Orchid Tree:
a phylogeny of epiphytes (mostly) on the tree of life

University of Florida Herbarium

Project Description

Results from Prior NSF Support

1. DEB-0108100 to KM Cameron, *Phylogenetics and plastid genome evolution of Vanilloideae (Orchidaceae).* 2001-2004. $131,417. Field collecting of plant material took place in East Asia, USA, Mexico, and Australia. Sequencing of ca. 200 taxa of Orchidaceae including all available material from Vanilloideae was completed for *rbcL, atpB,* and *psaB*. Sequencing of all available vanilloid orchids was completed for *nad1*b-c, *psaB, psbB, psbC, atpB, rbcL*, 26S, 5.8S, and 18S rDNA. The early stages of plastid genome degradation within achlorophyllous vanilloid taxa have been documented. Anatomical /morphological studies of floral organs also have been completed. Publications: Cameron (2002, 2003, 2004 a-d), Chase et al. (2003).**2. DEB-9615437 to JV Freudenstein,** *Cladistics of Orchidaceae and critical anther characters: evidence from molecular, developmental and morphological investigations.* 1997-2000. $170,198. A morphological data matrix for 100 genera was assembled, and over 400 sequences were collected for these genera, sampling all of the subfamilies. We obtained data sets from four loci. A hypothesis of rapid radiation at the base of Epidendroideae was supported. The use of a mitochondrial locus, the *nad1*b-c intron, was demonstrated and was used as a case study for examining indel coding. Two graduate students, a postdoctoral scientist, a technician and an undergraduate were supported and trained. The following publications have resulted: Freudenstein & Rasmussen (1999), Freudenstein et al. (2000), Goldman et al. (2001), Freudenstein & Chase (2001), Simmons & Freudenstein (2002), Freudenstein et al. (2002), Chase et al. (2003), and Freudenstein et al. (2004), plus 28 seminars and professional meeting presentations. **3. DEB-0415920 to JV Freudenstein,** *Relationships among gene lineages, morphology, geography and fungal associations in Corallorhizinae (Orchidaceae).* $177,100. 2004-2007. We have thus far focused on fieldwork in North America to obtain populations of Corallorhiza and their fungi. DNA sequences have been collected for both the plants and fungi. One talk has been presented and the first manuscript is about to be submitted. Two graduate students have received training. **4. DEB-0234064 to NH Williams and WM Whitten,** *Systematics of Maxillariinae (Orchidaceae): Generic delimitation, pollinator rewards, and pollination.* 2003-2006. $300,000. Field work was conducted with 3 graduate students in Panama, Costa Rica, and Ecuador. Over 650 accessions representing over 350 species have been photographed, vouchered, and sequenced for ITS, *matK*, and the *atpB-rbcL* intergenic spacer. Keys are being constructed using Lucid software. Publications: Williams & Whitten 2003; Ojeda et al. 2003; Whitten et al. 2005. Results have been presented at international conferences in Ecuador, Costa Rica, and each year at BSA meetings. A generic/sectional re-classification of Maxillariinae will be submitted by the end of 2006. **5. DEB-9815821 to NH Williams,** *Molecular and morphological systematics of Oncidiinae (Orchidaceae).* 1999-2003. $25,000. Sampling within Oncidiinae was increased to 634 species representing about 90 generic concepts; ITS and *trnL-F, matK, atpB-rbcL* intergenic spacer were sequenced for a subset of 190 taxa. Well supported cladograms have allowed clarifications/ changes to the taxonomy and have revealed that floral traits are highly homoplasious in deceit-pollinated clades. Publications: Williams et al. 2001; Sosa et al. 2001; Chase & Williams 2001; Chase et al. 2005; Williams & Whitten 2001; Williams, Chase, & Whitten 2001; Dressler & Williams 2000; Koehler et al. 2002; Williams et al. 2005. Although not supported by this grant, the MS

thesis work of Katia Silvera was based on the work from this project. Results have been presented at international conferences in Ecuador (two different conferences), Costa Rica, and each year at BSA meetings. **6. DBI-0317335 to M Lauria, co-PI (PI: R Bundschuh).** *Hybrid Alignment: improving sensitivity of biological databases searches.* 2003-2006. $350,141. A version of the PSI-BLAST tool for iterative protein searches is being developed with enhanced sensitivity/coverage properties. The new version will take advantage of the favorable statistical properties of the so-called hybrid algorithm for sequence alignment. A partial implementation of the enhanced PSI-BLAST has been built and is undergoing evaluation and testing using SCOP and other protein databases. Two graduate students are being supported and trained. Publications: Li, Y., M. Lauria, & R. Bundschuh (2004).

RESUBMISSION: Responses to criticisms from previous submission 1. Expand evolutionary hypotheses to be tested. We have more thoroughly described the questions and hypotheses that can be tested given a tree for the orchid family. We have not proposed to explore all of these ourselves, however, because our focus here is on generating the tree. **2. Morphological aspects of the proposal needs greater discussion.** This is elaborated in the body of the proposal and reference is given to a website that more fully describes the characters. **3. Choice of gene regions was questioned, especially ribosomal coding regions and spacer regions with alignment concerns.** We agree and have decided to use a two-tiered analysis: 1) combined family-level analyses utilizing six easily-aligned coding genes to define highly supported clades; and 2) addition of ITS data for analyses within these clades. The problem with resolution in Fig 2 was caused by missing data for several taxa, now omitted from the preliminary analyses. Our combined consensus tree (Fig. 2) is well resolved. It is true that the individual dataset trees were not highly resolved, but that emphasizes the value of combined datasets as is shown in our revised figure. Additional elaboration in the project description. **4. Some web site links did not work.** Outdated/nonfunctional links have been corrected. The freeware demo version of the StarTree software was not compatible with a number of browsers; however, the purchased version, which we now have, is compatible with most modern browsers. Older incompatibilities are noted on our web site. Reviewers should carefully note the operating system /browsers that are supported for StarTree. **5. Division of labor questioned by one reviewer.** We believe our approach of dividing the project by taxa rather than by gene region is the most logical. A major portion of the project will involve graduate student training that will involve taxon based studies and morphology. We believe this will be more productive and lead to more productive use of time and student enrichment than a gene region based approach. **6. Minority recruitment.** See **III. Education, Training, and Outreach Undergraduate and Postgraduate Opportunities** for our plan to recruit underrepresented minorities.

Introduction, rationale and expected significance for this project - Charles Darwin began his second book, *On the various contrivances by which British and foreign orchids are fertilised by insects* (1862), by stating that the diversity of orchids' reproductive structures are "as varied and almost as perfect as any of the most beautiful adaptations in the animal kingdom" and "universally acknowledged to rank among the most singular and most modified forms in the vegetable kingdom." Given this enormous diversity, the challenge is to understand how it fits together in a phylogenetic pattern. Orchidaceae, though a single angiosperm family, represent an uncommonly significant branch of the Tree of Life. With approximately 800 genera /25,000 species, the family is not only perhaps the largest among plants (ca. 10% of all flowering plants and ca 1/3 of the monocots), but is more diverse than many well-known animal groups (more orchid species than all amphibians, "reptiles", birds, and mammals combined). The family is clearly monophyletic and is

now firmly positioned as a member of Asparagales and sister clade to the remainder of that order (Dahlgren & Rasmussen 1983; Fay et al. 2000; Janssen & Bremer 2004). This position indicates a relatively ancient origin (probably Early Cretaceous), despite the nearly complete absence of a fossil record, as suggested by molecular clock estimates (Cameron & Chase 1999; Bremer, in press; Janssen & Bremer 2004).

We agree with Chase et al. (2003) who stated that orchids are "one of the premier groups of flowering plants for evolutionary studies…" The huge diversity of ecological, morphological, and life history adaptations in the family makes them a rich system in which to address broad evolutionary and ecological questions. Understanding of this diversity is possible only within the framework of a robust phylogenetic hypothesis, which is not a trivial undertaking. Collaborator R. Bateman wrote (see letter of support) "Although it may be tempting to use AToL funding to fill major lacunae…it is my view that there is an equally strong case to be made for building upon success in selected groups that can be promoted as model systems-the pinnacle of phylogeny reconstruction. The chosen groups should be especially charismatic or intellectually challenging or economically important, and the orchid family clearly meets all three of these requirements…Orchids could become the first species-rich group of organisms to have well-formulated phylogeny at the genus level, and for many groups such as the European genera, at the species level". Although it is outside the purview of an ATOL proposal (because of its primary focus on the tree-building process) to investigate all the hypotheses and questions that can be explored with a robust phylogeny, we wish to describe some of these briefly because they argue for the importance of choosing the orchids for the ATOL program.

Because orchids exhibit a high diversity of vegetative and reproductive morphology, they are an excellent system in which to explore the relationship between character transformations and species diversity. For example, is it the rise of epiphytism and/or highly specialized pollination strategies or some other feature that correlates with increased diversity among orchids? Because of their diverse floral morphology, orchids provide an exciting system in which to study evo-devo questions, such as the nature of the column. Indeed, orchids have already become the focus of evo-devo floral studies using genomic techniques by several groups and may soon be the best model system for studies of floral development among non-graminoid monocots; (Gravendeel & Kramer/Harvard; Goh/Singapore).

Orchids provide an amazing system in which to study plant-pollinator relationships. Floral rewards are diverse and include nectar, oils, resin/waxes, fragrances (for male euglossine bees) and pseudopollen, but the majority of orchids may exhibit deceit pollination (no reward). Euglossine pollination and pseudocopulatory strategies in many diverse lineages are but two of the homoplasious syndromes that can be studied in the orchids. Orchids are one of the most striking examples of interaction between plant and fungus - all known species require a fungus at least to germinate. This system represents an unsurpassed opportunity to explore such interactions - do co-evolutionary patterns predominate or is it a story of opportunistic fungal utilization?

A fungal ATOL is already underway; an orchid tree will allow us to derive increased benefit from the fungal tree. Such orchid-fungal patterns are being examined in small groups of orchids, but will require a family-wide tree to understand the broader picture. Because of their fungal interactions, orchids appear to be preadapted to a shift from autotrophy to heterotrophy. Dressler (1993) hypothesized that the shift to leafless heterotrophy arose at least ten times in Orchidaceae. Such frequent shifts to heterotrophy are unheard of among angiosperms and thus present a unique opportunity to examine the changes that

accompany this nutritional shift - for example, when lineages go leafless, do they always follow the same pattern in terms of morphological and genomic changes? Many examples in this family allow us to seek generalizations about such changes. Orchids exhibit a range of photosynthetic mechanisms between C3 and CAM occurring in the family. A recent project was funded by NSF to examine the latter in the orchid subtribe Oncidiinae (Cushman lab; Univ. Nevada). With one published orchid chloroplast genome, several more in progress, and the advent of relatively inexpensive pyrosequencing, the orchid ATOL is crucial to intelligent sampling for genomic comparisons. Lastly, because they are a cosmopolitan group, orchids allow us to test biogeographic hypotheses involving almost any collection of land masses.

In addition to scientific hypotheses and questions, a robust orchid phylogenetic pattern will provide the basis for a new taxonomy of the group and resulting applied uses. Chase et al. (2003) published a recent skeletal revised classification (Fig.1), but our study will be much more comprehensive. A family-wide phylogenetic pattern will facilitate testing key morphological characters and lead to improved morphological characterization of clades. Orchids elicit great popular interest and are economically important (the industry being worth more than $110 million annually in the US alone); a phylogenetic classification will be significant in guiding hybridization and genetic engineering programs. All orchids are regulated under CITES at generic level; identification of non-flowering plants to genus is crucial for regulation of trade and enforcement. Our data will facilitate enforcement by providing the molecular database for rapid identification of plant material to generic level. Also, two of the genes we are using (*matK* and *rpoC1*) are currently being evaluated by the Plant Working Group of the Consortium for the Barcode of Life as candidate genes for plant barcoding; our data will complement this effort. Finally, the popular appeal of the group also makes them a captivating subject for fostering public understanding of evolution and of many facets of biology, and we will exploit this aspect through outreach programs.

Current status of orchid taxonomy - Reconstruction of phylogenetic relationships across the Orchidaceae was attempted by Burns-Balogh & Funk (1986) and by Freudenstein & Rasmussen (1999) using morphological characters analyzed by cladistic methods. Resolution of the trees was poor, but these studies demonstrated the value of such information, especially with regard to translating the orchid phylogeny into a working classification.

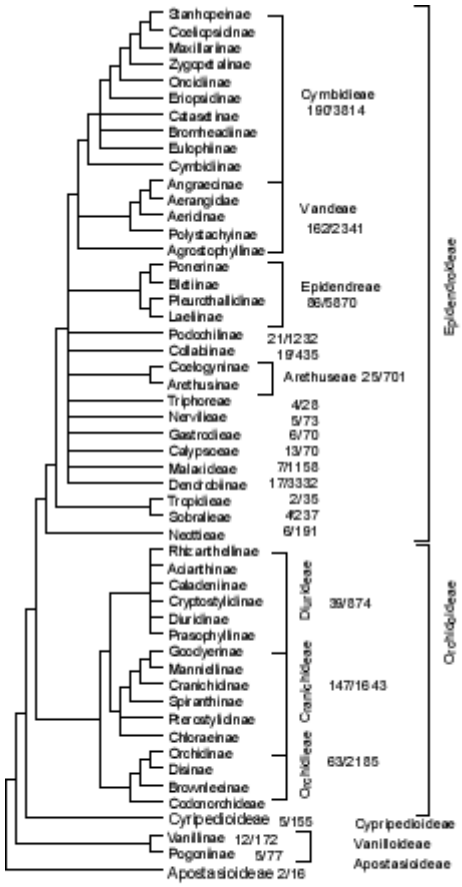


Fig. 1. A summary tree representing the current status of orchid systematic relationships from Chase et al. (2003). Note that only positions of subfamilies, tribes, and subtribes are indicated. Numbers are genera/species for each group.

Cameron (in press) reviewed all orchid molecular systematics studies to date and found that 4262 orchid DNA sequences had been deposited in GenBank during the first decade of sequencing (1994-2004) from approximately 75 published papers. The first molecular papers on orchids used plastid sequences and were focused at the family and subfamily levels (e.g., Albert 1994; Chase et al. 1994; Yukawa et al. 1996; Neyland & Urbatsch 1996a,b; Kores et al. 1997; Cameron et al. 1999).

Chase et al. (1994) employed plastid *rbcL* sequences from 33 orchids plus 62 other lilioid monocots and established the monophyly of five subfamilies: Apostasioideae, Cypripedioideae, Orchidoideae (including the diurid and spiranthoid orchids), Epidendroideae (including the vandoid and many neottioid orchids), and Vanilloideae (a surprise, since these orchids traditionally had been classified as a primitive lineage of Epidendroideae). Cameron et al. (1999) expanded the study to include 158 ingroup and 13 outgroup taxa. A few studies have focused on the entire Orchidaceae using genes other than *rbcL* (e.g., *ndhF* by Neyland & Urbatsch 1995, 1996a,b; 18S rDNA by Cameron & Chase 2000), but only the mitochondrial *nad1*b-c intron published by Freudenstein et al. (2000) and Freudenstein & Chase (2001) and the *psaB* study (Cameron 2004) have considered enough taxa (ca. 100) to adequately depict higher level orchid relationships. Most recently, Cameron (2004) and Freudenstein et al. (2004) have increased efforts to reconstruct higher-level relationships within the family using *atpB, psaB*, and *matK* plastid gene sequences singly and in combination with the rbcL data. However, these studies have been limited to fewer than 150 orchid genera (20% of the total) and have failed to resolve higher-level relationships with high levels of confidence.

Recent lower-level studies have been focused on orchid groups popular in horticulture, making sampling across the family uneven. For example, nine papers

on molecular systematics of subtribe Orchidinae (which includes most of the European terrestrial orchids) have been published since 1997. Eight have been published on the commonly cultivated Oncidiinae, yet none have been published on Podochileae. The more rapidly evolving nuclear ITS region (often with data from plastid *trnL-F* or *matK*) has been used to investigate species-level questions (e.g., Bateman et al. 2003; Clements et al. 2002; Cox et al. 1997; Douzery 1999; Pridgeon et al. 1997; van den Berg 2000). These studies are challenging a number of long-held ideas regarding evolution and classification of Orchidaceae at the species and genus levels, even though the overall picture of phylogenetic relationships within and among orchid subtribes, tribes, and even subfamilies remains unclear.

Although no consensus has been reached regarding exact relationships among the higher taxonomic levels of Orchidaceae, especially within the largest subfamily, Epidendroideae, Chase et al. (2003; including PIs Cameron and Freudenstein as co-authors) proposed an updated classification for the family. They fully admitted the ephemeral nature of their new classification and realized it would change as new data are generated. This system is depicted as a summary tree in Fig. 1, based on results from recent molecular and morphological studies of the family. Concurrent with new orchid classification is the publication of the first four volumes of Genera Orchidacearum (Pridgeon et al. 1999, 2001, 2003, 2005). This international, multi-authored series profiles each genus with a description, line illustrations, and a discussion of relevant literature. Authors note phylogenetic placement when that information is known, but in most cases this is based on poorly supported phylogenetic trees or traditional intuitive ideas.

Objectives - 1) Establish and coordinate more formally an international orchid phylogeny working group. **2**) Develop software/approach to automating alignment and tree search to explore the implications of different alignments for large data sets via parallel computing. 3) Construct phylogenetic hypotheses for Orchidaceae using an unprecedented amount of morphological data and molecular data from the nuclear, plastid, and mitochondrial genomes. **4)** Educate and train the next generation of postgraduate and postdoctoral orchid systematists, especially from under-represented groups and areas. **5)** Create an orchid diversity database and interactive website for the scientific community and general public. **6)** Begin to address fundamental questions regarding the evolution of orchids. **7)** Formulate educational and scientific outreach modules related to orchid phylogeny and conservation. **8)** Disseminate the results of our study to a broad audience in the form of workshops, lectures, symposia publications (print and Web-based) and museum/botanical garden exhibits.

Relation to work in progress - The PIs and collaborators involve most workers in orchid molecular systematics today in Europe, UK, USA, Mexico, Central America, Brazil and Australasia. Each of the PIs has received funding in the past and/or is being funded currently (e.g., by NSF or the American Orchid Society) to investigate questions of orchid phylogeny within specific subclades. For example, Freudenstein, Whitten, and Williams have been funded by NSF to investigate relationships within the largest orchid subfamily, Epidendroideae; Cameron was funded by NSF to examine relationships within subfamily Vanilloideae and has been doing so by increased taxon and gene sampling in coordination with Freudenstein. Williams, Whitten, and collaborators are reconstructing relationships within Cymbideae (including Maxillariinae) and other groups (Carlsward et al. 2003, 2006; Whitten et al. 2005; Williams et al. 2005) based on molecular and morphological data. The four orchid PIs overlap in their research with each other or with other orchid systematists (e.g., M. Chase at RBG Kew). However, there has been no formal attempt to coordinate these individual programs of investigation or to expand the focus or sampling beyond more than a few hundred taxa and one or two DNA

regions. Only through support by a program such as "Assembling the Tree of Life" could this integrative goal of processing thousands of taxa and characters be achieved.

Two current ATOL projects could provide fertile interactions with our proposed orchid project. First, the ATOL grant 0228671 "Assembling the Fungal Tree of Life" will produce a phylogenetic framework for fungi. Because all orchids are dependent upon fungal symbionts for seedling establishment, there is great potential for studies of orchid/fungal partnerships in an evolutionary framework. Robust phylogenetic trees for both orchids and fungi will greatly stimulate research on orchid mycorrhizae (e.g., work in progress by collaborator Clements in Australia; Hibbett at Clark University; Taylor at Fairbanks). Second, the ATOL grant 0334832 "A Phylogenomic Toolbox for Assembling the Tree of Life Molecular Sequence Databases" should provide tools of great utility as we try to integrate our ATOL dataset with the growing number of subtribal/generic level orchid projects (letter from Sanderson). We will exchange data with any interested workers and will stay abreast of developments in these fields. We are aware of the proposal on the monocots being submitted by Tom Givnish et al. and if both of these projects are funded we will collaborate with that group (see letters).

II. Plan for sampling and data collection

Scope of the analysis and approach to resolving relationships: Although a number of studies have focused on one or a small number of loci and have sampled orchid diversity to varying degree, no study has included sufficient sampling of species or incorporated enough data to resolve relationships substantially at all levels above the genus across the family. This project has been designed to do just that. It is clear that both taxon sampling and numbers of characters are important for phylogeny reconstruction (Graybeal 1998; Zwickl & Hillis 2002). We plan to construct a dataset with tenfold the number of taxa included in previous studies and more than three times as many base pairs of sequence data. The total size of our largest combined analysis will be 2000 taxa by 7 loci (ca. 12,900 bp), for a total of 2.58 X 107 bp of sequence (plus 2000 ITS sequences used in sub-analyses). Sequence data collection will be partitioned taxonomically rather than by locus, with the three labs each working on approximately one-third of the genera. This is done to avoid the need to send aliquots of all DNAs to all labs and to allow individual labs to focus on particular orchid groups in which they have expertise. It will also allow us to generate intermediate results for smaller groups prior to the total analysis.

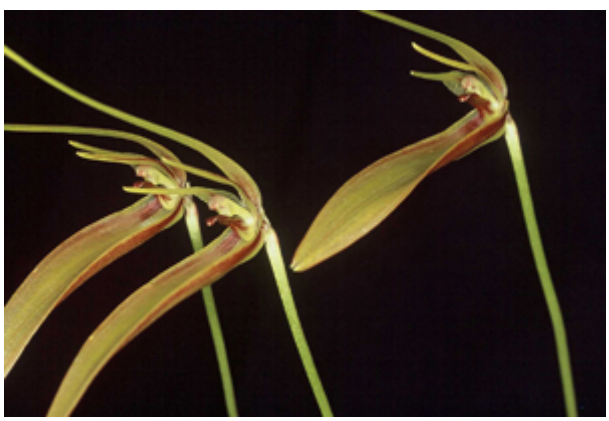
Choice of Taxa and Data

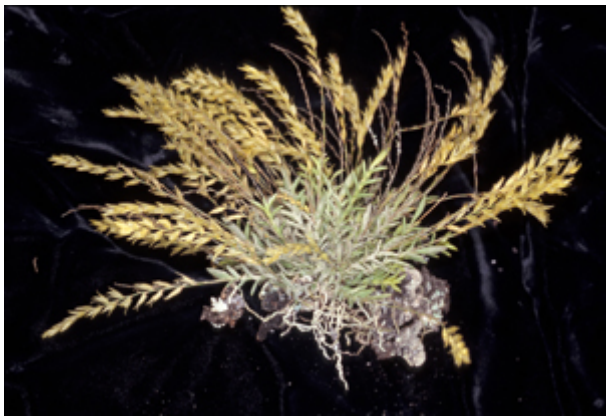
Taxa: We have planned a careful sampling strategy for **2000 taxa** that is informed by our own and others' previous phylogenetic work within the family. As our reference for current generic taxonomy for the orchids we are using The Royal Botanic Gardens,Kew Kew Checklist of Monocotyledons (http://www.kew.org/wcsp/home.do/). The largest phylogenetic studies of orchids published thus far have sampled fewer than 150 genera for two or three loci (Cameron 2004; Freudenstein et al. 2004). The subfamilies of orchids are well circumscribed at this point (Chase et al. 1994; Cameron et al. 1999; Freudenstein and Rasmussen 1999; Freudenstein et al. 2004); it is many of the relationships among those subfamilies and within them that are uncertain and on which we will focus. We will sample a minimum of two species from each genus that we obtain (except those 173 that are monotypic). Beyond this, we will add additional species for large and morphologically diverse genera to represent this diversity, although the goal of this project is not to perform definitive studies at the generic level. We plan to include a total of 2000 species and will use the results of previous generic-level studies to the extent that they exist to guide our selection of species in large

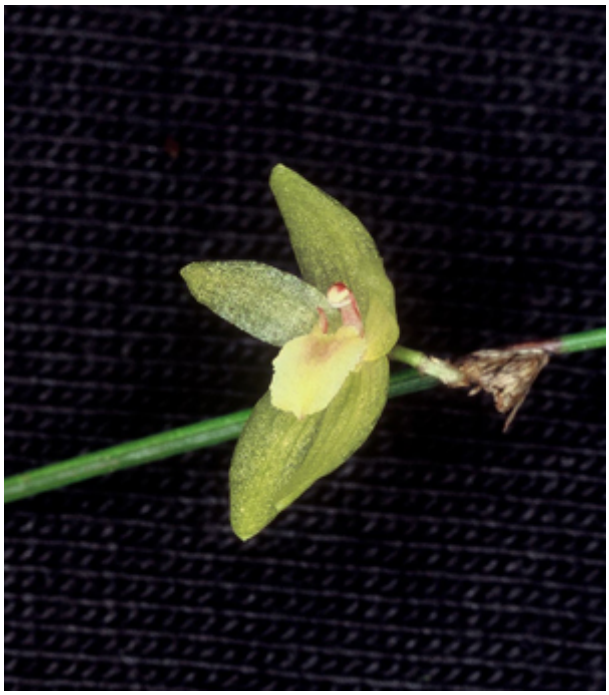
genera. Whenever possible, we will include the type species for a genus in our sampling. Of the approximately 800 genera that are currently recognized in Orchidaceae, we already have vouchered collections of silica-dried leaf material or isolated DNA for 77% of them. Of the remaining ±190 genera, nearly all are segregates of larger genera; many are rare and difficult to collect, and 84 that we lack are monotypic. We will use members of five genera as outgroups - *Borya, Blandfordia, Lanaria, Astelia, Anigozanthos, Maranta,* and *Hypoxis*. These genera represent seven families that, based on the most comprehensive analyses of asparagoid monocots (Fay et al. 2000; Rudall et al. 1998), belong to (1) a basal clade that is sister to the rest of the Asparagales excluding the orchids, or (2) the clade that is sister to this group plus the orchids, thus bracketing the intermediate orchid clade. DNAs for all of these taxa are already in hand.

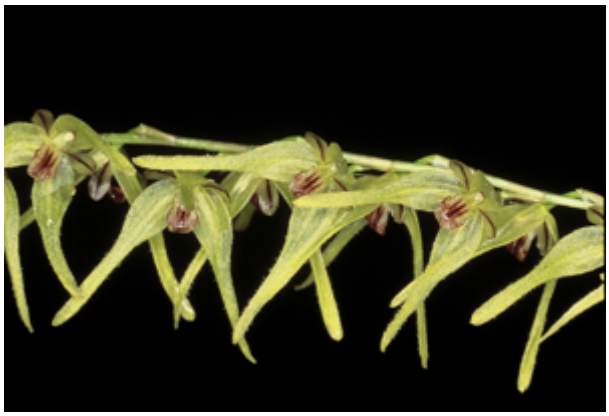
vandaceous

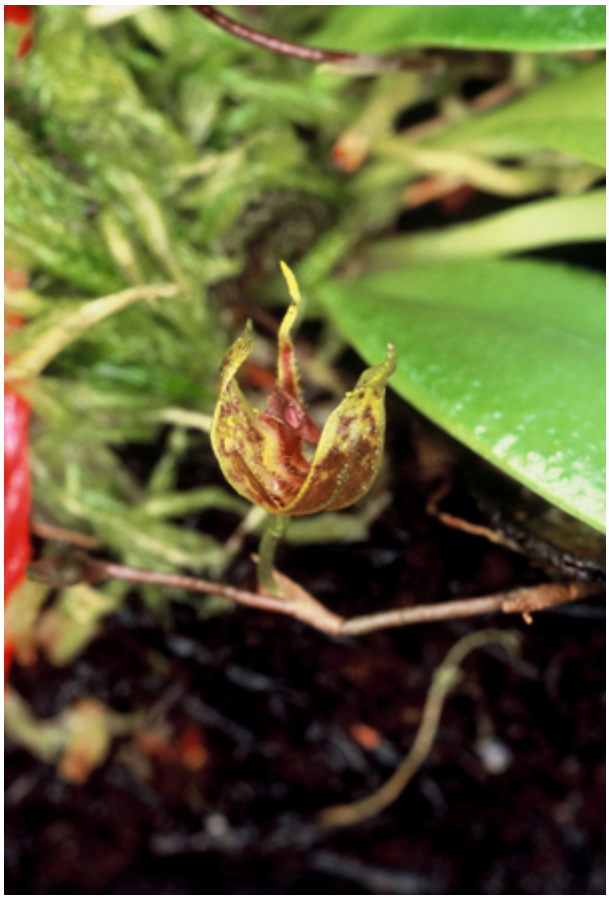
pleurothallids



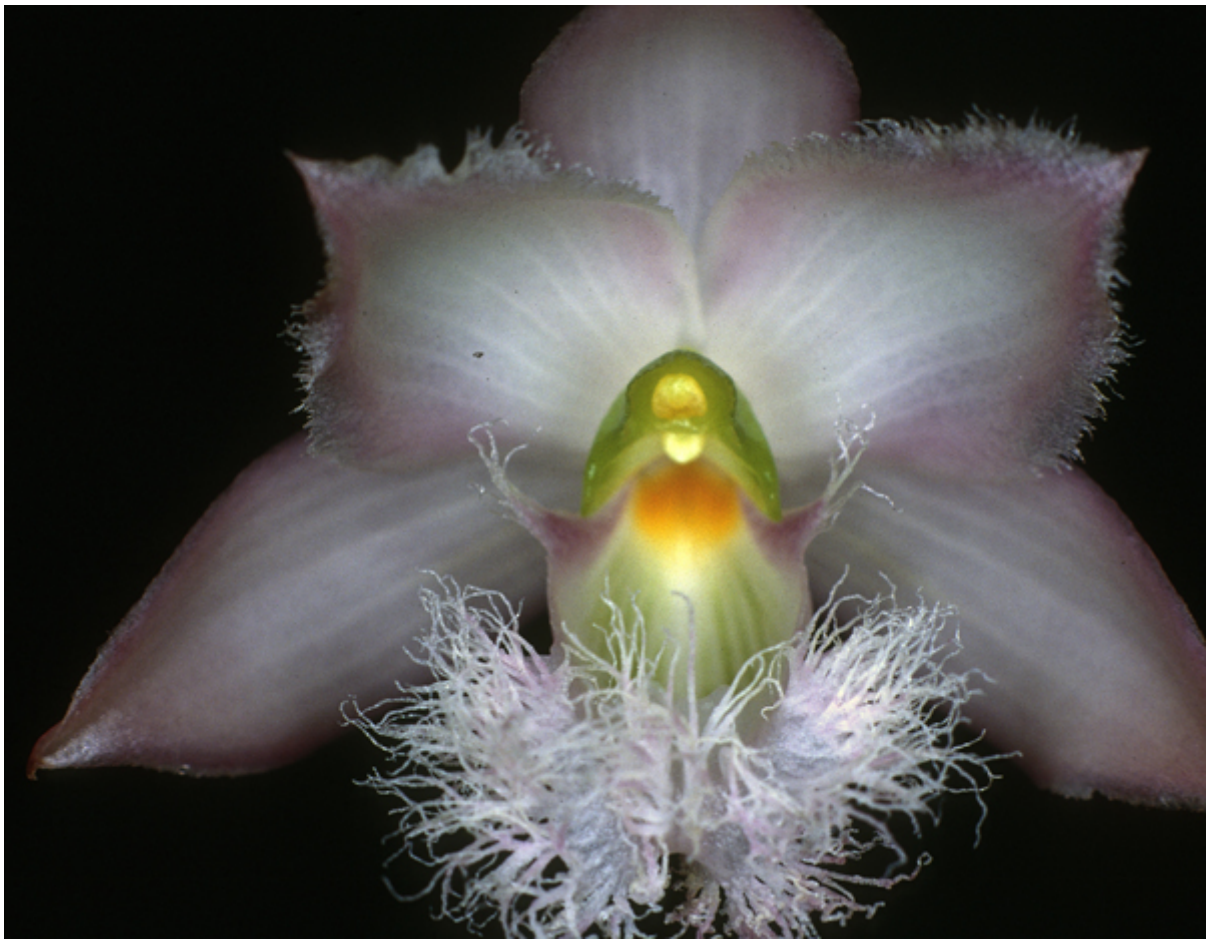








catasetinae



chysinae

coeliopsidinae

coelogyninae

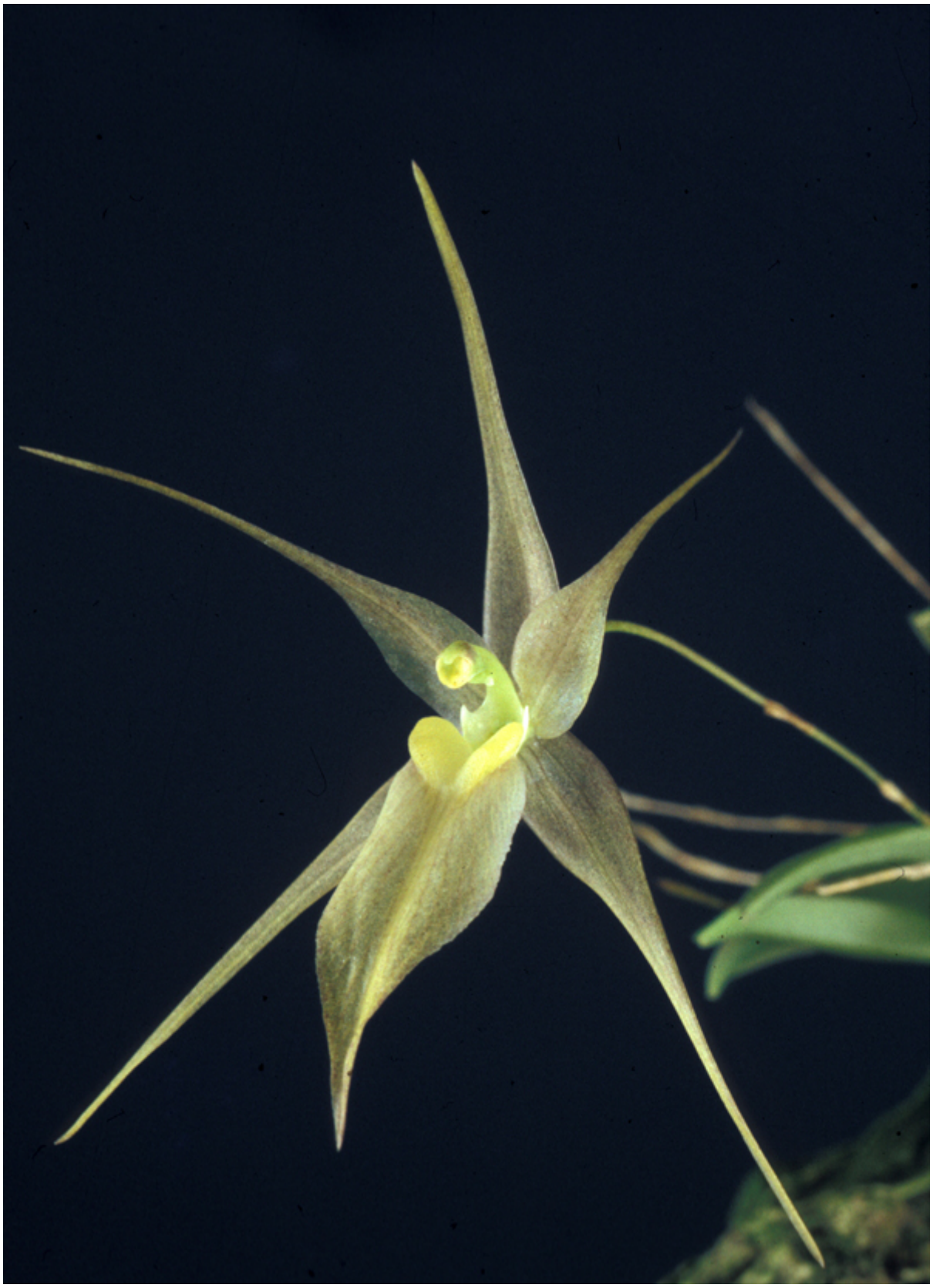
cyps

dendrobs

bulbophyllum

eriopsis

laeliinae



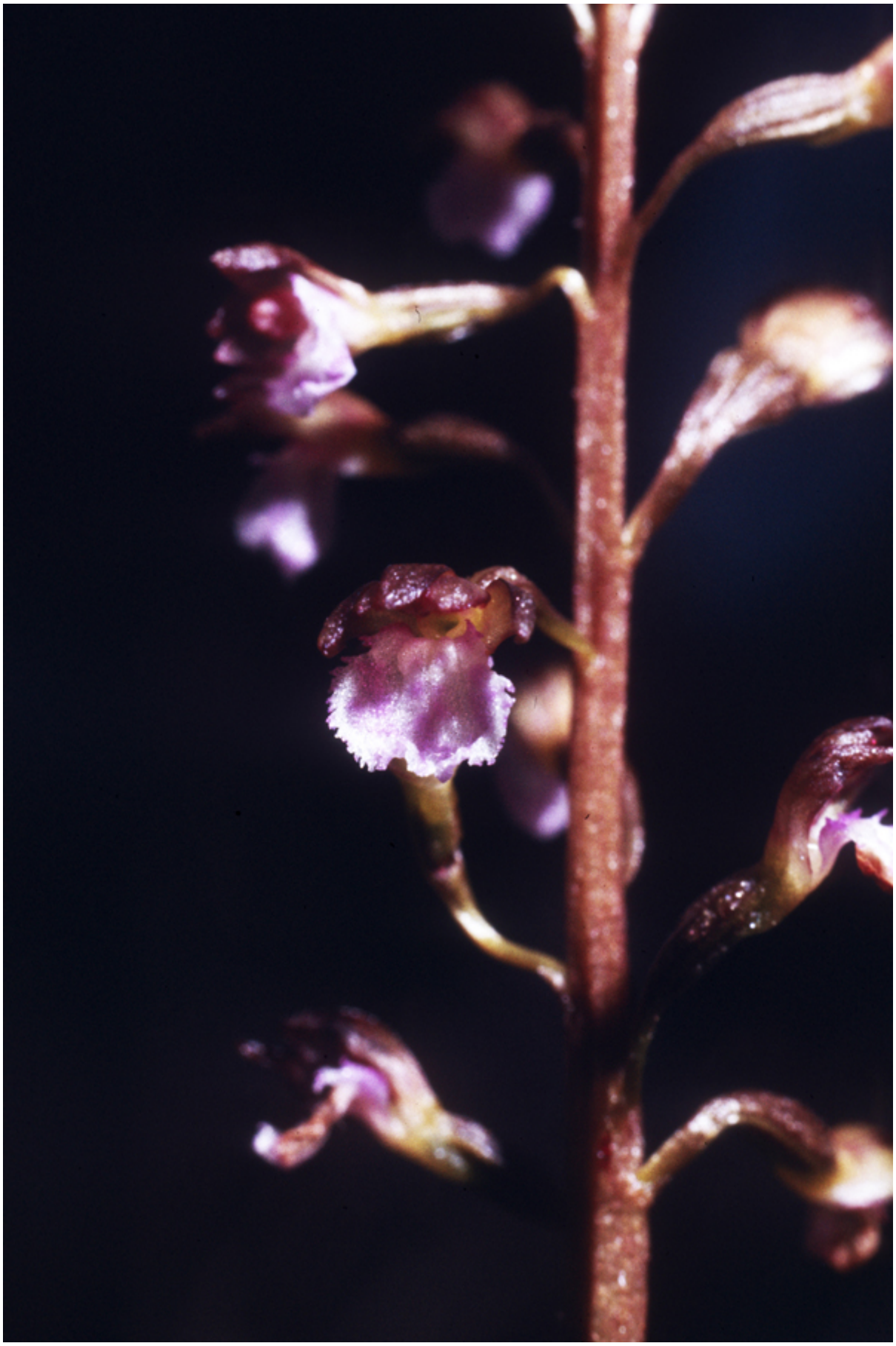
malaxidae

orchidoids

arethusinae

bletinae

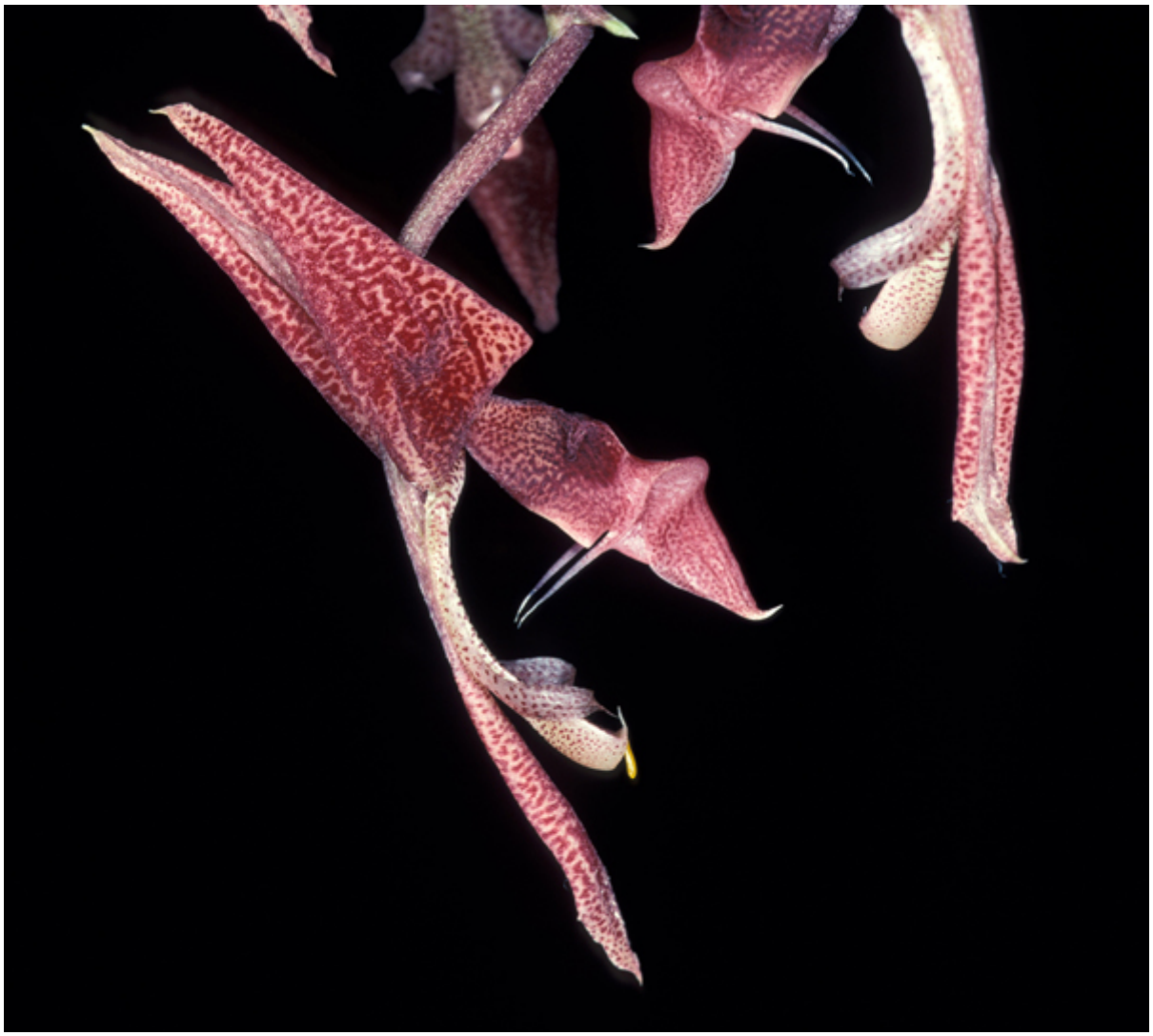
calypsoeae



coelinae

zygopets

stanhopeas



polystachyae

Morphological data: Morphological characters, in particular those of the flower and anther, have been the foundation for orchid classification since Swartz (1800). Characters such as anther number and orientation, pollinium number and orientation, along with types of pollinium stalks have been used to define taxa from subfamily to genus (Dressler 1993). Although recent molecular studies of the orchids have demonstrated the homoplastic nature of many of these characters (e.g., Freudenstein and Chase 2003), this level of homoplasy is not a problem per se, since even highly homoplastic data sets can still be highly structured (Källersjö et al. 1999) and useful in phylogenetic reconstruction and formal clade diagnosis. We consider the role of morphological data just as important to this project as we do the molecular data. In fact, one postdoc will be devoted exclusively to this component of the research, as described in greater detail below. All of the PIs have experience collecting morphological data and recognize the difficulty of homology assessment, parsing characters into states, making decisions about independence, and addressing issues related to character ordering and weighting. For these reasons, our method of data collection and analysis will be standardized and very similar to that described by Freudenstein and Rasmussen in their 1999 paper, "What does morphology tell us about orchid relationships?: a cladistic analysis." Previous cladistic analyses of morphological data scored relatively few characters for a handful of genera. We will use the 71 characters scored by Freudenstein and Rasmussen (1999) as a starting point, but since we will sample a much larger number of taxa than any previous morphological study of orchids, we anticipate a significant increase in the number of characters to be coded as well. For example, several characters were excluded from that 1999 study since they were autapomorphic, but these are now likely to be relevant as synapomorphies in our expanded matrix. Our OrchidTree webpage presents the complete starting list of characters and their states to be coded We will use species as terminals (Kron & Judd 1997) in both morphological and molecular analyses.

Although the total number of morphological characters will be small relative to the molecular data, they can still play an important role in affecting the topologies (e.g., Baker et al. 1998). In addition, the morphological data have another role that is

equally as important as their contribution to reconstructing the phylogenetic pattern - describing it. Careful morphological analysis will allow us to diagnose the clades recovered (e.g., subtribes) and characterize them in a user-friendly way, which cannot be done using molecular data alone.

Morphological data methods and analysis: Because this project does not emphasize fieldwork, it will not be possible to obtain enough fresh tissue to allow for anatomical and embryological study for a majority of taxa, or fruiting material for enough taxa to make scoring seed features possible. Instead we will focus on features that can be scored from herbarium specimens, living plants, and spirit-preserved material already in collections. The focus collections for morphological character scoring include the vast living and/or preserved specimen collections at the Royal Botanic Gardens, Kew (K), University of Florida (FLAS), Ames Orchid Herbarium at Harvard (AMES), The New York Botanical Garden (NY), the University of Copenhagen (C), Marie Selby Botanical Gardens (SEL), and Leiden University (L; see letters of support). We wish to emphasize our own examination of specimens to assure consistent interpretations of characters. Given the scope of this exercise, we can foresee this broad morphological work on Orchidaceae also helping to clarify and standardize terminology as applied to these plants.

The morphological matrix will be analyzed using Wagner parsimony applied through a heuristic search strategy, and involving limited random taxon addition followed by intensive branch swapping on the set of shortest trees recovered; all characters will be weighted equally. Jackknifing will be the method of choice for evaluating tree topology support.

Molecular data: We chose 8 loci to provide a range of variability to maximize resolution and support for clades at all hierarchic levels, as well as to represent the perspectives of all three genomes. Preliminary data for each demonstrate their level of variation; we show the results of the combined analysis in Fig. 2. Although sequences for some species /regions are already in GenBank, many of these earlier sequences are not explicitly vouchered and many were generated by manual radioactive sequencing and are less reliable. For these reasons and for logistical ease (96-well formats), we will sequence all 2000 taxa for all 8 DNA regions, assuring uniformity of source and sequence quality. Our colleague at UF, David Reed, is currently funded by NSF for development of BioCorder, a database for specimen/sequence acquisition and management; we will pilot use of this product as modules are completed.

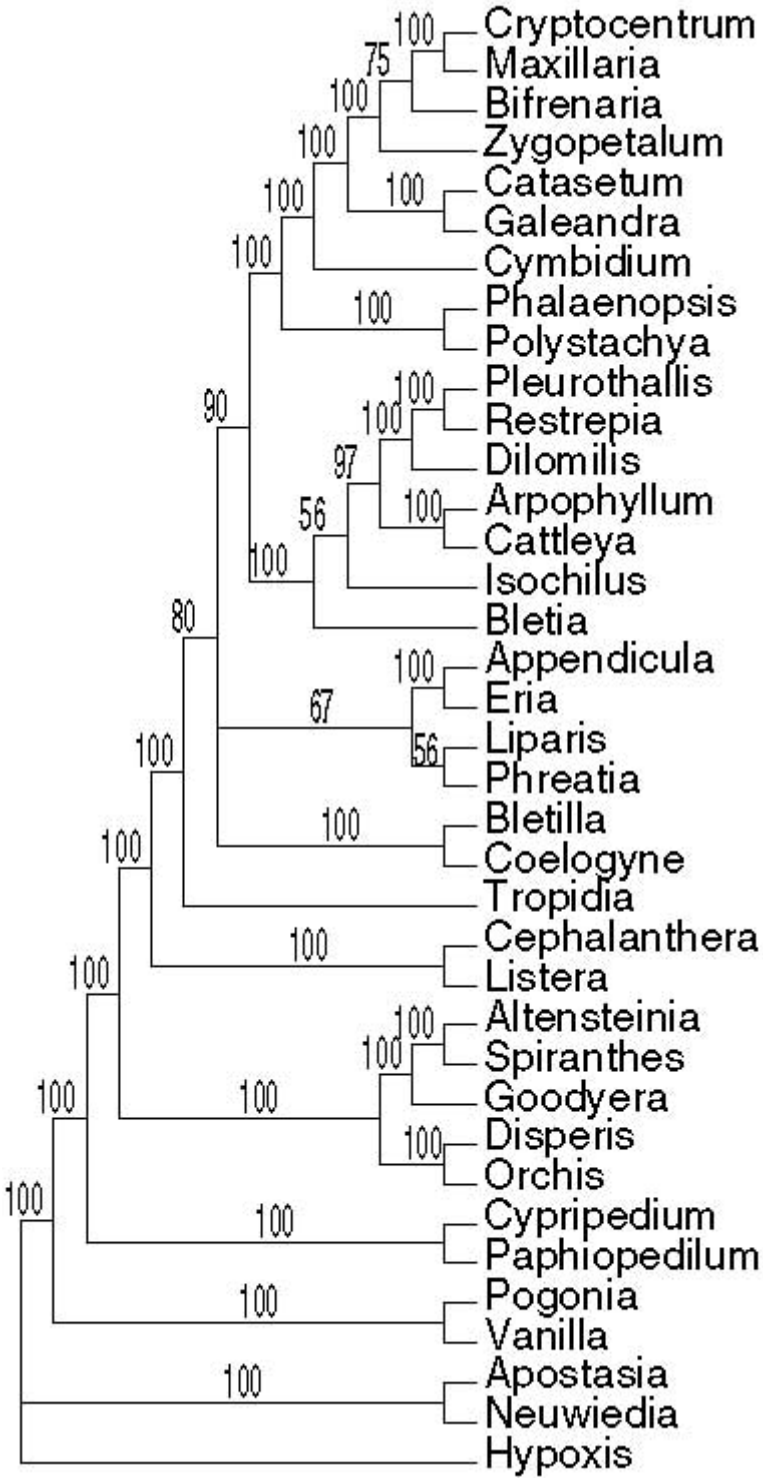


Fig. 2. Combined analysis with jackknife values for selected orchids based on *matK, rbcL, atpB* (plastid), 26S and ITS 1 & 2 (nuclear), and *nad1* (mitochondrial).

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rbcL (plastid;1428 bp; 10% divergence) is the most widely sequenced locus in plants and is perhaps still the most intensively sampled among plants. The first broadly sampled molecular study of orchids employed it (Cameron et al., 1999). Källersjö et al. (1999) found that while third base positions for *rbcL* are most homoplastic, they provide the majority of structure for the tree (RI = 0.74 vs. 0.26 for second position). Primers for *rbcL* are from Cameron et al. (1999).

matK/trnK (plastid;1720 bp; 22% divergence); *matK* has more variable positions

than *rbcL* in orchids and exhibits a number of indels, not always in triplets in orchids, suggesting that it could be a pseudogene. It is easily aligned and the few indels provide additional phylogenetic data. *matK* has been used in orchids by Kores et al. (2000), Whitten et al. (2000), Goldman et al. (2001) and Freudenstein et al. (2004). Primers for *matK* are from Goldman et al. (2001) and Whitten et al. (2000).

psaB (plastid;2200 bp; 7% divergence) has been used to investigate the phylogeny of distantly related land plant lineages (Nishiyama and Kato 1999). Of five plastid genes sampled in their study, *psaB* performed best alone, in that it produced the same tree topology as the total evidence tree. DNA sequences for *psaB* were obtained from 173 species of Orchidaceae (representing 150 different genera) and nine genera from Hypoxidaceae, Asteliaceae, Lanariaceae, and Boryaceae to serve as outgroups by Cameron (2004), who found that the locus compares to *rbcL* in its level of variation. Primers are from Nishiyama and Kato (1999).

atpB (plastid;1500 bp; 8% divergence) has been used in various angiosperm groups, from studies among genera of a subfamily or family (Stefanovic 2002; Pfosser et al. 2003), to among families (e.g., Savolainen et al. 2000; Anderberg et al. 2002; Soltis et al. 2002). Cameron (in press) found that its level of variation among orchids is comparable to *psaB*. Primers are from Hoot (1995).

rpoC1 (plastid; 2800 bp; 10% divergence) contains ca. 2000 bp of coding region and ca. 800 bp intron; this intron has been used in Apiaceae (Downie et al. 2000). Preliminary data for pleurothallid orchids (Whitten unpubl.) indicates the coding regions are easily aligned across orchids and contain strong phylogenetic signal (congruent with 5 other plastid genes).

26S (nuclear;1800 bp [5' half]; 10% divergence) has been used within families and across angiosperms (Kim et al. 2004). Kuzoff et al. (1998) found that it evolves about twice as fast as 18S. Many characters are derived from the expansion segments, a preponderance of which are found in the first half of the gene, which we are proposing to use. Freudenstein et al. (2000) used this locus in combination with ITS across Orchidaceae. Primers are from Kuzoff et al. (1998).

ITS rDNA (nuclear; 600 bp; 45% divergence), comprising ITS1, 5.8S, and ITS2, has been used extensively among studies of genera and species. It is the most variable locus that we have included and will provide significant variation among closely related terminals in our analysis. Primers are17SE and 26SE of Sun et al. (1994). Based on our collective experience with ITS in Orchidaceae, recovering more than one copy is rarely a problem; stringent PCR protocols (high denaturation and annealing temperatures, plus the use of betaine/DMSO) prevent the amplification of non-functional paralogs.

The ***nad1*****b-c** intron (mitochondrial;1420 bp; 7% divergence) is one of the few mitochondrial loci that have been used for plant systematics. It is a Type II intron with well-defined stem-loop structure (Wissinger et al. 1991). It has been used most successfully at higher levels as Porter and Johnson's (1998) analysis of relationships within Polemoniaceae. Freudenstein and Chase (2000) sampled it for ca. 100 species across Orchidaceae and found that the topology was in almost complete agreement with that obtained from *rbcL*. The sequences were easily aligned and numerous phylogenetically informative indels were scored.

Molecular Laboratory Methods: DNA isolation and PCR: In cases for which DNA has yet to be isolated, the total DNA CTAB method of Doyle and Doyle (1987) will be employed using ca. 1 g of fresh or dried leaf (or floral, in the case of leafless taxa) material. Target sequences will be amplified from genomic DNA using Taq polymerase according to established protocols in each lab.

Sequencing: We will use the University of Washington High Throughput Genomics Unit for sequencing. Using 96-well format, they provide PCR cleanup and cycle sequencing with 3730xl automated DNA sequencers. This is much more cost effective than each of us doing our own sequencing, both in terms of funds and person-power (see budget). All amplicons will be sequenced in both directions. Sequence contigs will be assembled and examined against their electropherograms using Sequencher (Gene Codes, Ann Arbor, MI) to check accuracy and correct any obviously incorrect base-calls. We expect to maintain a read-length of at least 700 bp per sequence reaction, a standard that has been easily achieved for the preliminary data.

Data Analysis: Because of the difficulty of alignment of ITS across the entire Orchidaceae, we will utilized a two-tiered analysis strategy. Family-level analyses using easily-aligned regions (all but ITS) will be used to resolve major clades (subfamilies and major divisions within them); ITS data will then be added to the matrix for further analysis of each of these smaller units utilized in restricted combined analyses of these clades, providing additional resolution. We realize that in any analysis of ITS beyond closely related species, ambiguous areas of alignment will occur; however, we believe that the variability of the region in parts that are confidently alignable argues for its use to help resolve relationships especially among genera and tribes. We have designed a procedure to assess the effects of alignment variability (see next section).

Sequence alignment: Machine alignments will be performed in ClustalW/X (Thompson et al., 1994, 1997). Alignment will be straightforward for six of the loci to be used here (matK, psaB, rbcL, atpB, rpoC1, 26S rDNA) because they are protein- or RNA-coding regions. For the nad1b-c intron, alignment of stem and small loop regions is straightforward because of the relatively low sequence divergence level; variation in large loop regions is also non-problematic because it is due mainly to deletions and easily recognized tandem insertions (Freudenstein and Chase 2000). The ITS region presents the greatest challenge for alignment because of its high level of variability, which is the same reason that it is useful at low taxonomic levels. However, Hershkovitz and Zimmer (1996) were able to align many regions of ITS2 even across angiosperms and we have been able to align the whole ITS region within subfamilies of orchids with little problem (Freudenstein and Williams, unpublished). Goertzen et al. (2003) aligned the ITS region across Asteraceae based on secondary structure.

We will not rely on our expectation of alignment tractability for ITS, however. Because of the size of these datasets, manual adjustment of machine alignments is impractical. Moreover, it introduces subjectivity into the procedure (Gatesy et al. 1993). In addition, it is unclear when using an alignment program such as Clustal how many equally optimal alignments exist, since only one alignment is output. In order to address this issue, we have designed a novel strategy to determine the implications of possible additional alignments and further to examine the effect of changing alignment parameters (i.e., sensitivity analysis of Wheeler 1995). In order to make it practical with large datasets, we will automate the procedure by writing code that links together the freestanding programs and implement it on a processor cluster. This procedure inputs a FASTA file of assembled sequences, aligns them in Clustal, then passes the alignment to TNT (Goloboff 2002), which performs a jackknife analysis to identify relatively strongly supported clades (e.g., >70%). The jackknife consensus tree is stored and the number of nodes resolved is recorded. The procedure is then repeated a minimum of 100 times, randomizing the taxon order each time before submitting to Clustal. The variation among numbers of nodes on the resulting jackknife trees provides an indication of the effects of the different alignments, but does not reveal whether there is conflict among the trees

or just different levels of resolution, so the next step consists of performing a consensus of all of the jackknife trees. If the number of nodes in the grand consensus is similar to the jackknife result with the fewest nodes, then there cannot be significant conflict among the jackknife trees. If, however, the grand consensus has many fewer nodes than the minimally resolved jackknife tree, notable conflict must exist and it will be important to examine the different alignments. This whole operation is then repeated, changing the alignment parameters to determine the extent to which differences might also result from choice of specific parameter values (gap opening and extension cost, transition/transversion weight). This software tool and approach will be made broadly available to the systematics community. It provides an alternative to the integrated approach of POY (Wheeler et al. 2002), for those who wish to maintain the homology (alignment) and tree building steps in analysis of molecular data as separate operations, yet wish to explore the implications of alternative alignments and not incur the effects of linking gap costs to ultimate weight of characters in the analysis that is inherent in POY (Simmons and Ochoterena 2000; Simmons 2004).

We will use indels as well as base substitutions as phylogenetic characters. Some have argued that indels are more reliable characters than base changes (Lloyd and Calder 1991). Simmons et al. (2001) supported the use of indels in phylogenetic studies, but they did not conclude that they were superior to base substitutions. Freudenstein and Chase (2001) found them to be a particularly important component of the *nad1*b-c dataset. At present, we have implemented the automated workflow procedure only for base substitutions, but we plan to incorporate a module that codes indels as well according to the simple gap scoring procedure of Simmons and Ochoterena (2000), because the complex approach, relying on step-matrices, would be potentially intractable for such large datasets with their high numbers of indel character states.

The computations needed for this project will be carried out using a general purpose system for the parallel execution of bioinformatic workflows under development by one of the PIs (ML). A prototype of the system has already been built and it was used for the preliminary experiments reported in this proposal. The design objectives of this system are: high throughput execution of large scale computations by means of workflow parallelization, ease of use and of customization by non-computer scientist users, and open source design. These objectives are met by an architecture that integrates a graphical user interface (GUI) with state-of-the-art Grid scheduling technology and implemented entirely in Python. The scheduling tool used in our prototype is the UCSD AppLeS Parameter Sweep Template (APST) metascheduler (Casanova et al. 2000), which is a general purpose metascheduler supporting a variety of Grid platforms (Foster and Kesselman 2004) ranging from ssh/scp to a full Globus environment (see The Globus toolkit) [http://www.globus.org].

Based on our experience with the prototype, there are a number of design issues that need to be solved in adapting Grid technology to the execution of large scale bioinformatic workflows and that we will study as part of this proposal. Specifically, we will seek solutions to the issues of the heterogeneity of middleware software, optimal interfacing to queuing systems such as PSB and Maui commonly found in production supercomputers, the load balancing of multi-site computations (using supercomputers at different institutions), and robustness to failures in subtask execution.

The issues described above are not adequately addressed by existing workflow management tools. Pegasys (Shah et al. 2004) is a flexible and customizable system built around a relational database which focuses on data integration, but the parallelization functionality is limited to the controlled environment of a local

cluster; its customization requires Java programming skills. Kepler (Ludäscher et al. in press) is an extension of the Ptolemy II system for heterogeneous, concurrent modeling and design. However the parallelization is at the level of the individual components of the workflow; customization here also requires Java programming skills. GridAnt (Amin et al. 2004) is based on the Ant build tool for Java platforms. It does not have a graphical interface and only runs in the Globus environment. Its use also requires Java and Globus programming skills.

Preliminary analysis with an early version of this tool employing 50 Clustal + TNT runs with a matK dataset of 443 taxa with approximately 1600 characters on a six-node cluster took 110 hours. This demonstrates the feasibility of the analysis approach, since essentially unlimited run time is available to us for larger data sets on clusters with many more nodes (see facilities statements). We found jackknife trees with a range of 159 to 177 nodes resolved at >70% jackknife support. This is relatively little variability (18 nodes out of a possible 442 = 0.04%), which is to be expected for matK because it has little in the way of indels. It directs us to which alignments should be examined for the cause of variation among even these similar alignments, however. We expect to see more significant variability among ITS alignments, for example, where this approach will be particularly important.

Phylogenetic analysis: We will analyze each dataset individually, combined by genome, and combined in a total analysis under our 2-tier approach. Conducting individual and various combined analyses will allow us to determine whether there is conflict at the level of locus or genome. Previous phylogenetic analyses of Orchidaceae utilizing sequences from the three genomes have not revealed notable incongruence (e.g., Cameron et al. 1999; Freudenstein & Chase 2001; Goldman et al. 2001). However, we will examine the data sets for incongruence using the ILD test (Farris et al. 1994) in order to have a more objective, quantitative approach in addition to our manual comparisons. While this procedure, which is implemented in TNT and PAUP*, has been criticized as not being an accurate indicator of congruence (Yoder et al. 2001; Darlu & Lecointre 2002; Dowton & Austin, 2002), it remains the best available tool for exploration. If we find incongruence we will attempt to localize it to particular taxa by performing an Adams consensus (Adams 1972) on trees from analyses for each gene locus done separately, since that procedure allows individually conflicting terminals to drop to the node of conflict without losing the remaining structure in the tree. This will allow us to identify and reexamine problematic taxa to determine if lab errors could be responsible for data disagreement.

Parsimony analysis under equal weights as implemented in TNT (Linux version; Goloboff et al. 2000) and run on a parallel cluster will be used as the primary tree search strategy for all analyses because this approach can handle large data sets in a reasonable time frame and because of its accommodation of heterogeneity among base positions relative to model-based approaches (Kolaczkowski & Thornton 2004). Heuristic searches will be performed using strategies to explore the data as completely as possible to search for islands of most parsimonious trees (Maddison 1991), given that the problem of local optima can be particularly troublesome for very large data sets (Goloboff 1999). In particular, TNT has implemented a number of innovative strategies such as the Parsimony Ratchet (Nixon, 1999), sectorial searches, tree-drifting, and tree-fusing (Goloboff 1999) that improve the thoroughness of tree search. Individual data sets will be combined directly (Miyamoto 1985; Cracraft & Mindell 1989; Kluge 1989; Kluge & Wolf 1993; Nixon & Carpenter 1996), as opposed to consensus combination (Penny et al. 1982; Bull et al. 1993; Jones et al. 1993), because it allows full character interaction, yielding, to the extent of the heuristic analysis, a maximally parsimonious solution. Given that each data set will undoubtedly have homoplasy, this can be important in revealing "underlying signal" among the data sets. This is done with the knowledge that

independent genomes could be functioning essentially as single characters (Doyle 1992; de Queiroz 1993) and that they may be reflecting different patterns due to incongruent transmission and fixation. Nonetheless, the combined analysis will represent our best phylogenetic estimate.

Because of the size of the datasets we will be analyzing, model-based approaches such as Maximum Likelihood (ML) and Bayesian analysis are computationally more problematic for use as primary tree search methods. However, we will employ these approaches in particular cases in which their properties might be especially useful. These include situations where long branches are to be expected (as with the leafless mycoheterotrophic taxa) and where the parsimony analyses indicate significant rate heterogeneity among branches. In these cases, smaller clades with potentially problematic taxa will be submitted for model-based analyses. Maximum Likelihood analysis will be performed using PAUP*. Modeltest (Posada & Crandall 1998) will be used to select the evolutionary model that best fits the data on starting trees obtained from the parsimony analyses (typically GTR + I + G), with parameter values estimated from the data. Bayesian implementation of likelihood in MrBayes (version 3.1; Ronquist & Huelsenbeck 2005) using the Metropolis Coupled Monte Carlo Markov Chain approach will also be used to reconstruct trees under the likelihood model.

Assessing tree robustness and support: Jackknife analysis (Farris et al. 1996) will be used to assess tree support for parsimony analysis of individual datasets and all combined analyses. A minimum of 5000 replicates will be performed, each of which will include a small amount of branch swapping in each jackknife replicate, as it can significantly improve results (Freudenstein et al. 2004). As stated above, Bayesian analysis also provides an indication of support in its posterior probability values. Sensitivity analysis will be used to explore the datasets for robustness to alignment variation, as described above.

Analytical and informatics resources and expertise: Each of the participating laboratories is fully equipped to perform DNA isolations, PCR amplification, DNA sequencing and contig assembly. Each also can perform routine phylogenetic analysis on desktop PCs and Macintoshes (see Facilities statements for details). Our primary resources for performing large analyses such as the combined alignment and tree search runs and analyses with large numbers of taxa are processor clusters and the supercomputers located at Ohio State University. Our Computer Science PI, Mario Lauria, will supervise use of this hardware and will develop the software infrastructure for the parallel execution of bioinformatic workflows linking free-standing programs (Clustal, TNT). Servers dedicated to the construction and maintenance of our website will be purchased by PI Williams for installation at the University of Florida.

Dissemination of results and archiving of data (products): Results will be disseminated in a number of ways. First, the most up-to-date results will be posted on our website ("OrchidTree") that will convey the results of our project using the large-scale phylogeny as an organizing tool. Because of the large number of taxa and nodes, we have chosen to employ special software to make viewing the tree and recovering information from it as easy as possible. Inxight VizServer software (already purchased at UF) allows users to navigate easily through the tree using a hyperbolic tree. By clicking and dragging on nodes, users can stretch portions of the tree and follow a path of their own choosing through the tree. Clicking on terminals and nodes brings up pages of information on those groups. These pages will include descriptions of the taxa, photographs of included species, literature references, links to GenBank accession numbers for individual DNA sequences, and links to other relevant orchid websites. These results will be integrated with the Genera Orchidacearum project based at Royal Botanic Gardens, Kew.

All data will be made available to the public as soon as they are collected and verified. DNA sequences will be deposited in GenBank and accession numbers will be provided on the appropriate taxon page on our website, which will be maintained at the University of Florida. All morphological character observations will be tied to voucher specimens, which will be documented and archived on our web site, as well as being archived on the OrchidTree website and ultimately deposited in TreeBase at (http://www.treebase.org/treebase/). The morphological data matrix will be input into the LucID software package (University of Queensland; http://www.lucidcentral.com) to create polyclave keys with illustrated character states posted on the web; this Delta-compatible key to genera can be easily revised as additional taxa or characters are added. A member of our group (Whitten) has already assembled such a key for the orchid genus *Maxillaria* for Central America.

We will make available to the scientific community the software tool that we develop for the management of large scale bioinformatic workflows that is easy to use, can take advantage of a range of computational resources (from collections of single PCs to production supercomputers) requiring only minimal or no preinstalled supporting software, and is highly customizable by anyone with only basic script writing skills. See the "Workshops, Symposia, Presentations" section below for further details on dissemination.

III. Education, Training, and Outreach Undergraduate and Postgraduate Opportunities: One of the most important components of this proposal is the educational opportunities available for undergraduate and graduate students. Funding is requested to provide stipends for undergraduates at UF and OSU as well as assistantships for at least one graduate student position at each institution. Although specific thesis topics have not yet been formulated, these students will be trained broadly with aspects of their research being integrated into this greater Orchid Tree project. In conjunction with a student's monographic work, she/he would complete the morphological coding and gene sequencing for their thesis group. In this way, each graduate student will have his/her own independent program of research and also will play an integral role in bringing the greater Orchid Tree project to fruition. It should be noted that the PIs have had great success attracting graduate students from Latin America. There are already a number of prospective students from Latin America who have expressed interest in pursuing graduate studies on orchids at these institutions; all efforts will be made to recruit students from this part of the world, Australasia, as well as underrepresented minorities from the United States. **To emphasize our commitment to recruiting minority and underrepresented groups,** PI Williams has a commitment from UF administrators to support a minority graduate student for four years. In the first year of the grant he will give talks/workshops at both Florida A & M University and Bethune-Cookman College to recruit minority graduate students - a person could be in either biology or computer science - to begin work on this project. Each year he will continue this program. See letters for elaboration.

Postdoctoral Opportunities: In training the next generation of plant systematists, educational opportunities will also be made available for two postdoctoral researchers - one under the direct supervision of Cameron and one working with Freudenstein, but both interacting with the entire team. Unlike the graduate research activities described above, these postdoctoral fellowships will be advertised to attract individuals for specific research topics that have been developed from the results of the Orchid Tree project. These are as follows:

1) A postdoctoral researcher will be recruited by Freudenstein at OSU **to A) assist with the coding of morphological characters** for each genus as discussed in the research plan above. Travel money for this postdoc will also be requested so that

he/she can spend time in the world-class collections at RBG, Kew and NYBG. This person will not simply be used as a technician to gather and code morphological data -- quite the contrary. We expect this postdoc **to B) employ the resulting phylogenetic trees in order to investigate the evolution of key morphological orchid features.** S/he will study how many times important features that have been implicated in the success of the family (such as pseudobulbs, lateral inflorescences, pollinium stalks and epiphytic habit) have been gained and lost As stated above, others have tried to tabulate some of these traits for the family but their specific patterns of evolution have never been documented explicitly in light of a robust phylogenetic hypothesis. At the same time as these trends in character evolution are evaluated, the postdoc will seek to identify correlations among these characters of form, life history, physiology, and pollination syndromes, and will seek correlations with species richness, in order to relate character transformation to diversification. The computer program DISCRETE (Pagel 1994) has been used to document the phenomenon of concerted convergence in monocots among such characters as climbing habit, reticulate leaf venation, and fleshy fruits (Givnish 2003). The software looks at the evolution of pairs of binary characters to test for significant correlations. This approach should uncover whether characters suspected to be correlated in Orchidaceae (e.g., trilocular ovaries and fleshy fruits) have evolved independently or in concert. It is worth noting that when this proposal was first submitted, a reviewer said that s/he was "pleased that a post-doc would be dedicated to creaming off he most interesting comparative analyses . . . I am sure there will be many, and . . . it is good that the applicant will get the first choice of nice evolutionary papers to write from the major effort which they are putting in." This is indeed our intention, and we continue to see this postdoc as essential to the success of our endeavor.

2) The second postdoc will work under Cameron's supervision at NYBG. This person will spend his/her fellowship in the area of science education and public outreach developing educational modules focused on orchid evolution, diversity, and conservation. We expect that the ideal candidate for this position may or may not have an exclusive background in plant biology, but instead would be equally likely to hold a degree in museum science, children's education, or horticulture. Past reviewers of our proposal considered this to be "an interesting and innovative approach."

Both NYBG and RBG, Kew are international museums of plants dedicated not only to science but also to horticulture and public education. For example, NYBG's Everett Children's Adventure Garden welcomes more than 125,000 children, their parents and teachers each year to learn basic information about plants and their role in our world. The postdoc would be expected **to A) develop at least one orchid diversity educational module directed toward K-12 school children.** For example, a weeklong program focused on "Vanilla: the ice cream orchid" could be crafted and implemented to educate young people about evolution, orchid biology, pollination, diversity, and conservation by using this economically important orchid as a centerpiece.

In a similar vein, both gardens organize an annual orchid show. These have been so popular that now nearly every botanical garden and museum in the country, including the Atlanta Botanical Garden, Missouri Botanical Garden, and Smithsonian Institution, offer similar exhibitions. Last year's orchid show at NYBG drew more than 66,000 visitors, and the Kew show is even more popular. At present, these shows contain little scientific content. To remedy this problem, our postdoc will work closely with the show organizers and the PIs to communicate the results of our Orchid Tree project to the public (see letter from M. Falk). We propose at least one specific project for the postdoc related to these orchid shows: **B) a script will be developed for the production of a self-guided audio tour and/or signage**

that can be implemented at a typical orchid show. Currently, NYBG offers a handheld wand that takes visitors on a self-guided tour of its annual exhibit. Most of the content is horticultural rather than scientific. Mr. B. Klein, Exec. Producer for Acoustiguide, the world's leading supplier of audiotours, has agreed to assist in working with the postdoc to develop this script (see his letter of support). It will offer numerous examples of common orchids that could be displayed in a show to highlight interesting orchid biology facts, and this script would be made available to any botanical garden, museum, or orchid society free of charge for developing their own customized set of signs and/or audiotour. Considering only the orchid shows at the New York, Smithsonian, Kew, and Atlanta Botanic Gardens, these each have a potential visitor base of tens of millions within a day's travel. See letter from Kress (Smithsonian) and Chase (Kew).

We have identified one additional outreach module for the postdoc to develop. The American Orchid Society offers to its approximately 24,000 members the free use of dozens of slide and video programs for local society meetings. According to the AOS, "many smaller societies use these educational programs for a few months of meetings. A recent Education Committee initiative has made the generation of new slide programs a high priority for the AOS, including PowerPoint presentations." They state that more than 800 requests for slide presentations have been made, yet not a single scientific presentation is offered. Our postdoc will have the unique opportunity to **C) develop PowerPoint/slide presentations for the AOS (and any interested societies)** on such topics as "Orchid Evolution and Classification", "Orchidology and the Molecular Revolution" and/or "Building the Orchid Tree of Life". These will be made available at no cost, with sponsorship from NSF clearly listed, not only to the AOS for distribution to its unparalleled membership base, but also to many of the 34 other orchid societies around the world.

Workshops, Symposia, Presentations: Our goal is to reach out to the general public and of course also to disseminate the information resulting from this project to professional plant biologists. To this end we will present results regularly at national and international scientific meetings at all stages of the project. These meetings will include Botanical Society of America and the International Monocot Symposium ("Monocots IV" in Copenhagen, 2008).

In addition, we will organize one workshop and two scientific symposia. The workshop will be scheduled in 2008 to coincide with the 19th World Orchid Conference in Miami, Florida. The WOC attracts several thousand commercial orchid growers, scientists, and hobbyists from around the world with lectures, plant sales, and exhibits. The PIs will attend this meeting and organize a workshop to introduce the Orchid Tree concept and an update on the progress of the project to this diverse group. Preliminary phylogenetic trees, databases, and web sites will be demonstrated, and the principles of molecular systematics, evolution, cladistics, and phylogenetic classification will be presented. The PIs have found that this audience of well-educated orchid enthusiasts is eager to adopt new ideas if they can be explained without the use of jargon or taught in a non-patronizing manner. For example, Cameron presented an introduction to DNA sequencing to a standing-room-only crowd at the 17th WOC.

We will organize one symposium in 2008 for the International Monocot Symposium ("Monocots IV" in Copenhagen, 2008). During the final year of the grant period, we will organize a formal orchid phylogeny symposium to present our research to the professional orchid systematics community. Such a meeting was held in 2000 at the Linnean Society in London. Personal communications through M. Chase (see letters) indicate that the Linnean Society would be willing to host this Orchid Tree Symposium. Our intention is to invite all orchidologists who have been involved in the five-year endeavor to deliver a paper on their orchid clade of specialization. A

Proceedings or a volume of the Bot. J. Linn. Soc. will be published based on the symposium. In this way, a dozen or more orchidologists (not only the PIs) will be able to make a contribution to the orchid systematics literature as a result of this large AToL initiative.

PI Cameron (the scientific collaborator for orchids for the TOLWeb) will coordinate our work with the TOLWeb at http://tolweb.org/tree/phylogeny.html. We will provide material for branches to insure that our work reaches the broadest possible audience.

The broader impacts of this project are numerous: Because orchids are such a popular and increasingly economically important group of plants, and in view of their diversity and myriad specializations, orchids are a natural group with which to capture the interest of the public and to convey the importance of systematics, evolution, biodiversity, and especially conservation. This project is designed to capitalize on this opportunity by providing outreach at various levels, including institutional (museums, universities, elementary and middle schools, local orchid societies) and most broadly through web-based tools. The project will provide for training of undergraduates, graduate students, and postdoctoral fellows, and will foster collaboration between scientists and museum outreach specialists. It will increase collaboration among a worldwide network of orchid systematists, including professionals from throughout Latin America. Male and female graduate students from Brazil, Peru, Ecuador, Colombia, Mexico and Costa Rica will broaden the representation of scientists from Central / South America and bring gender, ethnic and geographical diversity to this collaborative project. Our outreach to historical black colleges will, we hope, bring underrepresented minorities into the project. With respect to the orchid breeding and cultivation industry, now estimated to be worth more than $9 billion annually worldwide, the results of systematic studies such as this provide a context for planning breeding programs and for better understanding basic orchid biology. From a more academic point of view, orchids have been the subject of many evolutionary studies because of their diversity of form, intimate relationships with fungi, and elaborate pollination strategies. Such studies can only be properly interpreted within a phylogenetic context, and the ATol Orchid Tree will provide that framework, as well as the basis for a phylogenetically based classification. This will position the family well for increased opportunities for studying evolutionary and ecological questions. The software and approach to exploring multiple alignments and their implication will be of broad use to systematists studying any group.

Disclaimer: The FLMNH Office of Museum Technology is aware of the potential for revealing the identity of anonymous reviewers and panelists by visiting web sites that track visitor statistics. The web site associated with this proposal - /orchidatol/ - does not track any type of individual visitation usage and all associated logging for the pages on this site have been disabled on our server.

This ends what is in the actual grant proposal. The table of morphological characters and the table of taxa follow.

Table of morphological characters and character states to be used as a starting point.

The list of taxa to be sampled follows this table.

This table is taken from the paper by Freudenstein & Rasmussen and the comments refer to taxa and characters as studied in that paper.

Freudenstein, J. V. & F. N. Rasmussen. 1999. What does morphology tell us about orchid relationships? -- A cladistic analysis. *American Journal of Botany* 86: 225-248.

This list of characters represents the beginning of a morphological data set for all Orchidaceae. There are 71 character states organized into 53 characters.

0= root tubers 0=absent 1=present

Root tubers are thickened roots that serve as organs of perennation, producing shoots in subsequent seasons. These structures occur primarily in Diseae, Orchideae, and Diurideae and have been studied intensively (e.g., Irmisch, 1850; Germain de St.-Piérre, 1855; Prillieux, 1865; White, 1907; Ogura, 1953; Pridgeon & Chase, 1995). *Triphora* was also scored as having a true root tuber, but *Pogonia* and *Isotria* were not, even though their roots (unthickened) are known to produce shoots (Ames, 1922). Root thickenings are known from *Mesadenella* (Pabst & Garay, 1952), *Apostasia* (Stern & Warcup, 1994), *Wullschlaegelia* (Freudenstein, unpubl.), and possibly others, but these evidently do not produce new shoots.

1= roots 0=rhizodermis 1=velamen

2= exodermis 0=unthick 1=unif. thick 2=outer thick

3= exodermal cells 0=±isodiametric 1=elongate

4= velamen cell thickenings 0=absent 1=linear 2=circular

These characters derive primarily from the work of Porembski & Barthlott (1988). Instead of coding their ten velamen types as states for the terminals, which would necessarily have to be coded as unordered states of a single character, we chose to code these component features of the variation that they describe.

5= spiranthosomes 0=absent 1=present

This character was first described by Stern et al. (1993a). Spiranthosomes are specialized amyloplasts known only from the spiranthoid orchids. Most of the occurrences of spiranthosomes are taken from Stern et al. (1993a, 1993c) Many of the coded absences, outside of spiranthoid orchids, are assumed, since they have not been described before from other groups.

6= growth pattern 0=sympodial 1=monopodial

Most orchids follow the sympodial growth pattern common in monocots (Holttum, 1955). Notable exceptions to this are *Vanilla*, which is a monopodial vine with elongate internodes, and members of the Vandeae, which have monopodial growth, but shortened internodes.

7= thickened stem 0=absent 1=present

Stem thickening may be in the form of swelling in aerial stems, as in some species of *Dendrobium*, as corms (e.g., *Aplectrum*), or as pseudobulbs. If the stem is

notably but uniformly thickened, as in *Vanilla*, the taxon is not scored as having a thickened stem.

8= no. of thickened internodes 0=several 1=one

This character codes variation in numbers of internodes that comprise a pseudobulb. In many taxa, it is clearly one internode (e.g., *Bulbophyllum*), while in others (e.g., *Catasetum*), it can be several. Those taxa with no thickened stem are coded as unknown.

9= phyllotaxy 0=spiral 1=distichous

Phyllotaxy in orchids is described as either spiral or distichous, with the latter supposed to be the more advanced state (Dressler & Dodosn, 1960; Dressler, 1993). We found that in all species examined, the condition at the base of a stem, represented by the first bracts surrounding a bud, is distichous. In some taxa (e.g., *Apostasia, Neuwiedia*, many spiranthoids and diurids), the phyllotaxy soon shifts to more or less spiral, so that most of the leaves appear spirally arranged. In many of these, the arrangement is not strictly spiral, as was shown by Fuchs and Ziegenspeck (e.g., 1927a, b, c). In other taxa (e.g., species examined of *Dactylorhiza, Orchis*, many epidendroids), the majority of leaves show a distichous arrangement. Even in those species with distichous leaves, the inflorescence is generally spiral in floral arrangement. In some cases, even the infloresence is distichous (*Tropidia effusa* Rchb. f., *Liparis gibbosa, Phalaenopsis cornu-cervi*). Hence the basic model for orchid phyllotaxy appears to be a distichous basal condition on the shoot, with a possible shift to a spiral or pseudo-spiral condition at some point (before inflorescence or not). We scored states according to whether a taxon had spiral (or pseudo-spiral) or distichous phyllotaxy on the leaf-bearing portion of the shoot.

10= leaf morphology 0=flat, "herbaceous" 1=plicate 2= conduplicate

Variation among orchid leaves is more complex than it appears at first glance. Phyllotaxy plays a part in determining mature morphology, and since this was scored as a separate character, it is important not to duplicate this information. Leaves with convolute vernation usually are spirally arranged, while conduplicate leaves have duplicate vernation. The state found in many of the terrestrial spiranthoids and orchidoids has been termed by Dressler (1990c) "nonplicate herbaceous". It describes those leaves that are essentially flat (not conduplicate), without prominent midrib, usually fleshy in texture, and that are the result of convolute vernation. Plicate leaves may result from either convolute or duplicate vernation, and are characterized by the accordion-like pleating of the lamina. Conduplicate leaves, as coded here, are those with a single fold of the lamina at the midrib, and without any plication. There are some conduplicate plicate leaves (e.g., *Sobralia*), but, again, in order to avoid redundancy, these were simply scored as plicate. The states are unordered.

11= winter leaf 0=absent 1=present

Some genera of terrestrial orchids have a leaf that is produced at the end of the growing season, overwinters, is photosynthetically active when conditions are favorable, and withers at or before flowering. These are primarily members of Corallorhizinae (e.g., *Aplectrum, Calypso*; cf. Freudenstein, 1994), but occasionally other species show a similar modification (some *Spiranthes* have overwintering leaves [Rasmussen, unplubl.]).

12= leaf articulation 0=absent 1=present

This character has been discussed and the state noted for each orchid group by Dressler (1981) and reflects the presence or absence of an abscission zone at the base of a leaf. As a general rule, most epiphytic orchids are articulate, while terrestrial species are not.

13= stegmata 0=conical 1=spherical 2=absent

Most of the information on this character is from Møller & Rasmussen (1984). Additional scoring was derived from sources quoted in Solereder and Meyer (1930) and from Stern et al. (1993) for Spiranthoideae. The character seems to be largely uniform within genera, although Dressler & Cook (1988) found conical stegmata in *Eria javanica*, while other members of the genus are known to have spherical stegmata. Since the outgroup (*Hypoxis*) does not have stegmata present, the plesiomorphic state in the analysis is absence, although *Apostasia* and *Neuwiedia*, considered to be a basal lineage, do have conical stegmata. Because stegmata are always found in association with fibrous support tissue, absence may be due either to their loss of the stegmata themselves (where sclerenchyma is present), or to the loss of sclerenchyma. Since presence of fibrous support tissue in the leaf is scored as a separate character, those taxa that have no leaf sclerenchyma were scored as unknown for the presence of stegmata.

14= leaf fiber bundles 0=present 1=absent

Many orchid leaves have sclerenchyma associated with the vascular bundles, as well as independent fiber bundles, while other leaves have no scelerenchyma. Stern et al. (1993c) used this character in their analysis of Spiranthoideae. Presence of sclerenchyma is often, but not exclusively, associated with large leaf size.

15= leaf abaxial epidermal cells 0=straight 1=wavy

Lavarack (1971) utilized this character in his phenetic study of Australian Orchidaceae (Diurideae), and Stern et al. (1993c) surveyed it in the Spiranthoideae. The states are usually quite distinct, with either clearly sinuous anticlinal walls when viewed in paradermal section, or with straight-polygonal walls (cf. Dressler, 1993, fig. 2-6). Adaxial surface cells may also exhibit the condition, but it is most pronounced on the abaxial surface.

16= subsidiary cells 0=present 1=not distinguishable

Some detailed studies of subsidiary cells in orchids have been done (Williams, 1975, 1979; Rasmussen, 1981, 1987), but not on a broad enough scale to make it possible to score the different developmental patterns for many of our terminals. Hence, we have simply scored the presence/absence of subsidiary cells that are morphologically distinct from surrounding epidermal cells, without regard to their ontogeny.

17= inflorescence position 0=terminal 1=lateral

In most orchids this appears to be a relatively straightforward character, although, as shown by Andersen et al. (1988) for *Eria*, there may be variation within a genus and the true state can be difficult to ascertain.

18= floral abscission 0=absent 1=present 2=present, stalked

Many orchids have an abscission layer at the base of the pedicel; if the flower is not pollinated, it falls from the inflorescence. As opposed to perianth abscission, floral abscission seems to occur only in a more "advanced" group, the epidendroids. Within the Pleurothallidinae there is a further specialization, in that the abscission zone is located between the pedicel and ovary, so that after the flower falls a

distinct stalk remains (Dressler, 1993; Neyland & Urbatsch, 1993). This character was coded as ordered (equivalent to coding the stalked ovary as a distinct character).

19= perianth abscission 0=present 1=absent

As with leaves, the perianth may dehisce from the flower at the summit of the ovary. Dressler (1983) described this feature and noted its occurrence in the "basal" orchid groups.

20= calyculus 0=absent 1=Vanilloid 2=Polystachyoid

The calyculus is a series of small bractlike structures outside of the normal perianth. Because of its resemblance to a small perianth whorl, significant evolutionary implications sometimes have been ascribed to the structure -- such as Lindley's (1847) homologizing the calyculus with an additional floral whorl and consequent reinterpretation of the inner whorls. Soon thereafter, however, Crüger (1849) concluded that the calyculus is not an additional floral whorl. Additionally, Kurzweil (1987a) examined the development of these structures in *Neobenthamia*, and found no relation to the perianth whorls.

In this study two types of calyculus are recognized. *Epistephium* and *Lecanorchis*, and to a lesser extent, *Vanilla*, have a distinct collar below the perianth (Hashimoto, 1990; Dressler, 1993). The type known from *Neobenthamia*, and also observed here (and by Kurzweil, 1987a) in *Polystachya*, is different, being essentially a series of swellings on the ovary valves. A bractlike calyculus has also been reported from some species of *Bulbophyllum* (e.g., Lindley, 1838; Seidenfaden, 1979; Dressler, 1981).

21= lip slipper-shaped 0=absent 1=present

This distinctive labellum form has the middle portion greatly expanded and the apex and distal margin pulled back toward the column, forming a hollow, shoelike structure. This morphology is characteristic of the Cypripedioideae. Superficially similar structures occur occasionally in the Epidendroideae (e.g., *Calypso*), but do not include the inrolled margin that is present in Cypripedioids.

22= apiculate perianth 0=absent 1=present

The perianth apices of *Apostasia* and *Neuwiedia* are prolonged into distinctive tips, which are formed by the midrib. To our knowledge this feature has not been discussed before in a phylogenetic context, although it is visible in illustrations of the flowers (de Vogel, 1969), but it seems consistent within these genera.

23= petals carinate 0=present 1=absent

Rasmussen (1982; fig. 61d) illustrated an unusual feature in *Vanilla*, interlocking sepals and petals. This creates a keel on the adaxial surface of the petal along the midrib that is found in several putatively basal orchid groups.

24= lip-column marginal adnation 0=absent 1=present

In some vanilloid orchids the labellum is fused to the column marginally to varying degree. Other taxa sometimes have labellum column fusion of other types, but this is not included here.

25= dorsal median anther 0=present 1=absent

26= lateral inner anthers 0=present 1=absent

The homology of the functional anthers present in orchids was worked out by Brown (1833), Darwin (1862), Swamy (1948), and Rao (1974). Further investigation was not undertaken here; we assume that all monandrous orchids have A1, all diandrous orchids have a1 and a2, and triandrous orchids have A1, a1, and a2. No evidence has ever been presented to substantiate Garay's (1960) claim that two lateral anthers of the outer whorl are present in *Satyrium*. Occasional unusual situations, such as the presence of a third functional anther in *Phragmipedium lindenii* and the putatively peloric forms observed by Chen (1982) were not included.

27= anther orientation 0=erect 1=bend late 2=bend early

Anther bending (incumbency) during ontogeny has been discussed by Hirmer (1920) and Dressler (1981, 1986b, 1993). Although there has been some question of whether "advanced" epidendroids bend at all (Dressler, 1981), further study suggests that these taxa bend very early (Dressler, 1986; Kurzweil, 1987a). We distinguish between early and late bending following Kurzweil's (1987a:438) stage 2-3 distinction, relative to the time of column elongation. This character is ordered based on ontogenetic information -- the erect state is more general than the incumbent state (cf. Kurzweil, 1987a).

28= operculate anther 0=absent 1=present

Typical lilioid anthers dehisce by slits, releasing pollen without also shedding parts of the anther wall. Some orchid anthers (Apostasioideae, Cypripedioideae, Orchidoideae, Spiranthoideae - **N.B.** this paper was written before we knew that the Spiranthoideae was included in the Orchidoideae and before Vanilloideae was recognized as a subfamily) also have basically this type of dehiscence. Incumbent anthers usually need to be physically disturbed to allow the pollinia to be removed. In some taxa with incumbent anthers, the anther develops in tight proximity to the clinandrium; in order for pollinia to be released, the anther (sometimes called the "anther cap") must be removed. This removable type of anther is called an operculate anther.

29= Endothecial thickenings 1 0=other 1=int 2=type II

30= Endothecial thickenings 2 0=other 1=type III/IV

Endothecial thickening morphology in orchids was surveyed by Freudenstein (1991). Here the distinctive type II thickening is coded as one character, and the types III/IV and their intermediates are coded as a second character. State 1 in character 29 is for those thickenings that are intermediate in morphology between types I and II. Because it is a eesentially a morphocline, the character is coded as ordered.

31= basal caudicles 0=absent 1=present

Caudicles are pollinium stalks that are composed of pollen and/or pollen-derived substances, as opposed to rostellar tissue (Richard, 1817; Rasmussen, 1986). In epidendroid and spiranthoid orchids they are produced apically in the anther, due to the bending of the anther or the apical position of the rostellum, respectively. In orchidoids the caudicles are basal extensions of the pollinia.

32= hammer stipe 0=absent 1=present

A distinctive stipe with a "hammer"-like morphology was identified by Rasmussen (1986) in *Sunipia*. We found this type also in *Genyorchis*.

33= epidermal stipe/tegula 0=absent 1=present

Rasmussen (1986) has distinguished between the tegula, a pollinium strap formed from the abaxial cuticle of the rostellum, and a hamulus, the apex of the rostellum itself. Rasmussen and Freudenstein (unpubl.) have since distinguished other varieties of pollinium strap. One that is common in epidendroids is an epidermal strap that consists of a varying number of epidermally-derived cell layers plus the cuticle. Because it is sometimes difficult to distinguish between this type and a true tegula, we have combined the two in this character.

34= pollen unit 0=monad 1=tetrad

Mature pollen unit was described by Schill & Pfeiffer (1977) for a large number of species; others were reported in Newton & Williams (1978), Ackerman & Williams (1980, 1981), and Hesse et al., (1989). Ackerman & Williams (1981) described cases of some diurids (e.g., *Caladenia*) in which both states occur. Wolter & Schill (1986) suggested that the occurrence of tetrads in orchid pollen may be due to paedomorphosis.

35= pollen tectum 0=reticulate 1=smooth

Pollen structure has been described by Schill & Pfeiffer (1977), Burns-Balogh (1983), Hesse et al. (1989) and Zavada (1990). Rather than focus on details of the tectal structure, which has not been studied in enough genera, we simply scored the appearance of the pollen grains, whether reticulate or smooth.

36= pollen apertures 0=colpate/sulcate 1=porate 2=inaperturate 3=polyporate

The aperture state of orchid pollen was taken from Erdtman (1944, 1966), Schill & Pfeiffer (1977), Newton & Williams (1978), Ackerman & Williams (1980), and Burns-Balogh et al. (1987). The greater proportion of orchid pollen is inaperturate (Schill & Pfeiffer, 1977), but putatively basal groups have sulcate-colpate or porate pollen (Newton & Williams, 1978; Burns-Balogh et al., 1987). Some of the vanilloids (*Vanilla, Epistephium, Lecanorchis*) have been reported to have polyaperturate pollen (Erdtman, 1944, 1966; Schill & Pfeiffer, 1977; Ackerman & Williams, 1980), a feature otherwise unknown among orchids.

37= operculate colpus 0=absent 1=present

Burns-Balogh & Funk (1986) utilized this character in their analysis. This pollen feature was described and illustrated by Schill (1978) and Newton & Williams (1978) and only appears in *Apostasia* and *Neuwiedia*.

38= massulae 0=absent 1=orchidoid 2=epidendroid 3=arethusoid

Sectile pollinia have been shown to vary with respect to layering and regularity of massulae (Vermeulen, 1965; Freudenstein & Rasmussen, 1996). Orchidoid sectile pollinia have a single layer of uniform massulae, while epidendroid pollinia have variable numbers of layers of irregular massulae. Pollinia of *Arethusa* and *Calopogon* are hollow at maturity (cf. Pace, 1909; Freudenstein & Rasmussen, 1996), while most other orchids have solid pollinia.

39= pollinium texture 0=loose 1=hard

Pollinium texture is largely a continuous character (Dressler, 1986a), but it is possible to distinguish those pollinia that are truly coherent from those that are soft enough to be easily crushed when touched. The structural basis for this difference has been elucidated by ultrastructural studies of pollinia (Chardard, 1958, 1969; Cocucci & Jensen, 1969;Schill & Pfeiffer, 1977; Wolter & Schill, 1986; Yeung, 1987; Hu & Yang, 1989; Zavada, 1990; Pandolfi et al., 1993). The most important difference is whether or not exine is deposited on internal pollen grains; if not, a

more cohesive, calymmate pollinium results, while those that do have exine on all grains are termed acalymmate and are much more friable (van Campo & Guinet, 1961).

40= poll. no.: 2 0=absent 1=present

41= poll. no.: 8 0=absent 1=longitudinal 2=transverse

The primary pollinium numbers in orchids are 2, 4, and 8. Other numbers are sometimes reported (e.g., in Laeliinae), depending upon whether additional small masses of pollen that sometimes are found along the caudicles are interpreted as pollinia. Four is the predominant number, and is found in the putatively basal orchid groups, as well as in outgroups (where there are four anther locules). Freudenstein & Rasmussen (1996) found that there are at least two ways to produce eight pollinia -- by longitudinal or transverse division of embryonic pollen masses.

42= pollinium orientation 0=juxtaposed 1=superposed

Variation in pollinium orientation was first recognized by Dressler & Dodson (1960). They described the superposed state, where, when four, the pairs of pollinia are stacked one on another, as opposed to the juxtaposed (Freudenstein & Rasmussen, 1996) condition, in which the pollinia are arranged side by side. We have detected two distinct ways in which the superposed state can occur -- either by inward or outward "rotation" of anther thecae (Freudenstein and Rasmussen, unpubl.), but as we have not yet been able to complete developmental study on a sufficient number of taxa, we have here coded all superposed pollinia as the same state.

43= ovary locule number 0=one 1=three

Most orchids have a single ovary locule, while a very few putatively basal groups have three locules, as does the outgroup. Transverse sections of each type are shown in Atwood (1984) and, in diagrammatic form, in Garay (1960).

44= stigma 0=convex 1=concave

Variation in stigma morphology was discussed by Dressler (1981, 1993), Rasmussen (1982) and Dannenbaum et al. (1989). In many taxa portions of one or more stigma lobes are protruded, form a raised triangular or circular mass, or are of other shape (cf. Rasmussen, 1982, fig. 73). These morphologies are grouped here under the term "convex". A concave stigma is a sunken, usually circular depression, which, as Dressler (1993) has suggested, appears to be specialized to receive hard pollinia (cf. Rasmussen, 1982, fig. 73:2.1.2); this type is usually encountered in Epidendroideae.

45= stigma receptive cells 0=various other 1=finger 2=prosenchymatic

This character derives from the work of Dannenbaum et al. (1989). It describes the shape of the receptive cells of the stigma that are usually hidden under stigmatic secretions in living plants.

46= viscidium 0=none 1=diffuse 2=detachable

Most orchids have some sort of adhesive associated with insect transfer of pollen masses. This may be either in the form of a glue that is transferred to the insect before it contacts pollen (diffuse), or a more elaborate cellular structure composed of rostellar tissue that is attached either directly, or via a stalk, to the pollinia (detachable). Some controversy over terminology involving the viscidium exists, with Dressler (1986a) and Dressler & Salazar (1991) arguing for restricting use of the term to a detachable structure, while Rasmussen (1982) used the term more

broadly to include also any secreted adhesive. Here it is used in the broad sense simply for convenience, with the two senses of the term being the states. The character is coded as ordered because all detachable viscidia also have adhesive secretion.

47= endocarpic trichomes 0=absent 1=present

Endocarpic trichomes, or Schleuderhaare, were described by Beer (1857), and have been little discussed since. Hallé (1986) illustrated them in transverse sections of ovaries. An SEM of a hair from *Pteroceras* appears in Pedersen (1993). Their function is suspected to be in seed dispersal. Variation in shape (hairlike or flattened) occurs among genera. According to Malguth (1901), their presence is correlated with epiphytism, although not all epiphytes have them and some terrestrial orchids do. The only genus in which we found them outside of Epidendroideae is *Prasophyllum*.

48= seed lat. compressed walls 0=absent 1=present

49= seed testa cell shape 0=all iso 1=end iso, med elong 2=all elong

50= seed striations 0=absent 1=trans/ret 2=longitudinal

51= seed intercellular spaces 0=absent 1=present

52= seed wax caps 0=absent 1=present

53= seed covered cell border 0=absent 1=present

External morphology of seeds has provided a promising new set of data for orchids (Barthlott, 1976; Dressler, 1986b, 1990a, 1993; Molvray & Kores, 1993) These characters derive from the work of Ziegler (1981). Much of the scoring was done from the plates in Ziegler (1981), Tohda (1983, 1985, 1986), Chase & Pippen (1988, 1990), and Kurzweil (1993). Rather than coding seed morphology as types, such as those recognized by Ziegler (1981) and Dressler (1993), we coded component features to the extent possible. Laterally compressed testa walls refers to the extremely narrow cell lumen seen in some taxa (e.g., Vandeae), resulting from the close positioning of lateral anticlinal testa walls. Some seeds have distinct variation in testa cell size depending on location -- with either all cells isodiametric, the cells at both ends isodiametric and those in the center elongate, or all cells elongate (unordered). When present, striations on the sunken testa cell lumina may be either transverse/reticulate or straight-longitudinal (unordered). Distinct spaces occur among the cells in some taxa. "Wax caps" are present at the ends of testal cell protrusions in some members of Cymbidieae (Ziegler, 1981; Chase & Pippen, 1990). In some seeds the abuttment of adjacent testal cell walls is clearly evident, while in others, the line of demarcation between them is covered by tissue from one or the other cell (a covered cell border).

THIS IS THE END OF THE MORPHOLOGICAL TABLE.
THE TAXON TABLE FOLLOWS.

List of genera of Orchidaceae from the The Kew World Checklist of Monocotyledons (slightly modified), number of species in parentheses, dna available or obtainable, and number of species to sample.

ORCHIDACEAE (24,888 species)	Tribes or subtribes (# of species)	Genera (# of species)	# to sample
Subfamilies (# of		* = no DNA currenlty in hand, but if a	

species)		number is in next column, we can get DNA of this genus	
APOSTASIOIDEAE (16)		Apostasia (7)	2
		Neuwiedia (9)	1
VANILLOIDEAE (249)	Pogoniieae (77)	Cleistes (63)	3
		Duckeella (3)	1
		Isotria (2)	1
		Pogonia (7)	2
		Pogoniopsis* (2)	0
	Vanillineae (172)	Clematepistephium (1)	1
		Cyrtosia (5)	1
		Dictyophyllaria* (1)	0
		Epistephium (23)	2
		Eriaxis (1)	1
		Erythrorchis (3)	1
		Galeola (7)	1
		Lecanorchis (14)	1
		Pseudovanilla (8)	1
		Vanilla (109)	11
CYPRIPEDIOIDEAE (154)		Cypripedium (50)	7
		Mexipedium (1)	1
		Paphiopedilum (77)	25
		Selenipdeium (5)	1
		Phragmipedium (21)	8
ORCHIDOIDEAE (4699)			0
	Cranichideae (1643)		0
	Goodyerinae (651)	Aenhenrya* (1)	0
		Anoectochilus (45)	2
		Aspidogyne (19)	2
		Chamaegastrodia* (5)	0
		Cheirostylis* (47)	0
		Cystorchis* (21)	0
		Danhatchia (1)	0
		Dossinia (1)	2
		Erythrodes (88)	8
		Eurycentrum* (7)	0
		Evrardianthe* (1)	0
		Gonatostylis* (2)	1
		Goodyera (96)	10
		Gymnochilus* (3)	0
		Halleorchis (1)	0
		Herpysma* (1)	0

		Hetaeria (32)	2
		Hylophila* (10)	0
		Kreodanthus* (6)	2
		Kuhlhasseltia* (12)	2
		Lepidogyne* (1)	0
		Ligeophila (9)	1
		Ludisia (1)	1
		Macodes* (9)	2
		Microchilus (2)	0
		Moerenhoutia* (13)	0
		Myrmorchis (16)	0
		Odontochilus (3)	0
		Orchipedum* (2)	0
		Pachyplectron (3)	1
		Papuaea* (1)	0
		Platylepis (8)	1
		Platythelys (10)	2
		Pristiglottis* (22)	1
		Rhamphorhynchus* (1)	0
		Rhomboda (19)	0
		Stephanothelys* (2)	0
		Tubilabium* (13)	0
		Vrydagznea* (41)	2
		Zeuxine (76)	2
	Spiranthinae (470)	Aracamunia* (1)	0
		Aulosepalum (6)	2
		Beloglottis (7)	1
		Brachystele* (20)	1
		Buchtienia* (3)	0
		Coccineorchis (4)	1
		Cotylolabium (1)	0
		Cybebus* (1)	0
		Cyclopogon (82)	8
		Degranvillea* (1)	0
		Deiregyne (19)	2
		Dichromanthus (1)	1
		Discyphus* (1)	0
		Eltroplectris (13)	0
		Eurystyles (18)	2
		Funkiella (4)	0
		Hapalorchis* (8)	0
		Helonoma* (2)	0
		Kionophyton (4)	0
		Lankesterella (11)	2

		Lyroglossa* (2)	0
		Mesadenella (7)	0
		Mesadenus (6)	1
		Microthelys (6)	0
		Odontorrhynchus (6)	0
		Pelexia (77)	3
		Physogyne* (3)	0
		Pseudogoodyera* (2)	0
		Pteroglossa* (9)	0
		Sacoila (5)	1
		Sarcoglottis (41)	4
		Sauroglossum* (12)	1
		Schiedeella (21)	2
		Skeptrostachys* (12)	0
		Spiranthes (24)	4
		Stalkya (1)	0
		Stenorrhynchos (7)	2
		Stigmatosema* (12)	0
		Svenkoeltzia (2)	0
		Thelyschista (1)	0
		Veyretia (9)	0
		Wallnoeferia* (1)	0
	Manniellinae (1)	Manniella (1)	1
	Cranichidinae (268)	Aa (27)	2
		Altensteinia (7)	2
		Baskervilla* (12)	2
		Coilochilus (1)	1
		Cranichis (54)	5
		Exalaria (1)	0
		Fuertesiella* (1)	0
		Gomphichis* (25)	3
		Myrosmodes* (10)	2
		Nothostele* (1)	0
		Ponthieva (57)	5
		Porphyrostachys* (2)	1
		Prescottia (31)	3
		Pseudocentrum* (7)	0
		Pseudocranichis* (1)	1
		Pterichis* (20)	2
		Solenocentrum* (4)	0
		Stenoptera (7)	1
	Pterostylidinae (160)	Pterostylis (160)	16
	Chloraeinae (84)	Bipinnula (11)	1
		Chloraea (51)	5

		Gavilea (14)	2
		Geoblasta (2)	0
		Megastylis (in part) (6)	2
	Galeottiellinae (3)	Galeottiella (3)	1
	Diurideae (874)		0
	Acianthinae (170)	Acianthus (26)	2
		Corybas (125)	12
		Cyrtostylis (6)	1
		Stigmatodactylus (11)	1
		Townsonia (2)	0
	Caladeniinae (281)	Adenochilus (2)	1
		Aporostylis (1)	1
		Caladenia (250)	25
		Cyanicula (10)	1
		Elythranthera (2)	1
		Eriochilus (12)	1
		Glossodia (2)	1
		Leptoceras (1)	1
		Praecoxanthus (1)	0
	Cryptostylidinae (26)	Coilochilus (1)	1
		Cryptostylis (25)	3
	Thelymitrinae (166)	Arthrochilus (10)	0
		Burnettia (1)	0
		Caleana (1)	1
		Calochilus (23)	5
		Chiloglottis (27)	5
		Drakaea (9)	3
		Leporella (1)	1
		Lyperanthus (2)	1
		Megastylis (in part) (1)	1
		Paracaleana (9)	0
		Pyrorchis (2)	0
		Rimacola (1)	1
		Spiculaea (1)	1
		Thelymitra (78)	8
	Diuridinae (61)	Diuris (58	6
		Epiblema (1)	0
		Orthoceras (2)	1
	Prasophyllinae (168)	Genoplesium (45)	5
		Microtis (18)	2
		Prasophyllum (105)	12
	Rhizanthellinae (2)	Rhizanthella (2)	1
	Codonorchideae (2)	Codonorchis (2)	1
	Orchideae (2185)		0

		Ponerorchis (17)	2
	Brownleeinae (83)	Brownleea (7)	2
		Disperis (76)	2
	Disinae (220)	Ceratandra (6)	0
		Corycium (15)	2
		Disa (169)	4
		Evotella* (1)	0
		Huttonaea (5)	1
		Pterygodium (18)	2
		Schizodium (6)	0
	Orchidinae (1865)	Aceratorchis* (2)	0
		Amerorchis (1)	1
		Amitostigma* (28)	2
		Anacamptis (13)	3
		Androcorys* (6)	1
		Aorchis* (2)	0
		Arnottia* (4)	0
		Barlia (2)	2
		Bartholina* (2)	1
		Benthamia* (31)	2
		Bonatea (17)	2
		Brachycorythis (36)	3
		Centrostigma* (3)	0
		Chamorchis (1)	1
		Chusua (20)	0
		Comperia (1)	1
		Cynorkis (158)	16
		Dactylorhiza (48)	5
		Diphylax* (4)	1
		Diplomeris* (5)	0
		Galearis (1)	1
		Gennaria (1)	1
		Gymnadenia (24)	2
		Habenaria (848)	85
		Hemipilia (18)	2
		Herminium (28)	2
		Himantoglossum (7)	2
		Holothrix (44)	3
		Megalorchis* (1)	0
		Neobolusia* (4)	0
		Neotinea (4)	1
		Neottianthe (8)	1
		Ophrys (60)	6
		Orchis (27)	5

		Pachites* (2)	0
		Pecteilis (4)	1
		Peristylus (102)	11
		Physoceras* (11)	0
		Platanthera (135)	14
		Platycoryne* (17)	0
		Porolabium* (1)	0
		Pseudorchis (1)	1
		Roeperocharis* (5)	0
		Satyrium (82)	8
		Schizochilus* (11)	2
		Serapias (14)	2
		Smithorchis* (1)	0
		Stenoglottis (4)	2
		Steveniella (1)	1
		Symphyosepalum* (1)	0
		Thulinia* (1)	0
		Traunsteinera (2)	2
		Tsaiorchis* (1)	0
		Tylostigma* (8)	0
EPIDENDROIDEAE (19770)			0
		Claderia* (2)	1
	Neottieae (191)	Aphyllorchis* (21)	2
		Cephalanthera (20)	2
		Epipactis (64)	2
		Limodorum (3)	2
		Neottia (63)	6
		Palmorchis (20)	2
	Sobralieae (237)	Elleanthus (106)	12
		Epilyna* (3)	2
		Sertifera* (8)	3
		Sobralia (120)	15
	Tropidieae (35)	Corymborkis (6)	2
		Tropidia (29)	2
	Triphoreae (28)	Diceratostele (1)	1
		Monophyllorchis (2)	2
		Psilochilus* (7)	1
		Triphora (18)	2
	Nervilieae (73)	Nervilia (65)	2
		Epipogium (4)	1
		Silvorchis* (1)	0
		Stereosandra* (1)	0
		Xerorchis (2)]	1
	Gastrodieae (70)	Auxopus (3)	0

		Didymoplexiella (6)	0
		Didymoplexis (17)	2
		Gastrodia (41)	4
		Neoclemensia* (1)	0
		Uleiorchis (2)	0
	Calypsoeae (70)	Aplectrum (1)	1
		Calypso (1)	1
		Changnienia (1)	1
		Corallorhiza (11)	2
		Cremastra (3)	1
		Dactylostalix (1)	1
		Didiciea (2)	0
		Ephippianthus (2)	1
		Govenia (19)	2
		Oreorchis (18)	2
		Tipularia (5)	2
		Yoania* (3)	1
		[Wullschlaegelia (3)]	1
	EPIDENDREAE (5870)		0
		Chysis (8)	2
		Coelia (5)	2
	Ponerinae (22)	Helleriella (2)	1
		Isochilus (12)	2
		Ponera (8)	2
	Bletiinae (48)	Basiphyllaea (7)	2
		Bletia (34)	4
		Hexalectris (7)	2
	Pleurothallidinae (3999)	Acianthera (131)	15
		Anathallis (89)	10
		Andinia (24)	4
		Anthereon (6)	2
		Barbosella (18)	2
		Brachionidium (65)	7
		Chamelophyton (1)	0
		Dilomilis (5)	1
		Diodonopsis (5)	1
		Dracula (111)	12
		Dresslerella (9)	4
		Dryadella (42)	4
		Echinosepala (8)	2
		Frondaria (1)	1
		Lepanthes (931)	90
		Lepanthopsis (38)	5

		Masdevallia (507)	50
		Myoxanthus (44)	10
		Octomeria (143)	10
		Phloeophila (14)	0
		Platystele (91)	10
		Pleurothallis (813)	80
		Pleurothallopsis (30)	5
		Porroglossum (34)	5
		Restrepia (48)	5
		Restrepiella (1)	1
		Scaphosepalum (41)	5
		Specklinia (90)	10
		Stelis (490)	50
		Teaguia (10)	10
		Tomzanonia (1)	0
		Trichosalpinx (122)	15
		Trisetella (22)	5
		Zootrophion (12)	5
	Laeliinae (1788)	Acrorchis (1)	1
		Alamania (1)	1
		Arpophyllum (4)	1
		Artorima (1)	1
		Barkeria (15)	3
		Brassavola (20)	5
		Broughtonia (6)	2
		Cattleya (54)	5
		Caularthron (4)	2
		Dimerandra (6)	2
		Dinema (2)	1
		Domingoa (2)	1
		Encyclia (154)	15
		Epidendrum (1125)	112
		Euchile (2)	2
		Hagsatera (2)	0
		Hexisea (5)	2
		Homalopetalum (4)	2
		Isabelia (1)	1
		Jacquiniella (6)	2
		Laelia (11)	2
		Lanium (9)	2
		Leptotes (6)	2
		Loefgrenianthus (1)	0
		Meiracyllium (2)	1
		Myrmecophila (10)	2

		Nageliella (2)	1
		Nanodes (2)	1
		Neocogniauxia (2)	1
		Nidema (2)	1
		Oerstedella (29)	5
		Oestlundia (11)	2
		Orleanesia (9)	0
		Pinelia (4)	0
		Platyglottis (1)	1
		Prosthechea (93)	10
		Pseudolaelia (8)	2
		Psychilus (15)	2
		Pygmaeorchis* (2)	0
		Quisqueya (4)	1
		Renata (1)	1
		Rhyncholaelia (2)	2
		Scaphyglottis (63)	6
		Schomburgkia (15)	2
		Sophronitis (57)	12
		Tetramicra (14)	2
	Podochileae(1232)		0
	Eriinae (725)	Ascidieria (1)	1
		Ceratostylis (145)	15
		Cryptochilus* (4)	0
		Epiblastus (22)	2
		Eria (404)	4
		Mediocalcar (24)	2
		Porpax (13)	0
		Pseuderia (19)	0
		Sarcostoma (5)	2
		Stolzia (15)	2
		Trichotosia (73)	2
	Podochilinae (208)	Appendicula (142)	14
		Chitonochilus* (1)	0
		Poaephyllum* (6)	1
		Podochilus (59)	2
	Thelasinae (299)	Chitonanthera* (24)	0
		Octarrhena* (41)	0
		Phreatia (201)	2
		Rhynchophreatia (9)	1
		Ridleyella (1)	0
		Thelasis (23)	1
	Arethuseae (701)		0
	Arethusinae (9)	Anthogonium (1)	1

		Arethusa (1)	1
		Arundina (1)	1
		Calopogon (5)	2
		Eleorchis (1)	0
	Coelogyninae (692)	Bletilla (5)	2
		Bracisepalum (2)	0
		Bulleyia (1)	0
		Chelonistele (12)	1
		Coelogyne (182)	20
		Dendrochilum (264)	28
		Dickasonia (1)	0
		Dilochia (8)	1
		Entomophobia (1)	0
		Geesinkorchis (2)	1
		Glomera (127)	13
		Ischnogyne (1)	0
		Nabaluia (3)	1
		Neogyna (1)	0
		Otochilus (5)	0
		Panisea (8)	1
		Pholidota (43)	2
		Pleione (20)	2
		Thunia (5)	2
	Malaxideae (1158)	Crossoglossa (21)	2
		Hippeophyllum* (13)	0
		Liparis (418)	4
		Malaxis (395)	4
		Oberonia* (308)	30
		Orestias* (3)	0
		Risleya* (1)	0
	Cymbidieae (3814)		0
	Bromheadiinae (28)	Bromheadia* (28)	2
	Catasetinae (367)	Catasetum (157)	16
		Clowesia (7)	3
		Cycnoches (33)	5
		Cyrtopodium (44)	4
		Dressleria (10)	4
		Galeandra (34)	2
		Grobya (4)	2
		Mormodes (78)	8
	Cymbidiinae (67)	Cymbidium (51)	12
		Grammatophyllum (11)	2
		Graphorkis (4)	2
		Porphyroglottis* (1)	0

	Eulophiinae (316)	Acriopsis (7)	2
		Acrolophia* (7)	0
		Ansellia (1)	1
		Cymbidiella (3)	2
		Cynaeorchis* (2)	1
		Dipodium (24)	2
		Eulophia (211)	20
		Eulophiella* (5)	2
		Geodorum* (13)	2
		Grammangis (2)	2
		Oeceoclades (38)	2
		Thecopus* (2)	0
		Thecostele (1)	1
	Eriopsidinae (5)	Eriopsis (5)	2
	Oncidiinae (1589)	Ada (16)	5
		Amparoa (1)	1
		Antillanorchis* (1)	0
		Aspasia (7)	7
		Brachtia (7)	2
		Brassia (34)	5
		Caluera (2)	0
		Capanemia (14)	2
		Caucaea (20)	3
		Centroglossa (5)	0
		Chytroglossa (3)	0
		Cischweinfia (11)	2
		Cochlioda (7)	4
		Comparettia (7)	3
		Cuitlauzina (1)	1
		Cypholoron* (2)	0
		Cyrtochiloides (3)	3
		Cyrtochilum (119)	20
		Diadenium* (2)	2
		Dignathe (1)	1
		Dunstervillea (1)	0
		Eloyella (6)	2
		Erycina (7)	2
		Fernandezia (9)	3
		Gomesa (17)	2
		Goniochilus (1)	1
		Hintonella (1)	1
		Hofmeisterella (1)	1
		Hybochilus (1)	1
		Ionopsis (6)	3

		Leochilus* (11)	5
		Lockhartia (27)	6
		Macradenia (11)	2
		Macroclinium (38)	4
		Mesospinidium (7)	3
		Miltonia (10)	4
		Miltoniopsis (5)	3
		Neokoehleria* (10)	2
		Notylia (58)	6
		Odontoglossum (69)	12
		Oncidium (336)	50
		Ornithocephalus (44)	8
		Osmoglossum (7)	2
		Otoglossum (13)	5
		Pachyphyllum (39)	4
		Palumbina (1)	1
		Papperitzia* (1)	1
		Pfitzeria (1)	1
		Phymatidium (8)	2
		Platyrhiza (2)	0
		Plectrophora* (10)	2
		Polyotidium* (1)	1
		Psychopsiella* (1)	1
		Psychopsis (5)	5
		Pterostemma* (2)	1
		Quekettia* (7)	0
		Raycadenco* (1)	0
		Rodriguezia (48)	5
		Rodrigueziella (6)	2
		Rodrigueziopsis* (2)	1
		Rossioglossum (4)	2
		Rauhiella (3)	0
		Rhynchostele (16)	5
		Sanderella* (2)	1
		Saundersia* (3)	1
		Scelochiloides* (3)	2
		Scelochilopsis (1)	0
		Scelochilus* (48)	8
		Seegeriella (1)	1
		Sigmatostalix (56)	8
		Solenidiopsis (4)	2
		Solenidium* (2)	2
		Stellilabium (34)	5
		Stictophyllorchis* (2)	0

		Stigmatorthos (1)	0
		Suarezia* (1)	1
		Sutrina* (1)	1
		Symphyglossum (4)	2
		Systeloglossum (5)	2
		Telipogon (133)	8
		Thysanoglossa (2)	0
		Ticoglossum (2)	2
		Tolumnia (36)	10
		Trichocentrum (69)	7
		Trichoceros (9)	2
		Trichopilia (26)	7
		Trizeuxis (1)	1
		Warmingia (6)	2
		Zelenkoa (1)	1
		Zygostates (19)	2
	Maxillariinae (749)	Anguloa (11)	2
		Anthosiphon (1)	1
		Bifrenaria (26)	16
		Chrysocycnis (4)	2
		Cryptocentrum (19)	6
		Cyrtidiorchis (4)	1
		Horvatia* (1)	0
		Ida (?)	2
		Lycaste (50)	5
		Maxillaria (552)	60
		Mormolyca* (8)	2
		Neomoorea (1)	1
		Pityphyllum (5)	5
		Rudolfiella (6)	2
		Scuticaria (8)	2
		Teuscheria* (7)	2
		Trigonidium (14)	4
		Xylobium (32)	4
	Stanhopeinae (257)	Acineta (15)	5
		Archivea (1)	0
		Braemia (1)	1
		Cirrhaea (9)	2
		Coryanthes (38)	5
		Embreea (1)	1
		Gongora (58)	5
		Horichia (1)	1
		Houlletia (9)	2
		Kegeliella (3)	2

		Lacaena (2)	1
		Lueckelia (1)	1
		Lueddemannia (1)	1
		Paphinia (16)	5
		Polycycnis (15)	5
		Schlimmia (8)	2
		Sievekingia (16)	3
		Soterosanthus (1)	1
		Stanhopea (55)	5
		Trevoria (6)	2
		Vasqueziella (1)	1
	Coeliopsidinae (18)	Coeliopsis (1)	1
		Lycomormium (6)	2
		Peristeria (11)	2
	Zygopetalinae (418)	Aganisia* (3)	1
		Batemannia (5)	2
		Benzingia* (2)	1
		Bollea* (12)	4
		Chaubardia* (5)	5
		Chaubardiella* (8)	5
		Cheiradenia* (1)	0
		Chondrorhyncha (30)	5
		Chondroscaphe (12)	5
		Cochleanthes* (14)	5
		Cryptarrhena (4)	2
		Dichaea (110)	25
		Dodsonia* (2)	1
		Galeottia* (12)	2
		Hirtzia (2)	0
		Hoehneella* (1)	0
		Huntleya* (13)	4
		Kefersteinia* (61)	8
		Koellensteinia (19)	2
		Neogardneria* (1)	0
		Otostylis* (4)	1
		Pabstia* (6)	1
		Paradisanthus* (4)	1
		Pescatorea (16)	4
		Promenaea* (19)	2
		Stenia* (18)	2
		Vargasiella* (2)	0
		Warrea (4)	2
		Warreella* (2)	1
		Warreopsis* (4)	1

		Zygopetalum (14)	2
		Zygosepalum* (8)	2
	Vandeae (2341)		0
	Polystachyinae (228)	Hederorkis* (2)	0
		Imerinaea* (1)	1
		Neobenthamia (1)	1
		Polystachya (224)	25
	Aeridinae (1352)	Abdominea* (1)	1
		Acampe* (7)	2
		Adenoncos* (16)	2
		Aerides* (25)	12
		Amesiella* (3)	1
		Arachnis* (11)	4
		Armodorum* (3)	0
		Ascocentrum* (13)	2
		Ascochilopsis* (2)	1
		Ascochilus* (6)	0
		Ascoglossum* (1)	1
		Biermannia* (10)	0
		Bogoria* (4)	0
		Brachypeza* (7)	0
		Calymmanthera* (5)	0
		Ceratocentron* (1)	0
		Ceratochilus* (2)	1
		Chamaeanthus* (2)	0
		Chiloschista* (20)	2
		Christensonia (1)	1
		Chroniochilus* (4)	0
		Cleisocentron* (3)	0
		Cleisomeria* (2)	0
		Cleisostoma (87)	9
		Cordiglottis* (7)	0
		Cottonia* (1)	0
		Cryptopylos* (1)	0
		Dimorphorchis* (2)	1
		Diplocentrum* (2)	0
		Diploprora (2)	1
		Dryadorchis (4)	0
		Drymoanthus (4)	0
		Dyakia (1)	1
		Eparmatostigma (1)	0
		Esmeralda* (2)	1
		Gastrochilus* (55)	6
		Grosourdya* (9)	1

		Gunnarella* (9)	0
		Haraella* (1)	1
		Holcoglossum* (10)	2
		Hygrochilus* (1)	0
		Hymenorchis* (10)	1
		Lesliea* (1)	0
		Loxomorchis* (3)	0
		Luisia* (39)	2
		Macropodanthus* (6)	0
		Malleola* (29)	2
		Megalotus* (1)	0
		Micropera* (18)	2
		Microsaccus* (13)	0
		Microtatorchis* (50)	2
		Mobilabium* (1)	0
		Neofinetia (2)	1
		Nothodoritis* (1)	0
		Omoea* (2)	0
		Ornithochilus* (3)	0
		Papilionanthe* (10)	2
		Papillilabium* (1)	0
		Paraphalaenopsis* (4)	2
		Parapteroceras* (5)	0
		Pelatantheria* (7)	2
		Pennilabium* (10)	0
		Peristeranthus* (1)	0
		Phalaenopsis (62)	12
		Phragmorchis* (1)	0
		Plectorrhiza* (3)	0
		Pomatocalpa* (33)	4
		Porphyrodesme* (3)	0
		Porrorhachis* (2)	0
		Pteroceras* (24)	2
		Renanthera* (17)	2
		Renantherella* (2)	0
		Rhinerrhiza* (1)	0
		Rhynchogyna* (3)	0
		Rhynchostylis* (3)	2
		Robiquetia* (38)	2
		Saccolabiopsis* (12)	0
		Saccolabium* (10)	0
		Sarcanthopsis* (1)	0
		Sarcochilus* (25)	2
		Sarcoglyphis* (12)	1

		Sarcophyton* (3)	1
		Schistotylus* (1)	0
		Schoenorchis (26)*	2
		Sedirea* (2)	0
		Seidenfadenia* (1)	1
		Smithsonia* (3)	0
		Smitinandia* (3)	1
		Staurochilus* (14)	2
		Stereochilus* (7)	2
		Taeniophyllum* (185)	10
		Thrixspermum* (144)	2
		Trichoglottis* (64)	6
		Tuberolabium* (12)	2
		Uncifera* (6)	0
		Vanda* (57)	6
		Vandopsis* (5)	2
		Ventricularia* (2)	0
		Xenikophyton* (1)	0
	Angraecinae (445)	Aeranthes (47)	5
		Ambrella* (1)	0
		Angraecum (219)	22
		Bonniera* (2)	1
		Calyptrochilum* (2)	1
		Campylocentrum (73)	5
		Cryptopus* (4)	2
		Dendrophylax (9)	5
		Harrisella (3)	1
		Jumellea (58)	2
		Lemurella* (4)	1
		Lemurorchis* (1)	1
		Listrostachys* (2)	1
		Neobathiea* (5)	2
		Oeonia (6)	2
		Oeoniella (2)	1
		Ossiculum* (1)	0
		Sobennikoffia (4)	2
	Aerangidinae (315)	Aerangis (49)	5
		Ancistrorhynchus* (16)	2
		Angraecopsis* (21)	2
		Beclardia* (1)	1
		Bolusiella* (6)	1
		Cardiochilos* (1)	0
		Chamaeangis* (10)	2
		Chauliodon* (1)	0

		Cribbia* (4)	1
		Cyrtorchis* (15)	2
		Diaphananthe (24)	2
		Dinklageella* (3)	0
		Distylodon* (1)	0
		Eggelingia* (3)	0
		Eurychone* (2)	1
		Margelliantha* (5)	1
		Microcoelia* (29)	2
		Microterangis* (7)	1
		Mystacidium* (9)	2
		Nephrangis* (2)	1
		Plectrelminthus* (1)	1
		Podangis* (1)	1
		Rangaeris* (7)	1
		Rhaesteria* (1)	0
		Rhipidoglossum* (37)	0
		Solenangis* (6)	1
		Sphyrarhynchus* (1)	1
		Summerhayesia* (2)	0
		Taeniorrhiza* (1)	0
		Triceratorhynchus* (1)	0
		Tridactyle* (43)	2
		Ypsilopus* (5)	1
	Unplaced subtribes within Epidendroideae (3963)		0
	Agrostophyllinae (196)	Adrorhizon* (1)	1
		Aglossorhyncha* (13)	1
		Agrostophyllum (91)	9
		Earina (6)	1
		Glossorhyncha* (80)	0
		Ischocentrum* (2)	0
		Sepalosiphon* (1)	0
		Sirhookera* (2)	1
	Dendrobiinae (3332)	Bulbophyllum (1789)	180
		Cadetia (60)	2
		Chaseella* (1)	0
		Dactylorhynchus* (1)	0
		Dendrobium (1184)	120
		Diplocaulobium (99)	2
		Drymoda* (3)	0
		Epigeneium (38)	4
		Flickingeria (69)	7

		Genyorchis* (7)	2
		Jejosephia* (1)	0
		Monomeria* (3)	1
		Monosepalum* (3)	0
		Pedilochilus* (35)	0
		Saccoglossum* (5)	0
		Sunipia* (22)	2
		Trias* (12)	2
	Collabiinae (435)	Acanthephippium (12)	2
		Ancistrochilus (2)	2
		Aulostylis* (11)	0
		Calanthe (187)	2
		Cephalantheropsis* (5)	1
		Chrysoglossum* (4)	1
		Collabium (14)	2
		Diglyphosa* (3)	1
		Eriodes* (1)	0
		Gastrorchis* (9)	2
		Hancockia* (1)	1
		Ipsea* (3)	0
		Mischobulbum (9)	2
		Nephelaphyllum (12)	2
		Pachystoma* (1)	1
		Phaius (48)	2
		Plocoglottis* (39)	2
		Spathoglottis (45)	2
		Tainia (29)	2
	Incertae sedis	Thaia (1)	0
	total		±2000