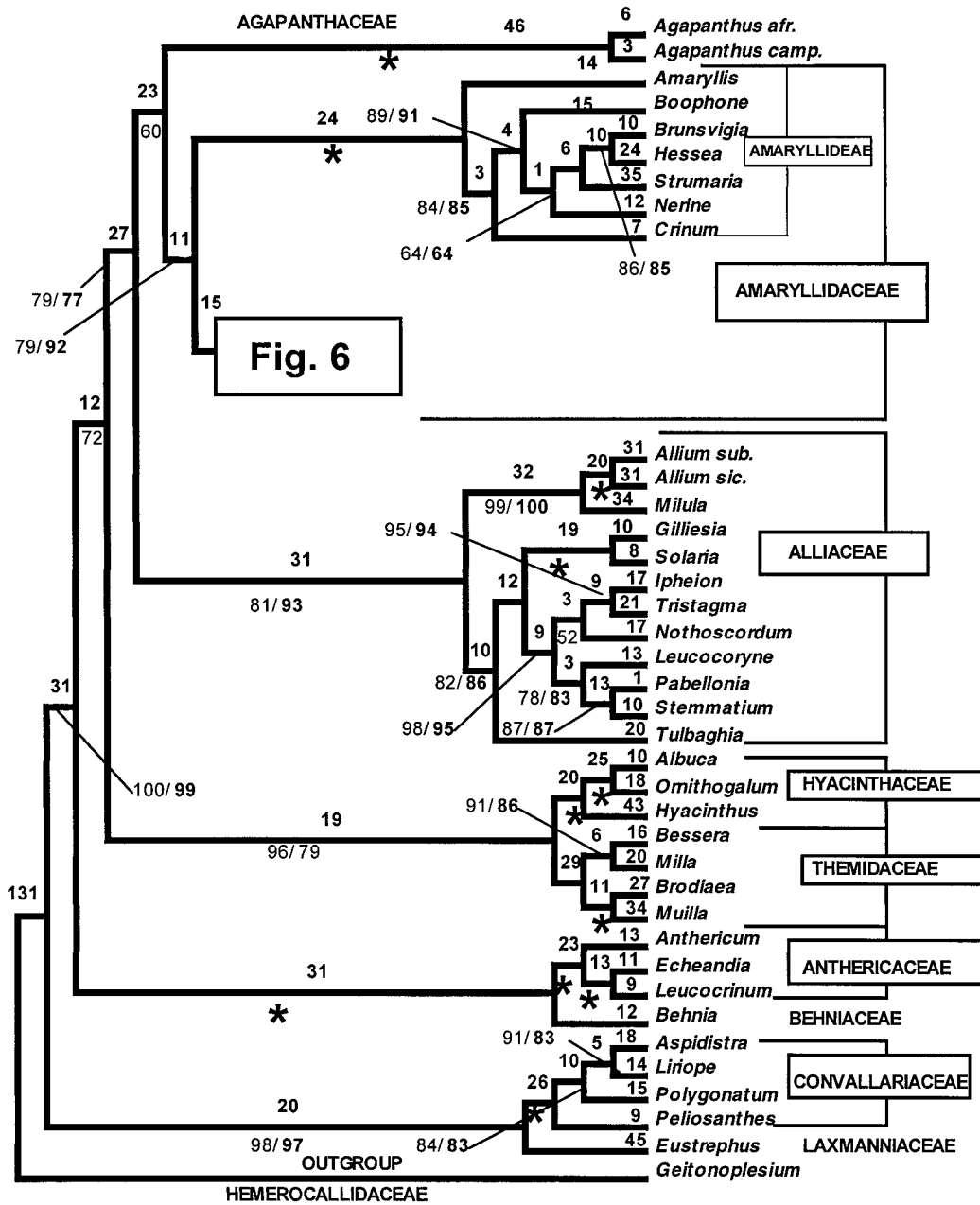


Fig. 5. One of 5000 equally parsimonious trees generated by cladistic analysis of successively weighted combined *rbcL* and *trnL-F* sequence matrix for Amaryllidaceae and other Asparagalean genera. Numbers above branches are branch lengths. Bootstrap (plain) and jackknife (boldface) percentages are below branches supported by one or both. An asterisk below a branch signifies that both bootstrap and jackknife = 100%. “*Agapanthus afr.*” = *A. africanus*, “*Agapanthus cam.*” = *A. campanulatus*, “*Allium sub.*” = *A. subhirsutum*, “*Allium sic.*” = *A. siculum* var. *bulgaricum*. The tree is continued in Fig. 6.

(Fig. 4). The petiolate Andean clade, which appears in the *rbcL* consensus, loses two members, *Eucharis* and *Rauhia*. Hymenocallideae are not resolved, and *Leptochiton* and *Pamianthe* are resolved as sister genera with moderate bootstrap and strong jackknife support. Hippeastreae (less Griffineae) appear with low bootstrap support (61) but with different internal resolution than with *rbcL*. Again, short branch lengths are characteristic of most of the internal nodes of the American clade (Fig. 4).

The combined matrix—More than 5000 equally most parsimonious trees were found of length = 2546 with CI = 0.64 and RI = 0.71. SW found more than 5000 equally parsimonious trees of length = 1194297 (Fitch = 2546) with CI = 0.89 (Fitch = 0.64) and RI = 0.89 (Fitch = 0.71). The strict consensus of the weighted trees is more resolved than the initial Fitch consensus. *Agapanthus* is sister to Amaryllidaceae in the combined topologies (Fig. 5), albeit with low bootstrap support (60%). A monophy-

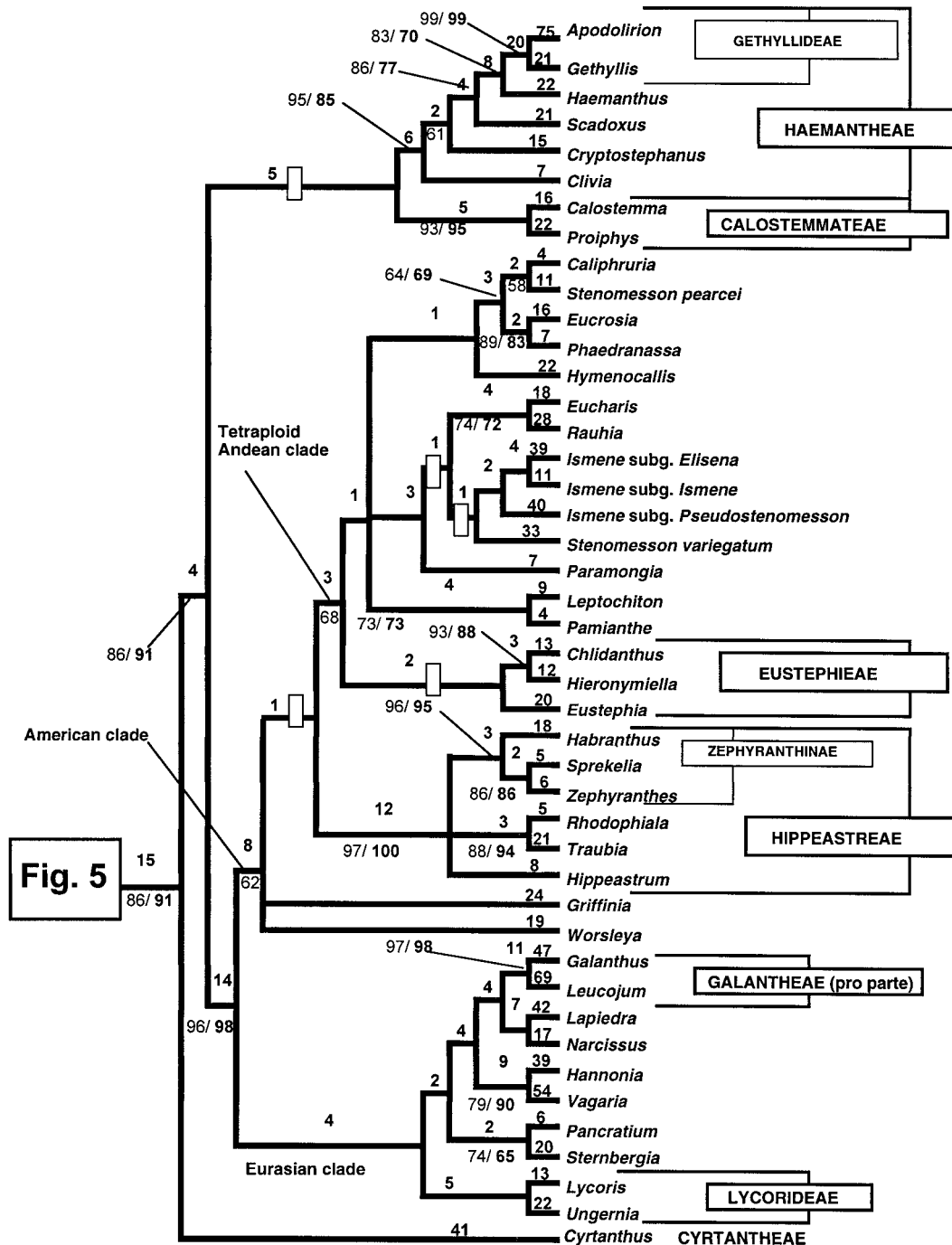


Fig. 6. One of 5000 equally parsimonious trees generated by cladistic analysis of successively weighted combined *rbcL* and *trnL-F* sequence matrix for Amaryllidaceae and other Asparagalean genera. Numbers above branches are branch lengths. Bootstrap (plain) and jackknife (boldface) percentages are below branches supported by one or both. The tree is continued in Fig. 5.

letic Alliaceae is sister to the former clade, with a bootstrap of 79% and jackknife of 77%. Both Amaryllideae and Haemantheae are well-supported tribal clades (Figs. 5–6), with higher bootstrap and jackknife percentages than in either of the separate analyses, and the former resolves as sister to the rest of Amaryllidaceae s.s.

Within Amaryllideae, most of the included genera resolve in a grade with *Amaryllis* and then *Crinum* as the

successive sister taxa to the rest (Fig. 5). Within Haemantheae, a well-supported, monophyletic Gethyllideae is again sister to *Haemanthus* (Fig. 6). As in both the individual analyses, Calostemmatae and Cyrtantheae remain as part of the polytomy inclusive of Haemantheae and the large Eurasian/American clade.

In the combined analysis, the Neotropical (American) and Eurasian genera are sister groups with strong boot-

strap and jackknife support (95, 98%), but of the two, only the American clade has weak bootstrap support (Fig. 6). *Galanthus/Leucojum*, *Hannonia/Vagarica*, and *Pan-crati-um/Sternbergia* are supported sister genera, but the remaining relationships, consistent in all trees, have no support.

Within the American clade (Fig. 6), a distinct Andean subclade has weak bootstrap support (68). Eustephieae have no consensus, bootstrap, or jackknife support. A well-supported Hippeastreae are in a polytomy with *Griffinia*, *Worsleya*, and the Andean clade. In Hippeastreae, a distinct Zephyranthinae and *Rhodophiala/Traubia* are well supported. Within the Andean clade, the resolution of Hymenocallideae, observed in the *rbcL* trees (Fig. 2), is lost, with *Ismene*, however, remaining monophyletic. *Leptochiton* and *Pamianthe* are weakly supported sister taxa. *Eucharis* and *Rauhia* fail to join the rest of a weakly supported petiolate-leaved subclade that is sister group to *Hymenocallis* (marked by a single synapomorphy).

DISCUSSION

Suprafamilial relationships of Amaryllidaceae—Fay and Chase (1996) presented a smaller *rbcL* analysis and argued for the inclusion of *Agapanthus* as a monotypic subfamily within Amaryllidaceae. The sister-group status of *Agapanthus* to Amaryllidaceae s.s. is only weakly supported by our combined matrix (bootstrap = 60%). Based on these data, it would be possible to argue for recognizing Amaryllidaceae in a modified Hutchinsonian (1934) sense, i.e., with three subfamilies, Allioideae, Agapanthoideae, and Amarylloideae.

Backlund and Bremer (1998) discussed the issue of monogeneric families and how best to treat them. They generated a set of guiding principles for classification: (1) primary principle of monophyly and (2) a set of secondary principles: (a) maximizing stability, (b) maximizing phylogenetic information (= minimizing redundancy), (c) maximizing support for monophyly, and (d) maximizing ease of identification. Principle 1 is considered most important by Backlund and Bremer (1998), as it is by most modern systematists. However, the secondary principles will vary in importance among different taxa.

Monophyly is maximized by either treating *Agapanthus* as a monogeneric family or accepting Amaryllidaceae in the Hutchinsonian sense. However, the support for a broad concept of Amaryllidaceae (including Alliaceae and Agapanthaceae) is only moderate (bootstrap = 79%, jackknife = 77%). The only morphological character that unites all three families is the pseudo-umbellate inflorescence (homoplasious with Themidaceae), whereas the Alliaceae are readily marked by their solid styles and sulfonated compounds, and the Amaryllidaceae have inferior ovaries and unique alkaloid chemistry. Maximizing stability in this case seems rather moot, given that *Agapanthus* has been maintained for years as part of Alliaceae, a classification that violates the primary principle of monophyly, and Amaryllidaceae and Alliaceae have been united on and off again over the last two centuries. However, maximizing phylogenetic information and ease of identification are best served by treating *Agapanthus* as the sole genus of a separate family (Agapanthaceae

Voight), while maintaining the independent status of Alliaceae.

The combined analysis supports most of the other relationships hypothesized by Fay and Chase (1996). The sister-group status of Themidaceae and Hyacinthaceae is confirmed with good support, and there is bootstrap support in the combined analysis for this clade as sister group to Amaryllidaceae/Alliaceae/Agapanthaceae, although Antheridaceae/Behniaceae resolves outside of this clade. It should be noted that, with this level of sampling of the broader Asparagales, these relationships are largely a matter of outgroup selection.

Relationships within Amaryllidaceae—Within Amaryllidaceae s.s., several groups are well supported within all of the analyses, some of which correspond to traditionally accepted tribes of the family. The most unexpected resolution concerns the sister status of the Eurasian/American clades. This is only supported in the combined analysis, and resolution of this group in relation to the remaining African and Australasian clades is still elusive because of short branch lengths in this portion of the trees (Figs. 2, 4, 6).

In a survey of internal morphology of American and African Amaryllidaceae, Arroyo and Cutler (1984) noted several characters that separated American genera from African. All American species surveyed have scapes with collenchyma, a one-layered rhizodermis, and obvolvate bracts. All Amaryllideae (entirely African with the exception of pantropical *Crinum*) have schlerenchyma in the scape, a multilayered rhizodermis, and equitant bracts. *Haemanthus* and *Cyrtanthus* exhibit scape and root anatomy of the American species but the equitant bracts of Amaryllideae (Arroyo and Cutler, 1984). Calostemmatae (*Calostemma* and *Proiphys*), which were not discussed by Arroyo and Cutler (1984), have equitant bracts. Many of the Eurasian genera have fused spathe bracts, which obscures the pattern of their coherence, but both *Lycoris* and *Pan-crati-um* species with free bracts show the equitant condition. Obvolvate bracts may thus be a synapomorphy of the American clade.

Two American subclades are found in the consensus of the combined analysis (Fig. 5), with both *Griffinia* and *Worsleya* forming a polytomy with them. The more weakly supported Andean subclade (tribes Eucharideae, Stenomessae and Eustephieae) is characterized by $2n = 46$ chromosomes, which has been interpreted as a tetraploid derivation from an ancestral $2n = 22$ (Meerow, 1985, 1987a, c, 1989). The strongly supported Hippeastreae is characterized for the most part by $x = 6$ or 11, with diploid chromosome numbers of 22, 24 or less. The short branch lengths and numerous polytomies in the Andean group (Fig. 6) may indicate that they are a relatively young clade with an evolutionary history tied closely to the geologically recent Andean uplift (Meerow, 1987c).

Four recognized tribes of Amaryllidaceae are consistently resolved by the plastid DNA sequences, and all receive strong bootstrap and jackknife support in at least the combined analysis. These are the Amaryllideae, Haemantheae, Calostemmatae, and Hippeastreae.

Amaryllideae—This tribe, with much of its generic diversity confined to South Africa is sister to the rest of

the Amaryllidaceae and has high bootstrap and jackknife support. Compared to other tribes in Amaryllidaceae, Amaryllideae are marked by a large number of synapomorphies (Snijman and Linder, 1996): extensible fibers in the leaf tissue, bisulcate 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 Asia, Australia, and America. Snijman and Linder's (1996) phylogenetic analysis of the tribe based on morphological, 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*). Müller-Doblies and Müller-Doblies (1996) recognized four subtribes with little discussion and no phylogenetic analysis: Crininae, Boophoniinae, Amaryllidinae, and Strumariinae, the latter two containing several segregate genera from *Hessea* and *Strumaria* (Table 1). Our sampling of this tribe is incomplete, and therefore we feel it is premature to attach a great deal of confidence to the generic sister relationships seen here. Four genera of Snijman and Linder's (1996) Amaryllidinae do form a weakly supported clade (Fig. 5) with *Amaryllis* as sister to the rest of the tribe in the *rbcL* and combined analyses. Identical positioning of *Amaryllis* occurred in Snijman's (1992) cladistic analyses if tribe Hippeastreae was used as the outgroup, with Haemantheae as outgroup (Snijman and Linder, 1996), and also both outgroups used (Snijman, 1992). *Amaryllis* resolves as sister to a clade containing the other genera they ultimately placed, with *Amaryllis*, in subtribe Amaryllidinae. Müller-Doblies and Müller-Doblies' (1996) concept of Amaryllidinae [*Amaryllis*, *Nerine*, and *Namaquanula* (= *Hessea*)] would make their subtribe Strumariinae paraphyletic and Amaryllidinae polyphyletic.

Haemantheae—This baccate-fruited tribe is another morphologically well-marked group with strong molecular support. The limits of the tribe, however, have been controversial. Müller-Doblies and Müller-Doblies (1996) insisted on retaining *Cyrtanthus* in the tribe, albeit as a monotypic subtribe, Cyrtanthinae. The basis for uniting *Cyrtanthus* with the Haemantheae has always been weak, chiefly the shared chromosome number with *Haemanthus* ($2n = 16$; Ising, 1970; Vosa and Snijman, 1984) and its strictly African range. This diploid number also occurs in some Hippeastreae (Flory, 1977; Grau and Bayer, 1991). Uniting *Cyrtanthus* with Haemantheae has no molecular support in our analyses, and we believe that *Cyrtanthus*, the only solely African genus with the flattened, winged, phytomelanous seed so common in the American clade, should be recognized as a monotypic tribe (Traub, 1963; Dahlgren, Clifford, and Yeo, 1985; Meerow and Snijman, 1998). Recognition of Gethyllideae as a distinct tribe (Müller-Doblies and Müller-Doblies, 1996; Meerow and Snijman, 1998), however, is not supported by the molecular data. Although the large, elongate, baccate fruits and small hard seeds of *Apodolirion* and *Gethyllis* are a departure from the berries and large succulent seeds

of the rest of Haemantheae, the two genera, though resolved as sister taxa (Figs. 4, 6), are firmly embedded within Haemantheae. Recognizing them as a distinct taxon would render the rest of Haemantheae paraphyletic. Haemantheae are the only tribe of Amaryllidaceae that contain rhizomatous genera (*Cryptostephanus* and *Scadoxus* in part), a condition that occurs in the sister family Agapanthaceae. This has generally been conceived as a plesiomorphy within the family (Nordal and Duncan, 1981; Meerow, 1995, 1997; Müller-Doblies and Müller-Doblies, 1996). In the *rbcL* and combined consensus trees, the three "bulbless" genera form a grade at the base of the Haemantheae, which would support this hypothesis, although *Cryptostephanus*, the only member of the tribe with the ancestral state of a phytomelanous testa, is not the first branch in the grade. *Haemanthus* and *Scadoxus*, which have been treated as one genus in the past (e.g., Hutchinson, 1934, 1959; Traub, 1963), are sister genera only in the *rbcL* topologies (Fig. 2). The position of *Scadoxus*, the only genus of the tribe polymorphic for the rhizomatous state, as the final terminal taxon in the "bulbless" grade seems reasonable. In any event, all three matrices render recognition of a subtribe Cliviinae for *Clivia* and *Cryptostephanus* by Müller-Doblies and Müller-Doblies (1996) as paraphyletic. Any further insight on the internal relationships within Haemantheae requires additional sampling.

Calostemmateae—Calostemmateae, treated as part of a polyphyletic Eucharideae by Hutchinson (1934, 1959), Traub (1963), and Dahlgren, Clifford, and Yeo (1985), were first suggested as a distinct lineage by Meerow (1989) and formally recognized by Müller-Doblies and Müller-Doblies (1996). The tribe consists of two Australasian genera (*Proiphys*, forest understory herbs of Malaysia, Indonesia, the Philippines and tropical Australia, and *Calostemma*, endemic to Australia). A few species of *Crinum*, with the broadest distribution of any genus in the family, are the only other members of Amaryllidaceae present in Australia. The indehiscent capsules of both genera are similar in appearance to the unripe berry-fruits of *Scadoxus* and *Haemanthus* (Haemantheae), but early in the development of the seed, the embryo germinates precociously, and a bulbil forms within the capsule and functions as the mature propagule (Rendle, 1901). The two genera exhibit the equitant bract condition of the African and Eurasian genera.

Hippeastreae—All but two of the genera treated by Meerow and Snijman (1998) as part of Hippeastreae are resolved as a well-supported monophyletic clade in all the analyses (Figs. 2, 4, 6). The two genera that lie outside of this clade are *Worsleya* and *Griffinia*, both Brazilian endemics, exhibiting the rare character of blue-range pigmentation in the flowers. The variable positioning of these two in the various analyses is interesting in itself. In the *trnL-F* topologies (Fig. 4), *Worsleya* is part of the basal polytomy within the Eurasian/American clade, whereas *Griffinia* weakly resolves as sister to the Mediterranean *Hannonia* (the latter on a long terminal branch). In the *rbcL* consensus (Fig. 2), *Worsleya* resolves as sister to *Chlidanthus* (Eustephieae), whereas *Griffinia* remains unresolved along with the rest of Eus-

tephieae. In the combined analysis (Fig. 6), both are positioned within the American clade, but unresolved with either Hippeastreae s.s. or the weakly supported tetraploid Andean clade. The failure of *Worsleya* or *Griffinia* to resolve as part of Hippeastreae in any of the analyses casts doubt on Müller-Doblies and Müller-Doblies' (1996) submergence of *Worsleya* in *Hippeastrum* and weakens Meerow and Snijman's (1998) retention of both genera in tribe Hippeastreae.

Another unexpected indication of relationship occurs within the tetraploid Andean clade, where a distinct petiolate-leafed subclade is resolved in the *rbcL* topologies (Fig. 2). This resolution is not retained by the *trnL-F* and combined analyses in which *Eucharis* and *Rauhia* are pulled from this group. Nonetheless, a core of petiolate genera remain monophyletic, with weak support in the combined analysis. Despite the fact that petiolate leaves have evolved independently several times elsewhere in the Amaryllidaceae (Amaryllideae, Calostemmataceae, Haemantheae, Hymenocallideae, and Hippeastreae), the molecular data begin to indicate that it may be a synapomorphy for this group. Hymenocallideae as a distinct tribe receive weak support (55%, one synapomorphy) in the *rbcL* matrix only, and the rest of Stenomessaeae is poorly resolved by all matrices.

Within the Eurasian clade of the combined analysis (Fig. 6), Lycorideae appears as sister to the rest, although without support. This tribe represents the more or less temperate Asian component of the family, with *Lycoris* ranging from Korea, through China, Myanmar, and Japan, and *Ungernia* restricted to the mountains of central Asia. Müller-Doblies and Müller-Doblies (1978) described similarities in the bulb anatomy of *Ungernia* and *Sternbergia*, a possible synapomorphy between Lycorideae and the rest of this clade. One genus of the Eurasian clade, *Pancratium*, is represented throughout Africa, tropical Asia, as well as Mediterranean Europe and the Middle East, a distribution that could signify a more ancestral position within the Eurasian clade. The current data, however, do not support this resolution for *Pancratium*, with only a single species from the Canary Islands represented in the analyses. The presence of *Pancratium* in Africa may thus be secondary, though it is the only genus outside of the African tribes Amaryllideae and Haemantheae with external trichomes (Björnstad, 1973).

Sister relationships of *Hannonia* and *Vagaris* receive good bootstrap and jackknife support, as does the traditional alliance of *Galanthus* and *Leucojum*. Crespo et al. (1995), using ITS sequences, refuted Müller-Doblies and Müller-Doblies' placement of *Lapiedra* in *Pancratieae*, and our data support a closer relationship with Galantheae or Narcisseae for this genus. However, concepts of Galantheae, Narcisseae, and Pancratieae presented in Müller-Doblies and Müller-Doblies (1996) or Meerow and Snijman (1998) are not resolved in any of the three analyses, and we believe that caution should be used before categorical statements are made about tribal lineages within this group.

The low internal branch lengths throughout the Amaryllidaceae, except in some of the deepest branches, are a striking contrast to the other asparagalean families included in the analysis (Figs. 1, 3, 5). The significance of this is not clear. It could mean that a great deal of the

modern diversity in the family is of relatively recent occurrence (as is likely, for example, within the Andean clade), or else base substitution rates in the chloroplast genome are lower within the family than for other Asparagales.

Character state evolution in the Amaryllidaceae—By optimizing morphological or other "traditional" characters onto a gene tree, one is able to gain insight about putative transformation series or state polarities that have characterized the evolution of the group under study. This can be useful for constructing a character state matrix for an ingroup in which rampant homoplasy in such characters confounds the endeavor. Certain characters that have been used to justify older intrafamilial classifications of Amaryllidaceae do show stability within some of the clades resolved by the combined analysis, while others appear extremely homoplasious (Fig. 7).

The most notable correlation between evolutionary depth as resolved by plastid DNA sequences and morphological synapomorphies is found in the Amaryllideae (Fig. 7). All of the characters listed are synapomorphous for the tribe, which terminates the longest internal branch within Amaryllidaceae on our gene trees (Figs. 1, 3, 5).

Presence or absence of bulbs—The bulbless condition occurs in the sister group to Amaryllidaceae, the monogeneric Agapanthaceae. It is also the character state for the only South African subfamily of Alliaceae, Tulbaghoideae (Fay and Chase, 1996). In Amaryllidaceae, the absence of bulbs characterizes only three genera, *Clivia*, *Cryptostephanus*, and *Scadoxus*, but the latter also includes species that form a true bulb. If this is a symplesiomorphy as most have interpreted it (Nordal and Duncan, 1984; Müller-Doblies and Müller-Doblies, 1996; Meerow and Snijman, 1998), the bulbous state has evolved at least three times in the family, in Amaryllideae, Haemantheae, and within the ancestral stock for the rest of the family.

Petiolate leaves—Petiolate or, more accurately, pseudopetiolate leaves are widespread throughout the Asparagales, and this character exhibits a great deal of homoplasy within Amaryllidaceae (Meerow and Snijman, 1998). At the extreme, one-to-few petiolate species occur in otherwise lorate-leafed genera (e.g., *Crinum*, *Hymenocallis*). The state may occur throughout a genus, but renders a tribe polymorphic (Calostemmataceae, Haemantheae, Griffineae). In the tetraploid Andean clade, a subclade is defined by the synapomorphy of a petiolate leaf in the *rbcL* trees, but *Eucharis* and *Rauhia* pull away with *trnL-F* and in the combined analyses.

Mesophyll palisade—It has been suggested that the presence or absence of a distinct palisade layer in the leaf mesophyll may have systematic significance (Arroyo and Cutler, 1984; Artyushenko, 1989). Petiolate-leafed taxa never have palisade chlorenchyma (Meerow and Snijman, 1998). It is characteristic of Amaryllideae (*Crinum* is polymorphic), but absent in Haemantheae (the state is unknown for *Gethyllis* and *Apodolirion*). In Calostemmataceae, it is present in *Calostemma* but absent in the petiolate *Proiphys* (Meerow, unpublished data). Palisade almost universally occurs in the Eurasian clade. It is ab-

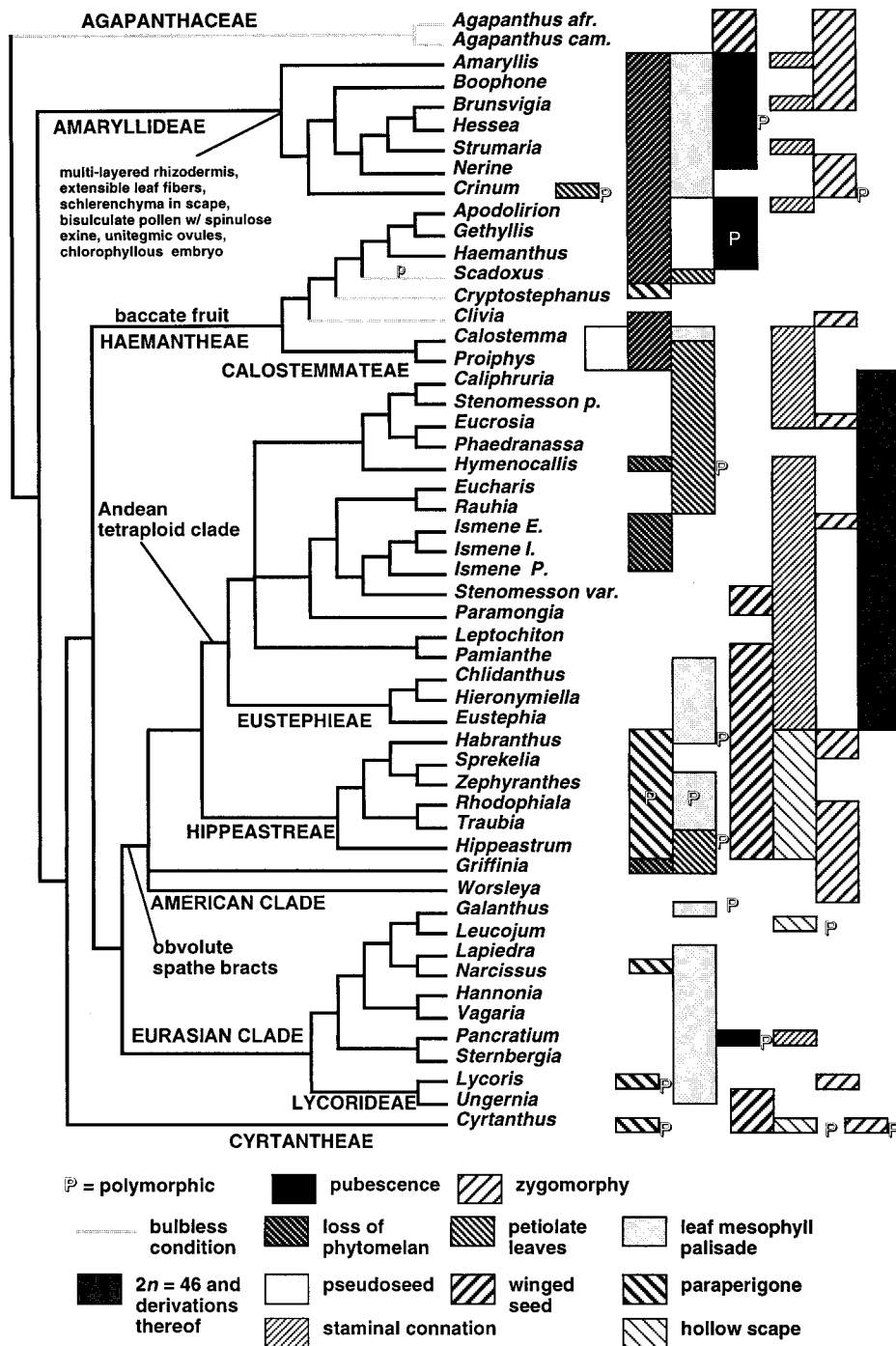


Fig. 7. Amaryllidaceae/Agapanthaceae clade from one of 5000 equally parsimonious trees generated by cladistic analysis of the successively weighted combined *rbcl* and *trnL-F* sequence matrix for Amaryllidaceae and other Asparagalean genera with selected morphological and karyological states optimized on the tree. A "P" to the right of a character bar or box refers to the state being polymorphic in the adjacent taxon; if superimposed on the character bar itself, polymorphy is widespread among all adjacent taxa. "*Agapanthus* afr." = *A. africanus*, "*Agapanthus* cam." = *A. campanulatus*, *Ismene* E = *I.* subg. *Elisena*, *Ismene* I = *I.* subg. *Ismene*, *Ismene* P = *I.* subg. *Pseudostenomesson*, *Stenomesson* p. = *S. pearcei*, *Stenomesson* var. = *S. variegatum*.

sent in *Leucojum* (Artyushenko, 1989), and *Galanthus* is polymorphic (Davis and Barnett, 1997). Within the American clade, it is wholly characteristic of the Eustephieae (Arroyo and Cutler, 1984; Meerow and Snijman, 1998) but occurs only sporadically within the Hippeas-

treae (Arroyo and Cutler, 1994). The state of *Worsleya* is not known. Outside of Eustephieae, a distinct palisade is absent from the Andean tetraploid clade (Meerow, 1987a, 1989). The inference based on the distribution of this character state on our topology (Fig. 7) is that a distinct

palisade is plesiomorphic within the family, though the state within Agapanthaceae, sister to Amaryllidaceae, has not to our knowledge been reported.

Pubescence—The presence of trichomes on the external parts of Amaryllidaceae is common only in some Amaryllideae and Haemantheae and one African species of *Pancreatum* (Arroyo and Cutler, 1984; Meerow and Snijman, 1998). It is completely unknown in the American clade, Cyrtantheae, and Calostemmataeae. It may have evolved independently in the three clades within which it occurs.

Scape characters—Solid scapes are the predominant condition in Amaryllidaceae as occurs in Agapanthaceae as well. Hollow scapes are almost universally characteristic of Hippeastreae, and thus appears to be a synapomorphy for that tribe. The only other genera within which hollow scapes occur are *Leucojum* and *Cyrtanthus* both of which are polymorphic for the character (Traub, 1963; Reid and Dyer, 1984). As discussed previously, obvolute spathe bracts seem to be apomorphic for the American clade, and the presence of schlerenchyma in the scape is an autapomorphy for Amaryllideae.

Floral symmetry—Zygomorphic and actinomorphic flowers occur in the Amaryllidaceae, and several genera (*Crinum*, *Cyrtanthus*, *Phycella*) are polymorphic. Snijman and Linder (1996) consider actinomorphy the apomorphic condition in Amaryllideae. The flowers of Agapanthaceae are zygomorphic. Within Haemantheae, only *Clivia* is zygomorphic. In the American clade, zygomorphy is the rule in the “hippeastroid” subclade. *Pyrolirion* and *Zephyranthes* (including *Haylockia*) are the only genera characterized exclusively by actinomorphic flowers, while *Phycella* (not included in the sequence analyses) is polymorphic. In the Andean subclade, only *Eucrosia*, *Plagiolirion*, and *Ismene* subgenus *Elisena* are exclusively zygomorphic; *Rauhia* is polymorphic. The Eurasian clade is on the whole actinomorphic; only *Lycoris* is characterized by zygomorphic flowers. The mosaic occurrence of actinomorphy throughout the family (Fig. 7) and the occurrence of polymorphic genera suggest that transformations between the two states of floral symmetry may be easily modified by pollinator-mediated selection, and perhaps controlled by one or few genes.

Paraperigone—The “paraperigone” is an anomalous secondary outgrowth of the perianthal meristem with ramifying vasculature (Arber, 1939; Singh, 1972), not to be confused with a similar-looking structure formed by staminal connation (see below). It is most well developed (and typified) by the corona of *Narcissus*. Such a well-developed paraperigone occurs in only one other genus, the Chilean endemic *Placea* (Hippeastreae). However, a homologous series of fimbriae, scales, or a continuous callose ring occurs in *Cryptostephanus* (Haemantheae), one or two species of *Cyrtanthus*, and variably throughout Lycorideae and Hippeastreae. It has thus probably evolved at least three times (Haemantheae, Cyrtantheae, and the Eurasian/American clade), but from a meristematic potential that is deep rooted in the family. Polymorphism

for this character within genera may suggest that it is easily lost.

Staminal connation—The fusion of the staminal filaments with the single most important character for which Traub (1957, 1963) justified recognizing his “infracfamily” Pancratioidinae, a subfamilial taxon that in fact was glaringly polyphyletic. Though staminal connation is a widespread character state within the Andean tetraploid clade (Fig. 7), it is paralleled elsewhere in the family, particularly in Amaryllideae subtribe Amaryllidinae (Snijman and Linder, 1996), the Calostemmataeae, in *Gethyllis*, and some species of *Cyrtanthus* (Reid and Dyer, 1984). In the Eurasian clade it occurs in *Pancreatum*, the flower morphology in general of which bears striking resemblance to several Andean genera (Hymenocallideae pro parte, *Paramongaia*, and *Pamianthe*). Meerow and Dehgan (1985) attempted to link these so-called “pancratioid” genera by pollen morphology, but a more parsimonious explanation may be convergence for pollinator specificity (Morton, 1965; Bauml, 1979; Grant, 1983). However, *Pancreatum* and these Andean genera are monophyletic in a larger sense (as part of the Eurasian/American clade), and the exact position of *Pancreatum* within the Eurasian subclade is still not strongly resolved (Fig. 6).

Fruit and seed characters—Fruit and seed morphology have been an important focus of experimentation within the family. Baccate fruits have apparently evolved only once, despite the difference in gross morphology between the long, aromatic fruit of *Gethyllis* and *Apodolirion* and the berries of the rest of Haemantheae. Phytomelan [the ancestral state for all Asparagales (Huber, 1969)] has been lost from the testa as many as five times in the Amaryllidaceae: in Amaryllideae, Griffineae, Hymenocallideae, Haemantheae, and Calostemmataeae [in Calostemmataeae a true seed never forms, but an integumentary rudiment is present (Rendle, 1901)]. In both Haemantheae and Hymenocallideae, phytomelan is found around the seeds of one genus each (*Cryptostephanus* and *Leptochiton*, respectively). The loss of one integument [or both, as been controversially reported for some *Crinum* (Prillieux, 1858; von Schlimbach, 1924; Tomita, 1931; Markötter, 1936, but see Snijman and Linder, 1996)] is synapomorphic for Amaryllideae.

A flattened, winged seed, which occurs in Agapanthaceae, is very common in the American clade, but otherwise occurs only in *Ungernia* (Lycorideae) and Cyrtantheae. The most similar type of seed to this is the D-shaped seed of *Worsleya* and some *Pancreatum*. A dry, hard, wedge-shaped or irregularly round seed is characteristic of most of the Eurasian clade (except Lycorideae), frequently with an elaiosome at the chalazal end. Among all genera of the family, *Pancreatum* is the most polymorphic for seed type (Werker and Fahn, 1975).

Characterization of certain seeds of Amaryllidaceae as fleshy (regardless of whether phytomelan is present) has led, in the past, to false homologies (see discussion in Meerow, 1989). Truly fleshy seeds occur in Amaryllideae (in which case the bulk of the seed volume is endosperm; Rendle, 1901), Hymenocallideae (the fleshy portion is integumentary; Whitehead and Brown, 1940), and some

water-rich Haemantheae. But an "intermediate" state occurs in a number of genera in which the seed is round, turgid, but not really fleshy (the seed will burst under pressure rather than give way), and contains copious, oily endosperm. This type of morphology is found in *Cryptostephanus* (Haemantheae), *Lycoris* (Lycorideae), Eucharideae sensu Meerow (1989), *Griffinia*, and a single species of *Hippeastrum*.

Chromosome number—A chromosome number of $2n = 22$ is considered plesiomorphic in Amaryllidaceae due to the broad occurrence in many of the tribes of the family (Goldblatt, 1976; Flory, 1977; Meerow, 1984, 1987b). Andean-centered genera in the tribes Eucharideae, Eustephieae, Hymenocallideae, and Stenomessaeae are characterized by a somatic chromosome number of $2n = 46$ or presumptive derivations thereof (Di Fulvio, 1973; Flory, 1977; Williams, 1981; Meerow, 1987a, b). Resolution of these genera as a clade in our plastid DNA trees supports the interpretation of a monophyletic polyploid origin for these tribes from an ancestor with $2n = 22$ via chromosome fragmentation or duplication and subsequent doubling or vice versa (Satô, 1938; Lakshmi, 1978; Meerow, 1987b).

Biogeographic implications—Raven and Axelrod (1974) postulated a western Gondwanaland origin for Amaryllidaceae sensu Huber (1969), and this is supported by the plastid DNA phylogeny. The deepest branches of the topology originate in Africa, including the sister group of the family *Agapanthus*. Africa has also been the site of considerable innovation in the family's history as well, as typified by the Afrocentric tribes Amaryllideae, Haemantheae, and Cyrtantheae. Most of the diversity within those three tribes is, however, centered in South Africa, and thus may reflect radiation engendered by the more recent paleoclimatic and geological history of Africa encompassing Neogene and later times (Axelrod, 1972; Raven and Axelrod, 1974). The increased aridity of the African climate and the uplift of the continental mass beginning near the end of the Oligocene, further abetted by Quaternary climatic fluctuations, were catastrophic to many elements of the African flora, but it may have been a selective pressure for diversity among groups of geophytes capable of adapting to increasing drought. The geophyte richness of South Africa is well documented (Goldblatt, 1978), and the Cape region has been suggested as a possible refuge for certain African plant and animal groups as the tropical flora of the continent was impoverished (Raven and Axelrod, 1974). However, the three basal genera of the baccate-fruited Haemantheae according to our combined analysis (Fig. 6), *Clivia*, *Cryptostephanus*, and *Scadoxus*, are all forest understory taxa, do not form bulbs, and are at least in part (*Scadoxus*, *Cryptostephanus*) elements of tropical vegetation farther north. *Cryptostephanus* does not occur in South Africa at all, and this is the only genus of Haemantheae in which the plesiomorphic state of a phytomelanous testa occurs.

The Calostemmateae, the only exclusively Australasian element of the family, may have been isolated from the African lineages as Australia separated from western Gondwanaland (Raven and Axelrod, 1974). Direct migration between Africa and Australia may have persisted

up through the close of the early Cretaceous, although India and Madagascar may have provided a less direct corridor up until the late Cretaceous (Raven and Axelrod, 1974). That the Calosternmateae remains within the unresolved grade of otherwise African tribes would suggest relative antiquity for the lineage. *Crinum* is the only amaryllid that is known to occur on Madagascar, despite the island's probable role as a refuge for taxa decimated by the Neogene African extinctions, whereas indigenous Indian amaryllids are restricted to *Crinum* and two to three species of *Pancratium*. The adaptations of *Crinum* for long-distance dispersal have been demonstrated (Koshimizu, 1930), and *Pancratium* may have been able to directly enter India from either Africa or Eurasia during the late Cretaceous or early Eocene (Raven and Axelrod, 1974).

The sister relationship of the Eurasian/Mediterranean clade to the American genera raises the interesting question of when and where the Amaryllidaceae, in the main, entered the New World. It should be noted that this probably occurred at least twice, as the arrival of *Crinum* in the Americas via oceanic dispersal was undoubtedly an unrelated event (Arroyo and Cutler, 1984). Although migration between Eurasia and North America has been possible throughout most of angiosperm history (Raven and Axelrod, 1974), the hypothesized pathways have been for plants of temperate forest biota and not considered to be important for plants of subhumid or semiarid vegetation (Raven, 1971, 1973). However, eastern North America and western Europe may have shared a warm, seasonally dry climate from the late Cretaceous to the early Eocene (Axelrod, 1973, 1975), which might have allowed east/west movement of species, with island chains of the Mid-Atlantic ridge providing stepping stones. Such a Madrean-Tethyan hypothesis would have the initial entry of the Amaryllidaceae into the New World through North America. Although there are members of the family in Mexico and the southern United States, they are, with the exception of the ubiquitous *Crinum*, components of terminal subclades (*Zephyranthes*, *Habranthus*, *Hymenocallis*) in an overall American phylogeny based on nuclear DNA ITS sequences (Meerow, Guy, and Li, 1998), all of which are linked to more basal taxa endemic to South America. The validity of the Madrean-Tethyan hypothesis has more recently been questioned by various studies using isozyme, plastid DNA restriction fragment length polymorphisms (RFLPs), or cladistic analyses of taxa considered emblematic of the disjunction: *Buxus* (Köhler and Brückner, 1989), *Datisca* (Liston, Rieseberg, and Hanson, 1992), *Lavatera* (Ray, 1994), *Quercus* (Manos, 1992; Nixon, 1993), *Pinus* (Little and Crutchfield, 1969; Miller, 1993), and *Styrax* (Fritsch, 1996). In these cases, the hypothesized Madrean-Tethyan linkage is not resolved as monophyletic, the taxon itself is not monophyletic, or the estimated time of divergence does not fit the Madrean-Tethyan hypothesis.

Given the extant distribution of Amaryllidaceae in North America and the generic richness south of the equator, a northern latitude entry into the New World for the family would necessitate massive extinction in North America sometime after migration to South America took place. Glaciation would be the likely factor involved. Little migration of plants from North America to South

America probably took place before the Eocene (Raven and Axelrod, 1974). All indications are that the movement of extant Amaryllidaceae has been northward from South America (e.g., Meerow, 1987b, 1989). This does not necessarily preclude an earlier, initial arrival in North America, migration to South America, and a more recent, but secondary, return of some elements of the family to North America long after glaciation extirpated the founder populations. However, if a North American entry is hypothesized, this begs the question of why the Eurasian sister clade has been so successful in adapting to temperate habitats, which constitute the majority of the species in tribes Galantheae, Narcisseae, and Lycorideae, whereas the American clade is relatively depauperate of temperate climate adaptation. There is nothing in our data to prove or disprove an initial New World entry of the Amaryllidaceae into North America, and the issue is for the present unresolved.

In conclusion, our combined analysis of plastid DNA sequences *rbcL* and *trnL-F* provide good support for the monophyly of the Amaryllidaceae and indicate Agapanthaceae as its likely sister family. The Alliaceae are in turn sister to the Amaryllidaceae/Agapanthus clade. The origins of the family are African. The phylogenetic relationships with Amaryllidaceae s.s. resolve strongly along biogeographic lines. The tribe Amaryllideae, primarily South African and well supported by numerous morphological synapomorphies, is sister to the rest of Amaryllidaceae. The remaining two African tribes of the family, Haemantheae and Cyrtantheae, are well supported, but their position relative to the Australasian Calostemmateae and a large clade comprising the Eurasian/American genera, is not yet clear. The Eurasian elements of the family and the American genera are monophyletic sister clades. Internal resolution of the Eurasian clade only partially supports currently accepted tribal concepts, and few conclusions can be drawn on the relationships of the genera based on these data. A monophyletic Lycorideae (Central and East Asian) is weakly supported. *Galanthus* and *Leucojum* (Galantheae pro parte) are supported as sister genera by the Bootstrap. The American clade shows a higher degree of internal resolution. A monophyletic Hippeastreae (less *Griffinia* and *Worsleya*) is well supported, and a distinct subtribe, Zephyranthinae, is resolved as well. A distinct Andean clade marked by a chromosome number of $2n = 46$ and derivations thereof is resolved with weak support, and a distinct petiolate Andean subclade composed of elements of the tribes Eucharideae and Stenomessaeae is partially resolved with weak support. The lack of resolution of *Griffinia* and *Worsleya* in the overall American clade, and of *Eustephia* in the Andean subclade, may indicate that these genera represent more isolated elements of the American lineage.

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