Phylogenetic relationships within the dogwood genus *Cornus* have been highly controversial due to the great morphological heterogeneity. Earlier phylogenetic analyses of *Cornus* using chloroplast DNA (cpDNA) data (including *rbcL* and *matK* sequences, as well as restriction sites) and morphological characters suggested incongruent relationships within the genus. The present study generated sequence data from the nuclear gene 26S rDNA for *Cornus* to test the phylogenetic hypotheses based on cpDNA and morphological data. The 26S rDNA sequence data obtained represent 16 species, 13 from *Cornus* and three from outgroups, having an aligned length of 3380 bp. Both parsimony and maximum likelihood analyses of these sequences were conducted. Trees resulting from these analyses suggest relationships among subgroups of *Cornus* consistent with those inferred from cpDNA data. That is, the dwarf dogwood (subg. *Arctocraania*) and the big-bracted dogwood (subg. *Cynoxylon* and subg. *Syncarpea*) clades are sisters, which are, in turn, sister to the cornelian cherries (subg. *Cornus* and subg. *Afrocrania*). This red-fruited clade is sister to the blue- or white-fruited dogwoods (subg. *Mesomora*, subg. *Kranionipsis*, and subg. *Yinquania*). Within the blue- or white-fruited clade, *C. oblonga* (subg. *Yinquania*) is sister to the remainder, and subg. *Mesomora* is sister to subg. *Kranionipsis*. These relationships were also suggested by the combined 26S rDNA and cpDNA data, but with a higher bootstrap and Bremer support in the combined analysis. The 26S rDNA sequence data of *Cornus* consist of 12 expansion segments spanning 1034 bp. These expansion segments evolve approximately four times as fast as the conserved core regions. The study provides an example of phylogenetic utility of 26S rDNA sequences below the genus level.

**Key words:** 26S rDNA; combining data; Cornaceae; *Cornus*; cpDNA; molecular evolution; molecular phylogeny.

*Cornus* L. sensu lato consists of ~55 species that are mostly trees and shrubs and rarely perennial herbs with woody rhizomes. The genus is widely distributed in the northern hemisphere, with centers of diversity in eastern Asia, eastern North America, and western North America. Two species of the genus are endemic to South America and one species is endemic to the subtropical Africa (see Table 1). Species of *Cornus* are morphologically diverse. Various types of inflorescences are found within the genus, including open compound cymes with minute, nonmodified bracts; umbellate cymes with four basal, scale-like bracts; capitate cymes with four large, basal, and showy bracts; and minute compound cymes with four basal, showy bracts. In addition, the color of fruits also varies among species (see Table 1). Due to the great morphological diversity encompassed by the Linnaean circumscription of *Cornus*, the taxonomy and phylogenetic relationships of subgroups within the genus are highly controversial (see Eyde, 1988; Murrell, 1993; Xiang et al., 1996). The genus has been divided into several distinct genera (e.g., Rafinesque, 1838; von Berchtold and Opiz, 1838; Spach, 1839; Nakai, 1909; Hutchinson, 1942; Pojarkova, 1950; also see Murrell, 1993), or into various numbers of subgenera (e.g., Harms, 1898; Wangerin, 1910; Ferguson, 1966; Eyde, 1987, 1988; Xiang, 1987). Following the broad view of *Cornus*, a total of ten subgenera have been recognized by different authors at one time or another (see Table 1; Ferguson, 1966; Xiang, 1987; Eyde, 1988; Murrell, 1993).

In order to understand relationships within *Cornus*, several sets of data have been recently collected for the genus for phylogenetic analyses. These include molecular data from the chloroplast genome (Xiang et al., 1996; Xiang, Soltis, and Soltis, 1998) and morphological characters (Murrell, 1993). Phylogenetic analyses of these data identified five major lineages within *Cornus*: (1) *C. oblonga*, an enigmatic blue-fruited dogwood, (2) other blue- or white-fruited dogwoods, (3) the cornelian cherries, (4) the big-bracted dogwoods, and (5) the dwarf dogwoods (the herbaceous species). However, relationships among these groups suggested by cpDNA data are different from those suggested by morphological data. The cpDNA data (combined *rbcL* and *matK* sequences and restriction sites) suggested that the dwarf dogwoods are sisters of the big-bracted dogwoods with the cornelian cherries sister to them, and *C. oblonga* is a member of the blue- or white-fruited dogwoods and sister to the remainder of the group (see Fig. 1; Xiang et al., 1996; Xiang, Soltis, and Soltis, 1998). These results are consistent with the hypothesis of Eyde (1988) based on synthesis of information available without performing phylogenetic analyses. In contrast, cladistic analysis of 28 morphological, anatomical, chemical, and cytological characters of *Cornus* by Murrell (1993) suggested that the cornelian cherries and the big-bracted dogwoods are sister groups, and the dwarf dogwoods are sister to them, with *C. oblonga* being the first-branching lineage in *Cornus* (see Fig. 2; Murrell, 1993; Hardin and Murrell, 1997). To explore possible sources of incongruence between the cpDNA and morphological data, additional phylogenetic analyses, especially analyses of molecular data from the nuclear genome, are necessary.

Although the most widely used nuclear phylogenetic markers below the generic level have been the ITS (internal transcribed spacer) sequences of ribosomal genes (see reviews by Baldwin et al., 1995; Soltis and Soltis, 1998), our initial anal-

---

1. Manuscript received 29 June 2000; revision accepted 9 November 2000.

2. Author for reprint requests (Tel: 919 515-3345; FAX: 919 515-3436; e-mail: cfan3@unity.ncsu.edu).

3. Current address: Dept. of Botany, North Carolina State University, Campus Box 7612, Raleigh, NC 27695-7601 USA.
TABLE 1. Morphological characteristics of the subgenera of Cornus. All subgenera are woody and hermaphroditic with terminal inflorescence and opposite leaves except those indicated. Taxonomic treatment was synthesized from Ferguson (1996), Xiang (1987), and Murrell (1993). Common names of subgenera are provided below the scientific names.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Fruits and inflorescence</th>
<th>Size and distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subg. Yinquania (Zhu) Murrell (oblong-blue-fruited dogwood)</td>
<td>Blue, oblong fruits; open compound, cymes; bracts minute.</td>
<td>1 spp.; East Asia</td>
</tr>
<tr>
<td>Subg. Kraniosis Raf. (blue- or white-fruited dogwoods)</td>
<td>Blue or white, and round fruits; open compound cymes; bracts minute.</td>
<td>~30 spp.; mostly East Asia, North America; 2 or 3 Europe; 1 or 2 South America</td>
</tr>
<tr>
<td>Subg. Mesomora Raf. (alternate-leaved, blue-fruited dogwoods)</td>
<td>Blue fruits; alternate leaves; open compound cymes; bracts minute.</td>
<td>2 spp.; East Asia, eastern North America</td>
</tr>
<tr>
<td>Subg. Afrocrania Harms (African cornelian cherry)</td>
<td>Red fruits; umbellate cymes subtended by four nonshowy bracts; dioecious.</td>
<td>1 spp.; tropical Africa</td>
</tr>
<tr>
<td>Subg. Cornus (Cornelian cherries)</td>
<td>Red fruits; umbellate cymes terminal subtended by four nonshowy bracts.</td>
<td>3 spp.; East Asia, western North America, Europe</td>
</tr>
<tr>
<td>Subg. Sinocornus Q. Y. Xiang (Chinese cornelian cherry)</td>
<td>Red fruits; umbellate cymes axillary subtended by four nonshowy bracts.</td>
<td>1 spp.; China</td>
</tr>
<tr>
<td>Subg. Discocrania Raf. (Mexican disciformous dogwood)</td>
<td>Red fruits; capitulate cymes subtended by four early deciduous bracts.</td>
<td>1 or 2 spp.; Central America</td>
</tr>
<tr>
<td>Subg. Cynoxylon Raf. (American big-bracted dogwoods)</td>
<td>Red fruits; capitulate cymes subtended by four large, showy bracts; fruit separate.</td>
<td>2 or 3 spp.; eastern and western North America extended to Mexico</td>
</tr>
<tr>
<td>Subg. Syncarpea (Nakai) Xiang (Asian big-bracted dogwoods)</td>
<td>Red fruits; capitulate cymes subtended by four small, showy bracts; hermaphroditic.</td>
<td>4–12 spp.; East Asia</td>
</tr>
<tr>
<td>Subg. Arctocrania Endl. Ex Reichenbach (dwarf dogwoods)</td>
<td>Red fruit; minute cymes subtended by four small, showy bracts; herbaceous.</td>
<td>3 spp.; circumboreal</td>
</tr>
</tbody>
</table>

Fig. 2. The phylogenetic tree derived from cladistic analysis of 28 morphological, anatomical, chemical, and cytological characters modified from Murrell (1993).
Table 2. Species sampled in the study of 26S rDNA sequencing of Cornus. Species of Cornus are listed according to classifications of Ferguson (1966), Murrell (1993), and Xiang (1987).

<table>
<thead>
<tr>
<th>Species</th>
<th>Sources and location of vouchers</th>
<th>GenBank accession no.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgen. Mesomorpha</td>
<td>C. controversa Hemsl.</td>
<td>Arnold Arboretum 20458, WS.</td>
</tr>
<tr>
<td>Subgen. Kranipsis</td>
<td>C. racemosa Lam.</td>
<td>Xiang et al. 157, WS.</td>
</tr>
<tr>
<td>Subgen. Archoaemia</td>
<td>C. volkensii Harms</td>
<td>Arnold Arboretum 414-67-1, WS.</td>
</tr>
<tr>
<td>Subgen. Cornus</td>
<td>C. mas L.</td>
<td>Knox 2528, Africa.</td>
</tr>
<tr>
<td></td>
<td>C. officinalis Seib. et Zucc.</td>
<td>Boufford et al. 26065, GH.</td>
</tr>
<tr>
<td></td>
<td>C. sessilis Torr. Ex Durand</td>
<td>Terry M. Hardig, California 1994.</td>
</tr>
<tr>
<td>Subgen. Cryptoforma</td>
<td>C. canadiensis L.</td>
<td>Xiang et al. 198, WS.</td>
</tr>
<tr>
<td></td>
<td>C. unalaschekensis Ledeb.</td>
<td>Xiang 220, WS.</td>
</tr>
<tr>
<td></td>
<td>C. suecica L.</td>
<td>Chris Brochmann, 94-388, Norway.</td>
</tr>
<tr>
<td>Subgen. Cynoxylon</td>
<td>C. florada L.</td>
<td>Xiang 250, WS.</td>
</tr>
<tr>
<td>Subgen. Syncarpia</td>
<td>C. kousa Hance</td>
<td>Xiang 310, Ohio State University compus.</td>
</tr>
<tr>
<td>Outgroups</td>
<td>Nyssa ogeche Marsh.</td>
<td>U.S. National Arbortum, s.n.</td>
</tr>
<tr>
<td></td>
<td>Alangium platanifolium (Sieb. &amp; Zucc.)</td>
<td>Soltis 2543, Japan.</td>
</tr>
<tr>
<td></td>
<td>Schizophragma hydrangeoides Sieb. et Zucc.</td>
<td>Soltis 2516, Japan.</td>
</tr>
</tbody>
</table>

*The prefix GBAN- has been added to link the online version of American Journal of Botany to GenBank but it is not part of the actual accession number.

to gain insights into the evolution of some subgroup-diagnostic morphological characters in Cornus.

MATERIALS AND METHODS

Sampling—Thirteen species of Cornus L. representing all major morphological diversity of the genus and the five major lineages identified through earlier phylogenetic analyses of cpDNA were sampled in the 26S rDNA sequencing study (Table 2; also see Table 1). All subgenera except subg. Discocrania and subg. Sinocornus were included. These two subgenera, each represented by a single species, were not sampled because of lack of DNA. Previous cpDNA studies indicated that C. discifera (the only member of subg. Discocrania) and the big-bracted dogwoods and C. chinensis (the only member of subg. Sinocornus) are a member of the cornelian cherries (see Table 1, Fig. 1; Xiang et al., 1996; Xiang, Soltis, and Soltis, 1998). Thus, even without these two species our sampling represented well all major subgroups within the genus. Although subgenus Kranipsis is the largest subgenus in Cornus (~30 spp.), all species in the subgenus are morphologically very similar, and they formed a strongly supported monophyletic group in the cpDNA study (Xiang et al., 1996). Therefore, only two species were sampled from this subgenus. All of the species sampled were included in previous matK and rbcL sequencing and chloroplast DNA restriction site analyses, except C. suecica (a dwarf dogwood) and C. volkensii (the single species from Africa). These two species had not been included in the cpDNA studies because of the lack of materials of these taxa at the time the studies were completed. Three genera, Nyssa, Alangium, and Schizophragma (Hydrangeaceae), were chosen as the outgroups for the present study based on the results of broad phylogenetic analyses of matK and rbcL sequences for Cornaceae (Xiang, Soltis, and Soltis, 1998), which suggested that Alangium is the sister of Cornus; Nyssa and Hydrangeaceae are close relatives of Cornus.

DNA isolation, PCR amplification, and DNA sequencing of 26S rDNA—To maximize comparability between the present and previous studies, DNAs used herein were those isolated for previous rbcL and matK sequencing studies. The procedures of isolating DNA were described elsewhere (Xiang et al., 1993; Xiang, Soltis, and Soltis, 1998). The 26S rDNA sequences were amplified via PCR (polymerase chain reaction) from total DNA aliquots using the forward primer N-n26S1 (5'-CGACCCCAGTGACTGCGC-3') and the reverse primer 3331rev (5'-ATCTCACTGATCGTGCCAGC-3') following Kuzzoff et al. (1998) with slight modifications. Our PCR reactions contained the following: 5 L of 10X Mg free buffer, 6 L of 25 mmol/L MgCl2, 10 L of 2.5 mmol/L dNTP, 1.0 L of 20 mmol/L N-nc26S1 (forward primer), 1.0 L of 20 mmol/L 3331 rev (reverse primer), 5 L of DMSO (dimethyl sulfoxide), 0.3 L of Taq polymerase (Promega), 2.0 L of 20 ng/µL total DNA extract, and 19.7 L of deionized water. This PCR reaction mix was covered with two drops of mineral oil and run on a Robocyte PCR machine as the following: (1) 94°C for 3 min for one cycle; (2) 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 3 min, and (3) a terminal phase at 72°C for 5 min.

The double-stranded (DS) PCR products were subsequently purified via precipitation with 60 L of 20% PEG 8000/2.5 mol/L NaCl on ice for at least 1 h (Morgan and Soltis, 1993; Soltis and Soltis, 1997). The precipitated DNA pellets were washed with 1000 L of 75% ethanol (prechilled to 4°C) and centrifuged for 3 min. The DNA pellets were washed again with chilled 95% ethanol and centrifuged for 3 min at 4°C. The ethanol was decanted, and the pellets were dried in a vacuum. The dried DNA products were resuspended in 20–30 µL of ddH2O. One microliter of the clean PCR products was electrophoresed in a 1% agarose mini-gel for quantification.

The purified DS DNA products were used as the templates for sequencing on an ABI-377 automated sequencer following the standard protocol recommended by the company (Applied Biosystems, Foster City, CA 94404 USA).
Maximum likelihood analyses were first conducted using heuristic searches with random taxon addition of five replicates with default settings of the ML program (i.e., empirical base frequency, HKY [Hasegawa, Kishino, and Yano, 1985] two-parameter model variant for unequal base frequency, ti/tv = 2, and equal rates for all sites). The resulting tree was simultaneously used to estimate values of shape of the discrete gamma distribution of rates across sites, through maximum likelihood. A subsequent ML analysis using the estimated values of these parameters (A = 0.220264, C = 0.272649, G = 0.307582, T = 0.199505; ti/tv ratio = 1.343463 [kappa = 2.741222]; proportion of invariable sites = 0.704318; shape value for discrete gamma distribution = 0.272649, G = 0.848768) was then conducted to see whether adjusting these parameters to the estimated values resulted in significant differences in tree topologies.

Since the results of analyses of 26S rDNA sequences suggested phylogenetic relationships within Cornus are highly congruent with those achieved via cpDNA data (combined matK, rbcL sequences, and restriction sites), the 26S rDNA sequences were combined with cpDNA data for further parsimony and ML analyses to obtain a comprehensive view of relationships. The combined data matrix included ten species of Cornus and two outgroups (Alangium and Nyssa), each of 6338 characters, among which 3380 bp were from 26S rDNA, 1212 bp from matK, 1504 bp from rbcL, and 242 from restriction sites. Phylogenetic analyses of the combined 26S rDNA and cpDNA were conducted as above.

### RESULTS

**Sequence divergence**—The 26S rDNA sequences generated for the 16 species of Cornus varied from 3340 to 3370 bp in length before alignment. All sequences can be aligned easily by sight against the reference sequences from two angiosperm species, Saxifraga mertensiana Bong. (AFO36498, Kuzoff et al., 1998) and Tragopogon dubius Scop. (AFO36493, Kuzoff et al., 1998), obtained from GenBank. The aligned 26S rDNA sequences in Cornus and outgroups contained a total length of 3380 bp. Sequences for all species are complete except for C. controversa and C. sessilis. In C. controversa, 166 bp (bases 1542–1707) are missing and in C. sessilis, 476 bp (bases 1217–1692) are missing. Despite repeated efforts, these missing data in these two species could not be obtained due to potential primer divergence or heterogeneity of temperate DNA. Among the 16 sequences of Cornus and outgroups, 391 of the 3380 sites are variable (11.56%) and 137 sites (4.05%) are phylogenetically informative.

The alignment sequences of Cornus and outgroups required the addition of 20 small alignment gaps (1–5 bp in length).
Eleven of these gaps appear to be autapomorphies, and nine of them are potentially phylogenetically informative within *Cornus* (Table 3, also see Fig. 3).

It is noteworthy that a majority of these indels (15 of 20) occur in the expansion segments, regions of gene that evolve more rapidly (Clark et al., 1984; Dover and Flavell, 1984; Flavell, 1986). The location of expansion segments of 26S rDNA sequences appears to be highly conserved over a wide range of taxa (Bult, Sweere, and Zimmer, 1995). According to rDNA sequences appears to be highly conserved over a wide range of taxa (Bult, Sweere, and Zimmer, 1995).

**Phylogenetic relationships inferred from 26S rDNA sequences** — Phylogenetic analyses of the entire 26S rDNA sequences of *Cornus* using parsimony found a single shortest tree of 577 steps (CI [consistency index] = 0.780, excluding the uninformative characters, RI [retention index] = 0.619; Fig. 3); this single shortest tree is completely resolved. Major clades identical to those recognized by earlier phylogenetic analyses of cpDNA data were identified in the 26S rDNA tree: (1) all of the blue- or white-fruited dogwoods (represented by *C. floridana* and *C. kousa*); (2) the dwarf dogwoods (*C. canadensis, C. suecica,* and *C. unalaschkensis*); (3) the big-bracted dogwoods (represented by *C. florida* and *C. kousa*); and (4) the dwarf dogwoods (*C. canadensis, C. suecica,* and *C. unalaschkensis*). The dwarf dogwoods (clade 4) and the big-bracted dogwoods (clade 3) (all producing showy bracts on the inflorescence) were recognized as sisters. This showy-bracted dogwood clade was, in turn, sister to the cornelian cherries (clade 2). All of the blue- or white-fruited dogwoods formed a monophyletic group (clade 1) sister to the large clade consisting of the dwarf dogwoods, big-bracted dogwoods, and the cornelian cherries. Although all of the major clades (except the cornelian cherries) are strongly supported (with bootstrap value >82% support and > 6 decay index), the relationships among major groups described above are not strongly supported (with bootstrap value <50% and decay index <3) (Fig. 3). Within the dwarf dogwood lineage (with all of the three species sampled), *C. canadensis* and *C. unalaschkensis* are sisters. Within the cornelian cherry group (four of the five species were sampled, see Table 1), *C. mas* and *C. officinalis* are sisters, with *C. sessilis* sister to them, and *C. volkensii* is at base within the clade. Within the blue- or white-fruited lineage, *C. oblonga* is sister to the remainder of the lineage (Fig. 3).

The ML analysis using default setting (see MATERIALS AND METHODS) found a tree with the best score of -Ln 8531 with a topology identical to the parsimony tree (Fig. 4). The ML analysis using estimated values found a tree with a higher likelihood (-Ln 8219), but showing the same relationships as described above.

**Analyses of combined cpDNA and 26S rDNA sequence data** — The combined 26S rDNA and cpDNA data set contains 939 (14.82%) variable sites of which 347 are from 26S rDNA, 232 from *matK*, 153 from *rbcL*, and 207 from restriction sites; and 327 (5.16%) are phylogenetically informative sites, of which 98 are from 26S rDNA, 59 from *matK*, 65 from *rbcL*, and 105 from restriction sites. Parsimony analysis of the combined data set found a single most parsimonious tree of 1229 steps, with a topology nearly identical to that of 26S rDNA tree (Fig. 5). The only difference between the two trees involves the placement of *C. volkensii* within the cornelian cherries. In the combined tree, *C. volkensii* is placed as the sister
of *C. sessilis*, whereas, in the 26S rDNA tree, the species is recognized as a distinct lineage sister to the remainder of the cornelian cherries. Significantly, higher bootstrap and decay values for all clades were obtained for the tree derived from the combined data (Fig. 5).

**DISCUSSION**

**Phylogenetic potential of 26S rDNA sequences below the genus level**—The phylogenetic utility of 26S rDNA sequences in seed plants has been demonstrated only recently by a few studies of taxa at higher taxonomic levels (above the generic level) (e.g., Ro, Keener, and McPherson, 1997; Kuzoff et al., 1998; Stefanovic et al., 1998; Ashworth, 2000). Our phylogenetic study of *Cornus* using 26S rDNA sequences provides an example of phylogenetic utility of this gene at an intragenic level. Although the rate of evolution of 26S rDNA is relatively conservative (comparable to *rbcL*), the gene contains several expansion segments that evolve faster than the conserved core regions (see Bult, Sweere, and Zimmer, 1995; Kuzoff et al., 1998). The variable rate of evolution in the expansion segments and the conserved core regions makes the gene useful for phylogenetic analyses at different taxonomic levels depending on the regions employed (see Kuzoff et al., 1998). The expansion segments can be used at lower taxonomic levels, whereas the conserved core regions are suitable at higher levels. In addition, the large size of the gene provides more variable characters for phylogenetic analysis than *rbcL*; the low sequence variation as a result of the conservative rate of the gene is well compensated by its large size. Although only 11.56% of the sites are variable, and 4.05% is phylogenetically informative in the 26S rDNA matrix of *Cornus*, the total number of variable sites from the entire sequence is 391, and the number of total informative sites is 137. These numbers are nearly two times higher than those for *rbcL* and *matK*, respectively, in the combined data matrix (see RESULTS). Analyses of the 26S rDNA sequences of *Cornus* resulted in a completely resolved phylogeny of the genus and suggested relationships congruent with the cpDNA-based phylogeny (Figs. 3, 4). This suggests that the 26S rDNA sequences as a whole contain sufficient phylogenetic information at the intragenic level of *Cornus* and are useful for elucidating phylogenetic relationships within the genus.

To explore differential utilities of the expansion segments and conserved core regions of 26S rDNA sequences in *Cornus*, we conducted phylogenetic analyses of the sequences from the expansion segments and conserved core regions separately. The analyses of these portions of 26S rDNA did not produce topologies that were as fully resolved. The results indicated that the analysis of the expansion segments alone produced a weakly supported phylogeny inconsistent with both the cpDNA-based phylogeny and the morphology-based phylogeny. In this phylogeny, the monophyly of the cornelian cherries was unsupported and species of the group appear in different clades. Similar results were obtained from the analysis of the conserved core regions. In the trees resulting from the analysis of the conserved core sequences, the monophyly of *Cornus* was even unsupported. These odd results may be explained as the effect of insufficient variable characters in either the expansion segments or the conserved core regions. Although the expansion segments contain a higher percentage of phylogenetically informative sites (9.38%) than that of the entire 26S rDNA (4.05%), the total number of phylogenetically informative sites in the expansion segments is only 97, 40 sites less compared to 137 sites in the entire gene. The conserved core regions contain only 40 potentially phylogenetically informative sites. Moreover, the expansion segments have a significantly higher average G + C content (68.5%)
than that of the entire 26S rDNA (58.1%) and that of the conserved core regions (52.6%). The higher G + C content in the 26S rDNA expansion segments poses a potential problem for phylogenetic analysis if the algorithm used to construct a tree assumes equally abundant nucleotides (see Kuzoff et al., 1998).

**Phylogenetic relationships within Cornus**—The phylogenetic tree derived from the analysis of 26S rDNA sequences of *Cornus* shows a topology identical to the tree derived from cpDNA data (Fig. 1; Xiang et al., 1996; Xiang, Soltis, and Soltis, 1998), although some nodes in the 26S rDNA tree are not strongly supported by bootstrap and decay analyses (Figs. 3, 4). This 26S rDNA tree is also congruent with the tree derived from analyses of the combined 26S rDNA and cpDNA data (Fig. 5). Thus all of the molecular data from both nuclear and chloroplast genomes of *Cornus* are concordant. These data suggest that the genus diverged early into two large lineages: (1) the blue- or white-fruited group and (2) the red-fruited group. The red-fruited group subsequently separated into the cornelian cherries and a clade bearing showy bracts, which then diverged into the big-bracted dogwoods and the dwarf dogwoods. Within the blue- or white-fruited group, *C. oblonga* was the first lineage to branch off. This phylogenetic pattern is consistent with the scheme proposed by Eyde (1988), but at odds with the morphology-based phylogeny (Murrell, 1993). Phylogenetic analysis of morphological characters by Murrell (1993) placed the cornelian cherries (rather than the dwarf dogwoods as in the molecular tree) as the sister of the big-bracted dogwoods. This relationship was supported by five inflorescence characters that were treated as independent characters, including protective bracts, precocious peduncle, inflorescence preformed in the previous fall and developed from a mixed bud, reduced primary inflorescence branches, and reduced secondary inflorescence branches. However, three of these characters (protective bracts, precocious peduncle, and reduced secondary inflorescence branches) are homoplasious on the morphological trees, whereas they are reversed one or two times in some other clades or terminal taxa. In addition, these five inflorescence characters may not be independent because these characters may be developmentally correlated. Thus, differences in inflorescence may be overweighted in the morphological analysis, resulting in an incongruence between the morphological and molecular phylogeny. Based on the molecular phylogeny derived from multiple molecular data sets, the synapomorphies used to unite the cornelian cherries and the big-bracted dogwoods appear to be plesiomorphic characters evolved in the ancestor of the red-fruited group, but were later lost in the dwarf dogwoods (see discussion in Xiang et al., 1996). Alternatively, these features may have evolved independently in the big-bracted dogwoods and the cornelian cherries. However, these two hypotheses cannot be distinguished without fossils representing the ancestor of the red-fruited group.

The second major incongruence between the molecular and morphological phylogenies involves the placement of *C. oblonga* (subg. Yinquania). *Cornus oblonga* was placed as the basal group within *Cornus* in the morphological tree (Fig. 2; Murrell, 1993), whereas in the molecular phylogeny, *C. oblonga* is a distinct lineage sister to the remainder of the blue- or white-fruited dogwoods, a relationship strongly supported by bootstrap and decay analyses (Figs. 3, 5). A single morphological character (displaced bracts, present in the remainder of the genus, absent in *C. oblonga*) separated *C. oblonga* and the remainder of the genus in the morphological tree (Murrell, 1993). Based on the molecular phylogenies, this character is better explained as a plesiomorphic condition, and the nondisplaced bract in *C. oblonga* is a derived state (perhaps a reversal). Several morphological, anatomical, biochemistry characters (blue fruits, lack of iridoids, crassinucellate ovule, open cyme with minute bracts) also support the placement of *C. oblonga* as a member of the blue- or white-fruited group.

The tropical African species *C. volkensii* is the only dioecious species in *Cornus*. Due to its morphological uniqueness, the species was treated as a separate subgenus (subg. *Afrocrania* Harm.) (Hutchinson, 1942; Ferguson, 1966; also see Murrell, 1993; Xiang et al., 1993). Eyde (1988) considered *C. volkensii* as a member of the cornelian cherries based mainly on similarities in fruit morphology and inflorescence type between *C. volkensii* and other cornelian cherries. Our 26S rDNA sequence data and the combined 26S rDNA-cpDNA data strongly supported *C. volkensii* being a member of the cornelian cherries (Figs. 3, 5) and recognized it as either the sister of all of the other cornelian cherries (Fig. 3) or as the sister of *C. sessilis* (Fig. 5). However, neither of these placements of *C. volkensii* within the cornelian cherries are highly supported by bootstrap and decay analyses (Figs. 3, 5). At present, no synapomorphic morphological characters can be identified to support either of these relationships of *C. volkensii* in the cornelian cherries. Both Eyde (1988) and Murrell (1993) suggested that *C. volkensii* is sister to all of the other cornelian cherries (also Fig. 2) based on the autapomorphies found in *C. volkensii* (e.g., dioecy, reduced secondary inflorescence branches). Neither Eyde nor Murrell identified any synapomorphies to unite the rest of the cornelian cherries. Eyde (1988) further proposed that the divergence of *C. volkensii* from the other cornelian cherries might have occurred in the Paleocene or early Eocene based on morphological and fossil evidence. For example, *C. volkensii* has dioecious breeding system, spiny pollen, up to nine or ten ripe fruits per umbel, a broad apical depression in the fruit-stones, whereas all the other cornelian cherries have a synoecious breeding system, smooth pollen, only one to five ripe fruits per umbel, and no apical depression in the fruit-stones. According to Eyde (1988) fossil fruits of an ancient, extinct lineage of cornelian cherries, *C. multilocularis*, were found in the early Tertiary in the London Clay, suggesting that the divergence of this lineage and the extant cornelian cherries occurred in or before the early Tertiary; the divergence of *C. volkensii* from the other cornelian cherries was subsequent to this event. Therefore, the basal placement of *C. volkensii* within the cornelian cherries as suggested by 26S rDNA sequence data is concordant with hypotheses of Eyde (1988) and Murrell (1993).

The dwarf dogwoods are the only herbaceous members of the dogwood genus *Cornus*. This group comprises three species, *C. canadensis*, *C. suecica*, and *C. unalascakensis*. Evidence from cytology, phytogeography, and morphology suggests that *C. unalascakensis* may be an allotetraploid species derived from past hybridization between *C. canadensis* and *C. suecica* followed by chromosomal doubling (Love and Love, 1975; Bain and Denford, 1979; Murrell, 1994). The 26S rDNA sequence data suggested that *C. unalascakensis* is more closely related to *C. canadensis* than it is to *C. suecica*. This result may indicate that, if *C. unalascakensis* is indeed an allotetraploid, as has been hypothesized, the 26S rDNA in *C. unalas-
C. unalaschkensis has converted to the type of C. canadensis. However, more extensive analyses (e.g., analyses of both nuclear and cpDNA data including all three species with more extensive sampling) are needed to test the hypothesis and to determine whether C. unalaschkensis is of hybrid origin.

In summary, the entire 26S rDNA sequence of Cornus shows a low level of sequence divergence, but because of its great length, provides sufficient variable characters (compared to rbcL and matK) to resolve the phylogenetic relationships within Cornus. Analyses of 26S rDNA sequence data result in a phylogeny of Cornus that is congruent with that inferred from combined cpDNA data, but at odds with the phylogeny derived from morphological analyses. Combining data for phylogenetic analysis can minimize sampling error and maximize the explanatory power of the data if congruent hypotheses are generated from separate analyses (see review by Johnson and Soltis, 1998). This was also demonstrated in our analysis of the combined 26S rDNA and cpDNA data in Cornus, which not only suggested congruent phylogenetic relationships to those inferred from separate data analyses within Cornus, but also significantly increased supports for most of the clades recognized in the tree (compare Figs. 3 and 5).

LITERATURE CITED


