# A PHYLOGENETIC ANALYSIS OF THE ORCHIDACEAE: EVIDENCE FROM *RBCL* NUCLEOTIDE SEQUENCES[1]

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Cladistic parsimony analyses of *rbcL* nucleotide sequence data from 171 taxa representing nearly all tribes and subtribes of Orchidaceae are presented here. These analyses divide the family into five primary monophyletic clades: apostasioid, cypripedioid, vanilloid, orchidoid, and epidendroid orchids, arranged in that order. These clades, with the exception of the vanilloids, essentially correspond to currently recognized subfamilies. A distinct subfamily, based upon tribe Vanilleae, is supported for *Vanilla* and its allies. The general tree topology is, for the most part, congruent with previously published hypotheses of intrafamilial relationships; however, there is no evidence supporting the previously recognized subfamilies Spiranthoideae, Neottioideae, or Vandoideae. Subfamily Spiranthoideae is embedded within a single clade containing members of Orchidoideae and sister to tribe Diurideae. Genera representing tribe Tropideae are placed within the epidendroid clade. Most traditional subtribal units are supported within each clade, but few tribes, as currently circumscribed, are monophyletic. Although powerful in assessing monophyly of clades within the family, in this case *rbcL* fails to provide strong support for the interrelationships of the subfamilies (i.e., along the spine of the tree). The cladograms presented here should serve as a standard to which future morphological and molecular studies can be compared.

**Key words:** molecular; monocotyledon; orchid; Orchidaceae; phylogeny; *rbcL*.

The use of nucleotide sequence comparisons for estimating phylogenetic relationships has attracted widespread attention among botanists studying a variety of plant groups (Clegg and Zurawski, 1992). Few systematic studies of the Orchidaceae have employed molecular techniques, and only one (Chase et al., 1994) has addressed higher order relationships of this large and floristically important family. Other angiosperm families of comparable size, such as the Asteraceae and Poaceae, have already received the attention of analyses employing more than one molecular data set (see Kellogg and Linder, 1995; Kim, Lookerman, and Jansen, 1995).

As pointed out by Palmer et al. (1988), nucleotide sequences have the advantages of being rapidly produced and easily assessed for homology (this is particularly true for *rbcL* in which there is virtually no length variation). These attributes are especially attractive for the orchi-

dologist, given the complexity and overwhelming diversity of orchid flowers and vegetative structures, most of which have not been fully investigated. Moreover, floral characters, especially those relating to anther configuration and pollinarium structure, have been the primary basis for classification of orchids (Dodson, 1962; Romero, 1990). These floral features are hypothesized to be especially prone to selective pressure from pollinators and, hence, are likely to display high levels of convergence or parallelism (Dodson, 1962; Atwood, 1986).

For the reasons stated above, several researchers (e.g., Schlechter, 1926; Garay, 1960; and Dressler, 1993) have each recognized drastically different relationships for the Orchidaceae. Even the same author has radically different schemes (e.g., Dressler, 1974, 1981, 1993). Competing orchid classifications are presented in Arditti (1992). These systems, usually reflecting intuitive processes and often based on single characters such as column or anther organization, have produced considerable disagreement among not only subtribal and tribal concepts, but also subfamilial and even familial delimitations. Proposals for splitting the family into two or three separate families (Vermeulen, 1966; Rasmussen, 1985) continue to surface, as do reassessments of subfamilial concepts (e.g., Garay 1960; Dressler, 1974, 1981).

A phenetic analysis of Orchidaceae was presented by Clifford and Lavarack (1974). The results of this analysis

produced relationships that were obviously artificial and quite unsatisfactory. Employing cladistic methods, Burns-Balogh and Funk (1986) made a valiant effort to combat "the classic problems of intuitive or *gestalt* systematics" (Burns-Balogh and Funk, 1986), but this study received considerable criticism for its inaccurate character choices and coding (Garay, 1986; Dressler, 1987).

The most recent treatment of Orchidaceae is that of Dressler (1993). This system originated 35 years ago (Dressler and Dodson, 1960) and has been altered and modified periodically by Dressler as basic knowledge of orchid morphology, anatomy, and genetics has expanded (Dressler, 1979, 1981, 1986, 1993). The Orchidaceae, as defined by this system, comprise ~850 genera and 20 000 species. These are arranged in 70 subtribes, 22 tribes, and five subfamilies based principally on anther number and position. The subfamilies are: Apostasioideae, containing the two orchid genera with either three fertile anthers or two fertile anthers and a filamentous staminode; Cypripedioideae, composed of the five genera with two fertile anthers (diandrous), a shield-shaped staminode, and a saccate labellum; Orchidoideae, containing the orchids with a single, erect, basitonic fertile anther (monandrous); Spiranthoideae, comprising the monandrous orchids with erect, acrotonic anther; and Epidendroideae, including all remaining monandrous orchids with an incumbent to suberect anther. This last subfamily is by far the largest (576 genera, ~15 000 species), encompassing more genera and species than all the others combined.

Historically, Apostasioideae have been considered the most primitive subfamily, followed by Cypripedioideae. In fact, these two groups have been classified by some as distinct families, Apostasiaceae and Cypripediaceae, because of their multiple anthers (Rao, 1974; Dahlgren, Clifford, and Yao, 1985; Rasmussen, 1985). The three monandrous subfamilies have always been regarded as a natural group, with Spiranthoideae and Orchidoideae suggested as closest allies on account of their shared terrestrial habit, sectile pollinia, and erect anthers. The vandoid orchids, subfamily Vandoideae sensu Dressler (1981) or tribes Vandeae and Maxillarieae sensu Dressler (1993), are usually regarded as the most advanced in the Old and New Worlds, respectively.

In assessing the results presented here, we will refer to the most recent system of Dressler (1993). Dressler's studies have provided a wealth of insight, information, and inspiration for all students of orchidology, but he has conceded that there are many orchids that do not seem to fit into any currently circumscribed tribe or subfamily and that there remain many important, unanswered questions regarding orchid systematics. Pending the evaluation of these questions by classical techniques such as anatomy, cytology, and morphology (studies of which are in progress), new technology affords the opportunity to apply methods of molecular analysis, especially DNA sequencing, to orchid systematics.

The plastid gene *rbcL* has proven useful in addressing phylogenetic relationships at a variety of taxonomic levels within a number of taxonomic groups. Studies among genera and species of Cypripedioideae by Albert (1994) and Dendrobiinae by Yukawa et al. (1996) indicate that the amount of sequence divergence exhibited by *rbcL* is sufficient and appropriate for addressing relationships

within the orchid family at the genus level. Presented here is a large analysis of *rbcL* nucleotide sequences representing nearly all tribes of Orchidaceae in an effort to evaluate the monophyly and arrangement of the currently recognized subfamilial, tribal, and subtribal groupings within the family.

## MATERIALS AND METHODS

Table 1 lists the 171 taxa used in this analysis; these are representative of all currently recognized subfamilies, nearly all tribes, and most subtribes of Orchidaceae sensu Dressler (1993). In addition, 13 outgroup taxa were determined to be appropriate for this study based on the results of Chase et al. (1995) and Dressler and Chase (1995). These outgroup taxa are members of Hypoxidaceae, Asteliaceae, Lanariaceae, Blandfordiaceae, and Boryaceae. Most taxa were vouchered as either pressed herbarium specimens or spirit-preserved flowers.

Total DNA was extracted according to the procedures outlined in Palmer et al. (1988). Although liquid nitrogen was occasionally used to isolate DNA, it often reduced yield; the hot CTAB method (Doyle and Doyle, 1987) was the preferred technique. Tissue for these extractions primarily took the form of either fresh or silica-gel-dried leaves, although herbarium specimens (*Diceratostele*), fresh tubers (*Nervilia*), dried stems (*Vanilla, Erythrorchis*), and flowers were also used. All extractions were purified on CsCl gradients, and when not in use stored at −80°C.

Several methods of producing *rbcL* sequences have been used since the first orchid, *Oncidium excavatum*, was sequenced nearly 10 yr ago. At the beginning of this study, taxa (approximately ten) were amplified and ligated into Bluescript vectors, cloned by routine recombinant techniques, and sequenced according to standard procedures for dideoxy nucleotide sequencing. Most recently, sequences (~30) were produced by automated methods on an Applied Biosystems, Inc. 373A sequencer according to the manufacturer's protocols. The majority of sequences, however, were completed by manually sequencing purified double-stranded PCR products according to the procedure outlined below.

Templates were amplified with primers that correspond to the highly conserved first 20 base pairs (bp) of the *rbcL* coding sequence and to a 20-bp region beginning at position 1352 in the *rbcL* exon. Because of sequence divergence in many monocots, particularly orchids, it was not possible to use the reverse amplification primer located downstream of the stop codon at a ribosomal control site that is frequently employed for sequencing *rbcL*. Approximately 1330 bp of sequence were collected for each taxon.

Most double-stranded amplified products were purified and sequenced according to the procedure described by Chase et al. (1995) using Sequenase® and either $^{32}$P or $^{35}$S. Four or five internal primers were usually sufficient to determine the complete gene sequence with adequate overlap of primers to ensure accuracy. The resulting autoradiograms were scored by hand and manually entered into a computer database (these sequences have been accessioned into GenBank; copies of the complete matrix are available from the authors).

Phylogenetic analyses were conducted using the parsimony algorithm of the software package PAUP (Phylogenetic Analysis Using Parsimony, version 3.1.1: Swofford, 1993). Excluding the primer sites and missing data at the 5′ end of the gene, all positions from bp 31 to 1351 were used; these were easily aligned by eye as there were no insertions or deletions detected. Based on larger *rbcL* analyses (Chase et al., 1995), outgroup taxa were specified to be a monophyletic sister to the ingroup. Shortest trees were initially found using the routine outlined by Olmstead and Palmer (1994). Heuristic tree searches of 1000 random taxon-addition replicates under the Fitch (1971) criterion (unordered with equal weights) were executed. Tree bisection and reconstruction (TBR) swapping was used, with MULPARS in effect, but keeping only two trees for each replicate. The resulting trees consisted of at least two islands of maximum parsimony (Maddison, 1991) and were then used

TABLE 1.   Species analyzed. Arranged by family, subfamily, tribe, subtribe, and genus where applicable (Orchidaceae sensu Dressler, 1993).

| Family, subfamily, tribe, and subtribe | Genus and species | Voucher | Database accession[a] |
|---|---|---|---|
| Asteliaceae | *Astelia alpina* R.Br. | *Chase 1103* (K)[b] | GBANZ77261 |
| | *Collospermum hastatum* Skottsb. | *Adelaide Bot. G.* 875767 | GBANY14986 |
| | *Milligania stylosa* F. Muell. ex Benth. | *Chase 511* (K) | GBANZ73693 |
| Blandfordiaceae | *Blandfordia punicea* Sweet | *Chase 519* (K) | GBANZ73694 |
| Boryaceae | *Borya septetrionalis* F. Muell. | *Chase 2205* (K) | GBANY14985 |
| | *Alania endlicheri* Kunth | *Conran 707* (AD) | GBANY14982 |
| Hypoxidaceae | *Curculigo capitulata* Kuntze | *Chase 205* (NCU) | GBANZ73701 |
| | *Empodium veratrifolium* (Willd.) M.E. Thompson | *Kirstenbosch b42-84* | GBANY14987 |
| | *Hypoxis leptocarpa* (Engelm. & A. Gray) Small | *Chase 108* (NCU) | GBANZ73702 |
| | *Pauridia longituba* M.E. Thompson | *D. Snijman s.n.* (WBG) | GBANY14991 |
| | *Rhodohypoxis milloides* (Baker) Hilliard & B.L. Burtt | *Chase 479* (K) | GBANZ77280 |
| | *Spiloxene capensis* (L.) Garside | *UCI ARB 728* | GBANZ77281 |
| Lanariaceae | *Lanaria lanata* Druce | *Goldblatt 9410* (MO) | GBANZ77313 |
| Orchidaceae | | | |
| Apostasioideae | *Apostasia stylidioides* Rchb.f. | *Clements 4823* (CBG) | GBANZ73705 |
| | *Neuwiedia veratrifolia* Blume | *Clements 5910* (CBG) | GBANAF074200 |
| Cypripedioideae | *Cypripedium passerinum* Richards | *Albert 48* (NCU) | GBANAF074142 |
| | *Cypripedium irapeanum* La Llave & Lex. | *Albert 47* (NCU) | GBANZ73706 |
| | *Mexipedium xerophyticum* V.A. Albert & M.W. Chase | *Hegedus s.n.* (AMO) | GBANAF074193 |
| | *Paphiopedilum delenatii* Guillaumin | *Albert 141* (NCU) | GBANAF074208 |
| | *Paphiopedilum sukhakulii* Schoser & Senghas | *Albert 100* (NCU) | GBANAF074209 |
| | *Paphiopedilum bellatulum* (Rchb.f.) Stein | *Albert 140* (NCU) | GBANAF074207 |
| | *Phragmipedium longifolium* (Rchb.f. & Warsc.) Rolfe | *Albert 18* (NCU) | GBANAF074212 |
| | *Phragmipedium schlimii* (Linden & Rchb.f.) Rolfe | *Albert 128* (NCU) | GBANAF074213 |
| | *Selenipedium chica* Rchb.f. | *Albert 167* (NCU) | GBANAF074227 |
| Spiranthoideae | | | |
| Cranichideae | | | |
| Cranichidinae | *Cranichis fertilis* Schltr. | *Chase O-401* (K) | GBANAF074137 |
| | *Ponthieva racemosa* (Walter) Mohr | *Chase O-398* (K) | GBANAF074223 |
| Goodyerinae | *Goodyera pubescens* (Willd.) R.Br. | *Chase O-212* (K) | GBANAF074174 |
| | *Platythelys querceticola* (Lindl.) Garay | *Chase O-378* (K) | GBANAF074216 |
| Pachyplectroninae | *Pachyplectron arifolium* Schltr. | *Ziesing 22* (CBG) | GBANAF074205 |
| Prescottiinae | *Altensteinia paleacea* Kunth | *Chase O-535* (K) | GBANAF074105 |
| Spiranthinae | *Spiranthes cernua* (L.) Richard | *Chase O-402* (K) | GBANAF074229 |
| Diceratosteleae | *Diceratostele gabonensis* Sumerhayes | *Chase O-484* (K) | GBANAF074148 |
| Tropideae | *Corymborkis* sp. Thouars | *Chase O-542* (K) | GBANAF074136 |
| | *Tropidia* sp. Lindl. | *Chase O-211* (K) | GBANAF074237 |
| Orchidoideae | | | |
| Diseae | | | |
| Disinae | *Disa tripetaloides* (L.F.) N.E. Br. | *Cameron 1047* (NCU) | GBANAF074151 |
| Satyriinae | *Satyrium nepalense* D. Don | *Chase O-539* (K) | GBANAF074226 |
| Diurideae | | | |
| Acianthinae | *Acianthus exsertus* R.Br. | *Chase O-565* (K) | GBANAF074101 |
| | *Corybas diemenicus* (Lindl.) Rupp | *Chase O-564* (K) | GBANAF074135 |
| Caladeniinae | *Caladenia* cf. *caerulea* R.Br. | *Chase O-487* (K) | GBANAF074116 |
| | *Eriochlus cucullatus* (Labill.) Rchb. | *Chase O-566* (K) | GBANAF074166 |
| | *Glossodia major* R.Br. | *Chase O-568* (K) | GBANAF074173 |
| | *Lyperanthus nigricans* R.Br. | *Chase O-836* (K) | GBANAF074187 |
| Chloraeinae | *Chloraea* sp. Rchb.f. | *Chase O-551* (K) | GBANAF074125 |
| | *Megastylis glandulosus* Schltr. | *Ziesing 29* (CBG) | GBANAF074191 |
| Cryptostylidinae | *Cryptostylis subulata* (Labill.) Rchb. | *Chase O-332* (K) | GBANAF074140 |
| Diuridinae | *Diuris sulphurea* R.Br. | *Chase O-554* (K) | GBANAF074152 |
| | *Orthoceras strictum* R.Br. | *Chase O-571* (K) | GBANAF074204 |
| Drakaeinae | *Chiloglottis trapeziformis* Fitzg. | *Chase O-569* (K) | GBANAF074124 |
| Prasophyllinae | *Microtis parviflora* R.Br. | *Chase O-553* (K) | GBANAF074194 |
| Pterostylidinae | *Pterostylis nutans* R.Br. | *Chase O-533* (K) | GBANAF074224 |
| Thelymitrinae | *Calochilus robertsonii* Benth. | *Chase O-570* (K) | GBANAF074118 |
| | *Thelymitra* sp. Förster | *Chase O-489* (K) | GBANAF074232 |
| Orchideae | | | |
| Habenariinae | *Habenaria repens* Nutt. | *Chase O-381* (K) | GBANAF074177 |
| Orchidinae | *Ophrys apifera* Hudson | *Chase O-536* (K) | GBANAF074202 |
| | *Orchis quadripunctata* Cyrillo ex. Tenore | *Chase O-911* (K) | GBANAF074203 |
| | *Platanthera ciliaris* (L.) Lindl. | *Albert 54* (NCU) | GBANAF074215 |

TABLE 1.  Continued.

| Family, subfamily, tribe, and subtribe | Genus and species | Voucher | Database accession[a] |
|---|---|---|---|
| Epidendroideae | | | |
|  Neottieae | | | |
|   Limodorinae | *Cephalanthera damasonianum* (Miller) Druce | *Chase O-575* (K) | GBANAF074123 |
| | *Epipactis helleborine* (L.) Crantz | *Chase O-199* (K) | GBANZ73707 |
|   Listerinae | *Listera smallii* Wiegand | *Cameron 1001* (NCU) | GBANAF074184 |
|  Nervileae | *Nervilia bicarinata* Schltr. | *Chase O-580* (K) | GBANAF074199 |
|  Palmorchideae | *Palmorchis trilobulata* L.O. Williams in Woodson & Schery | *Chase O-462* (K) | GBANAF074206 |
|  Triphoreae | *Monophyllorchis* sp. Schltr. | *Chase O-435* (K) | GBANAF074195 |
| | *Triphora trianthophora* (Swartz) Rydb. | *Chase O-379* (K) | GBANAF074236 |
|  Vanilleae | | | |
|   Galeolinae | *Erythrorchis altissima* (Bl.) Bl. | *Cameron 1029* (NCU) | GBANAF074168 |
| | *Erythrorchis cassythoides* (Cunn. ex Lindl.) Garay | *Weston 1831* (NCU) | GBANAF074169 |
|   Vanillinae | *Clematepistephium smilacifolium* Hallé | *Ziesing 33* (CBG) | GBANAF074131 |
| | *Epistephium* sp. Humbert | *Chase O-432* (K) | GBANAF074159 |
| | *Epistephium* sp. Humbert | *Chase O-433* (K) | GBANAF074160 |
| | *Epistephium cf. lucidum* Cogn. | *Chase O-795* (K) | GBANAF074161 |
| | *Epistephium parviflorum* Lindl. | *Chase O-794* (K) | GBANAF074162 |
| | *Epistephium subrepens* Hoehne | *Chase O-815* (K) | GBANAF074163 |
| | *Eriaxis rigida* Rchb.f. | *Ziesing 5* (CBG) | GBANAF074165 |
| | *Vanilla africana* Lindl. | *Chase O-584* (K) | GBANAF074239 |
| | *Vanilla aphylla* Bl. | *Chase O-578* (K) | GBANAF074238 |
| | *Vanilla cf. barbellata* Rchb.f. | *Chase O-591* (K) | GBANAF074240 |
| | *Vanilla cf. planifolia* Andrews | *Chase O-170* (K) | GBANAF074242 |
| | *Vanilla imperialis* Kraenzlin | *Chase O-587* (K) | GBANAF074241 |
| | *Vanilla roscheri* Rchb.f. | *Chase O-540* (K) | GBANAF074243 |
| Cymbidiod phylad | | | |
|  Calypsoeae | *Aplectrum hymale* Torr. | *Chase O-104* (K) | GBANAF074108 |
| | *Calypso bulbosa* (L.) Oakes | *Grant 92-02165* (US) | GBANAF074120 |
| | *Tipularia discolor* (Pursh) Nutt. | *Freudenstein s.n.* | GBANAF047234 |
|  Cymbidieae | | | |
|   Catasetinae | *Catasetum expansum* Rchb.f. | *Chase O-224* (K) | GBANAF074121 |
| | *Dressleria eburnea* (Rolfe) Dodson | *Chase O-313* (K) | GBANAF074153 |
| | *Mormodes* sp. Lindl. | unknown | GBANAF074196 |
|   Cyrtopodiinae | *Ansellia gigantea* Rchb. f. | *Chase O-429* (K) | GBANAF074107 |
| | *Cymbidium ensifolium* (L.) Sw. | *Chase O-290* (K) | GBANAF074141 |
| | *Cyrtopodium andersonii* (Lamb. ex Andrews) R.Br. | *Chase O-341* (K) | GBANAF074143 |
| | *Cyrtopodium punctatum* (L.) Lindl. | *Chase O-126* (K) | GBANAF074144 |
| | *Galeandra devoniana* Schomb. ex. Lindl. | *Chase O-382* (K) | GBANAF074171 |
| | *Grammatophyllum speciosum* Blume | *Chase O-890* (K) | GBANAF074176 |
|   Eulophiinae | *Eulopha nuda* Lindl. | *Chase O-292* (K) | GBANAF074170 |
|   Goveniinae | *Govenia* sp. Lindl. ex. Loddiges | *Chase O-146* (K) | GBANAF074175 |
|  Malaxideae | *Liparis lilifolia* (L.) L.C.M. Rich ex Lindl. | *Chase O-214* (K) | GBANAF074183 |
| | *Malaxis spicata* Sw. | *Chase O-377* (K) | GBANAF074188 |
|  Maxillarieae | | | |
|   Cryptarrheninae | *Cryptarrhena* sp. Lindl. | *Chase O-307* (K) | GBANAF074138 |
|   Lycastinae | *Lycaste cruenta* Lindl. | unknown | GBANAF074185 |
|   Maxillariinae | *Bifrenaria harrisoniae* (Hook.) Rchb.f. | *Chase O-95* (K) | GBANAF074112 |
| | *Cryptocentrum peruvianum* (Cogn.) C. Schweinf. | *Chase O-115* (K) | GBANAF074139 |
| | *Maxillaria cucullata* Lindl. | *Chase O-85* (K) | GBANAF074190 |
| | *Xylobium* sp. Lindl. | unknown | GBANAF074245 |
|   Oncidiinae | *Oncidium excavatum* (Rchb.f.) Lindl. | *Chase O-86* (K) | GBANAF074201 |
|   Stanhopeinae | *Acineta chrysantha* Lindl. & Paxt. | *Chase O-251* (K) | GBANAF074102 |
| | *Coryanthes verrucolineata* G. Gerlach | *Chase O-510* (K) | GBANAF074134 |
| | *Houlletia sanderi* Rolfe | *Chase O-500* (K) | GBANAF074178 |
| | *Kegeliella kupperi* Mansf. | *Chase O-495* (K) | GBANAF074181 |
| | *Lycomormium squalidum* Rchb.f. | *Chase O-273* (K) | GBANAF074186 |
| | *Stanhopea ecornuta* Lemaire | *Chase O-255* (K) | GBANAF074230 |
|   Telipogoninae | *Stellilabium pogonostalix* (Rchb.f.) Garay & Dunst. | *Chase O-123* (K) | GBANAF074231 |
|   Zygopetalinae | *Dichaea riopalenquensis* Dodson | *Chase O-114* (K) | GBANAF074149 |
| | *Huntleya heteroclita* (Poepp. & Endl.) Garay | *Whitten 88023* (FLAS) | GBANAF074179 |
| | *Koellensteinia graminea* (Lindl.) Rchb.f. | *Chase O-159* (K) | GBANAF074182 |
| | *Zygopetalum intermedium* Hort. Petrop. ex Regel | *Chase O-160* (K) | GBANAF074246 |

TABLE 1. Continued.

| Family, subfamily, tribe, and subtribe | Genus and species | Voucher | Database accession[a] |
|---|---|---|---|
| Epidendroid phylad | | | |
| Arethuseae | | | |
| Arethusinae | *Arethusa bulbosa* L. | *Chase O-880* (K) | GBANAF074109 |
| Bletiinae | *Acanthephippium mantinianum* Lindl. & Cogn. | *Chase O-397* (K) | GBANAF074100 |
| | *Bletia* cf. *purpurea* (Lam.) DeCandolle | *Chase O-581* (K) | GBANAF074113 |
| | *Bletilla striata* (Thunb.) Rchb.f. | *Chase O-556* (K) | GBANAF074114 |
| | *Calanthe vestita* Lindl. | *Chase O-207* (K) | GBANAF074117 |
| | *Calopogon tuberosus* (L.) B.S.P. | *Chase O-876* (K) | GBANAF074119 |
| | *Phaius minor* Blume | *Chase O-325* (K) | GBANAF074210 |
| Chysiinae | *Chysis bractescens* Lindl. | *Chase O-436* (K) | GBANAF074126 |
| Coelogyneae | | | |
| Coelogyninae | *Coelogyne cristata* Lindl. | *Chase O-491* (K) | GBANAF074133 |
| Thuniinae | *Thunia alba* Rchb.f. | *Chase O-589* (K) | GBANAF074233 |
| Epidendreae I | | | |
| Arpophylliinae | *Arpophyllum giganteum* Hartweg ex Lindl. | *Chase O-586* (K) | GBANAF074110 |
| Coeliinae | *Coelia triptera* (Smith) G. don ex Steud. | *Chase O-324* (K) | GBANAF074132 |
| Laeliinae | *Cattleya dowiana* Batem. & Rchb.f. | *Chase O-282* (K) | GBANAF074122 |
| | *Dilomilis montana* (Sw.) Summerhayes | *Chase O-206* (K) | GBANAF074150 |
| | *Encyclia* sp. Hook. | unknown | GBANAF074157 |
| | *Epidendrum* sp. L. | unknown | GBANAF074158 |
| Meiracylliinae | *Meiracyllium trinasutum* Rchb.f. | *Chase O-202* (K) | GBANAF074192 |
| Pleurothallidinae | *Masdevallia infracta* Lindl. | *Chase O-294* (K) | GBANAF074189 |
| | *Pleurothallis endotrachys* Rchb.f. | *Chase O-306* (K) | GBANAF074217 |
| | *Restrepia* sp. Kunth | unknown | GBANAF074225 |
| Sobrallinae | *Elleanthus* sp. Presl | *Chase O-374* (K) | GBANAF074156 |
| | *Sobralia macrantha* Lindl. | *Chase O-200* (K) | GBANAF074228 |
| Epidendreae II | | | |
| Glomerinae | *Earina autumnalis* Hook.f. | *Chase O-298* (K) | GBANAF074155 |
| | *Glomera* sp. Blume | *Chase O-555* (NCU) | GBANAF074172 |
| Polystachyinae | *Polystachya pubescens* (Lindl.) Rchb.f. | *Chase O-152* (K) | GBANAF074222 |
| Podochileae | | | |
| Bulbophylliinae | *Bulbophyllum lobbii* Lindl. | *Chase O-474* (K) | GBANAF074115 |
| | *Bulbophyllum macranthum* Lindl. | *Shiraishi 610*[c] | D58405 |
| Dendrobiinae | *Cadetia taylori* (F. Muell.) Schltr. | *Shiraishi 132*[c] | D58406 |
| | *Dendrobium aggregatum* H.B. & K. | *Jarrell 15* | GBANAF074145 |
| | *Dendrobium crystallinum* Rchb.f. | *Shiraishi s.n.*[c] | D58407 |
| | *Dnedrobium kingianum* Bidw. | *Chase O-164* (K) | GBANAF074146 |
| | *Dendrobium taurinum* Lindl. | *Shiraishi 577*[c] | D58408 |
| | *Diplocaulobium arachnoideum* Schltr. | *Yukawa 79010*[c] | D58409 |
| | *Epigeneium acuminatum* (Rolfe) Summerh. | *Shiraishi 804*[c] | D58410 |
| | *Flickingeria fugax* (Rchb.f.) Seidenf. | *Yukawa 69013*[c] | D58411 |
| | *Pseuderia smithiana* Schweinf. | *Setoguchi 53* (TI) | D58412 |
| Eriinae | *Eria ferruginea* Teijsm. & Binn. | *Chase O-590* (K) | GBANAF074164 |
| | *Trichotosia ferox* Blume | *Chase O-396* (K) | GBANAF074235 |
| Podochilinae | *Podochlius cultratus* Lindl. | *Chase O-559* (K) | GBANAF074218 |
| Thelasiinae | *Phreatia* sp. Lindl. | *Chase O-203* (K) | GBANAF074214 |
| Vandeae | | | |
| Aeridinae | *Cleisostoma rolfeanum* (King & Pantling) Garay | *Jarrell 7* | GBANAF074130 |
| | *Neofinetia falcata* (Thunb.) S.Y. Hu | *Jarrell 3* | GBANAF074197 |
| | *Phalaenopsis equestris* (Schauer) Rchb.f. | *Jarrell s.n.* | GBANAF074211 |
| Angraecinae | *Aeranthes ramosa* Rolfe | *Jarrell s.n.* | GBANAF074104 |
| | *Angraecum sesqupedale* Thouars | *Jarrell s.n.* | GBANAF074106 |
| Aerangidinae | *Aerangis calligera* (Rchb.f.) Garay | *Jarrell s.n.* | GBANAF074103 |
| | *Diaphananthe rutila* (Rchb.f.) Summerh. | *Jarrell s.n.* | GBANAF074147 |
| Anomalous Epidendroideae | | | |
| Arundinae | *Arundina graminifolia* (D. Don) Hochr. | *Chase O-395* (K) | GBANAF074111 |
| Pogoniinae | *Cleistes divaricata* (L.) Ames | *Chase O-376* (K) | GBANAF074127 |
| | *Cleistes rosea* Lindl. | *Cameron 1038* (NCU) | GBANAF074128 |
| | *Cleistes* sp. (Brazil) L.C. Richard | *Chase O-430* (K) | GBANAF074129 |
| | *Duckeella adolphii* Porto & Brade | *Romero 3013* (AMES) | GBANAF074154 |
| | *Isotria verticillata* (Muhl. ex Willd.) Raf. | *Cameron 1030* (NCU) | GBANAF074180 |
| | *Pogonia japonica* Rchb.f. | *Cameron 1034* (NCU) | GBANAF074219 |
| | *Pogonia minor* (Makino) Makino | *Cameron 1033* (NCU) | GBANAF074220 |
| | *Pogonia ophioglossoides* (L.) Jussieu | *Chase O-437* (K) | GBANAF074221 |
| Anomalous | *Eriopsis biloba* Lindl. | *Chase O-502* (W) | GBANAF074167 |
| | *Neomoorea irrorata* (Rolfe) Rolfe | *Chase O-503* (K) | GBANAF074198 |
| | *Xerorchis amazonica* Schltr. | *Romero 3014* (AMES) | GBANAF074244 |

[a] Accession numbers prefixed by AF, Y, or Z are GenBank; those with D are DNA Data Bank of Japan (DDBJ). The prefix GBAN has been added for linking the on-line version of *American Journal of Botany* to GenBank and is not part of the actual GenBank accession number.

[b] Chase vouchers represent orchid DNA collection numbers and correspond to herbarium or spirit material (K) unless otherwise noted.
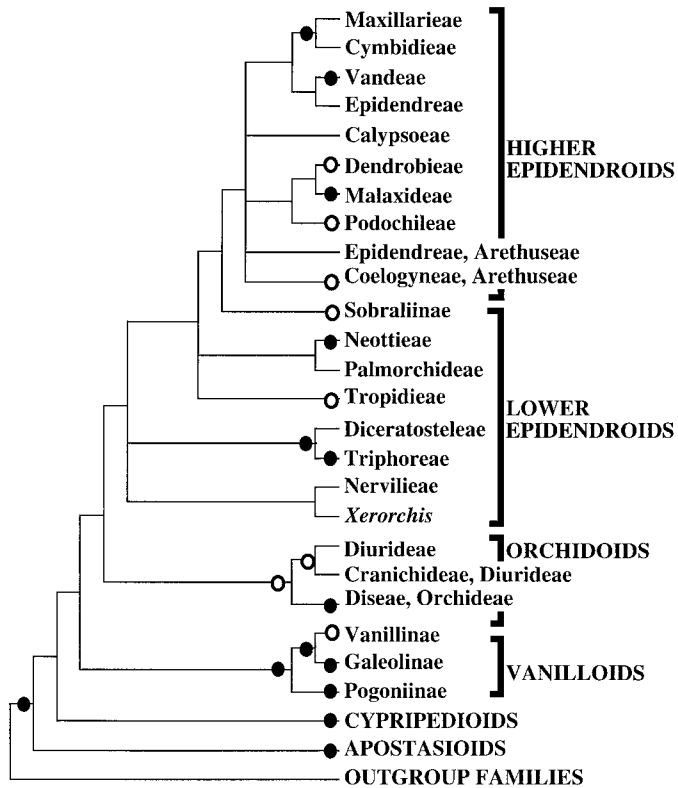
[c] Spirit collection of T. Yukawa.

Fig. 1.   Strict consensus summary of 6000 successively weighted *rbc*L trees for Orchidaceae. Length = 518 105 steps (2058 steps with equal weights); CI = 0.747; RI = 0.8418. Informal subfamily names are indicated, as are tribes sensu Dressler (1993). Solid circles indicate clades with high bootstrap support (75–100%). Open circles indicate clades with weak bootstrap support (50–74%). Tribes Nervileae, Diceratosteleae, and Palmorchideae are represented by single taxa.



Fig. 2.   One of 6000 equally parsimonious, successively weighted *rbc*L trees highlighting outgroup families, apostasioid, and cypripedioid orchids. Numbers above branches correspond to branch lengths (ACCTRAN optimization with equal weights). Arrows indicate clades that collapse in the strict consensus. Solid circles indicate clades with strong bootstrap support (75–100%). Open circles indicate clades with weak bootstrap support (50–74%).

as starting trees to find as many trees of maximum parsimony (MUL-PARS option in effect) as the computer memory would hold.

To improve the quality of the data matrix, successive weighting (Farris, 1969) was employed. Characters were assigned new weights using the "reweight characters" option based on the rescaled consistency index (RC) in PAUP 3.1.1 (Swofford, 1993) with a base weight of 1000. Successive rounds of heuristic searches were performed until two rounds of the same length trees were produced. Each round of successive weighting consisted of ten random replicates, after which the shortest trees were used as starting trees to generate several thousand trees upon which the next round of reweighting was based.

Bootstrap analysis (1000 replicates) was applied to the successively weighted matrix as an evaluation of topological robustness. All clades supported in at least 50% of these replicates are reported. Throughout the discussion, clades with at least 75% bootstrap support are considered "supported," those with 50–74% are considered "weakly supported," and clades found in the strict consensus, but with <50% bootstrap values, are considered "not supported."

## RESULTS

Of the 1320 characters in the data matrix, 641 were invariant and 485 were variable in two or more taxa. Equally weighted tree searches resulted in >5000 trees of 2046 steps. These trees are characterized by a consistency index (CI) of 0.39 and a retention index (RI) of 0.68. Successive weighting resulted in 6000 trees of length 518 105 steps (2058 Fitch steps) before computer
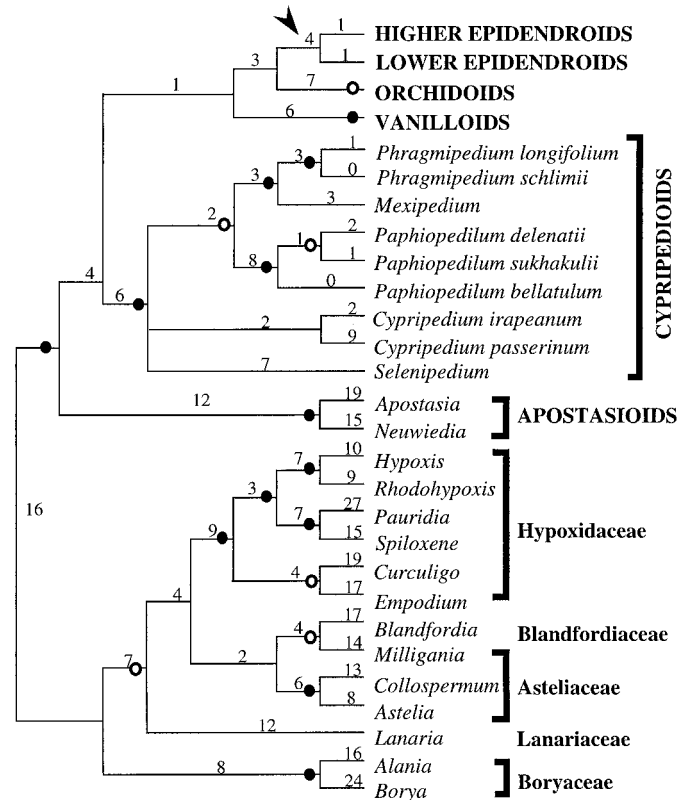
memory was exhausted. These trees are characterized by a CI of 0.75 and RI of 0.84. The strict consensus of these weighted trees is shown as Fig. 1. Representative single topologies highlighting each major subclade are presented as Figs. 2 through 7. Branch lengths (ACCTRAN optimization with equal weights) and bootstrap support are shown.

These data indicate that Orchidaceae are composed of five major monophyletic clades (Fig. 1). With a few exceptions, these major clades correspond to currently recognized subfamilies. They are the subfamilies Apostasioideae, Cypripedioideae, Orchidoideae, Epidendroideae, and Vanilloideae (sensu Szlachetko, 1995; part of Epidendroideae sensu Dressler, 1993). Informal names will be used in the remainder of this paper because, based solely on these data, we do not wish to propose an alternative orchid taxonomy. Confirmation of these results is first needed.

***Outgroup families***—Based on larger analyses of nucleotide sequences among monocotyledons (Chase et al., 1995), genera from Boryaceae, Blandfordiaceae, Asteliaceae, Hypoxidaceae, and Lanariaceae were included as outgroups (see Fig. 2). This analysis shows strong support for the monophyly of Boryaceae (*Alania* and *Borya*) as well as for Hypoxidaceae. Within Hypoxidaceae,
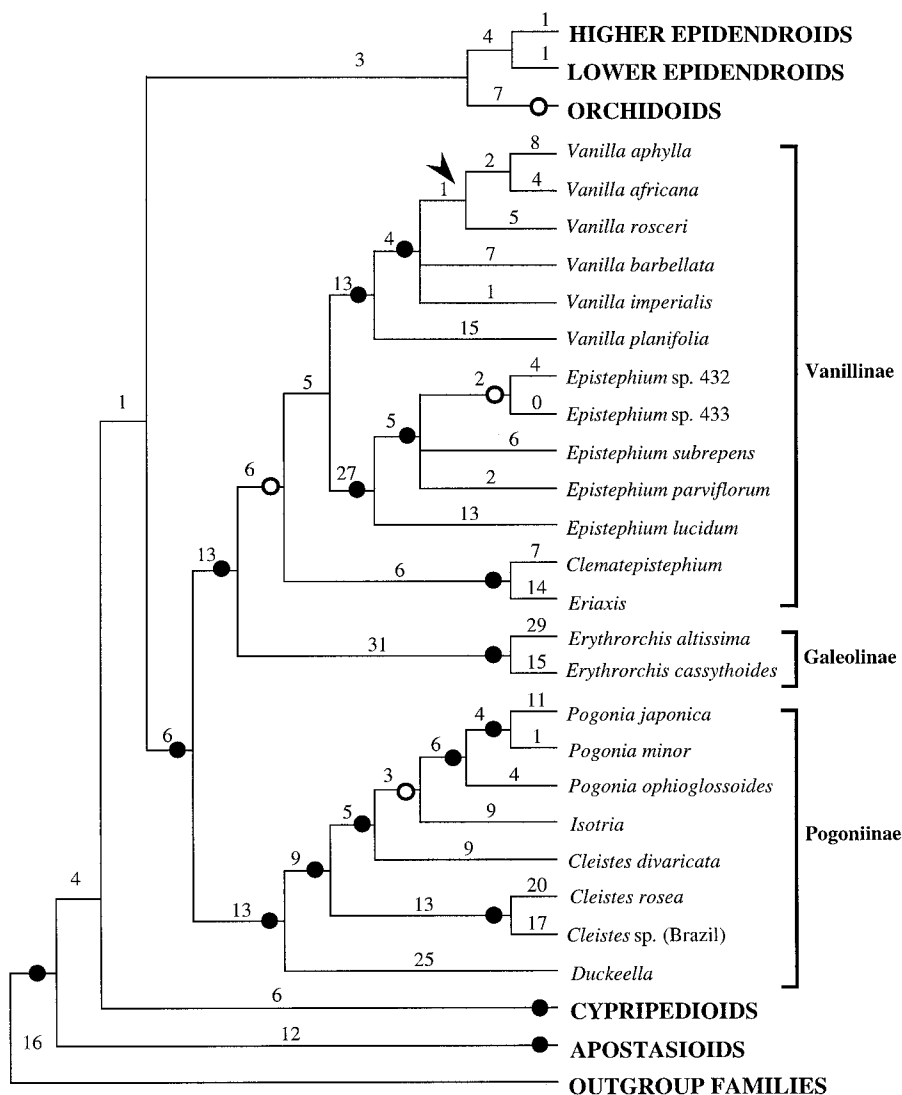
Fig. 3. One of 6000 equally parsimonious, successively weighted *rbc*L trees highlighting the vanilloid orchids. Numbers above branches correspond to branch lengths (ACCTRAN optimization with equal weights). Arrows indicate clades that collapse in the strict consensus. Solid circles indicate clades with strong bootstrap support (75–100%). Open circles indicate clades with weak bootstrap support (50–74%).

*Pauridia/Spiloxene* and *Hypoxis/Rhodohypoxis* are strongly supported by the bootstrap analysis.

***Apostasioid orchids***—This clade represents the subfamily Apostasioideae and includes the two genera of that subfamily, *Neuwiedia* and *Apostasia* (Fig. 2). There is strong support for the monophyly of this clade but no bootstrap support for its position as sister to the remainder of the Orchidaceae.

***Cypripedioid orchids***—A monophyletic clade containing all members of the diandrous subfamily Cypripedioideae, the slipper orchids, is well supported, but like the apostasioids, bootstrap support for its position as sister to all monandrous orchids (Fig. 1) is lacking.

Each of the five genera of the subfamily is represented, and each of those with more than a single species is monophyletic (Fig. 2). Although the exact relationship of *Selenipedium* is not clear in this large analysis, the to-

pology is in agreement with previous studies addressing relationships within the subfamily (Albert, 1994; Cox et al., 1997). In all most parsimonious trees, the conduplicate-leaved genera *Paphiopedilum, Mexipedium*, and *Phragmipedium* form a monophyletic unit with *Mexipedium* strongly supported as sister to *Phragmipedium*, and this pair sister to *Paphiopedilum*.

***Vanilloid orchids***—In the large intrafamilial analysis, tribe Vanilleae sensu Dressler (1990a), together with Pogoniinae, appear as sister to the remainder of the monandrous orchids (Fig. 1). Sequence divergence within this clade is extraordinarily high, even within genera (e.g., *Vanilla*) and is generally greater than for any other major clade. Within the clade (Fig. 3) there is bootstrap support for the monophyly of the three represented subtribes, Vanillinae, Pogoniinae, and Galeolinae (sensu Dressler, 1993).
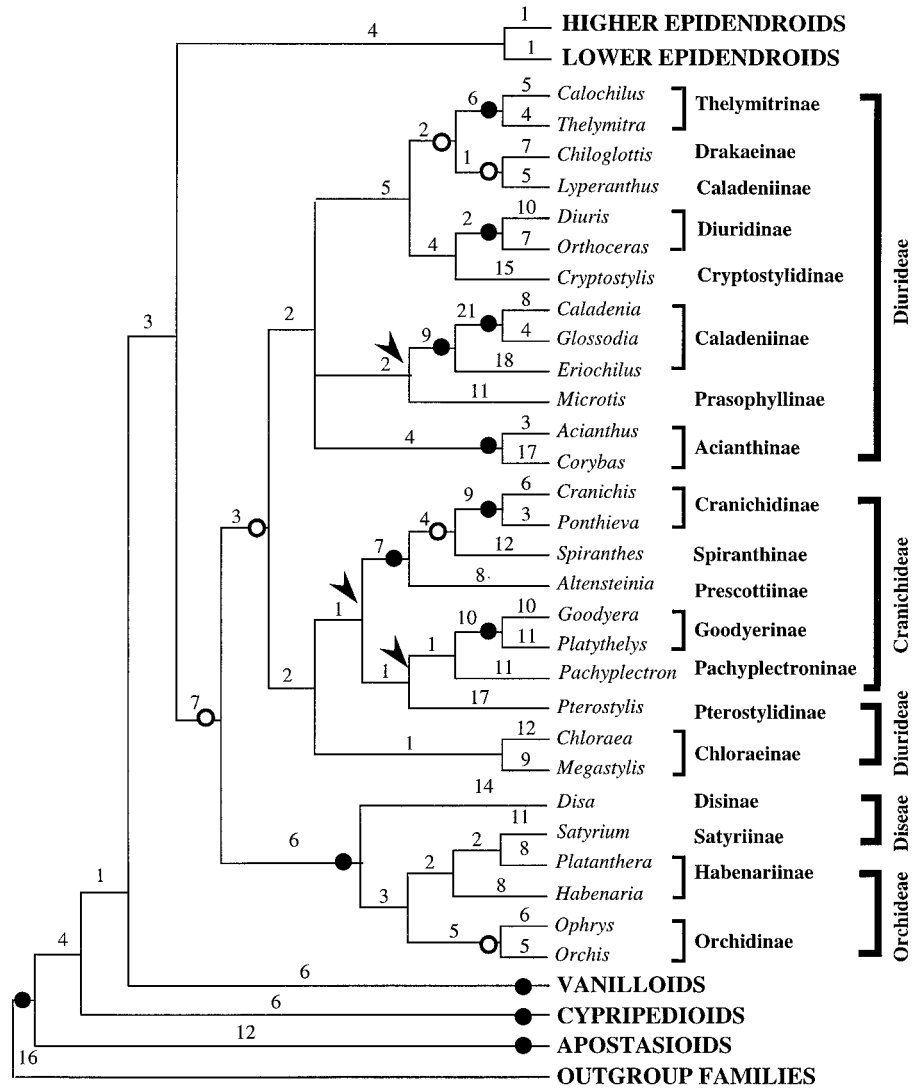
Pogoniinae are arranged with the South American ge-

Fig. 4. One of 6000 equally parsimonious, successively weighted *rbc*L trees highlighting the orchidoid orchids. Numbers above branches correspond to branch lengths (ACCTRAN optimization with equal weights). Arrows indicate clades that collapse in the strict consensus. Solid circles indicate clades with strong bootstrap support (75–100%). Open circles indicate clades with weak bootstrap support (50–74%).

nus *Duckeella* as sister to the rest. This is followed by two species of *Cleistes* from South America and a temperate clade containing the monophyletic taxa *Pogonia, Isotria,* and the North American *Cleistes* species, *C. divaricata. Cleistes* as a genus is not monophyletic. All but one branch in the subtribe receive strong bootstrap support.

Subtribe Vanillinae is monophyletic and consists of a strongly supported monophyletic genus *Vanilla* as sister to species of monophyletic *Epistephium*. Following this is a bootstrap-supported clade containing the two monotypic New Caledonian genera *Eriaxis* and *Clematepistephium*. Subtribe Galeolinae contains two species of the achlorophyllous genus *Erythrorchis* (on a long branch) as sister to Vanillinae.

***Orchidoid orchids***—The next diverging clade in the family contains the great majority of terrestrial, monan-

drous orchids typically assigned to Orchidoideae and Spiranthoideae (Fig. 1). There are two bootstrap-supported major subclades present as shown in Fig. 1, but these do not correspond to the two traditional subfamilies. The first contains tribes Orchideae and Diseae of Orchidoideae. The second is larger and includes Cranichideae of Spiranthoideae along with Diurideae of Orchidoideae. These results are in accord with those of Kores et al. (1997).

Tribe Cranichideae is polyphyletic, although its five constituent subtribes are each monophyletic despite the relatively low level of sampling (Fig. 4). These "core" spiranthoid orchids are embedded in a polyphyletic Diurideae and mostly sister to taxa from Pterostylidinae and Chloraeinae (Diurideae). The remaining Diurideae form a monophyletic unit, with some subtribes receiving strong bootstrap support (e.g., Acianthinae and Thelymitrinae). Sister to this large spiranthoid/diurid assemblage is a
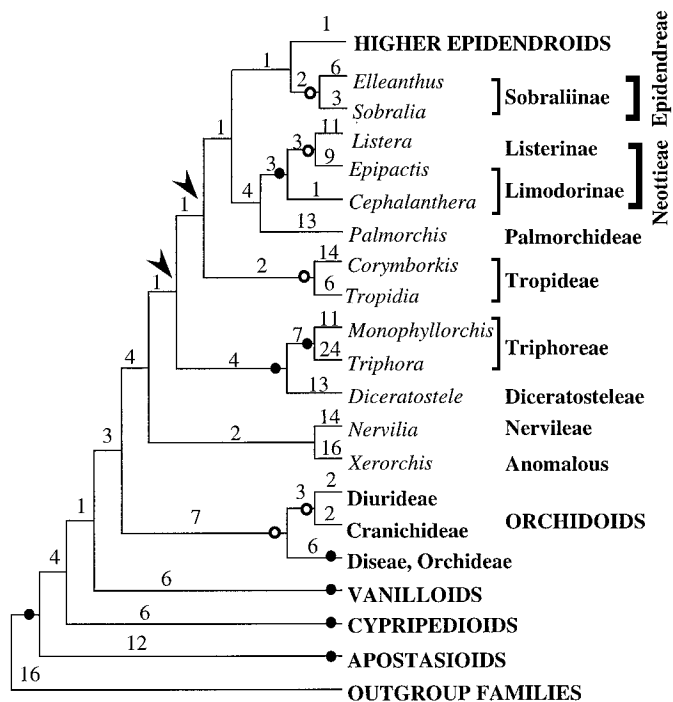
Fig. 5. One of 6000 equally parsimonious, successively weighted *rbcL* trees highlighting the "lower" epidendroid orchids. Numbers above branches correspond to branch lengths (ACCTRAN optimization with equal weights). Arrows indicate clades that collapse in the strict consensus. Solid circles indicate clades with strong bootstrap support (75–100%). Open circles indicate clades with weak bootstrap support (50–74%).

well-supported clade composed of genera from tribes Orchideae and Diseae. Neither tribe is monophyletic owing to the alliance of *Satyrium* with *Platanthera*, although the monophyly of subtribe Orchidinae does receive bootstrap support.

*Epidendroid orchids*—This clade contains taxa typically classified within subfamily Epidendroideae. Because of its large size and for convenience, it has been divided into "lower" and "higher" epidendroids as depicted in Figs. 1, 5, and 6.

A paraphyletic grade of taxa from the "lower" tribes Neottieae, Palmorchideae, Triphoreae, and Nervileae is sister to the remaining epidendroid genera (Fig. 5). Also included among these are Tropideae and Diceratosteleae, both of which have previously been classified in Spiranthoideae. The relationships of these "lower" epidendroids are unresolved, but in no trees do they form a monophyletic unit. Bootstrap support is evident, however, for the monophyly of Triphoreae, Tropideae, and Neottieae.

Within the "higher" epidendroids there is a paraphyletic clustering of tribes Coelogyneae, Arethuseae, Malaxideae, Podochileae, Dendrobieae, and part of Epidendreae (Fig. 6) that mostly corresponds to the tribes of Dressler's (1990b) epidendroid phylad. Dendrobieae, Podochileae, and Malaxideae are supported as monophyletic; Coelogyneae is paraphyletic; and both Arethuseae and Epidendreae are grossly polyphyletic.

The remaining "higher" epidendroid tribes—Calypsoeae, Vandeae, Cymbidieae, and Maxillarieae—correspond to the advanced "vandoid" orchids together with a Laeliinae–Arpophyllinae–Polystachinae clade of Epidendreae (Fig. 7). Of the tribes, Maxillarieae and Vandeae are monophyletic. Maxillarieae are sister to a grade of genera from Cymbidieae, and together these are well supported by the bootstrap analysis. Several of the "higher" epidendroid subtribes (e.g., Eriinae, Catasetinae, Aeridinae, and Pleurothallidinae) are monophyletic in these results, although only Catasetinae and Aeridinae receive bootstrap support.

DISCUSSION

One disadvantage of sequencing a plastid gene is the possibility that resulting phylogenetic hypotheses may not reflect the true phylogeny because of hybridization (Smith and Sytsma, 1990). Orchidaceae are well known as a family in which wide crosses are possible; interspecific and intergeneric hybrids are the basis for a thriving commercial market. This reputation is based on the great ease and frequency of artificial crosses, but, because of mechanical barriers and pollinator specificity, no parallel exists in nature. If one examines orchid floras, such as that of North America (Luer, 1975), documented hybrids at either the generic or specific level are not particularly frequent. No data exist to substantiate the claim that natural hybrids are more frequent in Orchidaceae than in other families. Hybridization is unlikely to be a factor at higher taxonomic categories within and between families because natural hybridization occurs only between closely related species, which for a moderately conserved gene like *rbcL* are likely to have little or no variation (Chase et al., 1995).

These data support Orchidaceae, defined in its broadest sense (i.e., including Apostasiaceae and Cypripediaceae), as a monophyletic family. Hypoxidaceae had been previously suggested as a potential sister group to the orchids (Hutchinson, 1959). Hence, its appearance near the orchids in previous molecular studies (Chase et al., 1995) was not unexpected. Many of the other outgroup taxa shown in Fig. 2, however, have never been suggested to have affinities with the orchids or to each other. Dressler and Chase (1995) discuss these findings, but it is worth noting that at least two of these outgroup taxa, *Alania* and *Borya*, are strongly mycotrophic, and many species of Asteliaceae are epiphytic like the orchids. Moreover, a plant combining broad, plicate leaves (as in *Curculigo* and some species of *Hypoxis*), parietal placentation (as in *Empodium*), simultaneous microsporogenesis (as in Asteliaceae), inferior ovary (as in Hypoxidaceae), and only three fertile anthers (as in *Pauridia*) could serve as a good model for an orchid ancestor.

Although the segregation of Apostasiaceae and Cypripediaceae from Orchidaceae would be permitted by cladistic classification, these data do not justify this move. Sequence divergence between Cypripedioideae/Apostasioideae and the remainder of the family is low relative to the divergence from the outgroups, and strong support for a clearly monophyletic unit falls at the level in the cladogram that favors a broadly defined Orchidaceae.

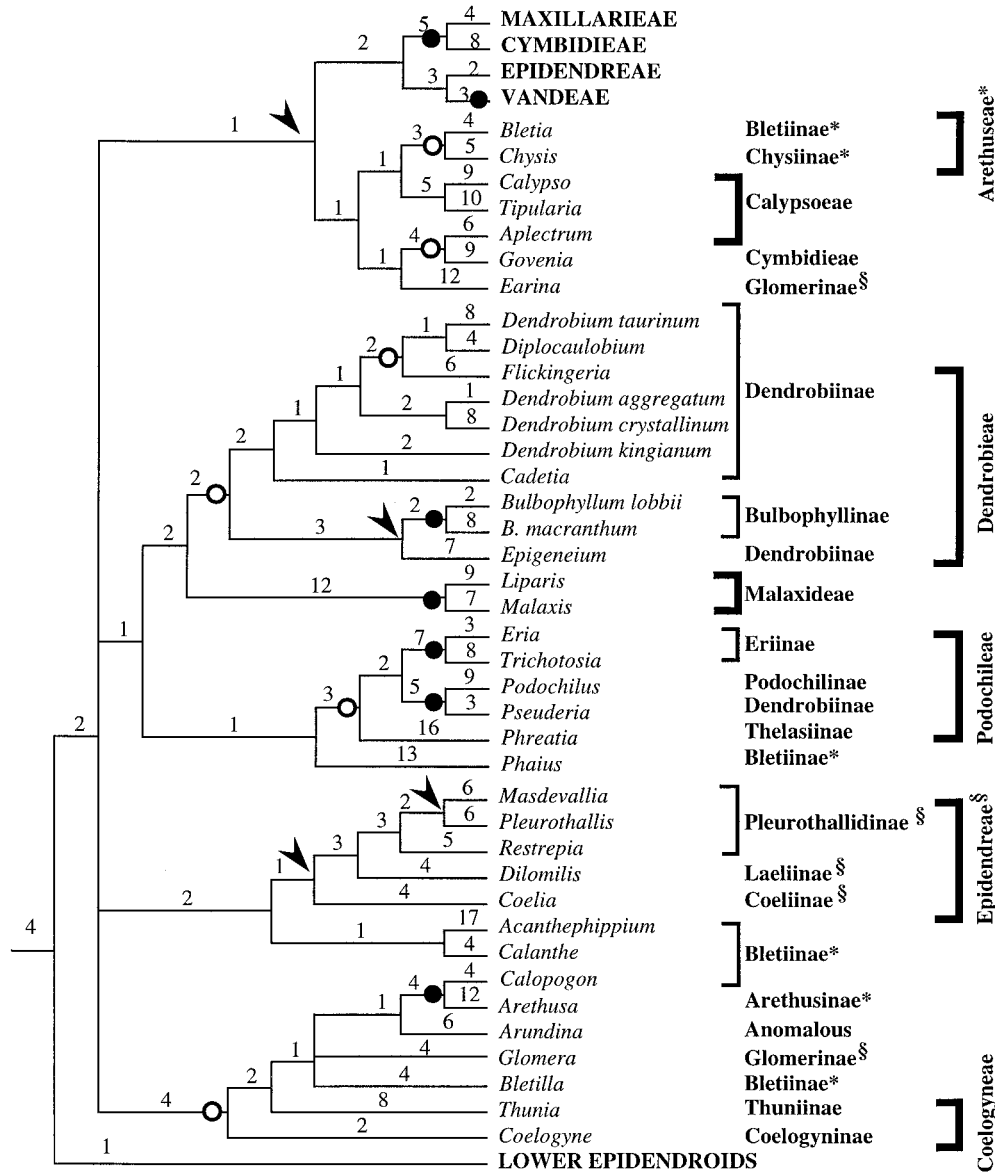Subfamilial relationships of Orchidaceae are, for the

Fig. 6. One of 6000 equally parsimonious, successively weighted *rbc*L trees highlighting most of the reed-stem phylad (sensu Dressler, 1993) of the "higher" epidendroid orchids. Numbers above branches correspond to branch lengths (ACCTRAN optimization with equal weights). Arrows indicate clades that collapse in the strict consensus. Solid circles indicate clades with strong bootstrap support (75–100%). Open circles indicate clades with weak bootstrap support (50–74%). Subtribes of Arethuseae are indicated by an asterisk. The symbol § is used to indicate subtribes within Epidendreae.

most part, in agreement with current thinking. Dressler's (1993) subfamilial cladogram (based on Hennigean argumentation) is nearly identical to this, with one important exception. The removal of the vanilloid orchids from the Epidendroideae and positioning of them as a clade sister to all other monandrous orchids distinguishes this topology. Their position is only weakly supported, but on the basis of molecular divergence, Vanilleae are clearly an isolated group of monandrous orchids. Lindley (1835) was the first to recognize the uniqueness of the vanilloid orchids and actually proposed a distinct family for them (Vanillaceae), and both Garay (1960) and Dressler (1990b) turned to these taxa when searching for primitive morphological characters in the family.

Only the incumbent anther of Vanilleae holds these taxa in association with Epidendroideae. Dogma has held that anther position is unidirectionally polarized from erect to incumbent. We suggest that this character is likely to be correlated with pollinator behavior, and, hence, likely to show parallelism or reversal due to selection. The anther of *Vanilla* is not only incumbent, but is hyperincumbent (Burns-Balogh and Bernhardt, 1985). If degree of anther bending is emphasized, then Vanilleae would have to be considered the most advanced Epidendroideae, a position never previously suggested. On the other hand, a suite of plesiomorphic characters almost exclusively absent in the orchidoid and epidendroid orchids but shared among Apostasioideae, Cypripedioideae,
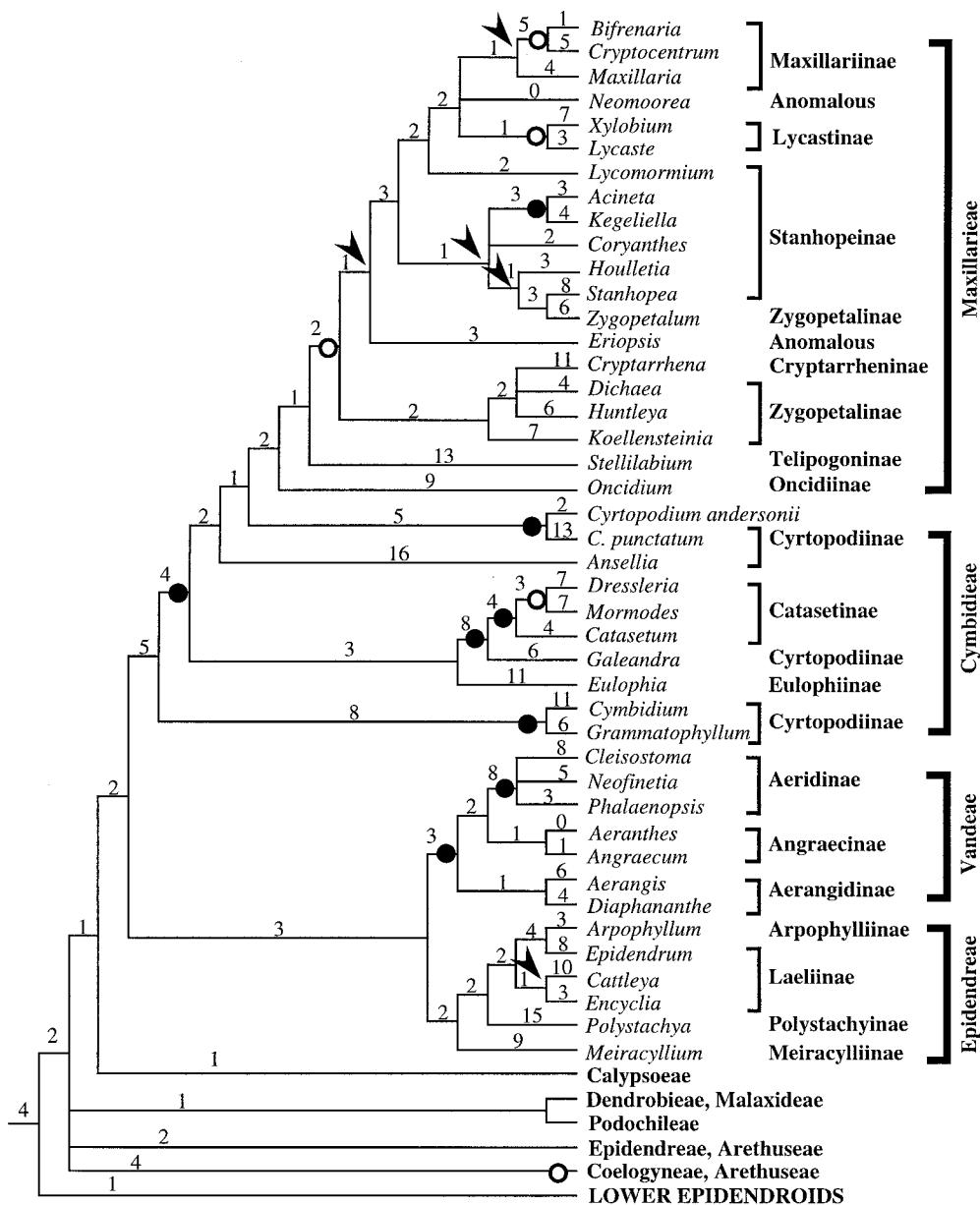
Fig. 7.   One of 6000 equally parsimonious, successively weighted *rbc*L trees highlighting most of the cormous phylad (sensu Dressler, 1993) of the "higher" epidendroid orchids. Numbers above branches correspond to branch lengths (ACCTRAN optimization with equal weights). Arrows indicate clades that collapse in the strict consensus. Solid circles indicate clades with strong bootstrap support (75–100%). Open circles indicate clades with weak bootstrap support (50–74%).

and Vanilleae—abscission layer between perianth and ovary, pollen shed as monads, endosperm formation, and trilocular ovary—supports the *rbc*L placement of the vanilloid orchids. We concede, however, that the Vanilleae lack obvious derived morphological features to distinguish them as a subfamily.

***Apostasioid orchids***—Burns-Balogh and Funk (1985) separated *Neuwiedia* and *Apostasia* into separate subfamilies based on a cladistic interpretation that *Apostasia* (with two fertile anthers) was more closely related to Cypripedioideae than to *Neuwiedia* (with three fertile an-

thers). With the exception of this case, the monophyly of Apostasioideae has been a feature of most orchid classification systems.

These analyses (Fig. 2) demonstrate strong support for the traditional interpretation that the two genera are members of a single subfamily but only weak support that they are sister to the remainder of Orchidaceae. Anatomical investigations (Stern, Cheadle, and Thorsch, 1993) confirm this viewpoint but clearly emphasize that these taxa should not be regarded as the living progenitors of the di- and monandrous orchids. Furthermore, it should be noted that the position of the Apostasioideae as sister

to the remainder of Orchidaceae cannot be assessed by outgroup comparison as attempted by Neyland and Urbatsch (1996).

The possession of 2-3 abaxial anthers is autapomorphous in the Apostasioideae. Evolutionary scenarios could hypothesize that *Neuwiedia* represents the intermediate stage in reduction from six fertile anthers to three and then to one, but in terms of cladistic analyses its condition is an autapomorphy and uninformative. Morphological support for this position rests solely on the lack of synapomorphies with the rest of the family. It can be hypothesized that the common ancestor of the orchids had six stamens (nearly all of the closest outgroups exhibit this condition; Chase et al., 1995), but the anther conditions of both the apostasioids (three anthers in *Neuwiedia* and two in *Apostasia*) and cypripedioids (two anthers) almost certainly were derived independently from the six-anther condition, raising the prospect that the monandrous orchids were also derived as well from a plesiomorphic ancestor with six anthers.

As pointed out by Stern, Cheadle, and Thorsch (1993), other characters of the apostasioids clearly indicate the highly autapomorphous nature of these two genera and their unsuitability to qualify as models for ancestral orchids. This same statement applies as well to the cypripedioids (Dressler, 1993), leaving us with the impression that it is likely that many plesiomorphic traits could have been retained in the monandrous orchids (i.e., the two other clades developed their own peculiar specializations). Thus, despite their relatively nested position in the *rbcL* tree, the possession of crustose seeds, endosperm formation, and multiseriate integuments (Cameron and Chase, 1998) in the vanilloid orchids could be retained plesiomorphies. The *rbcL* tree does not permit us to clearly evaluate these alternative ideas; all of them appear possible, and we must turn our hope for resolution to developmental studies.

***Cypripedioid orchids***—The five genera of slipper orchids comprising Cypripedioideae have received perhaps the most attention in systematic studies. Albert (1994) generated hypotheses of relationships using both molecular and morphological characters, and those relationships are substantiated by the larger analysis presented here. Current studies employing ITS sequences (Cox et al., 1997), likewise, show a similar pattern in which the conduplicate-leaved genera (*Paphiopedilum*, *Mexipedium*, and *Phragmipedium*) form a monophyletic clade that is sister to the plicate-leaved genera (*Cypripedium* and *Selenipedium*).

The only potential discrepancy between the topology depicted in Fig. 2 and that of others is the unresolved placement of *Selenipedium*. This genus is characterized by a number of plesiomorphic characters including trilocular ovary, crustose seeds, fleshy fruit, reed-stem habit, and pollen shed as free monads. One of two equally parsimonious explanations of the *rbcL* data is its placement as sister to the rest of Cypripedioideae, and this position is supported by analyses that include additional species (Albert, 1994; Cox et al., 1997).

***Vanilloid orchids***—This clade is composed here of subtribes Vanillinae, Galeolinae, and Pogoniinae (see Fig. 1); thus, it corresponds to tribe Vanilleae sensu Dressler (1990b rather than 1993). Attempts to amplify an intact *rbcL* gene from the achlorophyllous, mycotrophic Lecanorchidinae (one genus, *Lecanorchis*) have been unsuccessful, but ITS and 18S nuclear ribosomal DNA studies (Cameron and Chase, unpublished data) place that subtribe here as well.

Breaking with tradition, Dressler's (1993) most recent classification does not place the Pogoniinae in this tribe but next to it as a subtribe of uncertain position. This was done in light of Rasmussen's (1982) argument that Pogoniinae may be misplaced in Vanilleae because they lack derived characters shared with other Vanilleae. The *rbcL* data strongly support a relationship between *Pogonia* and its allies to Vanillinae and Galeolinae.

Within Pogoniinae the South American genus *Duckeella* is sister to the remainder of the clade (see Fig. 3). This poorly known genus produces a labellum that is only weakly differentiated from the other petals, a pair of projecting, staminode-like appendages on either side of the versatile anther, an abscission layer between perianth and ovary, and pollen shed as monads. These features are similar to what one might expect to find in an ancestral monandrous orchid. Following *Duckeella*, Pogoniinae are split into a South American and a temperate North American/Asian clade. The level of sequence divergence displayed among the three species of *Pogonia* is surprisingly high given the fact that some have considered *P. japonica* and *P. ophioglossoides* conspecific and *P. minor* to be a variety of that species (Ames, 1922). North American *Isotria* and *Cleistes divaricata* are strongly supported members of the temperate clade, making the genus *Cleistes* paraphyletic. Although it would be desirable to include additional species of *Cleistes* in the analysis, morphological features such as underground tuberoids, multiple-flowered inflorescences, and lack of raphide bundles in leaves (Cameron and Dickison, 1998) found in many of the tropical species vs. the slender roots and solitary flowers of *C. divaricata* corroborate the *rbcL* topology.

Representing Galeolinae are two highly divergent species of *Erythrorchis*. This divergence may be the result of both species being achlorophyllous mycoheterotrophs with reduced photosynthetic abilities. Since many achlorophyllous angiosperms do not contain functional copies of photosynthetic genes (dePamphilis and Palmer, 1990), it was not necessarily expected that an intact copy of *rbcL* would be found in these taxa.

Finally, the four genera of subtribe Vanillinae are monophyletic (Fig. 3). Both *Vanilla* and *Epistephium* are well-supported monophyletic genera, as is a clade containing the monotypic genera *Eriaxis* and *Clematepistephium*, both endemic to New Caledonia. The latter two genera were at one time classified as disjunct species of *Epistephium* because of shared reticulate leaf venation and winged seeds but were subsequently segregated (Hallé, 1977) on the basis of geographic distribution and trilocular ovaries. This move is upheld by the *rbcL* data. Once again, high levels of interspecific and intergeneric sequence divergence distinguish these morphologically peculiar orchids.

Since both Cypripedioideae and Apostasioideae are reasonably autapomorphous, orchid plesiomorphs could be found among more embedded clades such as the Van-

illeae. In this scenario, the ''primitive'' features noted among the vanilloid orchids (e.g., crustose seeds with endosperm) may not necessarily be character reversals.

*Orchidoid orchids*—The 29 sampled genera within this bootstrap-supported clade are divided initially into two bootstrap-supported subclades (Fig. 1). The first is a group corresponding to Orchideae and also includes *Disa* and *Satyrium* of Diseae (Fig. 4). Dressler (1993) asserts that of all the orchids, these two tribes are surely monophyletic, and he lists a number of morphological features to distinguish them. Indeed, *rbcL* shows the group as a whole to be monophyletic, but the individual tribes are not upheld as separate clades. *Platanthera*, of Orchideae, appears to be related more closely to *Satyrium* than does *Disa*. This contradicts the fact that both *Disa* and *Satyrium* share a similar seed type, whereas *Habenaria* has a seed type like that of *Orchis*. Further sampling is needed before any firm conclusions can be drawn concerning this clade, but no evidence for the separation of Orchideae and Diseae is present in the *rbcL* tree.

The second major grouping of orchidoids contains genera from Diurideae together with the spiranthoid tribe Cranichideae. This well-supported result is perhaps one of the most unexpected, although it is similar to the conclusion reached by Burns-Balogh and Funk (1986).

Cranichideae form a monophyletic unit of its constituent subtribal branchings only if *Pterostylis* (Pterostylidinae) is included within it. *Pterostylis* is confined to Australasia like all Diurideae and is well studied. It has always been classified in Diurideae of Orchidoideae with which it shares root-tubers and free pollen grains with reticulate exine. There are, however, a few species that produce an inflorescence of several, spirally arranged flowers like the ladies' tresses orchids (Spiranthoideae), and its seed is of the *Goodyera* type. It thus exhibits a mixture of ''spiranthoid'' and ''orchidoid'' features and, like Chloraeinae, appears to have closer affinities to Cranichideae than to Diurideae.

Before leaving the spiranthoid orchids, attention must be given to the genus *Diceratostele*, sole member of tribe Diceratosteleae, and the genera *Tropidia* and *Corymborkis* of Tropideae. These genera have always been classified as spiranthoids because of seed structure, sectile pollinia in *Corymborkis*, and columnar structure. However, their tall, nearly woody, reed-like stems, plicate leaves, and gross floral morphology are much out of place in that subfamily. Sequence data place these genera within Epidendroideae, apart from the other spiranthoid orchids (see Fig. 5), although without bootstrap support. Recent anatomical studies (Stern et al., 1993) confirm these results. *Tropidia*, *Corymborkis*, and *Diceratostele* are anatomically dissimilar from Cranichideae in lacking spiranthosomes, but similar to the epidendroid orchid *Palmorchis* given that they possess sinuous anticlinal walls of epidermal leaf cells and glandular hairs.

The remaining genera of orchidoids shown in Fig. 4 are all members of tribe Diurideae. These taxa are primarily Australian in distribution. Two complexes of diurid orchids have been suggested: the *Diuris* and *Caladenia* groups (Lavarack, 1976). Sampling presented here does not corroborate this notion. It does, however, provide evidence for the monophyly of the recognized subtribes

with one noticeable exception. Caladeniinae are polyphyletic due to the alliance of *Lyperanthus* with the genera of Drakaeinae and Thelymitrinae. Of special interest is the position of *Cryptostylis*. This genus has been problematic and included in either Spiranthoideae or Orchidoideae by various authors. Current evidence from anatomy (Freudenstein, 1991; Stern et al., 1993), as well as that presented here, confirms its position in Diurideae.

Moving the few misplaced genera from one tribe to another could be easily done and would be supported by other studies; however, recognition of the subfamilies Orchidoideae and Spiranthoideae as currently understood, is not justified by the *rbcL* tree. A new subfamily could be created for the majority of Diurideae (e.g., Thelymitroideae sensu Szlachetko, 1991), but the structure of the cladogram, lack of divergence in *rbcL*, and their highly similar habits and floral features (i.e., a mosaic pattern of seed types and root-tubers) do not support such a proposal. Rather, an enlarged, single subfamily, Orchidoideae, would accommodate better this entire monophyletic assemblage, as suggested by Kores et al. (1997).

*Epidendroid orchids*—This largest and most diverse subfamily has also been the most difficult to classify and interpret phylogenetically. For the most part, the group is easily recognized by the presence of fully incumbent anthers, hard pollinia, thickened stems, and typically epiphytic nature. However, there exist a number of genera, e.g., *Triphora*, *Epipactis*, and *Neottia*, that appear to be allied to the epidendroid orchids but lack these synapomorphic features. Dressler (1981, 1986) has made several attempts to break Epidendroideae into several natural groups. These attempts included segregating the orchids with erect anthers and soft pollinia into a distinct subfamily, Neottioideae, and placing those with stipes and superposed pollinia into Vandoideae. In his most recent treatment of Epidendroideae, Dressler (1993) elected to retain Neottioideae and Vandoideae within Epidendroideae, but proposed splitting the more advanced tribes into two large units: a cymbidioid phylad and an epidendroid phylad, containing a dendrobioid subclade, based on vegetative features rather than into epidendroid and vandoid orchids based primarily on floral characters. The cymbidioid phylad essentially corresponds to those orchids with a cormous growth habit and includes Maxillarieae, Cymbideae, Calypsoeae, and Malaxideae. The epidendroid phylad contains Arethuseae, Epidendreae, Coelogyneae, Glomereae, and dendrobioid subclade containing Dendrobieae, Podochileae, and Vandeae. Most members of this latter group possess a reed-stem growth form that Dressler interprets as secondarily derived (Dressler, 1990a). The overall result of this analysis, shown in Fig. 1, conflicts both with Dressler's (1981) previous system of Epidendroideae/Vandoideae and his current system of epidendroid/cymbidioid phylads (Dressler, 1993) but combines features of both.

At the base of Epidendroideae is found a paraphyletic assemblage of the ''lower'' epidendroid tribes Nervileae, Neottieae, Palmorchideae, and Triphoreae (Fig. 5); all of these possess soft pollinia or pollen shed as free monads, and most have erect or suberect anthers. The achlorophyllous Gastrodieae would be placed here as well (Molvray, Kores, and Chase, 1997), probably close to *Nervi-*

*lia*. Several attempts were made to amplify all or portions of *rbcL* from three different species of *Gastrodia*, but none was successful. Tropidieae and Diceratosteleae, considered members of Spiranthoideae as discussed above, are also found here. In addition, the anomalous genus *Xerorchis* occupies a position as sister to *Nervilia*. The affinities of *Xerorchis*, a little-known South American terrestrial, have never been clear. It has eight pollinia, similar to the more advanced Arethuseae or Epidendreae and consequently seems misplaced near *Nervilia*. Nevertheless, it has a habit reminiscent of a bambusoid grass, seed morphology of the *Limodorum* type, unthickened stem, and persistent leaves that have been interpreted as floral bracts (Sweet, 1970). These features are not out of place at the base of Epidendroideae among *Triphora*, *Tropidia*, and their allies.

Moving up the tree is a grade of taxa in monophyletic units (Fig. 6) that mostly correspond to the epidendroid (i.e., reed-stem) phylad of Dressler (1990b). In addition to lacking thickened stems, these taxa are characterized by eight pollinia, with a reduction to four or two in some subtribes. As a whole, the boundaries within this assemblage have been unclear, and attempts to construct a phylogeny of the phylad are usually prefaced with warnings as to the problems associated with it and lack of information for many of its constituents (Dressler, 1993). In these cladograms the middle epidendroid grade is paraphyletic and contains members of tribes Arethuseae, Epidendreae, Coelogyneae, Podochileae, Dendrobieae, and Malaxideae.

Tribe Arethuseae is grossly polyphyletic in the resulting cladograms, and further systematic investigations into these taxa are greatly needed. *Bletia* and *Chysis* are moderately supported sisters embedded within the Calypsoeae cluster (Fig. 7); *Phaius* is sister to Podochileae (Fig. 6); *Acanthephippium* and *Calanthe* show affinities to one of the Epidendreae clades; and *Arethusa*, *Calopogon*, and *Bletilla* are members of a clade that includes *Glomera* of Epidendreae, *Thunia* and *Coelogyne* of tribe Coelogyneae, and the anomalous *Arundina* (Dressler, 1993). These relationships are not all that surprising. Tan (1969) reported that seeds of hybrids between *Bletia* and *Bletilla* are infertile, suggesting that these two genera may have been erroneously allied in previous classification schemes. Dressler (1993) mentioned that the flowers of *Coelogyne* share petaloid columns and clam-shell stigmas with some Arethuseae and that a similar seed type in *Pleione* and *Bletilla* may indicate that the two groups share a common ancestry. Moreover, successful hybridization of *Bletilla* with *Arundina* (Tanaka, 1976), of *Calopogon* with *Arethusa* (Dressler, 1993), and similarity of vegetative morphology between *Arundina*, *Thunia*, and *Glomera* (i.e., tall, somewhat thickened stems with nonplicate, distichous, articulate leaves) point to a possible close relationship. It is within members of this clade comprising taxa with slender stems, thickened stems, corms, and pseudobulbs that a model of vegetative evolution within Orchidaceae might be investigated.

Tribe Epidendreae (Figs. 6 and 7) is also grossly polyphyletic and cannot be justifiably divided into Old World or New World clades. Subtribe Sobraliinae includes both *Sobralia* and *Elleanthus*. The latter genus has been problematic, for it has a distinctive seed type and pollinium,

both of which are unlike those in *Sobralia*. Despite these differences, the sequence data do support their monophyly and their sister group status to all other "higher" Epidendroideae. Subtribes Pleurothallidinae and Laeliinae have usually been considered sister groups linked together by a laelioid genus with eight pollinia, column-foot, and *Pleurothallis* seed type, such as *Dilomilis*. Rather than bridging the two tribes, which are never placed sister to each other in these trees, *Dilomilis* is excluded from the vicinity of Laeliinae. It is positioned sister to the Pleurothallidinae but does not bring its presumed Laeliinae allies along. Subtribe Laeliinae, encompassing the monotypic subtribe Meiracylliinae, is far removed from its traditional location near Pleurothallidinae and closely allied to Vandeae among the most advanced cymbidioid orchids (Fig. 7). This is perhaps the most difficult relationship to justify among these trees and may be due to undersampling or spurious attraction. Nevertheless, it is interesting to consider that Laeliinae displays a mosaic of lateral/terminal infloresences and reed/pseudobulbous stems, which could make them reasonable candidates to link the traditional vandoid and remaining epidendroid orchids. The close relationship of Polystachyinae (represented only by *Polystachya*), with its small stipes and either terminal or lateral inflorescences, to this group could also be a spurious result of undersampling, but, if correct, provides even more compelling morphological characters that might serve to link Vandeae with these "core" Epidendreae. It is also worthy to note that Polystachyinae and Laeliinae share a predominant chromosome number of $2n = 40$ and that Arends and van der Laan (1986) proposed this same number as an ancestral base number for Vandeae. From the perspective of Dressler's (1981) classification, the close association of Vandeae and part of the Epidendreae is ironic, since these tribes formed the bases for recognition of two distinct subfamilies. Dressler (1986) later argued that Vandoideae clearly represented a grade of advanced development, although the *rbcL* topology, in general, supports the original, advanced "vandoid" concept.

According to Dressler's (1993) phylad system, Vandeae are part of a dendrobioid subclade of the cymbidioid phylad near tribes Dendrobieae and Podochileae. As discussed, this relationship is not evident in the molecular phylogeny. Instead, Malaxideae occupies this position. Shared presence of spherical, as opposed to conical, silica bodies seems to be the primary character that links the Vandeae to Dendrobieae/Podochileae in Dressler's (1993) newest system. Møller and Rasmussen (1984) discussed the likelihood that silica bodies evolved independently on several occasions in the orchids and that this character is probably correlated to habitat. Likewise, defining a dendrobioid subclade by shared presence of upper, lateral inflorescences is questionable, as several members of Eriinae, Podochilinae, and Dendrobiinae display a terminal inflorescence. Although their taxon sampling was limited, Yukawa et al.'s (1993) restriction site analysis of Dendrobieae produces a set of relationships that has features in common with these cladograms. Yukawa's data also place *Malaxis* sister to the Dendrobieae. The notion that the naked pollinia found only in Malaxideae and Dendrobieae evolved once rather than two times indepen-

dently is certainly intriguing, and this is perhaps the best evidence for the relationship indicated here.

Dendrobieae are strongly supported as monophyletic only if *Pseuderia* is removed to Podochileae. Both subtribes Dendrobiinae and Bulbophyllinae (albeit the latter represented by only two species of *Bulbophyllum*) are monophyletic in some trees, although the strict consensus does not resolve the placement of *Epigeneium*. Further sampling within Bulbophyllinae is needed to confirm this result. The genus *Dendrobium* cannot be considered natural unless *Flickingeria* and *Diplocaulobium* are merged with it. These are virtually the same results found by Yukawa et al. (1993). With the inclusion of *Pseuderia*, tribe Podochileae and its constituent subtribes are monophyletic.

At the top of the tree are the remaining epidendroid orchids from tribes Calypsoeae, Cymbidieae, and Maxillarieae that essentially correspond to the cymbidioid (cormous) phylad of Dressler (1993). There is strong evidence for a close relationship between the tribes Maxillarieae and Cymbideae (Fig. 7). Within the monophyletic Maxillarieae, there is generally weak support for subtribal relationships. Subtribe Stanhopeinae, characterized by two pollinia and *Stanhopea* seed type, is polyphyletic owing primarily to the disassociation of *Lycomormium* (with *Maxillaria* seed type) from the majority of the subtribe. This result has also been found in other molecular studies (Whitten, unpublished data). Our sequences of *rbcL* alone are not sufficient to address the status of Zygopetalinae, Telipogoninae, Ornithocephalinae, or Oncidiinae, but they do provide long-awaited evidence for the relative position of the enigmatic genera *Cryptarrhena* (sole member of Cryptarrheninae) and *Eriopsis*. The former had been suggested to have affinities with such diverse taxa as Ornithocephalinae, Maxillariinae, and Coelogyneae; the latter has pollinia similar to Cyrtopodiinae but a *Maxillaria* seed type. The *rbcL* tree places both of these taxa deeply within Maxillarieae, most closely related to members of a broadly defined Zygopetalinae.

Tribe Cymbideae is polyphyletic in these topologies. Even the removal of monotypic subtribe Goveniinae to the vicinity of Calypsoeae (see Fig. 6) results in the remainder of the tribe being a paraphyletic grade. The only monophyletic subtribe here is Catasetinae, and the support of intergeneric relationships is high in that clade. Further sampling from unrepresented Bromheadiinae, Thecostelinae, and Acriopsidinae may help to resolve the relationships in Cymbideae, but a more quickly evolving gene phylogeny (e.g., from *matK*) is greatly needed.

Tribe Calypsoeae, especially the genus *Calypso* and genera associated with *Corallorhiza*, has been problematic in nearly all classifications because these members possess an unusual set of advanced floral features and are often reduced vegetatively. In these studies *Calypso* and *Tipularia* are sisters. *Aplectrum* is sister to *Govenia*, which, incidentally, has a tegular stipe like *Calypso*, suggesting that the alliance of these four genera may be reasonable. Their position near *Bletia*, *Chysis*, and *Earina* is less convincing, but these have been difficult orchids to classify in all systems.

***Conclusions***—The analyses of *rbcL* nucleotide sequences presented here provide a great deal of support for previous hypotheses of relationships within Orchidaceae and also indicate several new patterns as well. With minor changes in the placement of a few genera, slight rearrangement of particular tribes and subtribes, and the elevation of the vanilloid orchids to subfamilial status, the current system of Dressler (1993) would not look much different from the topology presented here. This fact clearly supports the utility of gene sequences in general, and *rbcL* in particular, for phylogenetic reconstruction. It is interesting that for this data set, *rbcL* has its greatest utility in inferring relationships at lower taxonomic levels despite its moderately conserved nature (Palmer et al., 1988). If only higher level relationships were of interest, then 20 or 30 sequences would have been sufficient to address that issue, since in this case more sequences have not improved the internal support of the overall tree "spine" (i.e., intersubfamilial relationships). However, increased sampling has substantially improved support for the monophyly of large clades (e.g., orchidoids/spiranthoids, vanilloids, Cymbidieae/Maxillarieae) and particularly for the composition of tribes and subtribes within them. Those clades that have been more fully sampled (e.g., Cypripedioideae, Vanilleae, Diurideae) are the best supported; the most weakly supported clades are those large ones that have been only superficially sampled. This final point is especially valid for Epidendroideae in which sampling and, consequently, bootstrap support is lower than elsewhere in the family. Additional *rbcL* sampling within Epidendroideae is encouraged for those interested in this large subfamily, but we concede that *rbcL* alone is not likely to provide robust estimates of phylogeny. Data from additional molecular and morphological sources and their combined analyses might compensate for sparse sampling, and both routes to uncovering more robust relationships should be followed.

The use of DNA nucleotide sequence data has awakened a quickly growing interest in orchid phylogenetics. Whereas this study represents the first in-depth molecular analysis of the entire family, sequences from *ndhF* (Neyland and Urbatsch, 1996), 18S (Cameron and Chase, unpublished data), *trnL* (Kores et al., unpublished data), ITS (Pridgeon et al., 1997; Kohnen et al., unpublished data), *rps4* (Whitten et al., unpublished data), and *matK* (Freudenstein et al., unpublished data) are being generated for comparable sets of taxa. These data, along with combined-gene studies, should be available soon. It is anticipated that the study presented here, as well as future studies, will generate discussion, debate, and reassessment of a family that unfortunately has lagged behind others of comparable size and importance as the focus of molecular systematic analyses.

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