PHYLOGENY OF AGAVACEAE BASED ON *ndh*F, *rbc*L, AND ITS SEQUENCES: IMPLICATIONS OF MOLECULAR DATA FOR CLASSIFICATION

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ABSTRACT

Great advances have been made in our understanding of the phylogeny and classification of Agavaceae in the last 20 years. In older systems Agavaceae were paraphyletic due to overemphasis of ovary position or habit. Discovery of a unique bimodal karyotype in *Agave* and *Yucca* eventually led to a reexamination of concepts and relationships in all the lilioid monocots, which continues to the present day.

Developments in cytogenetics, microscopy, phylogenetic systematics, and most recently DNA technology have led to remarkable new insights. Large-scale *rbc*L sequence studies placed Agavaceae with the core Asparagales and identified closely related taxa. Analysis of cpDNA restriction sites, *rbc*L, and ITS nrDNA sequences all supported removal of Dracaenaceae, Nolinaceae, and clarified relationships. Agavaceae s.s. presently consists of *Agave, Beschorneria, Furcraea, Hesperaloe, Hesperoyucca, Manfreda, Polianthes, Prochnyanthes,* and *Yucca*. In this paper we analyze recently obtained *ndh*F sequence data from Agavaceae and Asparagales and discuss the implications for classification. Parsimony analysis of *ndh*F data alone resolves most genera of Agavaceae and supports the inclusion of *Camassia, Chlorogalum, Hesperocallis,* and *Hosta* within Agavaceae s.l. Analysis of combined *ndh*F and *rbc*L data sets of selected Asparagales results in better resolution and stronger bootstrap support for many relationships. Combination of all available *ndh*F, *rbc*L, and ITS data in a single analysis results in the best resolution currently available for Agavaceae s.l. Implications for classification schemes past and present are discussed.

Key words: Agavaceae, Asparagales, classification, ITS, *ndh*F, phylogeny, *rbc*L.

INTRODUCTION

Agavaceae are a family of rosette-forming, often spiny plants centered in warm and dry areas of Mexico, the southwestern USA, and Caribbean basin. The family includes plants of great natural beauty, ornamental value, cultural significance, and economic importance. Although the family is easily recognized there have been questions about the circumscription of Agavaceae, how the genera are related to each other, and how Agavaceae are related to other families. In recent years, the family has been treated broadly with 18 genera (Cronquist 1981) and narrowly with nine core genera (Dahlgren et al. 1985; Verhoek 1998). The undisputed genera of Agavaceae s.s. are *Agave* L.*, Beschorneria* Kunth, *Furcraea* Vent., *Hesperaloe* Engelm., *Hesperoyucca* (Engelm.) Baker, *Manfreda* Salisb., *Polianthes* L., *Prochnyanthes* S. Watson, and *Yucca* L. Associated genera such as *Camassia* Lindl., *Chlorogalum* (Lindl.) Kunth, *Hesperocallis* A. Gray, and *Hosta* Tratt. have certain similarities to Agavaceae and have been considered closely related by some, but have never been fully integrated into the family.

Botanists of the 18th, 19th, and early 20th centuries relied on relatively few morphological characters to classify Agavaceae and its allies (Bentham and Hooker 1883; Engler 1888). In the 20th century, comparisons of karyotypes (McKelvey and Sax 1933; Satô 1935), embryology (Wunderlich 1950), anatomy and microcharacters (Huber 1969; Dahlgren and Clifford 1982), pollen (Álvarez and Köhler 1987), pollination and reproductive biology (Eguiarte 2000; Slauson 2001), and cladistic analysis (Dahlgren and Rasmussen 1983; Hernández 1995*a*) have all contributed to our understanding of Agavaceae and their relationships. The greatest advances in the last 20 years have been made using molecular markers, especially DNA sequence data (Eguiarte et al. 1994, 2000; Bogler and Simpson 1995, 1996; Chase et al. 1995). Advances in DNA sequencing technology have made it relatively easy for botanists to acquire molecular sequence data. Increased computer power has enabled researchers to combine sequence data from different gene regions and analyze very large data sets indeed (Fay et al. 2000; Soltis et al. 2000).

In this paper we review some of the major ideas in Agavaceae classification and report the results of phylogenetic studies utilizing recently acquired chloroplast *ndh*F sequences, alone and in combination with previously published *rbc*L and nuclear ITS rDNA data sets. The results are integrated with past and present ideas concerning classification of Agavaceae.

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Classification of Agavaceae Past and Present

Agavaceae s.s. are native to the New World, and so were unknown to Old World botanists before Columbus and other explorers ventured to the Americas and brought them back to Europe. The native peoples of Mesoamerica and the American Southwest were certainly very familiar with *Agave* and *Yucca,* and used these plants for food, beverages, medicine, fiber, cloth, ropes, and in rituals (Castetter et al. 1938). *Agave* was harvested directly from wild or semidomesticated and carefully cultivated populations, and perhaps transported long distances (Gentry 1982). Among the Aztecs, all *Agave* species were known as *metl,* with different prefixes appended to distinguish uses and forms. *Agave* was so important to the Aztecs that it acquired religious significance and was deified as the goddess *Mayahuel* (García-Mendoza 1998). *Agave* continues to be an important cultural icon in Mexico today, as well as the basis for the tequila industry. The first Europeans to encounter *Agave* were probably Christopher Columbus and his crew. In his voyages to the West Indies he must have encountered species of what we now know as *Agave* and *Furcraea. Agave* was widely cultivated in Europe and is illustrated in seventeenth century botany books and herbals. As the family Agavaceae had not yet been described, these specimens were classified as *Aloe* L., a well-known Old World genus with similar spiny leaves and tubular flowers, and *Agave* became known as American aloe. Linnaeus (1753) named and described four species of *Agave,* (transferred from *Aloe*), four species of *Yucca,* and *Polianthes tuberosa* L. Several of his species of *Agave* were later recognized as species of *Furcraea* and *Manfreda.* Linnaeus included these genera in Class Hexandria Monogyna, identified by having six stamens and a single pistil. Although Linnaeus' sexual system for identifying plants was simple, the higher categories he proposed were artificial and not widely adopted. Many of our modern plant families are instead traced directly back to the system of de Jussieu (1789). This system used a combination of characters such as ovary position, attachment of the stamens, and whether the petals are separate or united to create more natural groups. In de Jussieu's system, *Yucca* and other plants with six petals and a superior ovary were placed into the order Lilia, and those with an inferior ovary such as *Agave* in Bromeliae. This separation of *Agave* and *Yucca* must have seemed natural at the time, and persisted throughout the nineteenth and early twentieth century, until Hutchinson reunited them in 1934.

The first formal description of Agavaceae as a family-level taxon was provided by Endlicher (1841), who included *Agave, Furcraea,* and *Littaea* Tagl. in the order Agaveae—an order being equivalent to a family today. *Littaea* included those agaves with a spicate inflorescence and eventually became subgen. *Littaea* (Tagl.) Baker of *Agave.* Bentham and Hooker's (1883) system of classification in *Genera Plantarum* emphasized the importance of ovary position. *Agave* was placed in Amaryllideae, with *Hypoxis* and other taxa with an inferior ovary. *Yucca* went into Liliaceae, tribe Dracaeneae, with *Dasylirion* Zucc.*, Dracaena* Vand. ex L., and other lilioid plants with a superior ovary, more or less woody trunk and fibrous leaves. The treatments of *Agave* and *Yucca* by Engler (1888) and later Krause (1930) in *Die Natürlichen Pflanzenfamilien* were essentially the same as Bentham's.

Yucca was grouped with *Dasylirion* and *Dracaena,* whereas *Agave, Beschorneria, Furcraea,* and *Polianthes* went into Amaryllidaceae, subfamily Agavoideae. Lotsy (1911) was perhaps the first to refer to this group as Agavaceae, although still without *Yucca.*

Perhaps the greatest advance in the formal classification of Agavaceae came with Hutchinson's (1934) *Families of Flowering Plants,* in which *Yucca* was added to the family. In this system the xerophytic vegetative habit was considered as important as floral characters such as ovary position. Hutchinson's Agavaceae included *Agave, Beschorneria, Furcraea, Hesperaloe, Manfreda,* and *Yucca,* recognized as Agavaceae s.s. at the present time, and also *Cordyline* Comm. ex. R. Br., *Dasylirion, Doryanthes* Corrêa, *Dracaena, Nolina* Michx., *Phormium* J. R. Forst. & G. Forst., and *Sansevieria* Thunb. At about this time, the unusual bimodal $N = 30$ karyotypes of *Agave* and *Yucca,* with 5 large and 25 small chromosomes, were reported by McKelvey and Sax (1933), Whitaker (1934), and Satô (1935). The strong resemblance of the Agavaceae karyotype to bimodal karyotypes of *Camassia, Hesperocallis,* and *Hosta* was also noted and the possibility that these genera might be related to Agavaceae was suggested by Granick (1944).

In recent times, Cronquist (1981) adapted the broad treatment of Hutchinson (1934) with few changes, even though he realized some of the genera were out of place. Cronquist was concerned that if he couldn't use habit to recognize families, then some other families would fall. Besides, large families such as Liliaceae are simple to teach and remember. In contrast, Takhtajan (1980) recognized many small families, and a narrowly circumscribed Agavaceae, but he included *Hosta* in the family. Influenced by advances in anatomy, phylogenetic systematics, and cladistic theory, Dahlgren et al. (1985) also recognized many small families, but made more of an effort to recognize only families that were demonstrably monophyletic. Putting the monophyletic pieces back together into larger groups has proven to be quite challenging. Using primarily microcharacters of the seed coat, cuticle, endosperm, and embryo, Huber (1969) aggregated many of these small families into larger groups, which he informally identified as asparagoid, dioscoreoid, and so forth. Agavaceae were placed in the asparagoid group, which was treated as order Asparagales by Dahlgren et al. (1985). Many Asparagales are characterized by berry fruits or capsules, and seeds encrusted by a black pigment known as phytomelan.

The earliest molecular studies involving Agavaceae were the immunological studies of Chupov and Kutiavina (1981). Using serology and immunoelectrophoresis techniques on a wide array of lilioid monocots, they demonstrated that both *Camassia* and *Hosta* had a very strong serological affinity to *Agave* and *Yucca.* Their results went largely unnoticed in the West, but supported the ordinal rank of Asparagales (Dahlgren 1983). The chloroplast gene *rbc*L has been very useful in studies of monocot phylogeny (Chase et al. 1995). These *rbc*L data strongly supported Asparagales as a distinct clade separate from Liliales. Two constant groups were recognized within Asparagales, a lower paraphyletic grade of families characterized by simultaneous microsporogenesis, and a higher monophyletic clade characterized by successive microsporogenesis. The *rbc*L data provided some support for

potential family groups within Asparagales, but the bootstrap values were low and many branches collapsed in the strict consensus trees. The *rbc*L data showed that the family Agavaceae as circumscribed by Hutchinson (1934) and Cronquist (1981) was not monophyletic and that Agavaceae s.s. were on a separate clade from a Nolinaceae–Dracaenaceae clade and the other taxa (Eguiarte et al. 1994).

Chloroplast DNA (cpDNA) restriction site studies (Bogler and Simpson 1995) strongly supported and expanded the hypothesis that Agavaceae s.s. were on a separate clade from the Dracaenaceae–Nolinaceae–Convallariaceae (sensu Dahlgren et al. 1985) genera included in the family. *Beaucarnea* Lem., *Calibanus* Rose, *Dasylirion, Dracaena, Nolina,* and *Sansevieria* were all on a clade with berry-fruited, soft-leaved genera such as *Aspidistra* Ker Gawl.*, Maianthemum* F. H. Wigg., and *Polygonatum* Mill., included in Liliaceae by Cronquist (1981). The cpDNA also provided resolution for the genera of Agavaceae. *Hosta* was shown to share molecular synapomorphies with Agavaceae. *Hesperaloe* and *Yucca whipplei* Torr. (= *Hesperoyucca*) were demonstrated to be closely related. *Beschorneria* and *Furcraea* were also closely related, and together formed a sister group to *Agave* s.l. Subsequent studies using ITS rDNA sequence (Bogler and Simpson 1996) confirmed most of these relationships, and provided additional resolution. The ITS data strongly indicated a close relationship of *Camassia* and *Hosta* to Agavaceae. Molecular variation within *Agave* was very low in these studies.

One of the more innovative approaches to higher level classification of angiosperms that takes into account results of molecular phylogenies is the Angiosperm Phylogeny Group (APG 1998; APG II 2003). In this system all taxa are monophyletic, paraphyletic taxa are not accepted. There are no subclasses, only informal higher groups (e.g., asterids, eudicots, magnoliids, monocots, rosids, etc.), orders, and families. The system is based almost entirely on molecular phylogenies, with contributions from many people. It is mostly concerned with establishing monophyletic orders that refer directly to large clades, with a secondary focus on families. The first edition (APG 1998) recognized 40 orders and 462 families. Asparagales are recognized as an order with 29 families, Agavaceae among them. The APG (1998) family nomenclature is illustrated in Fig. 1 and 2. A potential problem with using the criterion of monophyly is that it tends to result in rampant splitting and a proliferation of families, making it difficult to remember or teach families, and creates practical problems for floristic botanists. Nevertheless, APG offers the hope of a system that will stabilize as more phylogenetic data are incorporated. The APG II (2003) update recognizes 25 families in Asparagales and suggests ways of reducing the number of families if the user so chooses. The core Asparagales contain 11 families, but can optionally be reduced to two families, Alliaceae and Asparagaceae, with Agavaceae going into the Asparagaceae (see Fig. 3).

In this paper we analyze the position of Agavaceae within Asparagales with new combinations of data, present evidence for including additional genera in Agavaceae, and draw together all the available sequence data into a single combined analysis of relationships of the genera of Agavaceae.

MATERIALS AND METHODS

The selection of taxa for this study was guided by our focus on family and generic relationships of Agavaceae and by the availability of sequence data. The sequences used in these analyses were obtained from a variety of sources (listed in Table 1). The *rbc*L sequences were all downloaded from GenBank. Many of the *ndh*F sequences were generated by J. Chris Pires in collaboration with Mark Chase, Sean Graham, and others using the protocol outlined in Pires and Sytsma (2000). Agavaceae *ndh*F sequences were generated by David Bogler at Florida International University and Fairchild Tropical Botanic Garden in the lab of Javier Francisco-Ortega. The *ndh*F sequences were amplified and sequenced in three overlapping pieces using various combinations of primers (listed in Table 2), derived from the paper by Terry et al. (1997) on *ndh*F sequence variation in Bromeliaceae.

Standard cycle sequencing techniques were used with an ABI 377 (Applied Biosystems, Foster City, California, USA) automated sequencer at FIU, using standard reagents and the manufacturer's protocols. The *ndh*F sequences were combined and aligned using CLUSTAL_X (Thompson et al. 1997). The data sets were analyzed separately and in various combinations with PAUP* vers. 4.0 (Swofford 1998). The *rbc*L sequences were all downloaded from GenBank and aligned using CLUSTAL_X. ITS 1 and ITS 2 sequences are the same ones used in a previous study of Agavaceae (Bogler and Simpson 1996). The ITS primers, sequencing technique, alignment procedures, GenBank accession numbers, and voucher specimens are provided in that paper.

Sequence data sets were assembled and combined using MacClade vers. 3.05 (Maddison and Maddison 1992) and PAUP*. Sequences in the data sets were trimmed at each end so that they began and ended at the same character. Parsimony searches were made using heuristic settings for all analyses, with all characters unordered and equally weighted, and tree bisection-reconnection (TBR) branch swapping in effect. Strict consensus trees were generated from the pool of most parsimonious trees found. Bootstrapping was carried out in PAUP* using 100 replicates with the number of trees saved per replicate limited to 100.

The first analysis examined sequence variation within the *ndh*F data set alone and its relative utility in resolving relationships of Agavaceae and selected Asparagales. Complete *ndh*F sequences were unambiguously aligned and analyzed with PAUP*. The *ndh*F data matrix included 38 taxa and a total of 286 parsimony informative characters.

For the second analysis, we focused on the relationships of Agavaceae within Asparagales using combined *ndh*F and *rbc*L data sets with a larger number of taxa representing major clades of Asparagales. In the majority of cases, the species were the same in both *rbc*L and *ndh*F data sets. In a few cases, the species in the *rbc*L data set differed from those in the *ndh*F data set, but were combined to represent the genus in the analysis. For a few taxa only partial *ndh*F sequences were available. The combined *rbc*L–*ndh*F data matrix included 60 taxa, with a total of 925 parsimony-informative characters.

In the third analysis, we attempted to summarize what is currently known of relationships within Agavaceae and all its closest relatives by combining all available data from all
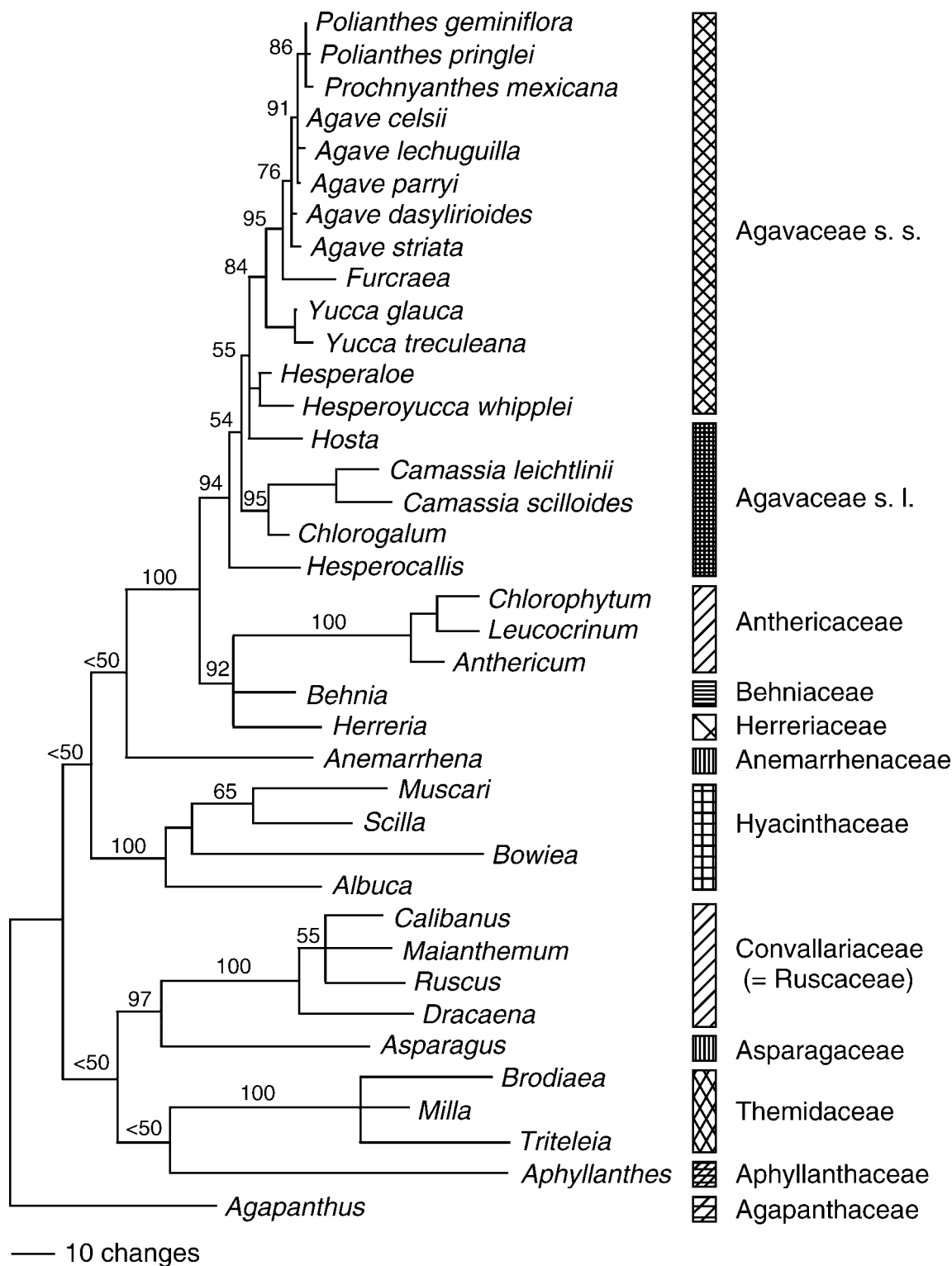
Fig. 1.—Strict consensus of 36 equally parsimonious trees from analysis of the *ndh*F data set for Agavaceae and related genera. Family names follow APG (1998). Numbers at nodes are bootstrap support values. Branch lengths correspond to distance.

three markers, *ndh*F, *rbc*L, and ITS. For some additional members of Agavaceae only partial *ndh*F sequences from the 3′ region were available. ITS sequences were available only for Agavaceae. Inclusion of partial data is justified because both *rbc*L and the 5′ region of *ndh*F are relatively conservative and exhibit little or no variation within Agavaceae s.s. (Chase et al. 1995; Eguiarte et al. 1994). ITS sequences are for the most part too variable to make reliable alignments outside Agavaceae s.s. (Bogler and Simpson 1996). Including ITS or other variable regions such as *trn*L–
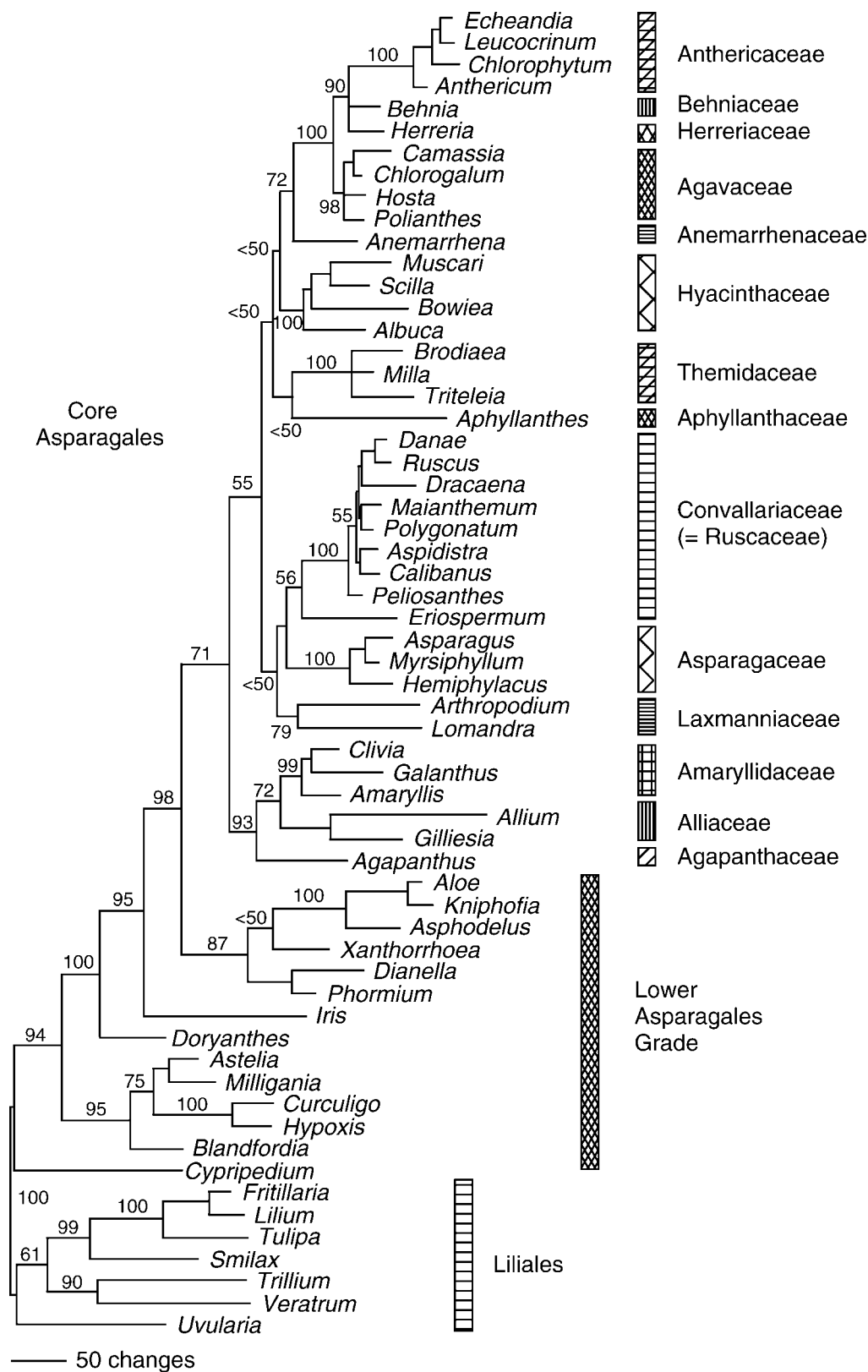
Fig. 2.—Strict consensus of 32 equally parsimonious trees found in analysis of combined *ndh*F and *rbc*L data sets from Asparagales and Liliales. Family names follow APG (1998). Numbers at nodes are bootstrap support values. Branch lengths correspond to distance.
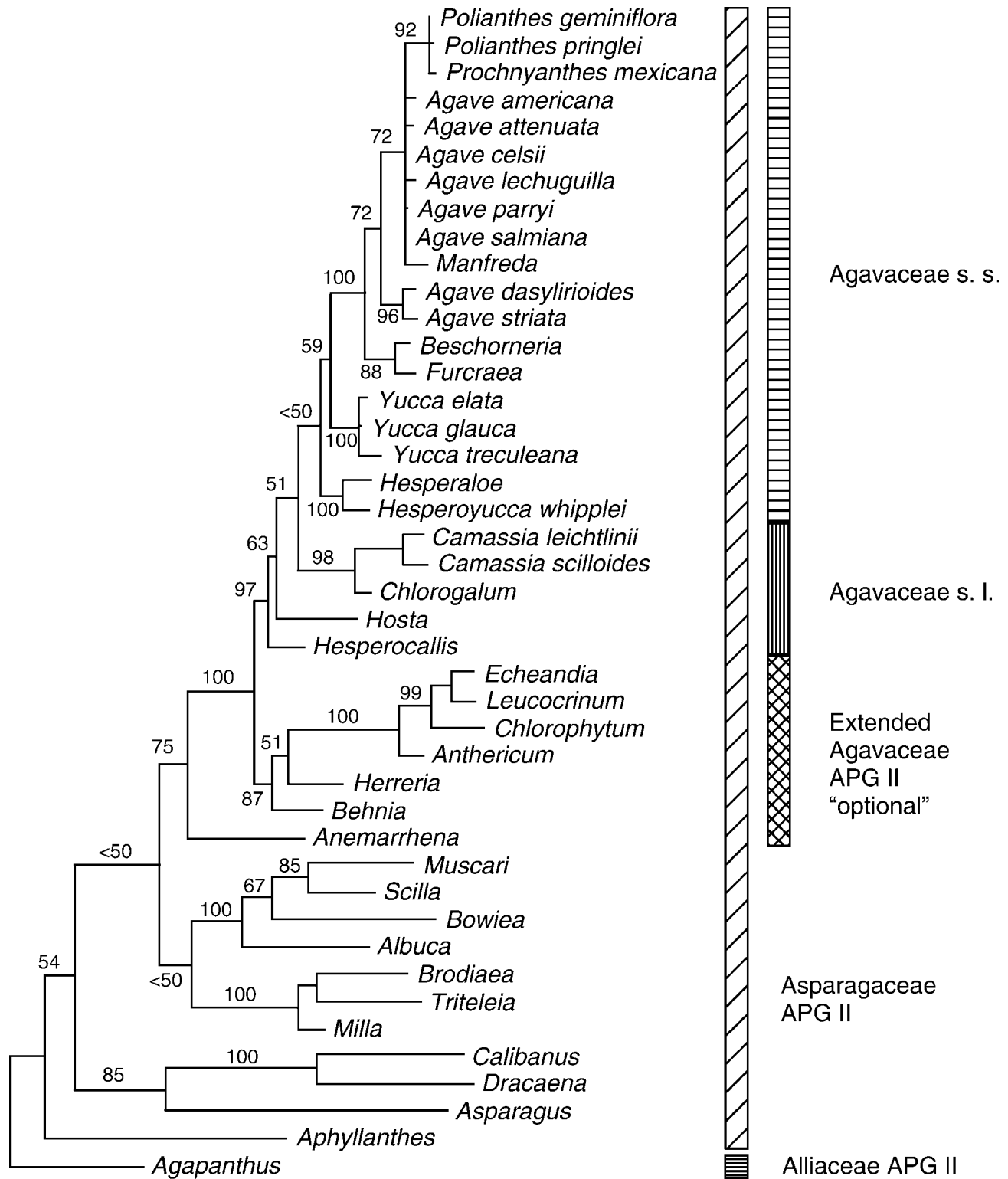
Fig. 3.—Strict consensus of equally most parsimonious trees found in analysis of combined *ndh*F, *rbc*L, and ITS data sets for Agavaceae. Family names follow APG II (2003), and illustrate the different options for recognizing family groups in Agavaceae and core Asparagales. Numbers at the base of the branches are bootstrap support values. Branch lengths correspond to distance.

F (Fay et al. 2000) in a large data set like this might actually decrease resolution and bootstrap support by introducing homoplasy due to misalignment or base saturation. The data matrix included 43 taxa and 578 parsimony informative characters.

RESULTS

Evaluation of ndh*F Sequence Data*

The relative utility of *ndh*F sequence data was assessed by parsimony analysis of complete *ndh*F sequences for 38 taxa of Agavaceae s.s. and selected core Asparagales. This data set included almost all genera of Agavaceae s.s. and basal Agavaceae s.l.—*Camassia, Chlorogalum, Hesperocallis,* and *Hosta.* This analysis resulted in 36 equally parsimonious trees, each with a consistency index of 0.655 and a retention index of 0.720. The strict consensus of these 36 trees is shown in Fig. 1. The tree is rooted using *Agapanthus* L'Hér., based on the results of previous studies. In general, resolution and support is very good in the more terminal nodes, and rather weak in some of the lower nodes. The extent to which Agavaceae are resolved by *ndh*F variation is about the same, or perhaps a little less, than that achieved with combined ITS 1 and ITS 2 data (Bogler and Simpson 1996), which is about one-fourth the length of *ndh*F. *Aphyllanthes* L.–Themidaceae are weakly supported as sister to Convallariaceae s.l. (following APG 1998, including Dracaenaceae, Nolinaceae, and Ruscaceae). Hyacinthaceae are on a strongly supported clade, and sister to *Anemarrhena* Bunge*, Herreria* Ruiz & Pav.–*Behnia* Didrichsen–Anthericaceae, and Agavaceae. The *ndh*F data strongly support *Hesperocallis, Camassia–Chlorogalum,* and *Hosta* as basal members of the Agavaceae clade (bootstrap 94%), although resolution of these taxa relative to each other is weakly supported. Agavaceae s.s. are more or less resolved by the *ndh*F data, and comparable to the resolution by cpDNA restriction sites and ITS data (Bogler and Simpson 1995, 1996). *Hesperaloe* and *Hesperoyucca* are resolved as sister taxa, although these are not on the same branch with *Yucca* as in the ITS analysis (Bogler and Simpson 1996). *Furcraea* is strongly supported (95%) as sister to *Agave* s.l. (including *Manfreda, Polianthes,* and *Prochnyanthes*). Resolution within *Agave* is very low. *Agave dasylirioides* and *A. striata* differed from other species of *Agave,* but were not strongly united as they were with ITS data. This is surprising because they share at least two unique *ndh*F synapomorphies. The *ndh*F sequence of *A. striata* contained a unique 142-base deletion in the 5′ region, not shared by *A. dasylirioides* or any other species in this small sample, and perhaps this deletion affects the resolution. *Polianthes* and *Prochnyanthes* are strongly united by several synapomorphies.

Combined ndh*F and* rbc*L Data Sets*

Analysis of combined *ndh*F and *rbc*L data sets for 60 selected taxa of Asparagales and Liliales resulted in 32 equally parsimonious trees, each with a length of 4431 steps, a consistency index of 0.453 and a retention index of 0.593. The strict consensus of these 32 trees is shown in Fig. 2. Overall, the topology is congruent with studies of *rbc*L data alone (Chase et al. 1995), but combining *ndh*F with *rbc*L

data resulted in greater resolution in the strict consensus tree and higher bootstrap support values. The topology is very similar to that obtained by Fay et al. (2000) in an analysis of combined *rbc*L, *atp*B, *trn*L intron, and *trn*L–F sequences. The taxa representing Liliales form a strongly supported clade that was designated as the outgroup for presentation purposes, although broader studies have shown that the ordinal relationships are not yet completely resolved (Chase et al. 2000). Asparagales are well supported by our data, as they are in studies of *rbc*L alone (Chase et al. 1995) and in combination with other markers (Fay et al. 2000; Soltis et al. 2000). At the base of Asparagales is a paraphyletic grade of clades often referred to as lower Asparagales. At the very base are the orchids, an extremely large group here represented by *Cypripedium* L., and a clade including *Blandfordia* Sm., *Curculigo* Gaertn. and *Hypoxis* L., and *Astelia* Banks & Sol. ex R. Br. and *Milligania* Hook. f., in Asteliaceae. The positions of *Doryanthes* and *Iris* L. are congruent with previous studies, as is the clade containing *Aloe* and its relatives, and *Xanthorrhoea* Sm. Of interest here is the observation that some of these lower Asparagales with woody trunks and fibrous leaves were once placed in Agavaceae or considered closely related (Cronquist 1981).

The so-called higher or core Asparagales clade receives bootstrap support of 71%. At its base is a clade containing the Agapanthaceae–Amaryllidaceae–Alliaceae alliance, supported by bootstrap values of 93%, and congruent with previous studies of *rbc*L alone and in combination. This is followed by a large clade containing Asparagaceae, Convallariaceae, and Laxmanniaceae, using the APG (1998) names for families. Support for this clade is rather poor (bootstrap value less than 50%), indicating a need for additional attention. The branch uniting woody taxa such as *Calibanus* and *Dracaena* with the smaller, herbaceous taxa such as *Maianthemum, Polygonatum,* and *Peliosanthes,* is very strong, with 100% bootstrap support, but variation and resolution within this clade are low. The branch supporting the odd sub-Saharan genus *Eriospermum* Jacq. ex Willd. as sister to Convallariaceae s.l. has relatively weak (56%) bootstrap support in our analysis, in contrast to the core support reported by Fay et al. (2000) and McPherson et al. (submitted) .

Agavaceae belongs to a complex of clades within the core Asparagales. The topology of this complex based on combined *ndh*F and rbcL data is congruent with previous analyses of *rbc*L data alone and in combination (Fay et al. 2000). The data strongly supports the close relationship of *Camassia, Chlorogalum,* and *Hosta* to Agavaceae s.s., here represented by *Polianthes.* The sister group of Agavaceae is a clade containing *Behnia, Herreria,* and representative Anthericaceae, with 100% bootstrap support. *Behnia* and *Herreria* are basal to Anthericaceae, with 90% bootstrap support, but the relationship between these taxa is unresolved in this tree. Anthericaceae are supported by 100% bootstrap values. *Anemarrhena* is supported as sister to this whole Agavaceae–Anthericaceae complex. Hyacinthaceae are well supported with 100% bootstrap support, as is Themidaceae. In this study, *Aphyllanthes* is weakly supported at the base of Themidaceae, although in other studies it is weakly supported at the base of Hyacinthaceae (Fay et al. 2000), sister to Laxmanniaceae (Pires et al. 2005) or sister to Agavaceae (McPherson et al. submitted) . Monte Carlo simulations have

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Table 1.   List of taxa included in sequence analyses and available GenBank accession numbers.

Taxon	*rbc*L	*ndh*F	*ITS1*	*ITS2*
Agapanthus campanulatus Leighton	Z69220	—	—	—
A. africanus (L.) Hoffmanns.	—	AF508405	—	—
Agave americana L.	—	—	U23997	U24017
A. attenuata Salm.	—	—	U23998	U24018
A. celsii Hook.	—	AF508398	—	—
A. dasylirioides Jacobi & Bouché	—	DQ071892	U23999	U24019
A. lecheguilla Torr.	—	DQ071893	U24000	U24020
A. parryi Engelm.	—	DQ071894	—	—
A. salmiana Otto	—	DQ071895	—	—
A. striata Zucc.	—	DQ071896	U24001	U24021
Alania endlicheri Kunth	Y14982	AY191170	—	—
Albuca setosa Jacq.	—	AF508391	—	—
Allium subhirsutum L.	Z69205	—	—	—
A. textile Nels. & Macbr.	—	AF547000	—	—
Aloe bakeri Scott-Elliot	Z73680	—	—	—
A. nyeriensis Christian	—	AY225054	—	—
Amaryllis belladonna L.	Z69219	AY225029	—	—
Anemarrhena asphodeloides Bunge	Z77251	AY191162	—	—
Anthericum liliago L.	Z69225	AF508402	—	—
Aphyllanthes monspeliensis L.	Z77259	AY191167	—	—
Asparagus officinalis L.	L05028	AY147788	—	—
Asphodelus aestivus Brot.	Z73682	AF508409	—	—
Aspidistra elatior Blume	Z77269	AY225010	—	—
Astelia alpina Banks & Solander	Z77261	—	—	—
A. banksii A. Cunn.	—	AY191164	—	—
Behnia reticulata Didr.	Z69226	AY191168	—	—
Beschorneria yuccoides Koch.	—	—	U24008	U24028
Blandfordia nobilis Sm.	Y14984	—	—	—
B. punicea Sweet	—	AY191169	—	—
Bowiea volubilis Harv.	Z69237	AF508392	—	—
Brodiaea coronaria (Salisb.) Engelm.	Z69210	—	—	—
B. elegans Hoover	—	AF508357	—	—
Calibanus hookeri Trel.	Z77276	AY225011	U24009	U24029
Camassia leichtlinii (Baker) Wats.	Z69238	—	—	—
C. scilloides (Raf.) Cory	—	DQ071897	U24010	U24030
Chlorogalum pomeridianum (DC) Kunth	Z69228	AY225049	—	—
Chlorophytum alismaefolium Baker	—	AY191163	—	—
C. comosum Baker	L05031	—	—	—
Clivia miniata Regel	L05032	U20539	—	—
Curculigo capitulata Kuntze	Z73701	AY225061	—	—
Cypripedium irapeanum La Llave & Lex.	Z73706	—	—	—
C. calceolus L.	—	AY225063	—	—
Danae racemosa (L.) Moench	Z73708	AY225013	—	—
Dianella ensifolia (L.) DC	M96960	AY225071	—	—
Doryanthes excelsa Corrêa	Z73697	AY225060	—	—
Dracaena aubryana Brongn.	Z77270	AY191186	—	—
Echeandia Ortega sp.	Z77258	AY225051	—	—
Eriospermum bayeri P. L. Perry	Z77277	AY225015	—	—
Fritillaria raddeana Regel	Z77293	—	—	—
F. meleagris L.	—	AF276003	—	—
Furcraea cahum Trel.	—	DQ071898	—	—
F. pubescens Tod.	—	—	U23977	U24037
Galanthus plicatus M. Bieb.	Z69218	AY225035	—	—
Gilliesia graminea Lindl.	Z69208	AY225027	—	—
Hemiphylacus latifolius S. Wats.	Z73688	AY225020	—	—
Herreria montevidensis Klotzsch	Z69230	—	—	—
H. salsaparilha Mart.	—	AY191178	—	—
Hesperaloe funifera (Koch.) Trel.	—	DQ071899	U23978	U24038
Hesperocallis undulata A. Gray	—	AY225050	—	—
Hesperoyucca whipplei (Torr.) Trel.	—	DQ071900	U23996	U24054
Hosta rectifolia Nakai	L10253	—	—	—
H. ventricosa Stearn	—	AF508401	—	U23980
Hypoxis glabella R. Br.	Y14989	—	—	—

Table 1. Continued.

Taxon	*rbc*L	*ndh*F	*ITS1*	*ITS2*
H. juncea Sm.	—	AY191179	—	—
Iris ensata Thunb.	D28332	—	—	—
I. tenax Dougl.	—	AY191181	—	—
Kniphofia uvaria Hook.	Z73689	AY225057	—	—
Leucocrinum montanum Nutt.	Z77252	AY225052	—	—
Lilium superbum L.	L12682	AY007655	—	—
Lomandra longifolia Labill.	L05039	AF547004	—	—
Maianthemum dilatatum (Wd.) Nels. & Macbr.	Z77272	—	—	—
M. racemosum (L.) Link	—	AY225016	—	—
Manfreda virginica (L.) Salisb.	—	DQ071901	U23984	U24043
Milla biflora Cav.	Z69216	AF508371	—	—
Milligania sp.	—	AY225053	—	—
M. densiflora Hook.	Y14990	—	—	—
Muscari neglectum Guss.	Z77278	—	—	—
M. comosum (L.) Mill.	—	AF547006	—	—
Myrsiphyllum Willd. sp.	Z77260	AY225021	—	—
Peliosanthes Andrews sp.	Z77273	AY225017	—	—
Phormium tenax Forst.	Z69232	—	—	—
P. cookianum Le Jolis	—	AY191177	—	—
Polianthes geminiflora (Lex.) Rose	Z69227	AY225048	U23989	U24047
P. pringlei Rose	—	DQ071902	U23990	U24048
Polygonatum humile Fisch	AB029828	—	—	—
P. pubescens (Willd.) Pursh	—	AY191191	—	—
Prochnyanthes mexicana (Zucc.) Rose	—	DQ071903	U23991	U24049
Ruscus aculeatus L.	Z77274	AY225018	—	—
Scilla biflora Ruiz & Pav.	Z69222	—	—	—
S. natalensis Planch.	—	AF508397	—	—
Smilax china L.	D28333	—	—	—
S. hispida Muhl. ex Torr.	—	AF276018	—	—
Trillium sessile L.	D28167	—	—	—
T. flexipes Raf.	—	AY191205	—	—
Triteleia bridgesii (S. Wats.) Greene	Z69198	—	—	—
T. grandiflora Lindl.	—	AF508380	—	—
Tulipa kolpakowskiana Baker	Z77292	—	—	—
T. pulchella Fenzl.	—	AF276010	—	—
Uvularia perfoliata L.	Z77315	—	—	—
U. sessilifolia L.	—	AF276023	—	—
Veratrum album L.	D28168	—	—	—
V. viride Aiton	—	AF276024	—	—
Xanthorrhoea hastilis R. Br.	Z73710	—	—	—
X. semiplana	—	AY191207	—	—
Yucca treculeana Carr.	—	DQ071904	U23995	U24053
Y. elata Engelm.	—	—	U23994	U24052
Y. glauca Nutt.	—	AF547014	—	—

demonstrated the potential for substantial bias in maximum parsimony-based inference of the phylogenetic position of Aphyllanthaceae (McPherson et al. submitted) .

Combined ndh*F,* rbc*L, and ITS Data Sets*

For the third analysis, we combined all the available *rbc*L, *ndh*F, and ITS sequences of Agavaceae and associated core Asparagales into a single data set. This data included complete and partial sequences, and some taxa for which one or another sequence was entirely lacking, as indicated in Table 1. Parsimony analysis resulted in well over 1000 equally parsimonious trees, each with a length of 1985 steps, a consistency index of 0.691, and a retention index of 0.698. The strict consensus of 1000 equally parsimonious trees is presented in Fig. 3, rooted with *Agapanthus.* The large number of trees found in the heuristic search is probably related to the inclusion of species of *Agave* with nearly identical sequences, and perhaps to the incomplete nature of the data sets. The overall topology is very similar to the other two analyses presented in this paper, which is not surprising since they are based on many of the same sequences, but the representation of Agavaceae taxa is more complete and the resolution better than in previous studies. Some of the differences in resolution and bootstrap support are probably influenced by the stronger resolution of Agavaceae s.s. afforded by addition of the ITS data set. The position of *Aphyllanthes* is somewhat unstable, as seen elsewhere (Pires et al. 2005; McPherson et al. submitted). Here it is sister to the rest of the taxa included in this study. In the previous two analyses it was sister to Themidaceae, and sister to Hyacinthaceae in

Table 2.   Primers used to amplify *ndh*F sequences in Agavaceae, derived from Terry et al. (1997).

*ndh*F	032F	5′-TACCTTTTCTTCCACTTCCAGTT-3′
*ndh*F	451F	5′-TGGGAACTTGTGGGAATGTG-3′
*ndh*F	745R	5′-CCTTCCATAGCATCAGGTAACCA-3′
*ndh*F	1101F	5′-GGAACCTATTGTTGGATATTCACC-3′
*ndh*F	1101R	5′-GGTGAATATCCAACAATAGGTTCC-3′
*ndh*F	1318F	5′-GGATTAACCGCATTTTATATGTTTC-3′
*ndh*F	1318R	5′-GAAACATATAAAATGCGGTTAATCC-3′
*ndh*F	1600R	5′-CATAGTATTGTCCGATTCGTGAGG-3′
*ndh*F	2110R	5′-CCCCCTATATATTTGATACCTTCTCC-3′

Fay et al. (2000). Asparagaceae–Convallariaceae are strongly supported and sister to the remaining taxa. Themidaceae and Hyacinthaceae are sister taxa in this tree, though bootstrap support for this relationship is less than 50%.

In all three analyses presented here, *Anemarrhena* is sister to the group containing Agavaceae s.l. and *Herreria–Behnia*–Anthericaceae, with moderate bootstrap support of 75%. For this reason, *Anemarrhena* and taxa above this node might be considered as a well-supported, monophyletic, extended Agavaceae family as suggested by APG II (2003). This clade consists of two major groups with very strong bootstrap support. One group contains *Behnia* and *Herreria,* resolved in this analysis, and the strongly supported clade containing Anthericaceae. In all analyses this group is the sister to Agavaceae. The other strongly supported clade is Agavaceae s.l. with its associated basal genera. *Hesperocallis,* for which only *ndh*F data was available, is strongly supported as a basal member of Agavaceae, with 97% bootstrap support. As in all previous molecular studies, *Camassia, Chlorogalum,* and *Hosta* are placed somewhere near the base of Agavaceae. The relative position of *Hosta* and *Chlorogalum–Camassia* is variable in these analyses and bootstrap support values are low in this part of the tree, indicating the need for additional study.

Hesperaloe and *Hesperoyucca* are strongly supported as sister taxa, congruent with previous cpDNA and ITS sequence studies (Bogler and Simpson 1995, 1996; Bogler et al. 1995). This pair comes out as basal to the rest of Agavaceae s.s., but with low bootstrap support. This position is similar to the tree derived from cpDNA restriction site analysis (Bogler and Simpson 1995), but in the combined ITS data tree *Hesperaloe* and *Hesperoyucca* are on a separate clade that includes *Yucca* and *Camassia.* At this point, it is difficult to say whether this difference is influenced by the amount or quality of data, or perhaps an artifact of combining partial data. The three species of *Yucca* are strongly supported. Agavaceae taxa with an inferior ovary comprise a very strongly supported clade with a bootstrap value of 100%. *Beschorneria* and *Furcraea* are joined, however, only ITS data was available for *Beschorneria.*

Agave s.l. falls with in the Agavaceae clade, with moderate bootstrap support of 72%. *Agave dasylirioides* and *A. striata* appear on a separate branch with 96% bootstrap support. This support is higher than it was in the analysis of ITS sequences alone, probably reflecting the contribution of *ndh*F support. There is very little variation in *ndh*F or ITS sequences in the other species of *Agave* and no resolution. Several species had identical sequences, but were included

anyway to emphasize the lack of variation. The only taxa that are consistently resolved are the two species of *Polianthes* and *Prochnyanthes mexicana,* which share a few unique *ndh*F and ITS characters. *Manfreda virginica* was not resolved from *Agave* in this analysis, though cpDNA linked *M. virginica* to *A. lechequilla* (Bogler and Simpson 1995) and ITS data alone linked it to *Polianthes–Prochnyanthes* (Bogler and Simpson 1996). The lack of sequence variation in *Agave* is frustrating, and has resulted in one of us screening cpDNA spacer sequence regions for variation (*atp*B–*rbc*L, *trn*T–*trn*L, *trn*H–*psb*A, *rpl*20–*rps*12, *psb*B–*psb*F), but with limited results (D. Bogler unpubl. data). Current efforts are directed toward using DNA fingerprinting techniques to study the groups of *Agave* recognized by Gentry (1982).

DISCUSSION

Asparagales

This study has provided insights into the phylogeny of Agavaceae at several levels. The overall topology of the Asparagales tree derived from combined *ndh*F and *rbc*L data sets (Fig. 2) is similar to those of other investigators (Chase et al. 1995; Fay et al. 2000). Lilioid monocots can be divided into several large groups that have been formerly named as orders, and Agavaceae clearly belong in the group known as Asparagales. All Agavaceae have the black phytomelan-encrusted seeds characteristic of Asparagales. Most Asparagales are hypogynous, but epigyny has evolved independently several times, as in the case of *Agave.* Our study supports the division of Asparagales into two parts, a paraphyletic grade of clades, and a large, monophyletic, clade. Although these have been referred to as lower Asparagales and higher Asparagales, we reject any notion of progress these terms may imply, and prefer to use the term core Asparagales for the monophyletic clade. A detailed discussion of the lower Asparagales grade is beyond the scope of this paper and can be found elsewhere (Fay et al. 2000; Reveal and Pires 2002; Graham et al. 2005; Pires et al. 2005). Here we only note that several of the woody taxa with fibrous leaves were once placed in or near Agavaceae in older systems of classification. Hutchinson (1934) included *Cordyline, Doryanthes,* and *Phormium* in Agavaceae. Cronquist (1981) placed *Aloe* and *Xanthorrhoea* in nearby families he considered ''parallel derivatives from the Liliaceae,'' which in a sense they really are. But the molecular data clearly show that *Aloe* and *Xanthorrhoea* are rather distantly related to Agavaceae s.s. (Bogler and Simpson 1996). An interesting corollary to this finding is that woody habit and fibrous leaves have arisen independently in a number of Asparagales in different parts of the world, possibly in response to herbivory or fire regime.

Core Asparagales

It is worthwhile to consider the core Asparagales in more detail. Sister group to the core Asparagales is a strongly supported clade that contains Agapanthaceae, Alliaceae, and Amaryllidaceae (following APG 1998) (Fig. 2). These families are generally characterized by having bulbs, scapes, and umbels subtended by bracts. *Agapanthus* and *Allium* L. are hypogynous and taxa of Amaryllidaceae are epigynous, a situation that parallels that of *Agave* and *Yucca* and has caused similar problems in classification. These taxa have

been classified as tribes of Liliaceae (Krause 1930) or, after certain disparate members have been removed, as separate families (Dahlgren et al. 1985; Kubitzki 1998*a*; Meerow and Snijman 1998; Rahn 1998). The phylogeny of these families has been extensively studied by Meerow et al. (2000). The first APG (1998) treatment listed these as separate families, but the second APG II (2003) update suggests it might be easier to optionally treat all three as the single family Alliaceae, recognized by the umbellate inflorescence subtended by two external bracts. In a similar fashion, the remaining families of core Asparagales might be reduced to the single family Asparagaceae, characterized by racemose inflorescences (see Fig. 3).

The remainder of the core Asparagales clade consists of two major groups. One group consists of Asparagaceae, Convallariaceae s.l. (following APG 1998, now Ruscaceae as per APG II 2003), and Laxmanniaceae. The genera in these clades have been placed in a bewildering assortment of families including Agavaceae and Liliaceae (Cronquist 1981), Dasypogonaceae (Dahlgren et al. 1985), Convallariaceae (Dahlgren et al. 1985; Conran and Tamura 1998), Dracaenaceae (Dahlgren et al. 1986; Bos 1998), Nolinaceae (Bogler 1998), Asparagaceae (Kubitzki and Rudall 1998), Eriospermaceae (Perry and Rudall 1998), and Ruscaceae (Yeo 1998; APG II 2003). This group is characterized by articulated pedicels, berry fruits or dry fruits derived from berries, small flowers, and tendency toward dioecy. The seeds of Convallariaceae s.l. have lost the black phytomelan pigment characteristic of many Asparagales. Phytomelan is also lacking in the seeds of *Eriospermum,* an enigmatic genus here included in Convallariaceae (Dahlgren et al. 1985). The woody members of Convallariaceae s.l. with fibrous leaves such as *Calibanus* and *Dracaena* in this analysis, and by extension *Beaucarnea, Dasylirion, Nolina,* and *Sansevieria,* were included in Agavaceae by Hutchinson (1934) and Cronquist (1981), but all molecular analyses indicate that they do not belong there. Convallariaceae s.l. (as Convallariaceae, Dracaenaceae, Nolinaceae) are discussed in more detail in Bogler and Simpson (1995, 1996), Bogler (1998), and Bos (1998). The molecular data strongly support a close relationship between *Asparagus* L. and *Hemiphylacus* S. Watson, but not between *Asparagus* and *Ruscus* L.

The other large group of core Asparagales contains the Agavaceae group, Hyacinthaceae, and Themidaceae. Somewhat isolated here is the monotypic genus *Aphyllanthes,* whose sole species occurs in the western Mediterranean region (Dahlgren et al. 1985; Conran 1998*b*). The leaves of this odd plant are reduced to scales and the photosynthetic organs are the scapes. The flowers are borne at the ends of the scapes in small, condensed spikes surrounded by scarious bracts. The fruits are capsules with a few black seeds. Based on morphology, it has been compared to Eriocaulaceae or Xanthorrhoeaceae, but all molecular analyses place *Aphyllanthes* with the core Asparagales. The *ndh*F data place *Aphyllanthes* near Themidaceae, but with low support. Given these anomalies, *Aphyllanthes* would be a good candidate for additional sampling and sequence verification. Themidaceae were once included in Alliaceae, but were ''resurrected'' as a distinct family on the basis of *rbc*L sequence studies (Fay and Chase 1996). Our data supports the separation of Themidaceae from Alliaceae, and the combined

data set analysis indicates a sister relationship to Hyacinthaceae, but with bootstrap support of less than 50%. Hyacinthaceae are characterized by having bulbs, leafless scapes, and racemes or spikes, but not umbels as in Alliaceae. *Camassia, Chlorogalum,* and several other genera formerly placed in Hyacinthaceae (Speta 1998) are here shown to be basal members of Agavaceae.

Agavaceae Senso Latissimo

The final clade to consider is the one containing all families and genera closely associated with Agavaceae in the broadest sense. It is not yet possible to identify unique morphological synapomorphies for this group. A lot of attention has been given to the karyotypes, since these have proven to be useful indicators of relationship to Agavaceae. The molecular data place *Anemarrhena* at the base of this clade, with moderately strong bootstrap support of 75% in the combined data trees. *Anemarrhena* is a monotypic genus from northern China and Korea. It is an understory herb, with a rhizome and short stem, linear, sheathing leaves, a pedunculate, compact panicle, mostly free tepals, three stamens attached to the tepals, superior ovary, and an ovoid capsule with one or two black seeds (Conran and Rudall 1998). The chromosome number is $N = 11$. The unusual reduction in stamen number has been used to associate *Anemarrhena* with *Hemiphylacus* and *Johnsonia* R. Br., but the molecular data do not support those relationships.

There is very strong bootstrap support (Fig. 1) for the sister group status of Herreriaceae–Behniaceae–Anthericaceae and Agavaceae s.l., with all the basal genera included. Both *ndh*F and *rbc*L strongly support this relationship. At the base of this clade are *Behnia* and *Herreria,* weakly resolved in our combined analysis. *Behnia* is a genus with a single species, *B. reticulata* from moist, shady forests in southern Africa (Conran 1998*c*). They are dioecious, slender, shrubby plants from short rhizomes with secondary thickening, thin erect branching stems, and shortly petiolate ovate leaves. The flowers are borne in small axillary cymose clusters or singly, campanulate, with partially united tepals, and six stamens adnate to the tepals. The fruit is a pale yellow or white berry with few angular, seeds; dark, but not phytomelan encrusted. With its broad leaves, campanulate flowers, berries, and lack of phytomelan, *Behnia* has been related to a variety of families including Asparagaceae, Luzuriagaceae, Ruscaceae, and Smilacaceae (Dahlgren et al. 1985; Conran 1998*c*). Of all the taxa that appear to be related to Agavaceae, *Behnia* is the most difficult to rationalize in terms of morphology, and is also a good candidate for independent verification.

Herreria is a genus of eight species in subtropical and temperate South America (Dahlgren et al. 1985; Conran 1998*d*). They are understory shrubs, acaulescent or caulescent with branching prickly stems several meters tall and secondary thickening. Leaves are linear to lanceolate, often clustered on short lateral shoots. Flowers are borne in small, scaly panicles. Flowers are hypogynous and bisexual. Fruits are trilobed thin-walled capsules, with flattened winged black seeds. The chromosome number is $N = 27$, with one large chromosome and 26 short ones (Satô 1942). This bimodal karyotype has been taken as a potential link to Aga-

vaceae. There is another genus of Herreriaceae, *Herreriopsis* Perrier in Madagascar, which differs in having sac-like tepal bases and numerous ovules per locule. Dahlgren et al. (1985) thought that *Herreria* resembled Asparagaceae or Ruscaceae, but the molecular data indicates they are not closely related.

Anthericaceae are strongly supported as a clade. As circumscribed by Conran (1998*a*), Anthericaceae s.s. are a family of 9 genera, about 200 species, and worldwide in distribution. They are somewhat herbaceous, from short rhizomes, with fibrous, fleshy or tuberous roots. The leaves are narrow, sheathing, and spirally arranged. Flowers are borne in erect scapose racemes or cymes. The flowers are bisexual, hypogynous, with mostly free tepals. The fruit is a dry elongated capsule with black angular somewhat flattened seeds (Conran 1998*a*). Chromosome numbers in the family are quite variable, with polyploidy and aneuploidy found in some taxa (Conran 1998*a*). In older systems Anthericaceae was a much larger family and included members of what are now classified as Boryaceae, Johnsoniaceae, and Lomandraceae. Dahlgren et al. (1985) tentatively positioned Anthericaceae near Asphodelaceae. *Leucocrinum montanum,* from western North America, has been associated with *Hosta* (Dahlgren et al. 1985) and with *Hemiphylacus* (Hernández 1995*b*). The flowers of *Leucocrinum* Nutt. ex A. Gray are tubular, with the ovary and capsule located below ground level. *Chlorophytum comosum* is a common houseplant called the "spider plant" because of the long, spreading inflorescence stalks. *Echeandia* occurs primarily in the New World and *Anthericum* L. in the Old World, but the generic limits of these two genera are uncertain.

Early Diverging Members of Agavaceae

The molecular studies described here provide strong support for the inclusion of *Camassia, Chlorogalum, Hesperocallis,* and *Hosta* in Agavaceae. Although these genera were known to have features in common with Agavaceae, they were usually placed in other families such as Hyacinthaceae because of their more generalized "liliaceous" appearance. These "basal Agavaceae" seem to have proliferated mostly in western North America, which is probably the area of origin of the entire clade. The *ndh*F data supports the position of *Hesperocallis* at the base of Agavaceae s.l. The single species, *H. undulata*, occurs in the Sonoran Desert. The leaves are narrow, linear, folded, with undulate margins, and are borne in a rosette from a small corm-like rhizome. The inflorescence is a bracteate raceme, with the flowers tending to be on one side. The tepals are partially united, forming a tube that withers and persists after flowering. The fruit is a three-lobed subglobose capsule, with flat black horizontally arranged seeds. The chromosome number is $N = 24$, with 5–6 very large and 19–18 small chromosomes (Kubitzki 1998*b*). Cave (1948, 1970) discussed similarities between embryology and karyotypes of *Hesperocallis* and Agavaceae. In the past, the classification of *Hesperocallis* was generally uncertain. Krause (1930) placed *Hesperocallis* in tribe Hemerocallideae, with *Blandfordia, Hemerocallis* L.*, Hosta, Leucocrinum,* and *Phormium.* Hutchinson (1934) included only *Hemerocallis, Hesperocallis, Hosta,* and *Leucocrinum.* The *ndh*F support for inclusion of *Hesperocallis* in Agava-

ceae is very strong, but additional sequence markers are needed to confirm the relationships among the early diverging genera.

Hosta is a genus of 23–50 species native to shady, temperate forests in China, Japan, and Korea. It is a popular ornamental plant and there are many cultivars. *Hosta* has clumpy rhizomes with fleshy roots. The leaves vary in shape from linear or oblong to lanceolate or broadly ovate, and sometimes are narrowed at the base forming a pseudopetiole. The inflorescence is an elongate bracteate raceme, with the flowers often on one side as in *Hesperocallis.* The flowers are hypogynous with a tubular perianth and six stamens. The fruit is an oblong to subglobose capsule with black flattened winged seeds, similar to those of *Agave.* Steroidal saponins are present, as in Agavaceae. The embryology and pollen resemble *Hesperocallis* (Kubitzki 1998*b*). The chromosome number is $N = 30$ for most species, with 4 large, 2 medium, and 24 small chromosomes. Triploids and aneuploids also are known. McKelvey and Sax (1933), Whitaker (1934), and Satô (1935) noted the similarity between the karyotypes of *Hosta* and Agavaceae many years ago. Granick (1944) suggested that *Hosta* was an early offshoot of the lineage leading to Agavaceae s.s., which appears to be correct, and that it must have been more widespread in the past, which is uncertain. It is interesting to speculate that the ancestor of *Hosta* might have originated in the New World and extended to China and Korea when there was a direct land connection.

The molecular data also support inclusion of *Camassia* and *Chlorogalum* as members of Agavaceae. These genera were formerly associated with subfamily Scilloideae of Liliaceae (Engler 1888; Krause 1930), and more recently included in Hyacinthaceae (Dahlgren et al. 1985; Speta 1998). *Camassia* has six species, mostly in western North America, with *C. scilloides* widespread in the central USA (Gould 1942). *Chlorogalum* has five species in western North America, mostly in California (Hoover 1940). *Camassia* and *Chlorogalum* both have bulbs, characteristic of Hyacinthaceae, but some also have a short rhizome. A densely fibrous tunic, similar to *Polianthes* and *Prochnyanthes,* surrounds the bulbs. The leaves are narrow, and sometimes keeled. The inflorescence is a slender raceme in *Camassia* or a branched raceme or panicle in *Chlorogalum.* The tepals are separate and persist in fruit. The stamens are inserted at the base of the perianth. The fruit is a subglobose capsule with 2–12 pyriform rugose black seeds. *Chlorogalum* has 2 seeds per locule, but *Camassia* has 10–12 seeds per locule. Steroidal saponins are especially abundant in *Chlorogalum,* with some species known as "soap plant." The chromosome number reported for *Camassia* is $N = 15$, bimodal with 3 large chromosomes and 12 small ones (Gould 1942; Fernández and Daviña 1990). The chromosome numbers reported for *Chlorogalum* are $N = 15, 17, 18, 30$ (Cave 1970). The karyotypes of *Chlorogalum* are also bimodal (Cave 1970). In the $N = 15, 17,$ and 18 populations there are 3 chromosomes larger than the others, and in the $N = 30$ species there are 5 chromosome larger than the others. Cave (1970) thought the karyotype data was good evidence for keeping *Camassia* and *Chlorogalum* together in Tribe Scilleae in Liliaceae. We think it is good evidence for a very close relationship to Agavaceae. The bimodal $N = 30$ karyotype of Agavaceae might have originated by a doubling of a bimodal $N = 15$

genome in the ancestral species, which may have looked something like *Camassia, Chlorogalum,* or *Hosta.*

Any discussion of *Camassia, Chlorogalum* and other basal Agavaceae must also consider several similar genera for which molecular data are not yet available. *Schoenolirion* Torr. is a genus of three species in the southeastern USA. It is similar to *Camassia,* but has a short vertical rhizome below the bulb, 1–2 seeds per locule, a more congested inflorescence, and persistent perianth that becomes twisted in fruit. *Hastingsia* S. Watson has four species in California and southern Oregon. It was formerly included in *Schoenolirion,* but has a shorter pedicel and dimorphic stamens. The chromosome number reported for *Hastingsia* is $N = 26$ (Cave 1970), bimodal with 5–6 large chromosomes and 21–20 smaller ones. *Fortunatia* J. F. Macbr. is a genus with four species in western South America that is also similar to *Camassia.* Cocucci (1969) thought it should be included in *Camassia,* however, Fernández and Daviña (1990) thought *Fortunatia* should be retained as separate, but Speta (1998) refers to it as the genus *Oziroë* Raf. and places it in a different subfamily than *Camassia.* The karyotype of *Fortunatia* is reported as $N = 15$ for three species by Fernández and Daviña (1990); bimodal with 3 large chromosomes and 12 small ones, and $N = 17$ for one species. Hopefully, all of these basal genera will some day be integrated into the phylogenetic analyses.

Agavaceae Sensu Stricto

The final group to discuss is Agavaceae s.s. Much of the background material on the phylogeny and morphology has been discussed and summarized elsewhere (Bogler and Simpson 1995, 1996; Verhoek 1998; Eguiarte et al. 2000). The basic outline of the tree derived from *ndh*F is similar to those derived from cpDNA and ITS data sets. The genera with a superior ovary, *Hesperoyucca, Hesperaloe,* and *Yucca,* are near the base of the clade, and the genera with an inferior ovary, *Beschorneria, Furcraea,* and *Agave* s.l., are united at the tip. In all molecular analyses *Hesperaloe* and *Hesperoyucca* are united as sister taxa with very strong support (Bogler et al. 1995; Bogler and Simpson 1995, 1996). This clade is extraordinarily diverse in pollination syndromes. *Hesperaloe* is a genus of 5–7 species in northern Mexico and south Texas with acaulescent rosettes of stiff, hard-fibrous leaves. The inflorescence is a terminal raceme or panicle from the rosette with flowers borne on small lateral spurs. Flower shape varies from tubular to campanulate, and are green, white, red, purple, pink, or yellow. Stamens are included or shortly exserted. The filaments are not swollen, and the style is filiform with a capitate, fringed stigma. The fruit is a short woody capsule with thin black seeds (Starr 1997). *Hesperaloe* species are pollinated by hummingbirds, hawkmoths, bees, and bats, and exhibit a wide range of associated floral forms, but have very low variation in molecular analyses. This would seem to indicate a very recent radiation of forms.

Hesperoyucca has one or two species in California, Arizona, and northwestern Mexico (Clary 2002). It has dense rosettes of hard-fibrous, spiny-tipped leaves. The inflorescence is a branched panicle borne on a massive, bracteate stalk. *Hesperoyucca* is monocarpic, flowering once and then dying. The flowers are campanulate with separate, thick, creamy-white tepals. Superficially, the flowers of *Hesperoyucca* resemble those of *Yucca* and are pollinated by a species of *Tegeticula,* the genus of yucca moths that pollinate all species of *Yucca.* The filaments are swollen as in *Yucca* flowers, which act as a support for the moths as they crawl around. The style is short with a capitate papillose stigma like *Hesperaloe,* not like the sunken cavity as in *Yucca* flowers. The fruits are short capsules with flat black seeds. The strong molecular evidence linking *Hesperaloe* and *Hesperoyucca* implies that *Hesperoyucca* evolved yucca-like floral traits involved in pollination following colonization by the yucca moth (Bogler et al. 1995).

Yucca is a large genus of about 40 species primarily distributed in arid regions of Mexico and the southwestern USA. They vary from acaulescent grass-like shrubs to massive, strangely branched tree-like forms. The leaves are hard fibrous, sometimes spiny tipped, and borne in dense rosettes. The inflorescence is a bracteate raceme or panicle. The tepals are mostly separate, fleshy and white. The filaments are swollen. The ovary is superior, with three stigma lobes that form a sunken cavity. The fruits are dry or fleshy oblong capsules with flat black seeds. The pollination of *Yucca* involves a famous and fine-tuned mutualistic association with several species of *Tegeticula,* the yucca moth genus (Baker 1986). The fertilized *Tegeticula* female enters a *Yucca* flower and gathers a mass of pollen with specialized tentacles. She then flies to another *Yucca* flower, oviposits her eggs in the ovary, and places the ball of pollen in the sunken cavity formed by the stigma. The developing larvae feed on some of the seeds before crawling out of the fruit and dropping to the ground to pupate. Each section of *Yucca* is pollinated by a different species or species complex of *Tegeticula,* as is *Hesperoyucca whipplei,* which was classified with *Yucca* in the past. *Yucca* s.s. appears to be monophyletic. In the ITS analyses (Bogler and Simpson 1996), *Yucca* appeared on a clade with *Camassia, Hesperaloe,* and *Hesperoyucca,* but in the more conservative cpDNA analysis (Bogler and Simpson 1995) and in the *ndh*F and combined analyses presented here, *Yucca* is resolved as a separate clade.

Agavaceae genera with an inferior ovary form a strongly supported clade in all analyses. *Beschorneria* and *Furcraea* are united by the ITS and cpDNA data, but *ndh*F sequences were obtained only for *Furcraea,* and independent confirmation of this relationship is desirable. *Furcraea* is a genus of about seven or eight species, widely distributed in the Antilles, Mexico, Central America, and in the Andes in South America (García-Mendoza 2000). *Furcraea* varies in habit from acaulescent to forming massive trunks several meters tall. Leaves are linear to narrowly lanceolate, and sometimes with a spiny margin. *Furcraea* is monocarpic, flowering once and then dying. The inflorescence is a massive, widely branched panicle. Flowers are pendulous, campanulate, with greenish or white tepals, an inferior ovary, and basally swollen filaments. Many species of *Furcraea* produce bulbils, and may depend on them for propagation. *Beschorneria* is a genus of seven species, all occurring in Mexico and Guatemala (García-Mendoza 1987). *Beschorneria* is generally acaulescent or less commonly caulescent, with a rosette of rather succulent, linear-lanceolate leaves. The inflorescence is a somewhat lax few-flowered raceme

with brightly colored bracts. The flowers are pendulous, red to greenish yellow, with separate tepals forming a tube, somewhat swollen filaments, an inferior ovary, and short capsule. Both *Beschorneria* and *Furcraea* produce pollen in tetrads.

The apex of the Agavaceae clade consists of *Agave* s.l., which contains *Agave* and subgroups that are sometimes recognized at the genus level such as *Manfreda, Polianthes,* and *Prochnyanthes. Agave* itself is a very large genus of about 200 species centered in Mexico, but widely distributed in the southwestern USA, Caribbean, Central America, and northern South America (Gentry 1982; García-Mendoza 2002). Most species of *Agave* s.s. are acaulescent xerophytic rosette plants, with only a few developing weak trunks. The fibrous leaves vary greatly in size, succulence, and development of marginal spines. The inflorescence is a small to large spike-like raceme or spreading panicle with flowers in broad subumbellate clusters, which is the basis for division into the two subgen. *Agave* and *Littaea.* Most species are monocarpic, the rosette flowering once then dying, but a few are polycarpic, flowering year after year. The flowers of *Agave* s.s. are borne in pairs, with a yellow, tubular, or campanulate perianth formed by six equal or unequal tepals. The stamens are exserted with slender filaments, and the inferior ovary has a long, exserted style. Most species are protandrous and pollinated by a variety of bats, bees, and hummingbirds (Verhoek 1998; Eguiarte et al. 2000; Slauson 2001). The fruit is a hard capsule with numerous flat black seeds. Gentry (1982) recognized 20 groups of *Agave* in continental North America, but the relationships of these groups are obscure and in need of further study. The molecular data seem to indicate the subgenera may be paraphyletic.

Several genera are often recognized within *Agave* s.l. (Rose 1899, 1903, 1906). *Manfreda* is a genus of about 26 species in the southeastern USA, Texas, Mexico, and Central America (Verhoek 1998), distinguished by thin or succulent, often mottled leaves and spike-like racemes with single flowers at the nodes. The tubular flowers are usually greenish yellow, white or pinkish, and have strongly exserted stamens and styles. *Polianthes* is a genus of about 14 species from Mexico (Verhoek 1998), distinguished by thin narrow leaves, flowers paired at the nodes, and stamens included in the tube. The flowers are white, pink, or red, sometimes strongly fragrant, and pollinated by moths and hummingbirds. Rose (1899) united *Bravoa* La Llave & Lex. with *Polianthes. Prochnyanthes* consists of a single somewhat variable species, *P. mexicana,* also from Mexico. It has thin, papery leaves, a lax raceme with paired, pink to greenish flowers that are often on a long pedicel, a greatly expanded floral tube, bent downward near the middle, included stamens, and is pollinated by sphinx moths and possibly bees. All of these genera have been included within *Agave* at various times in the past and sometimes in the present as well (Eggli 2001).

Molecular variation within *Agave* s.l. is rather low, but some groups were resolved in this small sampling. The ITS analysis (Bogler and Simpson 1996) provided some support for a basal clade containing *A. dasylirioides* and *A. striata.* Both species belong to Group Striatae as described by Gentry (1982). This group was considered to have a number of unusual features that might be considered generalized or

primitive within the genus (Gentry 1982). They are perennial and polycarpic, flowering repeatedly. The leaves are hard, and entire or finely serrate on the margin. The inflorescence is a simple spike, with paired flowers, nondimorphic tepals, an incompletely inferior ovary, and lack vegetative reproduction such as bulbils. Therefore, we think it is very significant to find the molecular data supporting the position of this clade at the base of the genus. The *ndh*F data for this relationship by itself were not quite as strong, but in the combined analysis the bootstrap support for this clade is strengthened. The other species of *Agave* s.s. were either not resolved by the *ndh*F data or weakly resolved into a single group by the ITS data. There is also both *ndh*F and ITS evidence for a close relationship between *Polianthes* and *Prochnyanthes,* which is not so surprising because of their many similarities. *Manfreda* is not resolved by the *ndh*F data, and there is only weak ITS support for a relationship to *Polianthes–Prochnyanthes.* There is simply not enough variation and too few taxa sampled to make any strong conclusions about relationships for most species. However, the finding of a clade supporting *A. dasylirioides–A. striata* raises some issues. If one chooses to recognize *Manfreda, Polianthes,* and *Prochnyanthes* as genera, then *Agave* becomes paraphyletic. The problem is solved by recognizing the entire clade as *Agave,* as was recently done by Eggli (2001), or by naming some additional genera. At present, it is probably premature to name new genera, but when more molecular data become available for the groups of *Agave,* this may be an attractive option.

CONCLUSIONS

These analyses have demonstrated the utility of *ndh*F sequence in resolving the relationships of Agavaceae. The advantage of using *ndh*F is that it has a relatively conservative 5′ region and a more variable 3′ region, which makes it well suited for large-scale studies and combination with other data sets. Analysis of *ndh*F alone provides support for inclusion of additional basal genera in Agavaceae and good overall resolution of the genera (Fig. 1). When *ndh*F is combined with *rbc*L data there is better resolution and stronger branch support for family level relationships within Asparagales (Fig. 2). When *ndh*F is combined with both *rbc*L and ITS data sets, the resulting tree is probably the best overall estimate of the phylogeny of Agavaceae currently available (Fig. 3). We think the addition of more taxa and sequence data will improve the resolution and stability of the relationships seen here. Adapting the nomenclature from previous systems of classification has not proven to be very easy, but with time this situation should also improve.

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