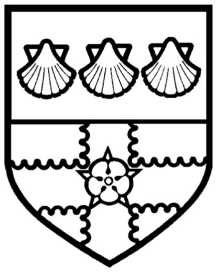

The University of Reading

MOLECULAR PHYLOGENETICS OF TRIBE EPIDENDREAE WITH EMPHASIS ON SUBTRIBE LAELIINAE (ORCHIDACEAE)

A thesis submitted to the University of Reading for the degree of Doctor of Philosophy

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ABSTRACT

In this project, the phylogenetic relationships of tribe Epidendreae, especially subtribe Laeliinae were assessed by using DNA sequence data. At the tribal level, I used data from three DNA regions, namely internal transcribed spacers of nuclear ribosomal DNA (ITS), and plastid *matK* (gene and spacers) and *trnL-F* (intron, exon and spacer). After individual and combined phylogenetic analysis using parsimony, it was possible to delimit Epidendreae as an exclusively Neotropical tribe (composed of subtribes Laeliinae, Pleurothallidinae, Ponerinae, Bletiinae and Chysinae). It is still unclear whether Coeliinae and Calypsoeae should be also included in Epidendreae. All Old World subtribes placed in Epidendreae in Dressler's (1993) system belong to different tribes of subfamily Epidendroideae. The revised subtribe Bletiinae is composed only of *Bletia, Hexalectris* and *Basiphyllaea.* All Old World genera previously placed in Bletiinae belong also to Old World groups. *Arpophyllum* (previously Arpophyllinae) and *Meiracyllium* (previously Meiracyllinae) should be included in Laeliinae. *Neocogniauxia* and *Dilomilis* belong to a clade sister to Pleurothallidinae. *Ponera, Isochilus* and *Helleriella* (previously in the *Scaphyglottis* alliance within Laeliinae) belong to a recircumscribed version of Ponerinae, which is sister to Bletiinae. Two other datasets were collected to investigate in more detail phylogenetic relationships within Laeliinae. The first dataset used 295 ITS sequences to assess generic delimitation and species phylogenies. Because the levels of variation were low, there was little resolution along the spine of the tree, and few generic groups achieved strong internal support. However, most species groups obtained were coincident with previous taxonomic groups at the infrageneric level, but several genera were found to be polyphyletic, including *Cattleya, Laelia, Encyclia,* and *Schomburgkia*. A second analysis of Laeliinae used the same three gene regions as in the Epidendreae study. This analysis found increased support for generic groups, confirmed polyphyly of several genera, and clarified unusual relationships in the ITS study. It also confirmed the suspicion that some ITS sequences were paralogous copies, although the underlying cause of the paralogy remains uncertain. Comparison of the three studies emphasise the importance of both taxon and character sampling in phylogenetic reconstruction.

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Introduction

The use of molecular data for systematic purposes has increased greatly in the last decade. Following the utilisation of isozyme electrophoresis in the 1960's and 70's, the application of methods that directly analyse DNA became predominant after 1985 (Crawford, 1990). These methods are particularly suitable for suprageneric taxonomy, at which level subjectivity in character choice makes more difficult the use of a classical morphological approach. The use of sequence data usually provides a large number of characters and the use of regions with different variability permits the methodology to be adapted for practically any group of organisms. However, the phylogeny of a given region may reflect only the evolution of that piece of DNA rather than organismic phylogeny. Accordingly, it is necessary to use more than one region and compare datasets to check the accuracy of the results (Crawford, 1990).

The use of plastid DNA for systematic purposes goes back to the early work of Vedel (1976), and there are good reviews of the results in Palmer et al. (1988) and Clegg and Zurawski (1993). The most commonly used gene has been *rbcL* (Chase et al., 1993). For the study of closely related genera it is generally necessary to use more variable regions, such as *trnL-F* and *matK*. The use of *matK* began with the work of Johnson and Soltis (1994, 1995). At the species level, it is often necessary to use even more variable regions, and recently the nuclear ITS region (internal transcribed spacers) has been widely used (see Baldwin, 1992, 1993; Suh et al., 1993; Kim and Jansen, 1994; Cox et al., 1997; Pridgeon et al., 1997; Ryan et al., 2000; Whitten et al., in press). ITS is bordered by conserved areas in the 18S and 26S ribosomal DNA that allows easy amplification by PCR.

Orchidaceae have been regarded as arguably the largest flowering plant family (Atwood, 1986; ca. 19,000 species). Some authors (e.g. Brieger 1961, 1976a, 1977) considered it a good model for the study of plant evolution, especially due to their great diversity and suitability for *ex-situ* collection. In spite of this, there has been comparatively less botanical research in this family than in other large families such as Fabaceae and Asteraceae. This fact could be explained by their morphological complexity and predominantly tropical distribution (Chase and Palmer 1993).

Until recently, little was known of orchid phylogeny, except some hypotheses based in morphology without an explicit cladistic analysis (Dressler and Dodson, 1960; Garay, 1960; Dressler, 1981). Burns-Balogh and Funk (1986) and Dressler (1990b, 1993) took into account a cladistic framework, especially for column and seed-type characters, but again did not perform explicit algorithm-based analyses. The first phylogenetic studies in orchids with molecular data were done with restriction-site variation (Chase and Palmer, 1989; Chase and Palmer, 1993; Yukawa et al., 1993). The information from these works was quickly improved with studies using DNA sequencing (Chase et al., 1994; Neyland and Urbatsch, 1996). Recent studies have dealt with the overall phylogeny of the whole Orchidaceae, both with molecular (Cameron et al., 1999) and morphological (Clements, 1995; Freudenstein and Rasmussen, 1999) data.

At lower levels, the delimitation of tribes, subtribes and genera has been in conflict, this being due especially to the arbitrary choice of a few morphological characters (e.g. Schlechter, 1926; Brieger et al., 1970-1984; Dressler, 1981). DNA studies at the species level have helped to begin a major redelimitation of these taxa (Cypripedioideae; Cox et al., 1997; Orchidinae, Pridgeon et al., 1997; Orchidoideae including Spiranthoideae, Kores et al., 1997, 2000; Vanilloideae and Pogoniinae, Cameron, 1996; Cameron et al., 1999; Maxillarieae, Whitten et al., in press)

When I first started to work with morphometrics and taxonomy of *Cattleya* (van den Berg, 1996; van den Berg and Martins, 1998) the need for phylogenetic information in Laeliinae soon became clear. I found two main problems in the subtribe, the first being the fact that Laeliinae genera had changed little from the original concepts proposed by Lindley (Lindley, 1821, 1830, 1853b). Researchers only removed morphologically unusual species to new, smaller genera without questioning the concepts of the larger genera, which were defined by simple clearcut morphological characters. A second problem was that there were recent revisions only for the genera considered horticulturally important (Braem, 1984; 1986; Withner, 1988, 1990, 1993, 1996, 1998, 2000), and these revisions were aimed primarily at horticulturists. They did not take into account a phylogenetic framework, and contained inaccurate nomenclature (van den Berg, 1999; van den Berg and Chase, 2000). Because these revisions were only a compilation of previous works, there was almost no change in the generic delimitation. I found that updating such nomenclature would make little sense without

first having a fresh understanding of the phylogeny of this group, and therefore dealing with broad reorganisation of the subtribal and generic limits. The only available data were the anatomical data of Baker (1972). Although his sampling was extensive, the results were less clear, and an attempt at analysing his data using cladistic methods produced almost no answers (van den Berg, unpubl.).

As I started to collect DNA data of ITS and *matK*, it soon became clear that a large amount of information could be generated. I chose initially to adopt two different approaches: a large dataset of ITS with as many taxa as I was able to sample (Chapter 3), and a more restricted sampling of taxa that allowed analysis of several DNA regions (ITS, *trnL-F* and *matK*; Chapter 4). The former approach was necessary to assure a clear delimitation for the genera as well as to identify subgroups in the genera to be assessed in the second analysis. The second analysis aimed at having more variation to resolve deeper nodes and assessing congruence between plastid and nuclear genomes. I tried also to include most subgroups that were used in previous taxonomic work. *Epidendrum* is a large genus (ca. 1000 spp.), composed of many widely recognised subgroups. Such a species-rich group would by itself constitute a subject for a thesis. Accordingly, I decided to have only limited sampling of various members of *Epidendrum* and a single or few species of related genera, to evaluate whether they would be distinct from the core Laeliinae in the study.

For the identification of sister groups and delimitation of Laeliinae, I included members of several subtribes on Epidendreae *sensu* Dressler (1993). Comparing my *matK* data (Chapter 4) with other orchid sequences available (D. Goldman, P. Kores, W. M. Whitten, A. Pridgeon, unpublished data) and with the study of Cameron et al. (1999) soon revealed the need for clearer delimitation among the subtribes of Epidendreae and between Epidendreae and other tribes. Because of this I collected three new datasets (ITS, *matK* and *trnL-F*; Chapter 1) for a selection of taxa in Epidendroideae, with emphasis on Epidendreae and Arethuseae. The need of a larger amount of data was justified by the lack of variable positions and poor taxonomic sampling in previous studies of single DNA regions (Neyland and Urbatsch, 1996; Cameron et al., 1999; Freudenstein et al., 2000).

At the level of nomenclature much has been debated in recent years on how to reflect phylogenetic relationships in the context of Linnaean nomenclature. A group of researchers defends the abolition of the present Code (de Queiroz and Gauthier, 1990,

1992, 1994; de Queiroz, 1996; van Welzen, 1998) and proposes a new one based uniquely on dichotomous splits of trees without formal ranks. This culminated in the text of the "PhyloCode' (Cantino and de Queiroz, 2000). However, this draft is far from acceptance in the scientific community. Another group of researchers defends keeping the present code (Brummit, 1996b), although there is a dispute between those who want to name paraphyletic groups as taxa (Brummitt, 1996a, 1997; Sosef, 1997; Brummitt and Sosef, 1998) and those who prefer to recognise only strictly monophyletic groups as acceptable for naming taxa. The latter group uses the rationale summarised by Freudenstein (1998), accepting that trees in phylogenetic analyses are relationship hypotheses, and therefore reconstructed ancestral nodes do not necessarily constitute taxa that need to be named or assigned a rank. In this thesis I try to apply this concept, using as a primary principle maximising monophyly and as secondary principles maximising stability, phylogenetic information, support and finally ease of identification by external morphological characters (as outlined by Backlund and Bremer, 1998).

The molecular phylogenetic studies in this thesis should provide a clear framework for future taxonomic revisions in Laeliinae as well as evolutionary studies in ecology, character evolution and speciation. It will also help to identify smaller scale phylogenetic questions that need to be examined in more detail with molecular, anatomical and morphological work.

Chapter 1 – Phylogeny and delimitation of the tribe Epidendreae

1.1. INTRODUCTION

1.1.1. TAXONOMIC HISTORY OF TRIBE EPIDENDREAE

Since their description by Kunth (1815), Epidendreae have had a complicated taxonomic history, in which their concept was gradually made narrower and the number of components reduced. In the original concept of Kunth, Epidendreae included most tropical epiphytic orchids as opposed to Orchideae, which contained the terrestrial, temperate genera. Lindley (1853b) presented a system with seven tribes, and in his concept placed only four subtribes in Epidendreae (Coelogyninae, Isochilinae, Laeliinae and Bletiinae). However, he did not provide descriptions for these subtribal names, which were only validly published later (Bentham, 1881; Szlachetko, 1991).

Bentham (1881) and Bentham and Hooker (1883) presented a system for the Orchidaceae with five tribes: Epidendreae, Vandeae, Neottieae, Orchideae and Cypripedieae. Epidendreae were composed of nine subtribes, namely Pleurothallidinae, Microstylinae, Liparidinae, Dendrobiinae, Eriinae, Bletiinae, Coelogyninae, Stenoglossinae and Laeliinae. In their system, *Epidendrum* was part of Laeliinae, whereas *Arpophyllum* and *Meiracyllium* were considered part of Pleurothallidinae.

Schlechter (1926) included *Epidendrum* in the tribe Kerosphereae, so this tribe would be the equivalent concept to Epidendreae. Schlechter's system within Epidendreae was highly artificial, and included 47 subtribes and several intermediate ranks, which correspond roughly to the subfamily Epidendroideae *sensu* Dressler (1993). Mansfeld (1937) provided a version of this same system with very little modification, but he split Kerosphereae into Epidendreae and Vandeae.

Dressler and Dodson (1960) published the first attempt to bring orchid classification in line with the International Code of Botanical Nomenclature, applying the correct suffixes and the principle of priority. Their system is in many aspects similar to that of Schlechter (1926) and Mansfeld (1937). Orchidaceae were composed of two subfamilies, Cypripedioideae and Orchidoideae. Epidendreae were one of three tribes in

Orchidoideae, and were a fusion of Epidendreae and Vandeae of Mansfeld (1937), including 27 subtribes, again with most of the Epidendroideae *sensu* Dressler (1993).

Garay (1972) presented a summary of all previous classifications and also his own. He applied the name Epidendroideae as a subfamily distinct from Orchidoideae and Neottioideae, although Epidendroideae was essentially the same group as Kerosphereae of Schlechter (1926). Within the subfamily there were only Epidendreae and Vandeae, so in this respect his system was nearly identical to that of Mansfeld (1937).

Brieger et al. (1970-1984) used a more restricted concept of Epidendreae, being one of three tribes (Podochileae, Arethuseae and Epidendreae) placed in subfamily Epidendroideae. Epidendreae consisted of 11 subtribes: Corallorhizinae, Pleurothallidinae, Dendrobiinae, Bulbophyllinae, Sobraliinae, Liparidinae, Thuniinae, Collabiinae, Bletiinae, Adrorhizinae and Coelogyninae. Some atypical placements in his system were Meiracylliinae in tribe Podochileae and *Hexalectris* in Corallorhizinae. *Eria* and *Trichotosia* were placed in Dendrobiinae rather than in Podochileae. Brieger's system also placed Maxillarieae and Vandeae in a separate subfamily, Vandoideae.

Dressler (1979) defined most of the subfamilial system later presented in detail in Dressler (1981). The concept of tribe Epidendreae of Dressler and Dodson (1960) changed to become a subfamily concept, whereas the tribe changed to a restricted number of subtribes. Epidendroideae of Dressler (1979, 1981) was much narrower than Epidendreae of Dressler and Dodson (1960). A great change from his previous systems was the removal of the vandoid orchids to their own subfamily, as in Brieger's system. Moreover, within Epidendroideae, subtribes that had previously been in Epidendreae were placed in new tribes, such as Vanilleae, Gastrodieae, Epipogieae, Arethuseae, Cryptarrheneae and Calypsoeae. Epidendreae retained ten subtribes: Eriinae, Podochilinae, Thelasinae, Glomerinae, Laeliinae, Meiracyliinae (erected by Dressler, 1971), Pleurothallidinae, Dendrobiinae, Bulbophyllinae and Sunipiinae. These included genera of the Old and New World. Although the subtribes of this system were somewhat similar to Brieger's, the generic composition of the subtribes was substantially different.

Burns-Balogh and Funk (1986) presented the first system to use a cladistic framework for the family. Their concept of Epidendroideae included ten subtribes: Gastrodieae, Triphoreae, Arethuseae, Vanilleae, Dendrobieae, Epidendreae,

Malaxideae, Coelogyneae, Maxillarieae, Vandeae and one informal group they called the "*Pleurothallis* group". Epidendreae were composed of only three subtribes, Laeliinae, Bletiinae and Sobraliinae, whereas Arethuseae were composed of the genus *Arethusa* alone. *Meiracyllium* was considered part of the "*Pleurothallis* group" rather than Epidendreae.

Dressler (1990b) re-united Epidendroideae with Vandoideae, and included Neottieae in this subfamily (it was placed in Orchidoideae by Dressler, 1981, and Neottioideae by Brieger et al., 1970-1984). Epidendreae were one of 17 tribes placed in Epidendroideae and only had five New World subtribes: Arpophyllinae (new), Meiracylliinae, Coeliinae (new), Laeliinae and Pleurothallidinae. Dendrobiinae, Bulbophyllinae and Sunipiinae were removed to Dendrobieae, whereas Eriinae and Podochilinae were placed in Podochileae. Dressler (1993) presented a similar system: Epidendroideae with 16 tribes grouped in three informal categories he called 'phylads'. His concept of Epidendreae was enlarged in relation to Dressler (1990b) to include Sobraliinae and some Old World subtribes such as Polystachyinae, Glomerinae and Adrorhizinae. Dressler divided the Epidendreae in two informal geographic groups that he called Epidendreae I (New World) and Epidendreae II (Old World).

The system of Szlachetko (1995) was substantially different from all previous systems. Arpophylliinae and part of Sobraliinae were removed to several subtribes in a new tribe Elleantheae. *Sobralia* was in a monogeneric subtribe, Sobraliinae, which he placed in Arethuseae (Vanilloideae). Polystachyinae was placed in Polystachyeae, Glomerinae in the Podochileae and Adrorhizon in Adrorhizeae. On the other hand, he placed Chysiinae in Epidendreae (which was placed in the Arethuseae of Dressler, 1993), and split the Laeliinae into three different subtribes: Laeliinae, Epidendrinae and Ponerinae.

1.1.2. PHYLOGENETIC AFFINITIES OF EPIDENDREAE

Dressler and Dodson (1960) gave a few hints of their ideas of the relationships of subtribes in Epidendreae, presented in the form of a diagram of relationships. Two main groups of subtribes were apparent. One had Laeliinae (as Epidendrinae) placed in the centre with three peripheral groups. In this first group, Dendrobiinae, Pleurothalidinae, Adrorhizinae, Coelogyninae and Bletiinae were considered closely related to Laeliinae, whereas Sobraliinae, Thelasiinae and Thuniinae were more distant

or of unknown relationships. Bletiinae was transitional to a second group of subtribes, which were primarily vandoid subtribes. It is unclear if they intended to suggest that Bletiinae were somehow ancestral to both groups of subtribes. Dressler (1981) presented a cladogram-like scheme for Epidendroideae, which placed the Epidendreae as sister to a group composed by the Malaxideae, Arethuseae and Coelogyneae. In the text he mentioned 'the Epidendreae seem clearly related to the Arethuseae, and surely have a common ancestry with that group', which contradicted in part the diagram. Dressler (1993) presented a diagram that showed an epidendroid 'phylad' with Arethuseae and Coelogyneae in the basal position and then places the Old and New World Epidendreae as a grade paraphyletic into Dendrobieae, Podochileae and Vandeae. Burns-Balogh and Funk (1986) suggested that the most primitive groups in Epidendroideae are Gastrodieae and Triphoreae. Epidendreae were sister to the '*Pleurothallis* group' near Dendrobieae, whereas Arethuseae were sister to Vandeae (Fig 1.1).

Chase et al. (1994) provided the first attempt of a phylogenetic analysis of Epidendroideae using *rbcL* sequence data. Epidendreae were not represented, but *Sobralia, Tropidia* and *Nervilia* were indicated as sister to the rest of the subfamily. Neyland and Urbastch (1996) provided another analysis based on the plastid coding *ndhF* gene. Their sampling was limited (36 taxa) and there was too little sequence divergence at the tribal and subtribal level. Although it was possible to identify *Sobralia* and *Listera* as lower epidendroids, their analyses produced an unresolved polytomy for most of the subfamily. Some patterns appearing in their trees were not present in Cameron et al. (1999), and among these, there is a clear Epidendreae composed of the New World subtribes Arpophyllinae, Laeliinae and Chysiinae. Bletiinae and Ponerinae were not sampled in their study, but the sister clade to the Epidendreae was a clade including *Coelia, Tipularia* (Calypsoeae) and *Dendrobium.* Their placement of *Polystachya* sister to Vandeae and Maxillarieae sister to Cymbidieae was also noteworthy. Freudenstein and Rasmussen (1999) presented the first morphological cladistic analysis of the whole Orchidaceae, in which members of Epidendreae *sensu* Dressler (1993) were placed in several different clades. *Sirhookera* (Adrorhizinae) and *Jensoa* (Calypsoeae) were successive sisters to a clade containing *Dactylostalix* (Calypsoeae), *Pleurothallis* (Pleurothallidinae), *Isochilus* (Laeliinae), *Glomera* (Glomerinae), *Agrostophyllum, Ceratostylis* and *Appendicula* (Podochileae),

Arpophyllum (Arpophyllinae), *Meiracyllium* (Meiracylliinae), *Epidendrum, Cattleya* and *Schomburgkia* (Laeliinae). Additionally, this clade was sister to another clade with two subclades, one with *Aplectrum, Calypso* and *Tipularia*, and the other with Cymbidieae, Maxillarieae, Polystachyinae and Vandeae. Sister to these four clades were *Calanthe, Plocoglottis* (Bletiinae) and *Liparis* (Malaxideae). The morphological analysis did not provide a clear distinction between Epidendreae and members of Calypsoeae and Podochileae. Cameron et al. (1999) performed a broad molecular study of the Orchidaceae based on the plastid gene *rbcL*. The levels of variation were enough to build a clear picture of the subfamilial relationships, but at the tribal and subtribal level there was low resolution. The epidendroid orchids were divided into a 'lower' grade and a set of 'higher' clades. Members of Epidendreae and Arethuseae were placed in several different clades, although without high bootstrap support. One moderately supported result was the placement of *Sobralia* and *Elleanthus* in the lower epidendroid clade, and therefore dinstantly related to the Epidendreae (in which they were placed by Dressler, 1993). Arethusinae and *Glomera* were placed with Coelogyninae, whereas *Dilomilis* was sister to the pleurothallids. *Bletia* and *Chysis* were in another clade, sister to *Tipularia* and *Calypso*, and Laeliinae had *Polystachya* embedded in it and was sister to Vandeae (Aeridinae, Angraecinae and Aerangidinae). Many of these relationships seem quite artifactual and differed from those in Neyland and Urbatsch (1996). However, they should not be taken as clear phylogenetic hypotheses because the levels of variation and bootstrap support in these trees were low. Another phylogenetic study was based on mitochondrial DNA data of the intron within the gene *nad1* (Freudenstein et al., 2000). It clearly placed *Cephalanthera* and *Epipactis* in the lower epidendroids. The topologies also showed a group with Laeliinae, Pleurothallidinae, *Bletia* and Calypsoeae, which are identifiable as Epidendreae. However, as in *rbcL*, the variation was low and consequently, only seven branches had bootstrap support above 70%. Many groups seem to be unlikely in the light of previous studies.

It seems quite difficult to discuss the placement of Epidendreae without comparing them to Arethuseae, and especially Bletiinae. Dressler (1993) suggested that the ancestor of advanced Epidendroideae was part of this group. Arethuseae display a mosaic of taxa showing gradual changes from soft, mealy pollen to hard, well-defined pollen masses. Whereas Brieger's system placed here several groups that based on recent molecular data (Cameron et al., 1999; Cameron and Chase, 2000, Goldman,

2000, Goldman et al., in press) belong to other subfamilies (Pogoniinae, Vanilinae), some are in the lower epidendroids (Gastrodieae, Nervilinae). Dressler's (1993) system used a restricted concept of Arethuseae, with only two subtribes: Arethusinae and Bletiinae. Although Goldman (2000) and Goldman et al. (in press) showed that Arethusinae are probably monophyletic, Bletiinae were indicated to be a completely artificial assemblage of genera (this pattern also emerged in Cameron et al., 1999). On the other hand, the *rbcL* and *matK* data used were probably not variable enough, and there has not been an extensive sampling of taxa in the Epidendreae and Coelogyneae to allow taxa in Arethuseae to be placed in these clades.

1.1.3. AIMS

In this study I aimed to delimit as clearly as possible Epidendreae by extensively sampling the putative component subtribes (based on the topologies of Neyland and Urbatsch, 1996; Cameron et al., 1999; Chapter 3, van den Berg et al. 2000, Chapter 4), and then using a broad sampling through Epidendroideae with an emphasis on Arethuseae. I used three lower epidendroids as outgroups and all major clades of advanced epidendroids were represented in the matrix in an effort to determine whether any genera currently considered to be Epidendreae in fact have different relationships, and belong to other clades.

1.2. MATERIAL AND METHODS

Plant material and voucher information for this analysis is given in Table 1.1. Outgroups *Epipactis helleborine* (L.) Crantz, *Cephalanthera damasonium* (Miller) Druce and *Listera smallii* Wiegand were chosen among the members of 'lower' Epidendroideae (Cameron et al., 1999; Freudenstein et al., 2000). Representatives of all other main clades of Epidendroideae were included. Within Epidendreae sampling aimed to have all a representation of all Old World and New World subtribes listed in Dressler (1993), with a larger sampling in subtribes that are more species rich, such as Laeliinae and Pleurothallidinae. Selected subgroups within Laeliinae were also sampled because we had previous knowledge of genera that were placed outside the subtribe such as *Ponera, Isochilus, Helleriella* (from Chapter 3 and 4), *Dilomilis* (Chapter 3 and 4, Cameron et al., 1999; Freudenstein et al., 2000), and *Basiphyllaea* (Goldman, 2000).

In Epidendreae I was unable to obtain material of *Adrorhizon* or *Sirhookera* and therefore the subtribe Adrorhizinae was not represented. *Sobralia* and *Elleanthus* were not included in the analysis because they have been shown to be distantly related to Laeliinae (Cameron et. al., 1999; Neyland and Urbatsch, 1999).

DNA was extracted mostly from fresh leaves, fresh flowers and silica gel dried leaves and flowers, using in most cases a modified version of the CTAB procedure of Doyle and Doyle (1987). For samples that presented difficulties due to polysaccharides, DNA was extracted using the Nucleon Phytopure Kit (Amersham Plc., Little Chalfont, UK). DNAs were purified either by caesium chloride/ethidium bromide gradient, or in QIAQuick silica columns (QIAGEN, Ltd.), and sometimes by a combination of both methods. Methodology for amplification and sequencing of ITS was as described in Chapter 3. For *trnL-F*, we used four universal primers (c, d, e, f) of Taberlet et al. (1991), and a program consisting of 28-30 cycles of 94 C denaturation for 1 min, 50C annealing for 30 s and 72 C of extension for 1 min. Most species were amplified and sequenced from primers c to f, but difficult samples had to be amplified in two halves with the consequent insertion of missing characters in the area corresponding to the primers d and e, which are a complements of each other. The *matK* region was amplified as a single piece, using the primers -19F (CGT TCT GAC CAT ATT GCA CTA TG; Molvray et al., 2000) and trnK-2R (AAC TAG TCG GAT GGA GTA; Johnson and Soltis, 1994). PCR conditions were a hot start with 2 min of initial denaturation at 94 C, followed by 28-30 cycles of 94 C denaturation, 52 C annealing for 45 s and 72 C for an initial time of 2 min 30 s with auto-extension of 8 s per cycle. Purification of PCR products was performed with QIAquick (QIAGEN, Ltd.) and Concert (Gibco BRL, Ltd.) silica columns. For ITS only, I added an extra wash with 35% guanidinium chloride solution to help in removing primer dimers. PCR products were sequenced in both directions, using the Big Dye Terminator Kit in an ABI 377 automated sequencer following manufacturer's protocols (PE Applied Biosystems, Inc., Warrington, Cheshire, UK). I used the same primers used in PCR and also matK-163F (AGT TTA GTR CTT GTG AAA CG; Molvray et al., 2000), matK-458F (CTA CTA ATA CCC YAT CCC ATC; Molvray et al., 2000), matK-556R (GAA GRA ACA TCT TTK ATC CA; Molvray et al., 2000), matK-731F (TCT GGA GTC TTT CTT GAG CGA; new), matK-881R (TTM TCA TCA GAA TAA GAG T; new), matK-877F (AGG AAC TCT TAT TCT GAT; Molvray et al., 2000), matK-1155F (TTC ACT TTT

GGT YTC ACC CT; new) and matK-1592R (TCA TGA ATG ATC CAC CAG A; Goldman, 2000). Electropherograms were assembled and edited using Sequencher 3.0 and 3.1 (Genecodes Inc., Ann Arbor, Michigan), and the resulting sequences were aligned by eye. Gaps were treated as missing characters, but I added a manually coded binary gap-matrix with all non-autapomorphic, unambiguous indels in the *trnL-F* and *matK* gene datasets. Gaps in the ITS dataset were not considered due to the less obvious alignment of this region among reasonably distant taxa. There were 30-40% of sequences missing in the upstream and downstream spacers of *matK*, and I decided not to code gaps in these regions. For the taxa available I added a matrix with *rbcL* data from Cameron et al. (1999). Analyses were performed using PAUP 4.0 (Swofford, 1998), with Fitch parsimony (equal weights, unordered; Fitch, 1971) as the optimality criterion. Initially, I performed two separate searches for the ITS dataset alone and for all plastid data (*trnL-F* regions, upstream and downstream *matK* spacers, *matK* gene and *rbcL*) combined. A third analysis included the data from all DNA regions. Each search consisted of 1000 random taxa-addition replicates, with the tree-bisection-reconnection (TBR) algorithm, and limited swapping on 15 trees per replicate to prevent extensive swapping on islands with many trees. The resulting trees were then used as starting trees for TBR swapping with an upper limit of 10,000 trees. Internal support was evaluated using 1000 replicates of character bootstrapping (Felsenstein, 1985), with simple taxon-addition and TBR algorithm, saving 15 trees per replicate. Sequences will be submitted to Genbank.

1.3. RESULTS

1.3.1. FEATURES OF THE DNA DATASETS

General features of the DNA regions used are presented in Table 1.2. The most variable dataset was ITS (64% potentially informative sites), but also the one with lower Consistency and Retention Index (CI=0.29 and RI=0.47). The *trnL-F* region, *matK* gene and spacers bordering *matK* had similar variation (around 25-27%), with CI between 0.51 (*matK* gene) and 0.62 (*matK* bordering spacers) and higher RIs than ITS (*trnL-F* region, 0.60; *matK* gene, 0.53; *matK* bordering spacers 0.62). *rbcL* was the least variable dataset with only 8.19% potentially informative sites, CI of 0.53 and RI of 0.47.

1.3.2. ANALYSIS OF INTERNAL TRANSCRIBED SPACER (ITS)

In the ITS analysis 648 trees of tree-length (L)=4764, CI=0.30 and RI=0.48 were found. *Basiphyllaea corallicola* and *Thelasis carinata* were excluded because I was unable to amplify and sequence ITS of these species. One of the trees (randomly chosen) is shown on Fig. 1.2. The strict consensus of all trees was well resolved and most branches that collapse occur only within subtribes. However, bootstrap percentages for most clades were below 50%. There is a clade recognisable as the New World Epidendreae, with *Chysis* as sister. Within these New World Epidendreae three main clades exist: the first contains Laeliinae, including *Arpophyllum* and *Meiracyllium*, the second Pleurothallidinae, including *Dilomilis* and *Neocogniauxia*, and the third Ponerinae plus Bletiinae (with only *Bletia, Hexalectris* and *Basiphyllaea*). The clade sister to Epidendreae included several subclades: Arethusinae (*Arethusa*, *Calopogon* and *Eleorchis*), most vandoid orchids (Vandeae, Maxillarieae, Cymbidieae and Polystachyiinae) and finally *Earina, Agrostophyllum* and *Nervilia*. The next are in a polytomy containing all the above plus a clade with most Coelogyneae, *Glomera*, Dendrobiinae and Bulbophyllinae, and another clade with Calypsoeae (*Calypso, Aplectrum* and *Govenia*) plus *Coelia*. The next clades on the tree consecutively are Podochileae, then *Ancistrochilus, Collabium* and *Nephellaphyllum*, then *Acanthephippium,* then *Calanthe* and *Phaius,* and finally a mixed clade with *Liparis*, *Malaxis, Angraecum* (vandoid) and *Phreatia*. Bootstrap support values for each clade are low and values larger than 50% occur in terminal groups rather than along the spine of the tree. Within Epidendreae some subtribes appear supported: Laeliinae (77%), Pleurothallidinae (76%), Ponerinae (89%) and Bletiinae (97%). The New World Epidendreae containing *Chysis* does not reach 50% support. In the rest of the tree fewer groups had internal support: Maxillarieae plus Cymbidieae (86%), Arethusinae (100%), Polystachyiinae (100%), Calypsoeae (70%) , Podochileae excluding *Ridleyella* (97%) and Phaiinae (*Phaius* plus *Calanthe*, 87%). *Angraecum magdalenae* had 96% support as sister to *Liparis* and *Malaxis* (this is likely to be a case of paralogy; see Discussion).

1.3.3. ANALYSIS OF PLASTID DATA (*TRNL-F, MATK* GENE AND SPACERS, *RBCL*)

In the analysis with plastid data I found more than 10,000 trees (limited in the search, L=4894, CI=0.56, RI=0.57). One of these trees (randomly chosen) is shown on Fig. 1.3. The strict consensus has many branches collapsing, but many terminal groups receive internal support. There is a large clade with New World Epidendreae, including also *Coelia* and *Chysis*. Laeliinae has as sister *Arpophyllum* (100% bootstrap), and includes *Meiracyllium* (embedded). The sister group (65% bootstrap) to this clade is Pleurothallidinae including *Dilomilis* and *Neocogniauxia* (97%). All the above are sister to a clade composed of Ponerinae and Bletiinae *sensu stricto*. *Chysis* and *Coelia* are consecutive sisters to the clades above. As sister to Epidendreae, there is a clade with *Govenia* and *Aplectrum* (New World) but also *Earina* and *Agrostophyllum* (Old World). *Calypso* is placed in an unresolved position. The remaining clades in Epidendroideae are in an unresolved polytomy with several internal clades. Among these, Vandeae is sister to Polystachyinae whereas Collabiinae (90%; including *Calanthe, Phaius, Collabium* and *Nephelaphyllum*) is sister to *Ancistrochilus* (less than 50% support). Arethuseae has two subclades: Coelogyninae (all taxa in Dressler, 1993, plus *Glomera, Thunia* and *Dilochia*) and Arethusinae (*Arethusa, Calopogon, Eleorchis, Anthogonium* and *Arundina*). These two latter relationships, however, had low bootstrap support. Another supported clade is Podochileae (88%), composed of Eriinae, Podochilinae, Thelasinae and Ridleyellinae. In unresolved positions were two small clades containing *Liparis* with *Malaxis* (100%; Malaxideae) and *Dendrobium* with *Bulbophyllum* (58%; Dendrobieae). Finally there is a vandoid clade (99% bootstrap) with Cymbidieae and Maxillarieae, and *Nervilia* is placed as sister to all Epidendroideae in the ingroup.

1.3.4. COMBINED ANALYSIS

A combined analysis with all the data from ITS and plastids produced 18 trees (Figs 1.4, 1.5, L=9818, CI=0.42, RI=0.51). Although there is a lack of internal support along the spine of the tree, the strict consensus is nearly fully resolved and the four branches that collapse are within subtribes. There is a clearly defined clade with the New World members of Epidendreae *sensu* Dressler (1993). The inclusion of *Chysis* in this clade has bootstrap support of 77%, but the inclusion of *Coelia*, although consistent

in all trees, receives less than 50% support. Within the Epidendreae, three main clades besides *Chysis* are clear: Laeliinae (including *Arpophyllum* and *Meiracyllium*, 100% support), Pleurothallidinae (99%), Bletiinae *sensu stricto* (100%) and Ponerinae (100%), the last two sister to each other (90%). The sister clade to Epidendreae has six subclades: Coelogyninae including *Glomera*, *Thunia* and *Dilochia*; the clade above sister to Arethusinae including *Anthogonium* and *Arundina*; and the two above sister to Dendrobieae. These are collectively sister to a clade that includes Cymbidieae plus Maxillarieae (88%), Vandeae plus Polystachyinae (less than 50% bootstrap) and *Earina* with *Agrostophyllum* (100%). To this larger clade, the successive sister groups are Podochileae (including *Ridleyella*; bootstrap less than 50%), then Malaxideae plus *Nephelaphyllum* and *Ancistrochilus*, then Collabiinae (syn. Phaiinae), and finally *Nervilia*.

1.4. DISCUSSION

1.4.1. MOLECULAR EVOLUTION

There is a noticeable difference in the levels of variation among the different DNA regions. As expected, the two ITS spacer regions have the fastest rate of change, being nuclear and non-coding. ITS1 and ITS2 have two times more variable sites than coding 5.8S, in relation to the total number of sites. The CI and RI of these regions are clearly lower than 5.8S and plastids, which can be explained in two different ways: one is a less obvious alignment of the sequences and the other taxon sampling. I favour the second, because the number of changes per site is three to four times higher than in the other DNA regions in this study, and therefore I expected both CI and RI to have substantially lower values when compared with the plastid regions. Increased taxon sampling might potentially increase RI values by recovering phylogenetic structure from most homoplasious characters. In all the plastid regions, there seems to be fewer differences among datasets. Although the alignment in the *matK* and *rbcL* datasets is unequivocal, *trnL-F* and the spacers bordering *matK* had higher RIs, suggesting they were more informative datasets in the combined analysis than the other regions. That could be explained for *rbcL* as being probably due to the low number of variable sites (and consequent low contribution to the tree topology), whereas in the *matK* gene the level of variation appears to be similar to the spacer regions. This fact in itself points out

how conserved the *trnL-F* region is for a non-coding piece of DNA (for a detailed discussion see Bakker et al., 2000) and also the surprisingly large number of variable sites in the *matK* gene. Although Kores et al. (2000) suggested that *matK* might be a pseudogene, I found little evidence of that, except the large percentage of variable sites. I found no internal stop codons, and all indels found were in triplets. First and second positions of *matK* evolved at similar rates, and there was an excess of third position substitutions, although by only 1.5 fold (against around 4-5 fold in *rbcL* and *atpB*; Savolainen et al., 2000). Transition/transversion ratio (ts:tv) in *matK* was around 1.0, whereas in *rbcL* it is 1.76, similar to other studies with plastid coding genes (e.g. Savolainen et al., 2000; *rbcL*=1.65, *atpB=2.09*). Despite these differences in the ts:tv ratio, it is unclear if I can conclude that *matK* would be a pseudogene from these parameters, without taking into consideration that *rbcL* and *atpB* are also much fewer variable sites.

1.4.2. COMPARISON WITH OTHER DNA STUDIES

As an overall comparison, my combined topologies of four genes were closer to the *ndhF* topologies of Neyland and Urbatsch (1996) and *nad1* intron of Freudenstein et al. (2000) than to the large *rbcL* study of Cameron et al. (1999). Although that is surprising considering that *rbcL* had a much more extensive sampling of taxa, it could be explained by the fact that *ndhF* has more variable positions, whereas *rbcL* has extremely few. The existence of Epidendreae composed of Pleurothallidinae, Laeliinae, Meiracyllinae, Chysiinae and the relationships of this cluster to *Coelia* and Calypsoeae were already clear in the *ndhF* maximum-likelihood tree and the parsimony tree of the *nad1* intron. Comparing these studies with our data shows clearly that the level of variation of *rbcL* was only satisfactory to resolve the subfamilies of orchids and relationships in groups that are supposed to have diverged early (e.g. Vanilloideae and lower epidendroids). It is unclear whether this is not pseudoresolution due to extiction on these old lineages, giving the impression of faster rates of change. In apparently more recently evolved groups such as Epidendroideae, the number of variable positions was low, and many unlikely placements might be due to character sampling error. A good example was the position of *Polystachya* embedded in Laeliinae and then sister to Vandeae, and the position of Pleurothallidinae with *Coelia*, *Acanthephippium* and *Calanthe.* It seems from the comparisons in our study that the best strategy to get even

more resolved topologies would be collecting more plastid spacers rather than coding genes like *rbcL* or *atpB*. To improve the information from ITS would require a more extensive sampling of taxa across Epidendroideae, because of the larger number of changes per site and also to clarify ambiguous sequence alignments.

1.4.3. DELIMITATION OF THE TRIBE EPIDENDREAE

From my results, Epidendreae appears to be composed exclusively of New World subtribes: Laeliinae, Pleurothallidinae, Ponerinae, Bletiinae and Chysinae. It is unclear whether Coeliinae should be included because although it is the sister group of Epidendreae in both the plastid and combined strict consensus, this relationships receives less than 50% booststrap support. In the ITS-only analysis, *Coelia* was placed together with Calypsoeae, distant from Epidendreae. Because the position of *Calypso, Govenia* and *Aplectrum* seems unstable, it is not clear whether *Coelia* belongs with them or with Epidendreae. Similarly, it is possible that these Calypsoeae could also be sister to Epidendreae.

The inclusion of *Arpophyllum* in Laeliinae and *Dilomilis* and *Neocogniauxia* in Pleurothallidinae receives high bootstrap support in all analyses. Both are small clades and sister to larger, species-rich subtribes. The branch length patterns among all the subtribes could support the maintenance of a separate subtribe for Arpophyllinae but probably not for 'Dilomilidinae'. The branch leading to most subtribes is around 50 steps, including the one placing *Arpophyllum* sister to Laeliinae. The branch separating *Arpophyllum* from the rest of Laeliinae is 27 steps, but the first internal branch in Laeliinae is just four steps, showing a clear demarcation. Similarly, the branch that makes 'Dilomilidinae' and Pleurothallidinae sisters is 51 steps, whereas the branch leading into 'Dilomilidinae' is 66 steps, but the first two next branches in Pleurothallidinae are 29 and 31 steps respectively.

These patterns are paralleled by morphology because 'Dilomilidinae' lack the generalised synapomorphy which defines the pleurothallids (e.g. articulation between ovary and pedicel). They basically have a reed-stem habit, whereas most Pleurothallidinae are not reed-stem (only *Frondaria* have a reed-stem-like habit that is not necessarily homologous to the one in other subtribes). In the case of *Arpophyllum*, plant habit is remarkably similar to some Laeliinae (e.g. *Laelia, Cattleya*), but also to *Octomeria* (pleurothallid). Its flower morphology is unique. Baker (1972) found that the

foliar anatomy of *Arpophyllum* is rather different from Laeliinae, and thus this similar external morphology could be interpreted as parallelism.

The generic composition of each subtribe has to be redefined in some cases, especially in Laeliinae and Bletiinae. ITS, plastid and the combined analyses gave a clear separation for all subtribes. Laeliinae should include all the genera listed in Dressler (1993), except *Isochilus, Ponera* and *Helleriella* (which should be included in Ponerinae), *Dilomilis* and *Neocogniauxia* (Pleurothallidinae) and *Basiphyllaea* (Bletiinae). Ponerinae is a resurrected subtribe with a new circumscription. Although the name was used in many systems (Schlechter, 1926; Brieger et al., 1970-1984; Szlachetko, 1995), it was always included all genera with column foot otherwise placed on Laeliinae. This study indicates that Ponerinae are a small subtribe including only *Ponera, Isochilus* and *Helleriella* (excluding *H. punctulata*). All other genera previously included (i.e. *Scaphyglottis* alliance and *Jacquiniella*) are part of Laeliinae, and *Helleriella punctulata* is in fact a member of *Scaphyglottis* (see Chapters 3, 4 for a discussion of this species). Bletiinae is part of Epidendreae rather than Arethuseae (as in Dressler, 1993). They are a small subtribe with only three New World genera: *Bletia, Hexalectris* and *Basiphyllaea.* All other genera placed in Bletiinae in Dressler (1993) and previous systems belong to different clades in Epidendroideae. Ponerinae and Bletiinae are placed sister to each other. They both have column foot and the main difference is that Ponerinae have a reed-stem habit, whereas Bletiinae are cormous. Meiracylliinae, placed outside Laeliinae in Freudenstein and Rasmussen (1999), Brieger et al. (1970-1984; Podochileae) and Dressler (1971, 1981, 1993) is deeply embedded in Laeliinae. The unusual column structure is considered a derived autapomorphic feature.

1.4.4. OTHER RELATIONSHIPS WITHIN THE EPIDENDROIDEAE

The relationships of the remaining Epidendroideae were more difficult to infer with my data due to limited sampling. Many interesting patterns are emerging because of the greater amount of data in this study compared with previous work. Although there were many differences between the individual and combined analyses, three clades appeared consistently. One of these was the Coelogyninae-Arethusinae clade (Fig 1.4). If these two clades are sister, they would represent a unique version of Arethuseae, different from that of any previous system. It is, however, a group that has similar morphological features, especially by having large heteroblastic pseudobulbs

and plicate leaves (or nearly so). *Arundina*, *Thunia* and *Dilochia* have a reed-stem habit, but share floral morphology with *Bletilla*. The Arethusinae clade, composed of *Arethusa, Calopogon* and *Anthogonium,* was also found in Goldman (2000) and Goldman et al. (in press), but the presence of *Arundina* as sister is unusual. The lack of bootstrap support might be an indication that this is sampling effect, and *Arundina* would move to the sister clade with *Thunia* and *Dilochia*, which seems more likely from a morphological standpoint. An alternative explanation would be that the morphology of these three genera is plesiomorphic in relation to both clades.

An unexpected result was the inclusion of *Glomera* in the Coelogyninae, a placement that occurred in Bentham's (1881) system, but no one has used subsequently. I sequenced a second species of the genus to confirm that the first had been correctly identified. Dressler (1993) had placed *Glomera* and many other Old World genera (such as *Earina* and *Agrostophyllum*) in Glomerinae (Epidendreae), which from my results is polyphyletic. The placement of Dendrobieae sister to this group is unusual and unsupported; it was present in the ITS dataset. Their placement with plastid data was unresolved.

The next sister clade to Arethuseae/Dendrobieae included the well-supported Maxillarieae/Cymbidieae (paraphyletic), together with Vandeae/Polystachyinae. This pattern clarifies the unsupported results of Cameron et al. (1999) which placed *Polystachya* near the Laeliinae, probably as consequence of character sampling error (see Discussion in section 1.4.2). Polystachyinae being sister to Vandeae and then sister to Cymbidieae with Maxillarieae is in complete agreement with the diagram presented in Dressler (1981, p. 155). All these taxa have complex pollinaria with well-developed stipes, caudicles and viscidia. Their columns are also similarly complex with arms and wings of various forms. Although this is a natural group, the levels of differentiation and embedded position in Epidendroideae demonstrate clearly that treating them as a separate subfamily Vandoideae (Dressler, 1981, non-1993) is inappropriate. *Earina* and *Agrostophyllum* (part of Glomerinae in Dressler, 1993) were placed without support as sister, but were themselves a well-supported group

The third clade that occurred consistently in the analyses was the Podochileae, including Podochilinae and Thelasinae, with good support. The existence of Eriinae as a separate subtribe from Podochilinae has to be analysed with increased sampling in this group. Ridleyellinae was placed sister to this clade in the combined analysis, although

without support, but this appears to be a clear relationship for it occurred in all three separate analyses. Ridleyellinae was placed here as a separate subtribe initially by Brieger et al. (1970-1984), considered to be a member of Thelasiinae by Dressler (1981) and again placed in a separate subtribe by Dressler (1993), although in the text he mentioned it probably was part of Thelasiinae.

Finally, Malaxidae, *Nervilia, Acanthephippium* and Collabiinae (*Collabium, Phaius* and *Calanthe*) were sister to all Epidendroideae included in this analysis. These position of these clades demonstrate the problem of finding the placement of all the genera in the Bletiinae *sensu* Dressler (1993). Although I was able to narrowly circumscript Bletiinae as a member of Epidendreae, the excluded genera are placed partly in Arethusinae and Coelogyninae (Arethuseae), and then in these other clades. The plastid analysis indicated the existence of Collabinae (90% bootstrap support; syn. Phaiinae) including *Collabium, Phaius, Calanthe, Nephellaphyllum* and *Acanthephippium*, whereas ITS data placed them paraphyletic to Malaxideae. Some of these genera appear superficially similar to Malaxideae (e.g. *Nephelaphyllum*) in habit and floral structure. Increased sampling in this group will be necessary to clarify the relationships between Collabiinae and Malaxideae. The position of *Ancistrochilus* was also unstable among the different analyses. It was placed in the Malaxideae/*Nephelaphyllum* clade with no support in the combined analysis (Fig. 1.4), due to the ITS characters which placed *Ancistrochilus* sister to *Nephelaphyllum* (Fig. 1.2). The plastid dataset placed it sister to Collabinae (Fig. 1.3) with less than 50% bootstrap support. *Ancistrochilus* has a very isolated geographic position in Central-Southern Africa, and these conflicting placements suggest the need for more DNA regions or closer taxa to solve its relationships. The presence of *Angraecum* sister to Malaxideae in the ITS study is probably due to paralogy. W. M. Whitten (unpublished data) found multiple copies in several species of Angraecinae and Aeridinae, and I found at least two. The second copy presented problems in sequencing (perhaps hairpins due to secondary structure) and I was unable to assemble a whole sequence. The partial sequence of this second copy was very sequence-divergent, suggesting that it is not the same copy as in other taxa in this study. Additionally, plastid data placed *Angraecum* together with other Vandeae with good support.

As a general conclusion from this study, Epidendreae can now be considered to be clearly delimited from Arethuseae and remaining Epidendroideae, and I achieved a

glimpse of the relationships within Epidendroideae, which was not possible with just single regions such as *rbcL*, *nad-1* intron, or *ndhF*. Clearly, the increased number of variable sites of plastid spacers and the faster rate of change of ITS increased significantly both the resolution and support for many groups. Increased taxon sampling and more DNA regions with variable positions will be necessary to achieve well-resolved, supported relationships among the different clades of Epidendroideae. Special attention should be given in future work to resolve groups here shown to be polyphyletic, such as Glomerinae, Collabinae and other genera formerly placed on Bletiinae.

Table 1.1. Plant material and vouchers used in this study.

Species	**Voucher**
Acanthephippium mantinianum L.Lind. & Cogn.	Chase O-397 (K)
Aeranthes grandiflora Lindl.	van den Berg C367 (K)
Agrostophyllum majus Hook.f.	Chase O-562 (K)
Amblostoma armeniacum (Lindl.) Brieger ex Pabst	Brieger Coll. 33081 (ESA)
Ancistrochilus rothschildianus J.O'Brien	Chase O-669 (K)
Angraecum magdalenae Schltr. & Perrier	Chase 9670 (K)
Anthogonium gracile Wall.	Chase O-538 (K)
Aplectrum hyemale Torr.	Chase O-104 (K)
Appendicula cornuta Blume	Chase O-560 (K)
Arethusa bulbosa L.	Goldman 446 (TEX)
Arpophyllum giganteum Hartw. ex Lindl.	Chase O-586 (K)
Arundina graminifolia (D.Don) Hochr.	Chase O-395 (K)
Basiphyllaea corallicola (Small) Ames	Ackerman and Axelrod 2381 (UPRRP)
Basiphyllaea hamiltonii ined.	Whitten 99108J-51 (FLAS)
Bletia catenulata Ruiz & Pav.	W. Forster 10 (ESA)
Bletia catenulata Ruiz & Pav.	E.L. Borba 590 (UEC)
Bletia purpurea (Lam.) DC.	van den Berg C342 (K)
Bletilla striata (Thunb.) Rchb.f.	Chase O-556 (K)
Broughtonia sanguinea (Sw.) R.Br.	Brieger Coll. 14440 (ESA)
Bulbophyllum lobbii Lindl.	Chase O-474 (K)
Calanthe calanthoides (A.Rich. & Gal.) Hamer & Garay	Chase O-819 (K)
Calanthe tricarinata Lindl.	Chase O-820 (K)
Calopogon oklahomensis D.Goldman	D. Goldman 553 (TEX)
Calypso bulbosa (L.) Oakes	Chase O-490 (K)
Cattleya labiata Lindl.	Brieger Coll. 5487 (ESA)
Cattleya violacea (Kunth) Rolfe	Brieger Coll. 28495 (ESA)
Caularthron bilamellatum (Rchb.f.) R.E.Schultes	Brieger Coll. 3690 (ESA)
Cephalanthera damasonium (Miller) Druce	Chase O-575 (K)
Chysis bractescens Lindl.	Chase O-436 (K)
Coelia macrostachya Lindl.	Chase O-817 (K)
Coelia triptera (Smith) G.Don ex Steud.	Chase O-324 (K)
Coelogyne cristata Lindl.	Chase O-491 (K)
Collabium sp.	Chase O-821 (K)
Cyrtopodium punctatum (L.) Lindl.	Chase O-126 (K)
Dendrobium kingianum Bidw. ex Lindl.	Chase O-164 (K)
Dendrochilum glumaceum Lindl.	Chase O-624 (K)
Dilochia sp.	Chase O-672 (K)

Table 1.1 (continued)

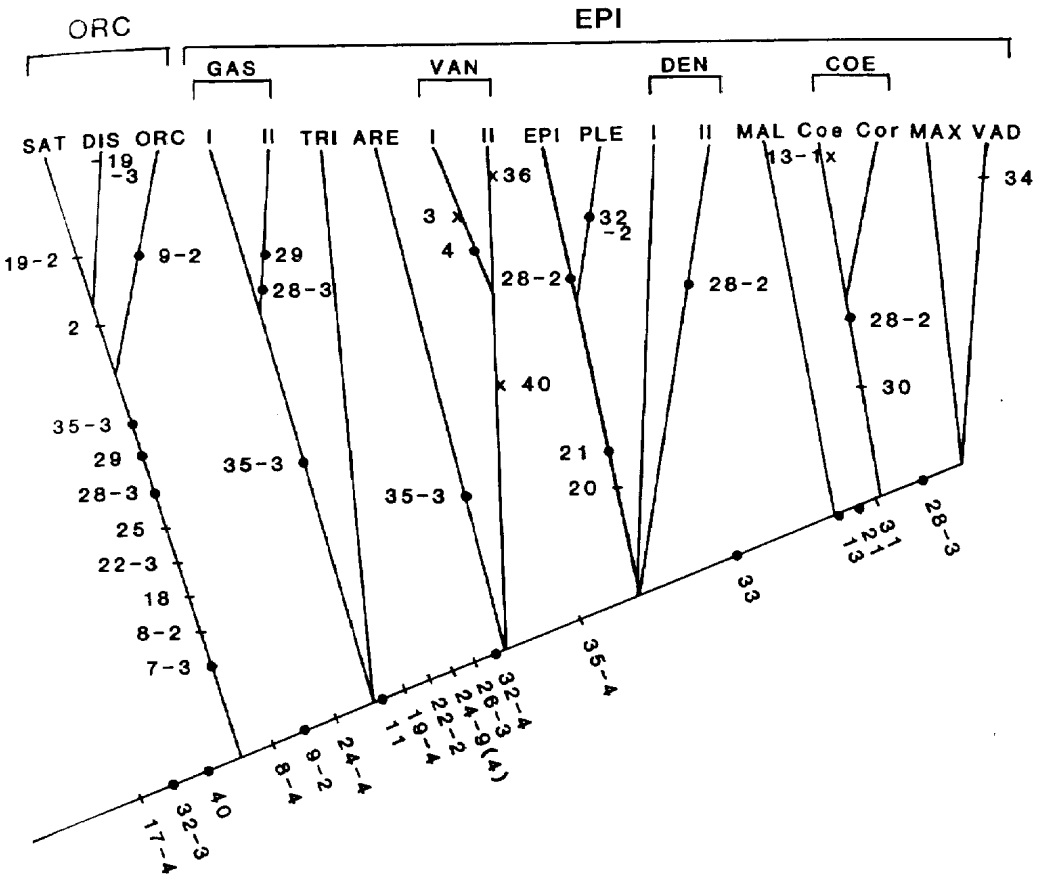
Species	**Voucher**
Dilomilis montana (Sw.) Summerh.	Chase O-206 (K)
Dinema polybulbon (Sw.) Lindl.	Brieger Coll. 6052 (ESA)
Dracula chimaera (Rchb.f.) Luer	Chase O-997 (K)
Earina autumnalis Hook.f.	Chase O-298 (K)
Earina valida Rchb.f.	Leiden 950080 (L)
Eleorchis japonica (A.Gray) Maekawa	Goldman 1103 (TEX)
Encyclia oncidioides (Lindl.) Schltr.	Brieger Coll. 5420 (ESA)
Entomophobia kinabaluensis Ames (de Vogel)	Leiden 970404 (L)
Epidendrum campestre Lindl.	E. L. Borba 553 (UEC)
Epipactis helleborine (L.) Crantz	Chase O-199 (K)
Eria ferruginea Teijsm. & Binn.	Chase O-590 (K)
Eulophia guineensis Lindl.	Whitten s.n. (FLAS)
Glomera pulchra J.J.Smith	Leiden 960835 (L)
Glomera sp.	Chase O-555 (K)
Govenia liliacea Lindl.	G. Salazar 6160 (K spirit)
Grammatophyllum speciosum Blume	Chase 89103 (K)
Helleriella guerrerensis Dressler & Hágsater	van den Berg C172 (K spirit)
Helleriella punctulata (Rchb.f.) Garay & H.R.Sweet	Chase O-299 (K)
Hexadesmia crurigera Lindl.	Chase O-336 (K)
Hexalectris revoluta Correll	D. Goldman 1364 (TEX)
Isochilus amparoanus Schltr.	Chase O-204 (K)
Isochilus brasiliensis Schltr.	Brieger Coll. 33696 (ESA 35553)
Laelia harpophylla Rchb.f.	Brieger Coll. 6687 (ESA)
Laelia rupestris Lindl.	Brieger Coll. 843 (ESA)
Laelia speciosa (Kunth) Schltr.	unvouchered Chase O-6088
Leptotes bicolor Lindl.	Brieger Coll. 1968 (ESA)
Liparis liliifolia (L.) L.C.Rich. ex Lindl.	Chase O-214 (K)
Listera smallii Wiegand	Cameron 1001 (NCU)
Malaxis spicata Sw.	Chase O-377 (K)
Masdevallia floribunda Lindl.	Chase O-296 (K)
Masdevallia uniflora Ruiz & Pav.	Kew 1997-5356 (K)
Maxillaria violaceopunctata Rchb.f.	SEL 1981-2139 (SEL)
Meiracyllium gemma Rchb.f.	M. Soto 8731 (AMO)
Meiracyllium trinasutum Rchb.f.	Chase O-202 (K)
Neobenthamia gracilis Rolfe	van den Berg C304 (K)
Neocogniauxia hexaptera (Cogn.) Schltr.	van den Berg C244 (K)
Nephellaphyllum pulchrum Blume	Chase O-668 (K)
Nervilia shirensis Schltr.	Kew 1981-4967 (K)

Table 1.1 (continued)

Species	**Voucher**
Octomeria gracilis Lodd. ex Lindl.	Hermans 2334 (K spirit 58256)
Phaius minor Blume	Chase O-325 (K)
Phalaenopsis manii Rchb.f.	van den Berg C345 (K)
Phreatia tahitensis Lindl.	Chase O-561 (K)
Pleione chunii Tso	van den Berg C290 (K)
Pleurothallis ochreata Lindl.	Kew 1974-1034 (K)
Pleurothallis ruscifolia (Jacq.) R.Br.	Hermans 2625 (K)
Podochilus cultratus Lindl.	Chase O-559 (K)
Polystachya galeata (Sw.) Rchb.f.	van den Berg C283 (K)
Ponera australis Cogn.	Brieger Coll. 33642 (ESA)
Ponera exilis Dressler	M. Soto s.n., Paracho, Michoacán (AMO)
Ponera striata Lindl.	Chase O-6178 (K spirit)
Prosthechea abbreviata (Schltr.) W.E.Higgins	Brieger Coll. 10092 (ESA)
Pseudolaelia vellozicola (Hoehne) Porto & Brade	São Paulo B.G. 13362 (SP)
Ridleyella paniculata (Ridl.) Schltr.	Leiden 31692 (L)
Scaphosepalum gibberosum (Lehmann & Kraenzl.) Schltr.	Hermans 2366 (K spirit 57891)
Sophronitis coccinea (Lindl.) Rchb.f.	São Paulo B.G. 9577 (SP)
Stelis argentata Lindl.	Kew 1984-4053 (K spirit 60886)
Tetragamestus modestus Rchb.f.	Brieger Coll. 2756 (ESA)
Thelasis carinata Blume	Leiden 932669 (L)
Thunia alba Rchb.f.	Chase O-589 (K)

Table 1.2. Features of DNA datasets used in this study, in relation to one of the trees resulting from the combined analysis.

DNA Region	aligned length	number of variable sites	number of potentially parsimony informative sites	number of changes/variable site	Fitch tree length	CI	RI	ts:tv
***trnL-F* region**	1544	735 (47.60%)	397(25.71%)	2.43	1789	0.5718	0.5962	0.79
trnL-F intron	834	372 (44.60%)	201 (24.10%)	2.41	896	0.5748	0.5359	0.86
trnL-F exon	49	21 (42.85%)	7 (14.28%)	2.19	46	0.5435	0.4324	0.39
trnL-F interg.spacer	661	342 (51.74%)	189 (28.59%)	2.48	847	0.5702	0.6497	0.82
ITS region	852	675 (79.22%)	548 (64.32%)	7.22	4870	0.2903	0.4652	1.71
ITS1	338	298 (88.16%)	263 (77.81%)	8.44	2516	0.2778	0.4428	1.54
5.8S	157	64 (40.76%)	28 (17.83%)	2.50	160	0.4813	0.6693	5.40
ITS2	357	313 (87.68%)	257 (71.99%)	7.01	2194	0.2908	0.4729	1.81
***matK* spacers**	618	363 (58.63%)	176 (28.48%)	2.22	805	0.6186	0.6200	0.33
***matK* gene**	1374	699 (50.87%)	381 (27.73%)	2.62	1828	0.5109	0.5287	1.02
matK (1st positions)		228	125	2.39	545 (29.82%)	0.5331	0.4940	-----
matK (2nd positions)		211	107	2.47	521 (28.50%)	0.5355	0.5560	-----
matK (3rd positions)		260	149	2.93	762 (41.68%)	0.4777	0.5318	-----
***rbcL* gene**	1428	234 (16.39%)	117 (8.19%)	2.14	502	0.5339	0.4670	1.76
rbcL (1st positions)		49	24	1.98	97 (19.32%)	0.5464	0.4500	-----
rbcL (2nd positions)		30	9	2.20	66 (13.15%)	0.5000	0.3654	-----
rbcL (3rd positions)		155	84	2.19	339 (67.53)	0.3985	0.4886	-----



--Cladogram of the Orchidaceae. Lines = synapomorphies, closed circles = homoplasies (parallel or convergent evolution), x = loss of a synapomorphy (reversal). NEU = Neuwiedioideae; APO = Apostasioideae; CYP = Cypripedioideae; SPI = Spiranthoideae, CRA = Cranichideae, Go = Goodyerinae, Tro = Tropidinae, PRA = Prasophylleae, DIU = Diurideae; NEO = Neottioideae, NEO = Neottieae, Lis = Listerinae, Lim = Limodorinae, GEO = Geoblasteae, Cal = Calademinae, Chl = Chloraeinae, PTE = Pterostylideae, THE = Thelymitreae; ORC = Orchidoideae, DIS = Diseae, SAT = Satyrieae, ORC = Orchideae; EPI = Epidendroideae, GAS = Gastrodieae, TRI = Triphoreae, ARE = Arethuseae, VAN = Vanilleae, EPI = Epidendreae, PLE = Pleurothallis group, DEN = Dendrobieae, MAL = Malaxideae, COE = Coelogyneae, Coe = Coelogyninae, Cor = Corallorhizinae, MAX = Maxillarieae, VAD = Vandeae.

Constancy index = 60%.

Fig. 1.1. Phylogenetic relationships of the Epidendroideae proposed by Burns-Balogh and Funk (1986). Numbers represent transformation series described in details in the text of the original publication.

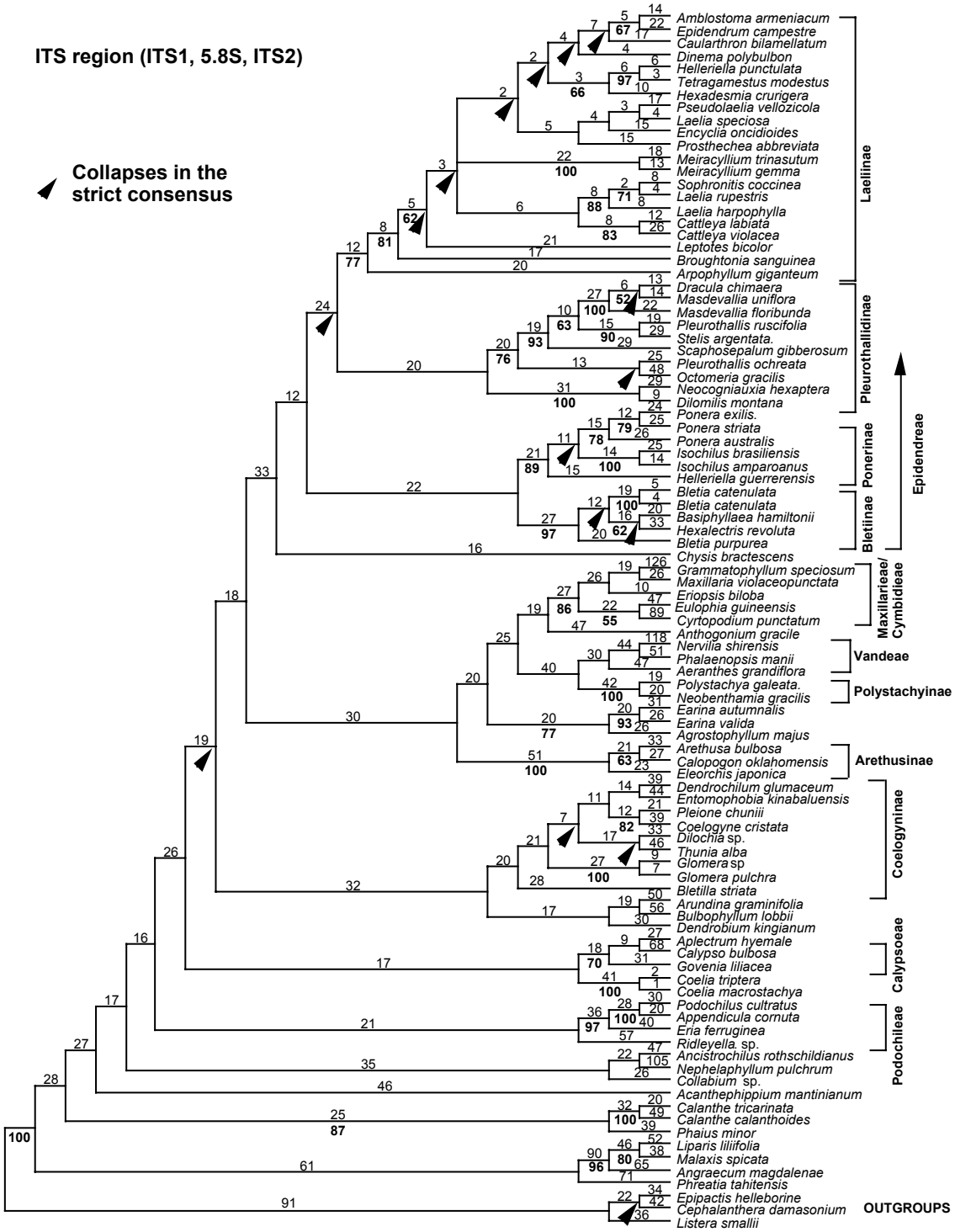


Fig. 1.2. One of the most parsimonious trees in the ITS-only analysis (L=4764, CI=0.30 and RI=0.48). The numbers above branches are Fitch lengths and bootstrap support percentages are indicated in bold below.

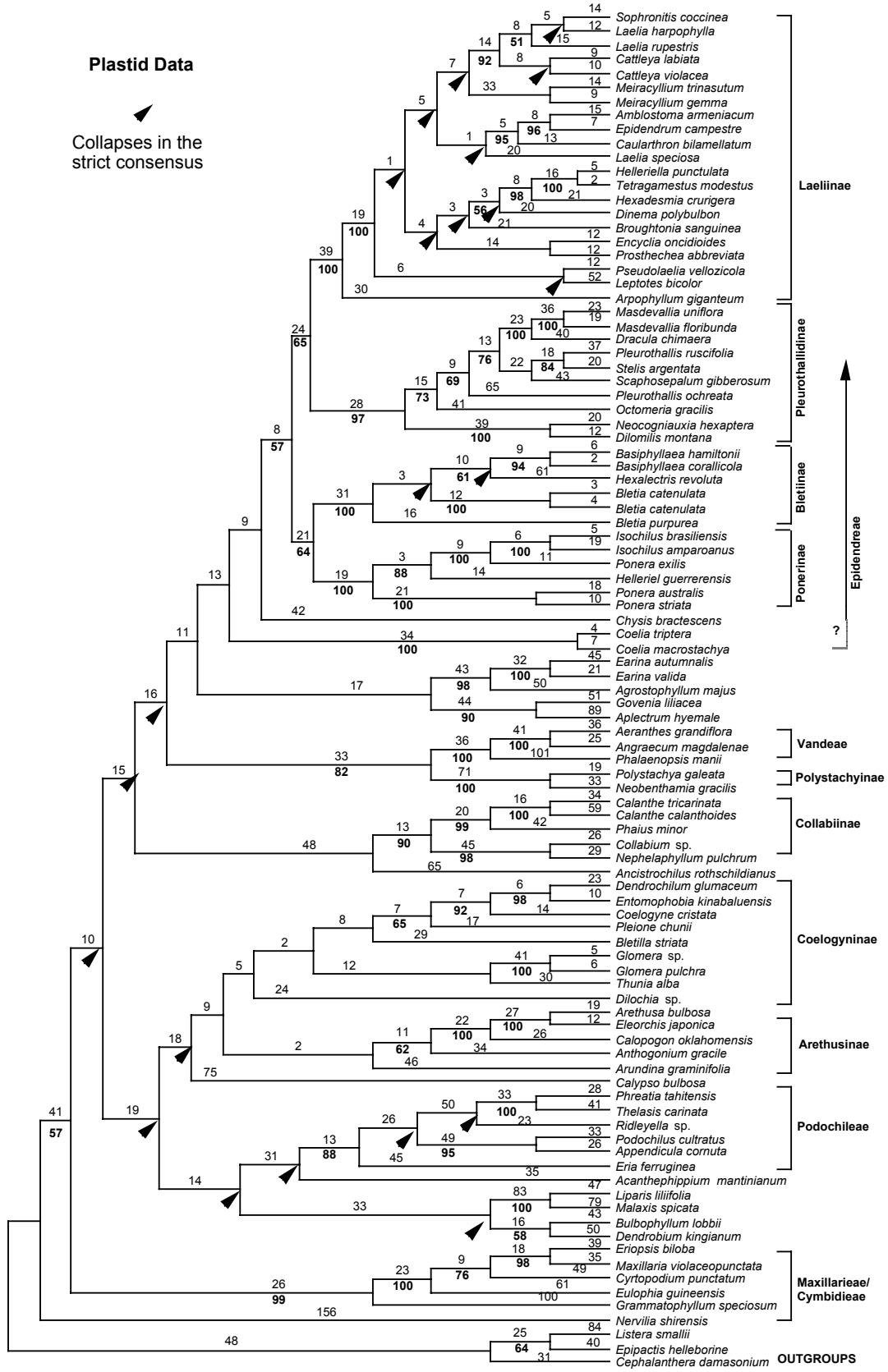


Fig. 1.3. One of the most parsimonious trees in the plastid-only analysis (L=4894, CI=0.56 and RI=0.57). The numbers above branches are Fitch lengths and bootstrap support percentages are indicated in bold below.

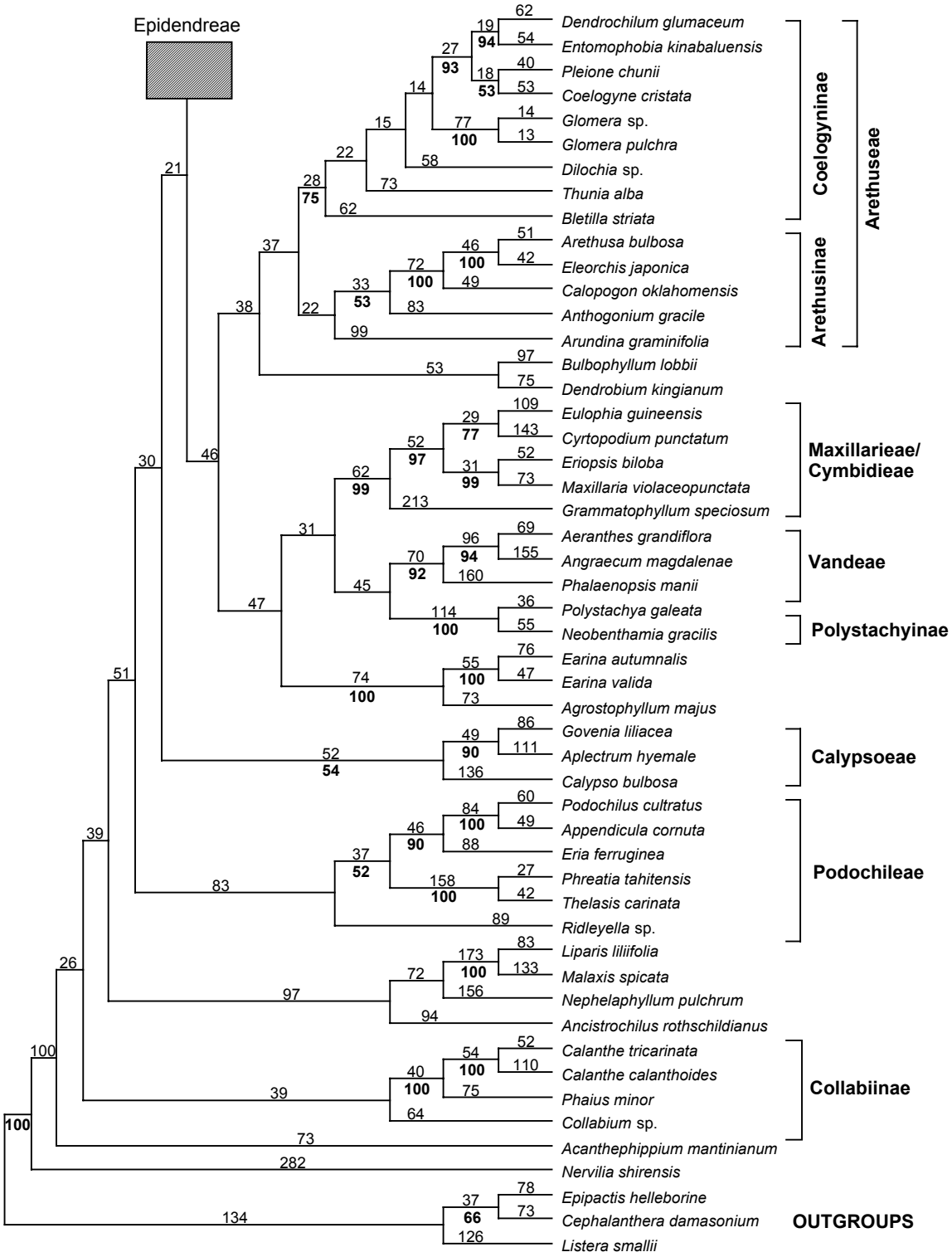


Fig 1.4. First part of one of the most parsimonious trees in the combined analysis (L=9818, CI=0.42 and RI=0.51). The numbers above branches are Fitch branch lengths and bootstrap support percentages are indicated in bold below.

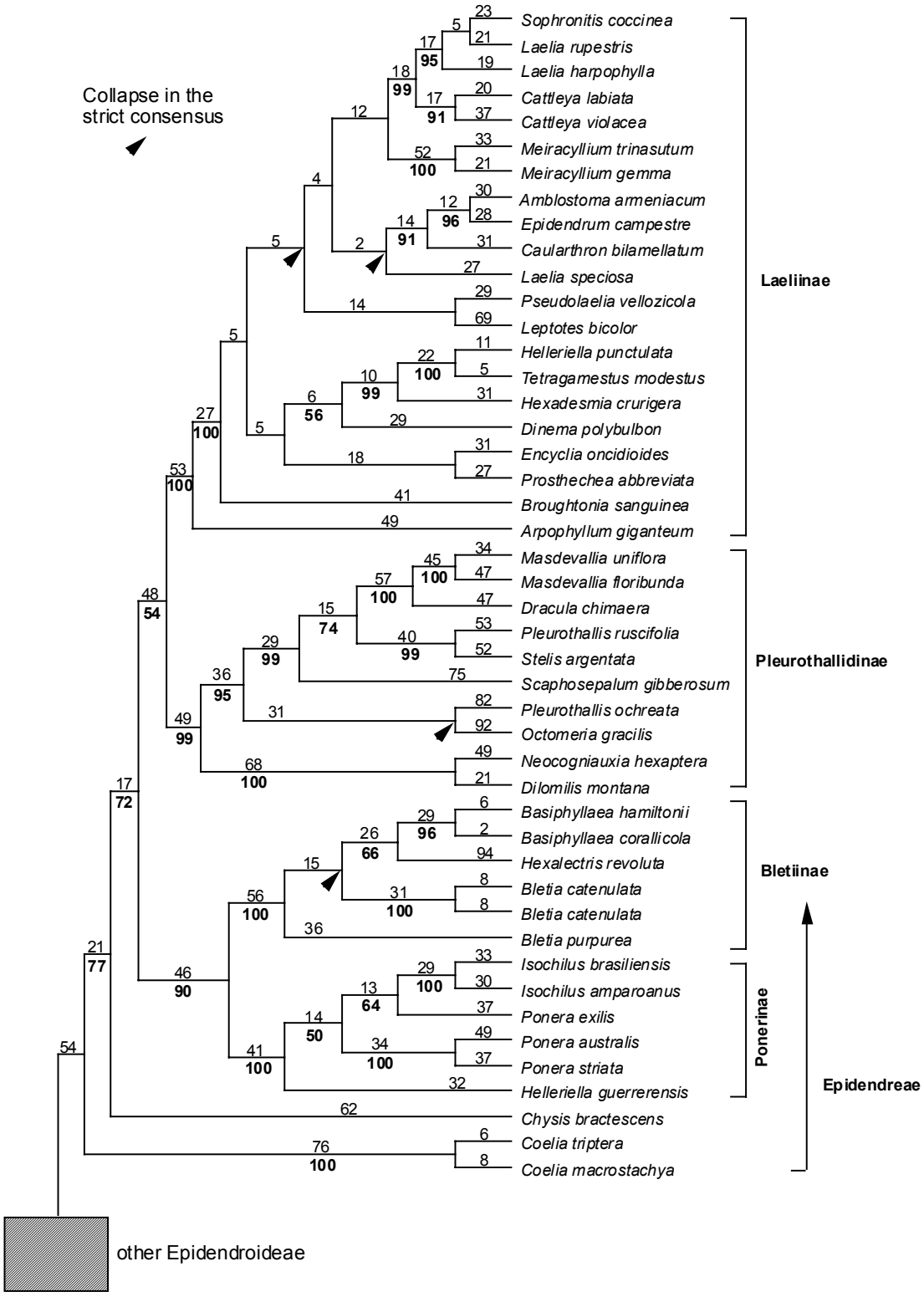


Fig 1.5. Second part of one of the same tree in Fig. 1.3. The numbers above branches are Fitch branch lengths and bootstrap support percentages are indicated in bold below.

Chapter 2 – An introduction to subtribe Laeliinae

2.1. INTRODUCTION

Laeliinae are an exclusively Neotropical subtribe that has been a primary focus of attention by orchid taxonomists and collectors since the 19th century. They include many ornamental genera, such as *Laelia* and *Cattleya*. They are one of the largest subtribes of the family (1466 species; Dressler, 1993), surpassed only by Pleurothallidinae (3021 species; Dressler, 1993), and are probably the most diverse in Epidendreae. Such diversity, especially in floral characters, has led to a situation in which, despite the many monographic treatments for several genera in the subtribe, old and fundamental doubts remain about the delimitation of the subtribe and many genera. Apparently, this diversity is correlated with the occurrence of different pollination syndromes, which include bees, moths, butterflies and hummingbirds. Dodson (1962) suggested that great selective pressure in floral morphology could have led to specialised flower types and increased considerably the likelihood of convergence in unrelated groups adapted to the same pollinators.

2.1.1. TAXONOMIC HISTORY

A detailed chronological view of the taxonomic history of Laeliinae is presented on Table 2.1. The subtribe was initially described by Bentham (1881), but some elements were placed in another subtribe, Stenoglossinae. Pfitzer (1889) merged the two subtribes of Bentham and Hooker's (1883) classification, Stenoglossinae (as 'Stenoglosseae') and Laeliinae (as 'Laelieae'), which were based on putative differences in the caudicles later found to be inaccurate. The new concept of Laeliinae had, however, two series: *Ponereae* for the genera bearing a column foot and *Cattleyeae* for the rest. This distinction was followed later by Schlechter (1926), who changed the respective names to *Ponereae* and *Laelieae*. Later, *Meiracyllium* (Dressler, 1971) and then *Arpophyllum* (Dressler, 1990b) were removed to their own subtribes.

2.1.2. CLASSIFICATION SYSTEMS

Several recent systems have been proposed for the classification of Laeliinae, as summarised in Fig. 2.1, some keeping the group as a single subtribe (Dressler,

1993); with generic alliances (Dressler, 1981); or splitting them into more subtribes (Brieger, 1977; Szlachetko, 1995). The latter two systems were based on the arbitrary choice of traditional floral characters, such as presence of column foot and fusion of the column and lip, to define the major groups. The system presented by Dressler (1981) was based on the relationships suggested by Baker (1972), by taking into account an informal interpretation of anatomical data.

2.1.3. PHYLOGENETIC RELATIONSHIPS AND TAXONOMIC PROBLEMS

According to Dressler (1993), Pleurothallidinae might be the sister group of Laeliinae, but the boundaries between these subtribes are not clear. In addition there are the closely related subtribes, Meiracylliinae and Arpophyllinae, established for *Meiracyllium* (Dressler, 1971) and *Arpophyllum* (Dressler, 1990b), both former genera of Laeliinae. Dressler (1993) suggested that the most primitive genus might be *Dilomilis* but also stated that this genus might instead be the ancestor of Pleurothallidinae. The genus *Isochilus* has been doubtfully associated with Laeliinae, and Szlachetko (1991) proposed the subtribe Isochilinae for it. Nevertheless, the same author included it in Laeliinae in his later system for Orchidaceae (Szlachetko, 1995). *Basiphyllaea*, a poorly known genus, has suffered similar ambiguity, and McCartney (1991, 1992) suggested it could be related to Bletiinae rather than to Laeliinae, contradicting its placement in all previous systems.

Although there is little congruence between the systems of classification (Fig. 2.1), a group of genera that could be referred to as the *Cattleya* alliance is one of the few that appears repeatedly among different systems.

There has been also controversy about the circumscription of genera in the *Cattleya* alliance. *Cattleya* and *Laelia* were traditionally kept as separate genera based only on the number of pollinia (four versus eight, respectively). Bechtel et al. (1980) and Dressler (1993) stated that this distinction was artificial. *Laelia* as defined previously has a disjunct distribution (Mexico and eastern Brazil). Section *Cattleyodes* appears more similar morphologically to the unifoliate *Cattleya* species than the rest of the genus. Likewise, Jones (1968b) separated *Laelia* section *Parviflorae* into the genus *Hoffmannseggella*. The number of pollinia has been shown not to be a good character in other orchid tribes in which it was emphasised, such as Maxillarieae (Whitten et al., in press). Frequently neglected vegetative

characters show good correlation with the molecular phylogenetic patterns found in both Whitten et al. (in press) and Pridgeon et al. (1997). A vegetative approach to Laeliinae could lead to a different classification, although there is a confusing mosaic among these characters as well (Withner and Adams, 1960).

Encyclia has experienced similar taxonomic and nomenclatural problems. The species were treated under *Epidendrum* before they were transferred to *Encyclia* (Schlechter, 1915). Bechtel et al. (1980), however, stated that *Encyclia* was scarcely distinguishable from *Cattleya*. There are probably at least two groups of species, but the separation of one of them to a genus under *Hormidium* (Brieger, 1960a, b) or *Anacheilium* (Pabst et al., 1981) failed due to nomenclatural problems. These species were finally placed in the resurrected concept of *Prosthechea* (Higgins, 1997). Moreover, although one groups of species (*Encyclia* subgenus *Encyclia*) appears to be morphologically similar to *Cattleya*, the other (subgenus *Osmophytum* = *Prosthechea*) might be more closely related to *Epidendrum*.

Schomburgkia also has two groups of species. The first one is similar to the Mexican species of *Laelia*, with one species intermediate between them (*Schomburgkia superbiens*). The second group has been sometimes treated under *Myrmecophila*, and does not appear to have a close relationship to *Laelia* (Dressler, 1993).

Similar problems occur in *Broughtonia*, *Cattleyopsis* and *Laeliopsis* (Sauleda and Adams, 1984; Dressler, 1993; Withner, 1996); *Isabelia*, *Neolauchea* and *Sophronitella* (Teuscher, 1968; Dressler, 1993); *Pseudolaelia* and *Renata* (Barros, 1994); the *Epidendrum* complex (Dressler, 1967, 1982b, 1984) and *Hexisea*, *Helleriella*, *Hexadesmia, Scaphyglottis*, and *Tetragamestus* (Adams, 1991; Dressler, 1994).

The infrageneric classification of *Cattleya* and *Laelia* has also had several different treatments, and these are not independent of the generic controversies. In *Cattleya* there are several species delimitation problems. All unifoliate species have been treated as single species with varieties (Hawkes, 1960), and in the bifoliate species several pairs (*C. loddigesii* and *C. harrisoniana*, *C. guttata* and *C. leopoldii*, *C. granulosa* and *C. schofiediana*, *C. walkeriana* and *C. nobilior*, *C. elongata* and *C. tenuis*) have been treated either as separate species or just two varieties of one species (Pabst and Dungs, 1975; Brieger, 1977; Fowlie, 1977; Braem 1984; Withner

1988). However, little data were offered to justify the decisions of these authors, and even the geographical distribution is rarely presented. This kind of subjective species treatment is also a problem in *Laelia* (especially the rupicolous species), *Schomburgkia* (the *S. crispa* and *S. undulata* groups), *Brassavola* and *Sophronitis*.

Tosello (1969) presented a study of *Cattleya* and *Laelia* floral flavonoids. The original work had only graphical analysis, and the flavonoid compounds were not characterised. However, by analysing only the presence/absence of bands in a phylogenetic analysis, I built the tree presented in Fig. 2.4. This analysis has two problems: there were no outgroups outside the two genera being analysed and the sampling was incomplete. Morphometric studies were performed in several genera such as *Cattleya* (Brieger et al., 1963; van den Berg, 1996), rupicolous species of *Laelia* (Cunha Filho, 1966; Resende, 1991) and *Brassavola* (Chacur, 1973). Although these studies helped in evaluating closely related species groups, they were of little value for resolving the overall phylogeny of the genera.

2.1.4. MOLECULAR SYSTEMATICS

In Laeliinae, there have been few molecular studies, although some general studies such as Neyland and Urbatsch (1996) dealt with delimitation questions. In a study with the plastid gene *ndhF*, they found *Meiracyllium* as the immediate sister group of Laeliinae as well as *Arpophyllum* as the sister group of Pleurothallidinae, and these two clades in a polytomy with *Chysis*. Cameron et al. (1999) included *Cattleya, Epidendrum* and *Encyclia*, as well as *Arpophyllum, Meiracyllium* and *Dilomilis*. Their data showed *Dilomilis* as sister to Pleurothallidinae rather than Laeliinae, whereas *Meiracyllium* was sister to the remaining Laeliinae plus *Polystachya*. *Arpophyllum* was embedded in Laeliinae, in a position sister to *Epidendrum*. Within Laeliinae, there were only the studies of Benner (1994) and Benner et al. (1995). They first examined the size of rDNA repeats for several genera in Laeliinae, which were difficult to interpret phylogenetically. The latter study, using RAPD markers in some *Cattleya* species, presents a similar problem because of the use of only one individual per species and less than ten species sampled overall from a genus of 50.

2.1.5. POLLINATION AND ECOLOGY

Although there are reports of the pollination of many members of Laeliinae (Table 2.2), these are infrequent considering the size of the subtribe (1,466 species). To make the situation worse, most of these published reports are anecdotal, and lack careful distinction between mere visitors and true pollinators (e.g. Dodson and Frymire, 1961). There are only a handful of studies performing a careful examination of the pollination mechanism (Rico-Gray and Thien, 1987; Mora and Valerio, 1988; Ackerman and Montalvo, 1990; Matias et al. 1996). In inspecting Table 2.2, one could get the impression that many Laeliinae are self-pollinating plants (also Catling, 1990). In fact, although I lack a precise figure, the percentage of self-pollinating species is expected to be as low as in other tropical epiphytic Orchidaceae (Catling, 1990). The large number of reports is probably because self-pollination is far easier to report than to carry out pollination studies in outcrossing species. Moreover, many of the self-pollinated species reported have also outcrossing populations (Catling, 1990).

Despite the different syndromes of pollination present in Laeliinae, there are two overall patterns: species that advertise and produce rewards such as nectar, and species that only advertise (deceptive pollination systems; Ackerman, 1986). In the second group fragrances play an important role in the attraction of pollinators and species must rely in a small number of visits before the insect learns there is no reward. These fragrances can vary greatly among different individuals and populations (Moya and Ackerman, 1993). Goss and Adams (1976) found that only males of a moth (Ctenuchidae) were attracted to *Epidendrum anceps* Jacq. and suggested that fragrances are used as precursors for pheromones such as pyrrolizidines, known in other plants. In an alternative system, the orchid might be a fragrance mimic of other plants actually providing the precursor for the insect. This seems to be a system similar to that in which male euglossine bees visit species of other orchid subtribes to collect fragrances (Dressler, 1968a, b, 1982a). In Laeliinae, however, the species pollinated by euglossine bees probably do not provide fragrances, but advertise nectar (e.g. *Myrmecophila tibicinis*, *Cattleya maxima, Cattleya mendelii,* and *Cattleya warscewiczii*). They have showy, attractive bee flowers and, as reported by Rico-Gray and Thien (1987) for *Myrmecophila*, the visits

were rapid (4-5 seconds) and not as elaborate as the behaviour of fragrance collection (Dressler, 1982a). Male euglossine bees visited *Cattleya granulosa* to collect fragrances (pers. obs.), but they scratched the petals and sepals and damaged the flower rather than pollinating it. The nectar and nectar-mimic systems for pollination are not as specific as fragrance collecting, and Laeliinae species are pollinated also by other large bees such as *Xylocopa* spp. (Anthophoridae). This is the case for *Schomburgkia splendida, Caularthron* sp., *Cattleya skinneri* and *Cattleya warscewiczii* (Dodson, 1965; van der Pijl and Dodson, 1966; Braga, 1977; Mora and Valerio, 1988). In other cases, the pollinator can be specific, as in *Constantia cipoensis*. Matias et al. (1996) found this small-flowered Brazilian species to be pollinated exclusively by *Xylocopa* cf. *artifex*, which nests in *Vellozia piresiana* and *V. compacta* (Velloziaceae). The orchid is endemic to a small area, and uses only these two species as phorophytes.

Another relevant aspect of orchid pollination is the low flower/fruit ratios found (Montalvo and Ackerman, 1987; Rico-Gray and Thien, 1987; Ackerman, 1989). The small number of fruits is due to short-term limitations (e.g. pollinator; Schemske, 1980), and long-term effects such as decreased growth and vigour in plants with fruits (Ackerman and Montalvo, 1990; Ortiz-Barney and Ackerman, 1999). However, Fowlie (1961) reported that *Broughtonia sanguinea* plants are easily pollinated, extremely abundant in wet lowland environments in Jamaica, and most plants produce 2-3 pods.

One interesting aspect of orchid ecology is the association between orchid species and their phorophytes. Most Laeliinae and other epiphytic orchids seem to have little specificity. In fact, some phorophytes seem to be good for the whole epiphytic guild (Zimmerman and Olmsted, 1992, five Laeliinae in an epiphytic community; Migenis and Ackerman, 1993, seven Laeliinae species in an epiphytic community). In these studies, the next important factor controlling the distribution of epiphytes was light limitation. However, in a group of Brazilian genera of Laeliinae, there are many species specialised for *Vellozia* spp., such as *Constantia cipoensis* (Matias et al., 1996) and several species of *Constantia* and *Pseudolaelia* (pers. obs.). We have little information about the macrogeographic distribution patterns of Laeliinae, although there is a group of species with broad distribution in the Neotropical lowlands (*Prosthechea cochleata, Prosthechea vespa, Epidendrum*

difforme, Epidendrum anceps, Jacquiniella globosa, Epidendrum nocturnum, Epidendrum ramosum, Epidendrum ciliare and *Epidendrum strobiliferum*). A study on altitudinal distribution of orchids in Suriname (Werkhoven, 1992) showed that Laeliinae occupied broader altitudinal ranges than orchids in other subtribes. Whereas they were only 11.4% of the orchid flora occurring exclusively from 0-200m, and no Laeliinae species occurred exclusively in the 200-350 m, 350-500 m, 200-700 m ranges, there were 42.8% in the 0-700 m range, 40% in the 0-1230 m, and 33% in the 700-1230 m.

2.1.6. CHROMOSOME NUMBERS, INTRA AND INTERGENERIC HYBRIDIZATION

A list of the chromosome numbers of Laeliinae is presented in Table 2.3. Chromosome numbers are remarkably constant when compared with other Orchidaceae, and generally 2n=40. The few species deviating from this number are generally autopolyploids (80, 120, 160; *Epidendrum ciliare, Cattleya bicolor, Cattleya elongata* and *Epidendrum nocturnum*), or species which had 2n=40 as well as the 2n=40 plus or minus one or two in multiple counts (*Cattleya bowringiana*, *Cattleya intermedia, Cattleya labiata, Cattleya mossiae, Cattleya trianaei, Encyclia cordigera, Epidendrum ciliare, Epidendrum nocturnum, Laelia longipes*). One large source of variation was the study of Chadard (1963), which seems unreliable because he always found different numbers from species counted by other authors. As a rule, there seems to be little taxonomic information in chromosome numbers for the subtribe, as most of the variation consists of species with both diploid and autopolyploid individuals reported, and the only group in which allopolyploidy has been suggested as a mode of speciation is the rupicolous *Laelia* species (Blumenschein, 1960).

This remarkable constancy of chromosome numbers could explain the number of fertile artificial interspecific and intergeneric hybrids produced so far. In the records of registered hybrids (Royal Horticultural Society, 1997), there were 5,000 hybrids with *Cattleya,* 9,000 *Laelia* with *Cattleya*, 1,700 *Brassavola* with *Cattleya*, 6,000 *Brassavola* with *Laelia* and *Cattleya,* and 2,000 *Sophronitis* with *Laelia* and *Cattleya*, among many others. Whereas there are several combinations of hybrids involving five genera, there are at least three with six genera, two with seven

and even a hybrid involving nine supposedly different genera. Part of this apparent fertility is due to the large number of seeds in each pod, such that a large number of seedlings is obtained even with a low percentage of fertility (Stort, 1984, 1986; Marin Morales and Stort, 1986). The number of reported natural hybrids is, however, much lower (Adams and Anderson, 1958; Dressler, 1981) and might indicate relationships between genera. This is true for *Brassavola* with *Cattleya,* and *Cattleya* with Brazilian species of *Laelia.* The limitation of these data is that phylogenetically related groups with different pollination syndromes (e.g. *Sophronitis* and *Cattleya*) do not produce natural hybrids, whereas some sympatric but distantly related genera which share pollinators do hybridise in nature (e.g. *Encylaelia*; Miranda, 1991).

2.1.7. ANATOMY

Baker's (1972) leaf anatomy study has provided the largest amount of comparative data for Laeliinae. Although there were some patterns, they are very difficult to code because most genera were polymorphic for the anatomical characters surveyed. It also seems that many leaf characters are potentially correlated with habitats, and many adaptations for xeric conditions are remarkably convergent. A strict phylogenetic analysis of Baker's data produced only an unresolved polytomy (van den Berg, unpublished), but the fact that he found *Meiracyllium* indistinct from other Laeliinae is noteworthy. He also found *Arpophyllum* very different from both Pleurothallidinae and Laeliinae. Pridgeon et al. (1983) studied the presence of tilosomes across Orchidaceae. Tilosomes are structures found in the roots that work as protective plugs and barriers against transpiration. In Laeliinae they were only of type I (lamellate) and occurred in *Encyclia, Prosthechea,* some *Epidendrum* and *Laelia anceps.* Inasmuch as this could indicate the relationship between *Encyclia* and *Prosthechea*, all species with tilosomes were from quite dry habitats, and therefore convergence cannot be discounted. Pridgeon (1987) reviewed the variation in velamen types in different groups of orchids. In Laeliinae both the number of layers and type of exodermal thickening was variable, and also polymorphic within genera, which again seems to be more correlated to habitat conditions than phylogeny.

2.2. CONCLUSIONS

This short review of the current knowledge of Laeliinae could be summarized in two main conclusions. First, although there appears to be a large body of data on several subjects, in fact there is a paucity of information when taking into account the large number of taxa belonging to the subtribe. A second problem is that even though I could compile here tables with data scattered through the literature, few of the datasets were collected under equal and standard conditions and therefore their comparability is questionable. The taxonomic history of Laeliinae is quite complex and many different classification systems were proposed. There have been hitherto only very preliminary molecular studies. Anatomical work was more extensive but provided few answers. Had more work been done in pollination, it would be possible to formulate clearer hypotheses for mechanisms of speciation acting upon these taxa. The amount and fertility of artificial and, to a lesser extent, natural hybrids show that there are only weak genetic barriers across the subtribe. This could mean an increased probability of hybrid speciation, and this should be taken into account. The effects of such hybrids in the wholly divergent branching model used in phylogenetic analysis has been explored in recent years (McDade, 1990, 1992, 1997). McDade found that F1 individuals generally are placed in the base of the clade that includes the parent with most derived character states, due to intermediacy in morphological features. In DNA sequence data the effect of hybridisation is different, basically reflecting the mode of inheritance of different genomes (e.g. plastid versus nuclear). Plastids from all orchids listed in Harris and Ingram (1991) were reliably maternally inherited and there are so far no reported cases of paternal inheritance. For ITS it could be expected that F1 plants contained both parental ITS sequences, and possibly a mixture by recombination in subsequent generations. However, as shown by Wendel et al. (1995), bidirectional interlocus concerted evolution may effectively remove one of the parental rDNA repeats from the hybrid genome of stabilized allopolyploid hybrids. Diploids backcrossing to one of the parents could cause a similar pattern. I found only one parental copy in diploid plants of three different natural hybrid swarms of *Pleione* (pers. obs.) and Chase et al. (in prep.) found a single copy coming unpredictably from the maternal or paternal sides in *Nicotiana* hybrids. In my case I am more interested in the behaviour of ITS in diploid hybrids, because chromosome counts published so far show a small number of polyploid taxa

and in fact only two species of *Laelia* sect. *Parviflorae* are considered to be allotetraploids. This would suggest that ecological barriers (van den Berg, 1996) are probably effective in preventing interspecific crossing, and hybridisation does not seem to occur often in nature. However, if hybridisation occurred in the past, it could be very difficult to detect, except by incongruence between plastid and ITS phylogenies. The latter cannot, however be taken as unique evidence because it could be the result of other events that cause gene and organism phylogenies to differ, such as lineage sorting. In this process co-existing alleles that predate speciation and are inherited in a paraphyletic way to some lineages and cause conflict between gene and organismal phylogenies. This concept could be traced back to Fisher (1930) but more clearly outlined in recent textbooks (e.g. Ayala, 2000).

Table 2.1. A chronological view of Laeliinae taxonomic history.

Year	Fact
1703	The first Laeliinae mentioned in Plumier's *Catalogus Plantarum Americanarum* as *Helleborine foliis rigidis, angustis, et canaliculatis,* for the species *Tetramicra canaliculata* (Aubl.) Urban, and *Helleborine cochleato flore,* for *Prosthechea cochleata* (L.) W.E.Higgins.
1707	Sir Hans Sloane illustrated *Brassavola cordata* and *Broughtonia sanguinea* in Plate 121 of *A Voyage to the islands Madera, Barbados, Nieves, S. Christophers and Jamaica*.
1753	Linnaeus in *Species Plantarum* 2: 939-954 described *Brassavola nodosa* (L.) R.Br. as *Epidendrum nodosum* L.
1758	*Tetramicra canaliculata* (Aubl.), *Psychilis atropurpurea* (Willd.) Sauleda, *Isochilus linearis* (Jacq.) R.Br. and *Epidendrum ciliare* L. were illustrated by Burman in Plumier's *Plantarum Americanarum,* Fasc. 8.
1759	Linnaeus described *Epidendrum ciliare* L. in *Systema Naturae*, 10th Ed. p. 1246.
1760	Jacquin described several Laeliinae in *Enumeration Systematica Plantarum*, such as *Epidendrum secundum* Jacq., *Epidendrum ramosum* Jacq., *Epidendrum difforme* Jacq., *Epidendrum rigidum* Jacq., *Epidendrum nocturnum* Jacq., *Epidendrum violaceum* Jacq., *Epidendrum anceps* Jacq., *Jacquiniella globosa* (Jacq.) Schltr. (as *Epidendrum globosum*), *Isochilus linearis* (Jacq.) R.Br. (as *Epidendrum lineare*). All of these species, but the last two, are still placed in *Epidendrum*. Jacquin's concept of *Epidendrum* shaped the concept adopted today.
1763	Linnaeus added *Brassavola cucullata* (L.) R.Br. and *Prosthechea cochleata* (L.) W.E.Higgins in the second edition of *Species Plantarum*.
1788	Swartz in *Nova Genera et Species Prodromus* described *Epidendrum diffusum* Sw., *Dilomilis montana* (Sw.) Summerh. (as *Epidendrum montanum*), *Epidendrum serrulatum* Sw., *Epidendrum sessile* Sw., *Epidendrum subulatum* Sw. (=*Brassavola??*), *Brougxhtonia sanguinea* (as *Epidendrum sanguineum* Sw.), *Jacquiniella teretifolia* (as *Epidendrum teretifolium*), *Epidendrum patens* Sw., *Scaphyglottis prolifera* (as *Epidendrum proliferum*), *Epidendrum nutans* Sw., *Dinema polybulbon* (Sw.) Lindl. as *Epidendrum polybulbon* Sw., *Prosthechea fragrans* Sw. (as *Epidendrum fragrans* Sw.), *Encyclia angustifolia* (Sw.) Schltr. (as *Epidendrum angustifolium*, *Epidendrum difforme* Jacq. (as *Epidendrum umbellatum* Sw.), *Homalopetalum vomeriforme* (Sw.) Fawc. & Rendle (as *Epidendrum vomeriforme*). *Epidendrum diurnum, Epidendrum verrucosum* Sw.

Table 2.1 (continued)

Year	Fact
1798	Ruiz and Pavon described several new *Epidendrum* in *Systema Vegetabilium Florae Peruvianae et Chilensis*.
1813	R. Brown described three new genera of Laeliinae for previously described species of *Epidendrum* in *Aiton Hortus Kewensis* 2nd Edn.: *Brassavola, Isochilus* and *Broughtonia.* He also described some new species such as *Epidendrum conopseum* R.Br.
1815	Several Laeliinae are described by Kunth in *Nova Genera et Species Plantarum*, among them *Laelia speciosa* (Kunth) Schltr. (as *Bletia speciosa*), which is the type species for the subtribe. He also described *Cattleya violacea* (Kunth) Rolfe (as *Cymbidium violaceum*), *Encyclia cordigera* (Kunth) Dressler (as *Cymbidium cordigerum*), *Cattleya candida* (Kunth) Lehm. (as *Cymbidium candidum*), *Epidendrum ibaguense* Kunth, *Epidendrum floribundum* Kunth, *Epidendrum longiflorum* Kunth.
1819	The first plant of *Cattleya loddigesii* appeared in a watercolor in *Loddiges' Botanical Cabinet* as *Epidendrum violaceum.*
1821	Lindley described the genus *Cattleya* in *Collectanea Botanica*, based on a plant imported in 1818 from Brazil and grown by a North London amateur called William Cattley.In the same work he also described *Cattleya loddigesii* (1823) and *Cattleya forbesii* (1823).
1825	La Llave and Lexarza described *Alamania* and *Arpophyllum* in *Novorum Vegetabilium Descriptiones*. They also described *Euchile citrina* (La Llave & Lex.) Withner, *Laelia autumnalis* (La Llave & Lex.) Lindl., *Encyclia adenocaula* (La Llave & Lex.) Schltr., *Encyclia adenocarpon* (La Llave & Lex) Schltr., *Barkeria scandens* (La Llave & Lex.) Dressler & Halbinger, *Prosthechea michuacana* (La Llave & Lex.) W.E.Higgins, *Prosthechea concolor* (La Llave & Lex.) W.E.Higgins.
1828	Lindley described *Sophronitis* based on *Sophronitis cernua* (Lindl.) Hook.
1828	W.J. Hooker described *Encyclia* based on *Encyclia viridiflora* Hook. No plants belonging to this species have since been found.
1831a, b, c	Lindley described the genus *Laelia* (Lindley, 1831a) based on *Laelia speciosa* (Kunth) Schltr., *Ponera* (Lindley, 1831b) based on *Ponera juncifolia* (Lindl.), *Tetramicra* based on *Tetramicra canaliculata* (Aubl.) Urban (as *Tetramicra rigida* (Willd.) Lindl.) and *Dinema,* based on *Dinema polybulbon* (Sw.) Lindl..
1833	Lindley described *Leptotes* based on *Leptotes bicolor* Lindl.
1834a, b	Lindley described *Hexisea* and *Diothonea* based on *Hexisea bidentata* Lindl. and *Diothonea iloensis* Lindl.

Table 2.1 (continued)

Year	Fact
1835	*Scaphyglottis* is described by Poeppig and Endlicher in *Nova Genera ac Species Plantarum*, based on five species. Dressler (1960) later lectotypified the genus with *Scaphyglottis graminifolia*.
1836	Rafinesque described *Dilomilis* based on *Dilomilis serrata* (now *Dilomilis montana*), and *Caularthron* based on *Caularthron bicornutum* Rafin.
1838	Knowles and Westcott described *Barkeria* and *Prosthechea,* based on *Barkeria elegans* Kn. & Westc. and *Prosthechea glauca* Knowles & Westc.
1838	Lindley described *Schomburgkia* based on *Schomburgkia crispa* Lindl.
1852	Lindley and Joseph Paxton described *Laeliopsis* based on *Laeliopsis dominguensis* Lindl. & Paxton.
1853a	Lindley described *Pinelia* based on *Pinelia hypolepta* Lindl.
1854	Lemaire described *Cattleyopsis* based on *Cattleyopsis lindenii* (Lindl.) Cogn. as *Cattleyopsis delicatula* Lem.
1877	Barbosa Rodrigues described *Isabelia* and *Constantia,* based on *Isabelia virginalis* Barb.Rodr. and *Constantia rupestris* Barb.Rodr.
1881	Bentham described *Octadesmia* based on *Octadesmia serratifolia* (Hook.) Benth., now known as *Dilomilis montana* (Sw.) Summerh.
1882	Barbosa Rodrigues described *Reichenbachanthus* based on *Reichenbachanthus modestus* Barb.Rodr. now *R. reflexus* (Rchb.f.) Brade.
1891	Barbosa Rodrigues described *Orleanesia* based on *Orleanesia yauaperyensis* Barb.Rodr.
1896	Rolfe described *Homalopetalum* based on *Homalopetalum jamaicense* Rolfe, now known as *Homalopetalum vomeriforme* (Sw.) Fawc. & Rendle.
1897	Kraenzlin described *Neolauchea*, based on *Neolauchea pulchella* Kraenzl.
1913	Schlechter described *Neocogniauxia* based on *Neocogniauxia monophylla* (Griseb.) Schltr. and *Domingoa* based on *Domingoa nodosa* (Cogn.) Schltr., previously a member of *Octadesmia* (=*Dilomilis*).
1917	*Myrmecophila* is described by Rolfe, for all the species of *Schomburgkia*. possessing hollow stems.
1918	Schlechter created *Rhyncholaelia* for two species previously placed in *Brassavola*, *R. digbyana* (Lindl.) Schltr. and *R. glauca* (Lindl.) Schltr.
1921	Schlechter described *Basiphyllaea* based on *Basiphyllaea sarcophylla* (Rchb.f.) Schltr., previously a member of *Bletia*.
1922	Schlechter described *Dimerandra* based on *Dimerandra stenopetala*.
1925	Schlechter removed *Sophronitis violacea* Lindl. to a new monospecific genus called *Sophronitella*.
1927	Hoehne described *Loefgrenianthus* based on *L. blanche-amesiae* (Loefgr.) Hoehne, which has originally been described as a *Leptotes* by Loefgren (1918)

Table 2.1 (continued)

Year	Fact
1935	Campos Porto and Brade described the genus *Pseudolaelia* based on *Pseudolaelia corcovadensis* Porto & Brade.
1939	Brade described *Pygmaeorchis* based on *Pygmaeorchis brasiliensis* Brade.
1940	L. O. Williams proposed the name *Nageliella* to replace *Hartwegia* Lindl., which was a later homonym of *Hartwegia* Nees (a synonym of *Chlorophyton* in the Anthericaceae).
1946	Ruschi described *Renata* based on *Renata canaanensis* Ruschi.
1960	Dressler lectotypified *Scaphyglottis* with the species *Scaphyglottis graminifolia* (Ruiz & Pavon) Poepp. & Endl. *(Fernandezia graminifolia* Ruiz & Pav.).
1960a, b	Brieger re-established part of *Hormidium* Lindl. ex Heynh. for a group of species of *Encyclia*.
1961	Summerhayes showed that *Dilomilis* Raf. is the an older name for the genus *Octadesmia* Benth.
1966	Hawkes described the genus *Helleriella* based on *Helleriella nicaraguensis* A.D.Hawkes (Heller and Hawkes, 1966)
1968b	H.G. Jones proposed the new genus *Hoffmannseggella* for the rupicolous species of *Laelia* (sect. *Parviflorae*), with the type species *Laelia cinnabarina* Batem.
1968	Senghas and Teuscher considered *Neolauchea* as a synonym of *Isabelia*, with the transfer *Isabelia pulchella* (Kraenzl.) Senghas & Teusch. (Teuscher, 1968)
1971	Dressler and Pollard described *Artorima* for *Epidendrum erubescens*. This species had been placed in *Encyclia* by Schlechter (1915).
1971	Dressler removed *Meiracyllium* to its own monogeneric subtribe Meiracylliinae
1974	González Tamayo described *Hagsatera* for *Epidendrum brachycolumna* L.O. Williams, and a new species *Hagsatera rosilloi* R.González. Dressler (1961) had considered the first species as a member of *Encyclia*.
1974	Garay and H. R. Sweet transferred *Ponera punctulata* to *Helleriella* for having a reed-stem habit. Dodson and Vásquez (1989) mention that in fact the pseudobulbs are cane like and branched only at the base, and prefer the combination *Scaphyglottis punctulata* (Rchb.f.) C.Schweinf.
1979	D.D. Dod described *Quisqueya* with the type species *Quisqueya karstii* D.D.Dod.
1980	Senghas created *Briegeria* for six species previously placed in *Jacquiniella*.
1981	Dressler presented Laeliinae with 43 genera in six alliances based on Baker (1972). *Meiracyllium* is kept in its separate subtribe and *Arpophyllum* is considered part of Sobraliinae. *Basiphyllaea* is considered part of Laeliinae.

Table 2.1 (continued)

Year	Fact
1981	Hágsater reestablished *Oerstedella* Rchb.f. as a genus separate from *Epidendrum*.
1981	Pabst, Moutinho and Pinto adopted a narrower sense of *Hormidium* than Brieger (1960) and re-established *Anacheilium* Hoffmans., transferring many other species to it.
1988	Sauleda re-established *Psychilis* Raf. for a group of Caribbean species previously placed in *Epidendrum* and *Encyclia*.
1990a	Dressler described *Acrorchis* based on *Acrorchis roseola* Dressler.
1990b	Dressler described a new monogeneric subtribe for *Arpophyllum.*
1991	Szlachetko published a new subtribe Isochilinae based on *Isochilus* R.Br.
1993	In *Phylogeny and Classification of the Orchid Family*, Dressler abandoned the alliances of Dressler (1981), and presented three subtribes: Meiracylliinae, Arpophyllinae and Laeliinae. *Basiphyllaea* is still included in Laeliinae.
1994	F. Barros considered *Renata* as a synonym of *Pseudolaelia*.
1995	Szlachetko placed the genera of Laeliinae sensu Dressler (1993) in three different subtribes in *Systema Orchidalium*: Laeliinae, Epidendrinae and Ponerinae. *Isochilus* was placed in Laeliinae rather than Isochilinae.
1997	W.E. Higgins re-established *Prosthechea* Knowles & Westc. and transferred to it all the species included in *Hormidium* and *Anacheilium*.
1997	M.A.Nir. describes the new genus *Tomzanonia,* based on *Dilomilis filicina*, after a morphological cladistic analysis.
1998	Withner raises *Encyclia* subg. *Euchile* to generic status, with two species *E. citrina* (La Llave & Lex.) Withner and *E. mariae* (Ames) Withner.

Table 2.2. Pollination systems in Laeliinae

Species	**Animal**	**Kind of Animal**	**Observer**
Barkeria dorotheae F.Halbinger	*Eulaema seabrae* (Euglossinae)	bee	Warford, 1993
Barkeria lindleyana Batem ex Lindl.	*Xylocopa tabaniformis* (Anthophoridae)	bee, skipper	Dodson, 1965
	Auglochlora sp.		
	Euglossa sp. (Euglossinae)		
	Skippers (4spp.)		
Barkeria obovata (Presl.) E.A.Christenson	facultative selfing		Warford, 1993
Basiphyllaea corallicola (Small) Ames	autopollination with the absence of a rostellum		Luer,1972
Brassavola sp.	Sphingidae	moth	Roebuck and Steinhart, 1978
Brougtonia sanguinea (Sw.) R.Br.	selfing		Sauleda and Adams, 1984
Cattleya aurantiaca (Batem. ex Lindl.) P.N.Don	selfing		Knudson, 1956; Thomale, 1958
Cattleya luteola Lindl.	*Melipona flavipennis*	bee	van der Pijl and Dodson, 1966
Cattleya maxima Lindl.	*Eulaema polychroma* (Euglossinae)	bee	Dodson and Frymire, 1961b
Cattleya mendelii Backh.f.	*Eulaema cingulata* (Euglossinae)	bee	van der Pijl and Dodson, 1966
Cattleya patinii Cogn.	selfing		Dressler, 1981
Cattleya skinneri Batem.	*Xylocopa tabaniformis* (Anthophoridae),	bee	Mora and Valerio, 1988
	Thygater sp. (Anthophoridae)		
Cattleya warscewiczii Rchb.f.	*Eulaema polychroma* (Euglossinae)	bee	van der Pijl and Dodson, 1966
Cattleya warscewiczii Rchb.f.	*Eulaema cingulata* (Euglossinae)	bee	van der Pijl and Dodson, 1966
	Xylocopa aff. *viridis* (Anthophoridae)		Dodson, 1965
Caularthron bilamellatum (Rchb.f.) R.E.Schultes	selfing		Catling, 1990.
Caularthron sp.	*Xylocopa* sp. (Anthophoridae)	bee	Braga, 1977

Table 2.2 (continued)

Species	Animal	Kind of Animal	Observer
Constantia cipoensis Porto & Brade	*Xylocopa* aff. *artifex* (Anthophoridae)	bee	Matias et al., 1996
Dimerandra emarginata (G.Mey.) Hoehne	selfing		Hamer, 1984
Encyclia bradfordii (Griseb.) G.Carnevali & I.Ramírez	selfing		Withner, 1970
Encyclia gravida Schltr.	selfing		Dressler and Pollard, 1976; Withner, 1970
Encyclia monticula (Fawc. & Rendle) Acuña	selfing		Withner, 1970
Encyclia sintenisii Britton	selfing		J. D. Ackerman pers. comm. in Catling, 1990
Epidendrum anceps Jacq.	*Lymire edwardsii* Grote, *Cisseps fulvicollis* Hubner (Ctenuchidae), *Oxydia vesulia* Cramer (Geometridae)	nocturnal moths, males only	Goss and Adams, 1976
Epidendrum cf. *acuminatum* Ruiz & Pav.	*Xylocopa frontalis* (Anthophoridae)	bee	Dodson, 1965
Epidendrum ciliare L.	*Pseudosphinx tetrio* (Sphingidae)	moth (Sphingidae)	Ackerman and Montalvo, 1990
Epidendrum cnemidophorum Lindl.	*Amazilia tzacatl*	hummingbird	Dodson, 1965
Epidendrum difforme Jacq.	*Amastus acona*	moth	Dodson and Frymire, 1961
Epidendrum difforme Jacq.	*Lymire edwardsii* Grote (Ctenuchidae) *Anticarsia gemmatalis* Hubner (Noctuidae) *Phyprosopus callitrichoides* Grote (Noctuidae)	nocturnal or crepuscular moths	Goss, 1977
Epidendrum eustirum Ames, F.T.Hubbard & C.Schweinf.	selfing		J. D. Ackerman pers. comm. in Catling, 1990
Epidendrum fimbriatum Kunth.	unidentified	fly	Dodson, 1962
Epidendrum funkii Rchb.f.	selfing		J. D. Ackerman pers. comm. in Catling, 1990

Table 2.2 (continued)

Species	Animal	Kind of Animal	Observer
Epidendrum latifolium (Lindl.) Garay & H.R.Sweet	selfing		Dressler, 1981; J. D. Ackerman, pers.comm. in Catling, 1990
Epidendrum latilabre Lindl.	*Amastus acona*	moth	Dodson, 1965
Epidendrum nocturnum Jacq.	selfing		Dressler, 1981; Luer, 1972; Ackerman pers. comm. in Catling, 1990; Stort and Pavanelli, 1985
Epidendrum pallens Rchb.f.	selfing		Catling, 1990; E. Hagsater pers. comm. in Catling, 1990
Epidendrum paniculatum Ruiz & Pav.	*Heliconia* sp.	butterfly	van der Pijl and Dodson, 1966
Epidendrum phragmites A.H.Heller & L.O.Williams	selfing		Hamer, 1982
Epidendrum pseudepidendrum Rchb.f.	unidentified hummingbird	hummingbird	van der Pijl and Dodson, 1966
Epidendrum radicans Pav. ex Lindl.	*Papilio* sp.	butterfly	Dodson, 1965
Epidendrum rigidum Jacq.	selfing		Catling, 1990; E. Hagsater pers. comm. in Catling, 1990
Isochilus carnosiflorus Lindl.	*Amazilia tzacatl*	hummingbird	Dodson, 1965
Isochilus linearis Jacq.	selfing		Northen, 1971
Laelia milleri Blumensch. ex Pabst	unidentified Hummingbird	hummingbird	van der Pijl and Dodson, 1966
Myrmecophila tibicinis (Batem.) Rolfe	*Eulaema polychroma* (Euglossinae)	bee	Rico-Gray and Thien,1987
Prosthechea baculus (Rchb.f.) W.E.Higgins	*Campsomeris columba*	wasp	van der Pijl and Dodson, 1966
Prosthechea boothiana (Lindl.) W.E.Higgins *var. erythronioides* (Small) W.E.Higgins	selfing		Luer, 1971; Dressler, 1981

Table 2.2 (continued)

Species	Animal	Kind of Animal	Observer
Prosthechea chacaoensis (Rchb.f.) W.E.Higgins	selfing		Dressler and Pollard, 1976
Prosthechea cochleata (L.) W.E.Higgins	*Campsomeris* sp.	bee	Dodson, 1972
Prosthechea cochleata (L.) W.E.Higgins var. *triandra* Ames	selfing		Luer, 1971; Dressler, 1981; J. D. Ackerman pers. comm. in Catling, 1990
Prosthechea cretacea (Dressler & G.E.Pollard) W.E.Higgins	selfing		Dressler and Pollard, 1976
Prosthechea ochracea (Lindl.) W.E.Higgins	selfing		Dressler and Pollard, 1976
Prosthechea pygmaea (Hook.) W.E.Higgins	selfing		J. D. Ackerman pers. comm in Catling, 1990
Prosthechea vespa (Vell.) W.E. Higgins	*Xylocopa frontalis*	bee	Dodson 1965
Prosthechea vespa (Vell.) W.E. Higgins	selfing		Hart, 1886
Prosthechea vespa (Vell.) W.E. Higgins	selfing		Catling, 1990
Prosthechea vespa (Vell.) W.E. Higgins	selfing		E. Hagsater pers. comm. in Catling, 1990
Prosthechea vespa (Vell.) W.E. Higgins	selfing		Fowlie, 1963
Prosthechea vespa (Vell.) W.E. Higgins	selfing		Jones, 1968a
Rhyncholaelia digbyana (Lindl.) Schltr.	Sphingidae	moth	Fuch, pers. comm. in van der Pijl and Dodson, 1966
Rhyncholaelia sp.	Sphingidae	moth	Roebuck and Steinhart, 1978
Scaphyglottis sp.	*Trigona* sp.	bee	Dressler, 1981
Schomburgkia lyonsii Lindl.	*Xylocopa* sp. (Anthophoridae)	bee	F. Bennet, pers. comm. in van der Pijl and Dodson, 1966
Schomburgkia moyobambae Schltr.	*Trigona nigrior*	bee	Dodson, 1965
Schomburgkia sp.	*Xylocopa* sp. (Anthophoridae)	bee	Dressler, 1981

Table 2.2 (continued)

Species	Animal	Kind of Animal	Observer
Schomburgkia splendida Schltr.	*Xylocopa lachnea* (Anthophoridae)	Bee	van der Pijl and Dodson, 1966
Sophronitis coccinea (Lindl.) Rchb.f.	10 spp. hummingbirds	Bird	Manuel et al., 1996

Table 2.3. Chromosome numbers in Laeliinae

Species	Gametic (n)	Somatic (2n)	Source
Brassavola cucullata (L.) R.Br.		40	Blumenschein 1960
Brassavola grandiflora Lindl.	20		Afzelius, 1943
Brassavola nodosa (L.) Lindl.		40	Kamemoto, 1950
Brassavola nodosa (L.) Lindl.		40	Blumenschein 1960
Brassavola nodosa 'gigas'		40	Kamemoto et al.,1961
Brassavola perrinii Lindl.	20		Afzelius, 1943
Broughtonia sanguinea (Sw.) R.Br.	20		Sagawa and Niimoto, 1961
Catteya intermedia Graham ex Hook. var. *aquinii*		40	Sagawa, 1962
Cattleya aurantiaca (Batem.) P.N.Don		40	Kamemoto, 1950
Cattleya aurea Linden		40	Sagawa, 1962
Cattleya bicolor Lindl.		40	Kamemoto, 1950
Cattleya bicolor Lindl.		40, 80	Blumenschein, 1960
Cattleya bicolor Lindl.		40	Blumenschein, 1961
Cattleya bicolor Lindl.		40	Stort, 1984
Cattleya bowringiana Veitch		40	Eftimiu-Heim, 1941
Cattleya bowringiana Veitch	20, 21	41	Kamemoto, 1950
Cattleya bowringiana Veitch		40, 42	Chadard, 1963
Cattleya dormaniana (Rchb.f.) Rchb.f.		40	Blumenschein, 1960
Cattleya dowiana Batem.	20	40	Kamemoto, 1950
Cattleya elongata Barb.Rodr. cv. 'nr. 1'		80	Kamemoto et al., 1961
Cattleya forbesii Lindl.		54-60	Chadard, 1963

Table 2.3 (continued)

Species	Gametic (n)	Somatic (2n)	Source
Cattleya gaskelliana Braem	20	40	Kamemoto, 1950
Cattleya guttata Lindl.		40	Kamemoto, 1950
Cattleya harrisoniana Batem. ex Lindl.	20	40	Kamemoto, 1950
Cattleya intermedia Graham ex		40	Kamemoto, 1950
Cattleya intermedia Graham ex Hook.		46, 55, 76	Li et al., 1992
Cattleya intermedia Graham ex Hook. var. *alba*		41+1F	Sagawa, 1962
Cattleya labiata Lindl.		40	Eftimiu-Heim, 1941
Cattleya labiata Lindl.	20	40, 42	Kamemoto, 1950
Cattleya labiata Lindl.	20	40	Vajrabhaya and Randolph, 1961
Cattleya labiata Lindl. cv. 'Westonbirt'		40	Kamemoto et al., 1961
Cattleya labiata Lindl. var. *amesiana*	20, 21	40, 41	Kamemoto, 1950
Cattleya lueddemanniana Rchb.f.	20	40	Kamemoto, 1950
Cattleya mossiae Hook.	20	40	Kamemoto and Randolph, 1949
Cattleya mossiae Hook. cv. 'Mrs.Butterworth'		ca. 60	Kamemoto, 1950
Cattleya mossiae Hook. cv. 'Verna'		40	Kamemoto, 1950
Cattleya mossiae Hook. var. *reineckiana* cv. 'Youngs'		41	Niimoto and Randolph, 1958
Cattleya mossiae Hook. var. *wageneri*		41	Niimoto and Randolph, 1958
Cattleya percivaliana O'Brien		40	Kamemoto and Randolph, 1949
Cattleya percivaliana O'Brien	20	40	Kamemoto, 1950
Cattleya rex O'Brien		40	Kamemoto, 1950
Cattleya skinneri Batem.		40	Kamemoto and Randolph, 1949
Cattleya skinneri Batem.	20	40	Kamemoto, 1950

Table 2.3 (continued)

Species	Gametic (n)	Somatic (2n)	Source
Cattleya skinneri Batem.		40	Chadard, 1963
Cattleya tigrina A.Rich.		40	Blumenschein, 1960
Cattleya trianaei Linden & Rchb.f.	20		Hoffmann, 1930
Cattleya trianaei Linden & Rchb.f.		40	Eftimiu-Heim, 1941
Cattleya trianaei Linden & Rchb.f.		40	Kamemoto, 1950
Cattleya trianaei Linden & Rchb.f. cv. ‘ Bloomhills’		40	Tanaka, 1964a
Cattleya trianaei Linden & Rchb.f. cv. ‘A.C. Burrage’		ca. 60	Kamemoto, 1952
Cattleya trianaei Linden & Rchb.f. cv. ‘A.C. Burrage’		60+-1	Kamemoto et al., 1961
Cattleya trianaei Linden & Rchb.f. cv. ‘Grand Monarch’		40	Kamemoto, 1959
Cattleya trianaei Linden & Rchb.f. cv. ‘Joan’		40	Kamemoto, 1950
Cattleya trianaei Linden & Rchb.f. cv. ‘Jungle Queen’		60	Sagawa, 1962
Cattleya trianaei Linden & Rchb.f. cv. ‘Llewellyn’		83	Kamemoto, 1950
Cattleya trianaei Linden & Rchb.f. cv. ‘Mary Fennell’		60	Sagawa, 1962
Cattleya trianaei Linden & Rchb.f. cv. ‘Mooreana’		ca. 60	Kamemoto, 1959
Cattleya trianaei Linden & Rchb.f. cv. ‘Naranja’		59	Sagawa, 1962
Cattleya trianaei Linden & Rchb.f.var. *alba*		40	Sagawa and Niimoto, 1961
Cattleya velutina Rchb.f.		40	Blumenschein, 1960
Cattleya walkeriana Gardner		40	Blumenschein, 1960
Cattleya warneri T.Moore		40	Kamemoto and Randolph, 1949
Cattleya warneri T.Moore		40	Kamemoto, 1950
Cattleya warscewiczii Rchb.f.	20	40	Kamemoto, 1950
Cattleya warscewiczii Rchb.f. cv. ‘Firmin Lambeau’		40	Kamemoto et al., 1961

Table 2.3 (continued)

Species	Gametic (n)	Somatic (2n)	Source
Caularthron bicornutum (Hook.) Raf.	20	40	Adair and Sagawa, 1969
Dimerandra emarginata (G.Mey.) Hoehne		40	Guerra, 1986
Encyclia cordigera (Kunth) Dressler	20	40	Kamemoto, 1950
Encyclia cordigera (Kunth) Dressler		80-90	Chadard, 1963
Encyclia mooreana (Rolfe) Schltr.		40	Eftimiu-Heim, 1941
Encyclia patens Hook.		40	Blumenschein, 1960
Encyclia patens Hook.		40	Chadard, 1963
Encyclia patens Hook. var. *serroniana*		40	Blumenschein, 1960
Encyclia tampensis (Lindl.) Small	20	40	Kamemoto and Randolph, 1949
Encyclia tampensis (Lindl.) Small	20	40	Kamemoto, 1950
Epidendrum appendiculatum T.Hashimoto		38	Nakata and Hashimoto, 1990
Epidendrum brachyphyllum Lindl.	30		Huynh, 1965
Epidendrum ciliare L.	20	40, 80, 160	Geitler, 1940
Epidendrum ciliare L.		40	Eftimiu-Heim, 1941
Epidendrum ciliare L.	20	40	Kamemoto, 1950
Epidendrum ciliare L.		40	Blumenschein, 1960
Epidendrum ciliare L.		40	Tanaka and Maekawa, 1983
Epidendrum conopseum R.Br.	20	40	Kamemoto, 1950
Epidendrum conopseum R.Br.		40	Chadard, 1963
Epidendrum cristatum Ruiz & Pav.		40	Blumenschein, 1960
Epidendrum denticulatum Barb.Rodr.		40	Blumenschein, 1960
Epidendrum difforme Jacq.		40	Chadard, 1963

Table 2.3 (continued)

Species	Gametic (n)	Somatic (2n)	Source
Epidendrum difforme Jacq.	40		Tanaka and Maekawa, 1983
Epidendrum diffusum Sw.	20	40	Kamemoto, 1950
Epidendrum ellipticum Graham		56	Blumenschein, 1960
Epidendrum elongatum Jacq.		56	Blumenschein, 1960
Epidendrum floribundum Kunth		40	Blumenschein, 1960
Epidendrum funckii Rchb.f.		40	Nakata and Hashimoto, 1990
Epidendrum lanipes Lindley		40	Tanaka and Maekawa, 1983
Epidendrum loefgrenii Cogn.		40	Blumenschein, 1960
Epidendrum longispathum Barb.Rodr.		40	Blumenschein, 1960
Epidendrum mosenii Barb.Rodr.		24	Blumenschein, 1960
Epidendrum nocturnum Jacq.	20		Hoffmann, 1929
Epidendrum nocturnum Jacq.	20		Hoffmann, 1930
Epidendrum nocturnum Jacq.		ca. 80	Kamemoto, 1950
Epidendrum nocturnum Jacq.		40, 80	Blumenschein, 1960
Epidendrum nocturnum Jacq.		74-85	Chadard, 1963
Epidendrum nocturnum Jacq. var. *guadetoupense*		42-38	Chadard, 1963
Epidendrum obrienianum Rolfe		40	Malla et al., 1977b
Epidendrum propinquum A.Rich. & Gal.		40	Kamemoto, 1950
Epidendrum purpureum Barb.Rodr.		56	Blumenschein, 1960
Epidendrum radicans Pavon ex Lindl.		40, 60	Kamemoto, 1950
Epidendrum radicans Pavon ex Lindl.		48-57	Chadard, 1963
Epidendrum radicans Pavon ex Lindl.	19		Mehra et al., 1970

Table 2.3 (continued)

Species	Gametic (n)	Somatic (2n)	Source
Epidendrum radicans Pavon ex Lindl.		64	Vijayakumar and Subramanian, 1994
Epidendrum radicans Pavon ex Lindl.		57, 62	Li and Chen, 1989
Epidendrum radicans Pavon ex Lindl.		57, 62	Li et al., 1992
Epidendrum raniferum Lindl.	20		Hoffmann, 1929
Epidendrum raniferum Lindl.	20		Hoffmann, 1930
Epidendrum rigidum Jacq.		40	Blumenschein, 1960
Epidendrum xanthinum Lindl.		40	Vij and Shekhar, 1985
Euchile citrina (La Llave & Lex.) Withner		40	Kamemoto, 1950
Euchile mariae (Ames) Withner		40	Tanaka, 1964
Hexisea reflexa Rchb.f.	19	38	Sau and Sharma, 1983
Laelia albida Batem. ex Lindl.		40, ca. 63	Kamemoto, 1950
Laelia anceps Lindl.		40	Blumenschein, 1960
Laelia autumnalis (La Llave & Lex.) Lindl.		41, 42	Kamemoto, 1950
Laelia briegeri Blumensch. ex Pabst		80	Blumenschein, 1960
Laelia caulescens Lindl.		80	Blumenschein, 1960
Laelia cinnabarina Batem. ex Lindl.		40	Blumenschein, 1960
Laelia cinnabarina Batem. ex Lindl.		40	Chadard, 1963
Laelia crispata (Thunb.) Garay (syn. *L. flava*)		40	Blumenschein, 1960
Laelia esalqueana Blumensch. ex Pabst		40	Blumenschein, 1960
Laelia gouldiana Rchb.f.		40, 60	Kamemoto, 1950
Laelia harpophylla Rchb.f.		40	Blumenschein, 1960
Laelia longipes Rchb.f.		40, 60, 80	Blumenschein, 1960

Table 2.3 (continued)

Species	Gametic (n)	Somatic (2n)	Source
Laelia lucasiana Rolfe		40	Blumenschein, 1960
Laelia milleri Blumensch. ex Pabst		40	Blumenschein, 1960
Laelia mixta Hoehne		40	Blumenschein, 1960
Laelia peduncularis Lindl.		40-44	Chadard, 1963
Laelia perrinii Batem.		40	Blumenschein, 1960
Laelia pumila (Hook.) Rchb.f.		40	Blumenschein, 1960
Laelia pumila (Hook.) Rchb.f.		40	Chadard, 1963
Laelia purpurata Lindl. & Paxton		40	Kamemoto, 1950
Laelia purpurata Lindl. & Paxton		40	Blumenschein, 1960
Laelia purpurata Lindl. & Paxton var. *semi-alba*		40+-1	Kamemoto et al., 1961
Laelia rubescens Lindl.	20	40	Kamemoto and Randolph, 1949
Laelia rubescens Lindl.	20	40	Kamemoto, 1950
Laelia rupestris Lindl.		80	Blumenschein, 1960
Laelia tereticaulis Hoehne		80	Blumenschein, 1960
Lanium avicula (Lindl.) Benth.		40	Blumenschein, 1960
Leptotes unicolor Barb.Rodr.		40	Blumenschein, 1960
Neohlemannia angustata (Hashimoto) Hashimoto		36	Nakata and Hashimoto, 1990
Prosthechea brassavolae (Rchb.f.) W.E.Higgins	20	40	Kamemoto, 1950
Prosthechea calamaria (Lindl.) W.E.Higgins		40	Blumenschein, 1960
Prosthechea campylostalix (Rchb.f.) W.E.Higgins	20	40	Kamemoto and Randolph, 1949
Prosthechea campylostalix (Rhcb.f.) W.E.Higgins		40	Kamemoto, 1950
Prosthechea cochleata (L.) W.E.Higgins	20	40	Kamemoto, 1950

Table 2.3 (continued)

Species	Gametic (n)	Somatic (2n)	Source
Prosthechea fragrans (Sw.) W.E.Higgins		40	Blumenschein, 1960
Prosthechea fragrans (Sw.) W.E.Higgins		40	Tanaka and Maekawa, 1983
Prosthechea glumacea (Lindl.) W.E.Higgins		40	Blumenschein, 1960
Prosthechea lindenii (Lindl.) W.E.Higgins		56	Blumenschein, 1960
Prosthechea linkiana (Klotzsch) W.E.Higgins	20		Hoffmann, 1930
Prosthechea linkiana (Klotzsch) W.E.Higgins	20	40	Kamemoto, 1950
Prosthechea ochracea (Lindl.) W.E.Higgins	20	40	Kamemoto and Randolph, 1949
Prosthechea ochracea (Lindl.) W.E.Higgins	20	40	Kamemoto, 1950
Prosthechea prismatocarpa (Rchb.f.) W.E.Higgins		40	Blumenschein, 1960
Prosthechea vespa (Vell.) W.E.Higgins		40	Blumenschein, 1960
Rhyncholaelia digbyana (Lindl.) Schltr.		40	Chadard, 1963
Scaphyglottis prolifera (R.Br. ex Lindl.) Cogn.		40	Nakata and Hashimoto, 1990
Schomburgkia crispa Lindl.		40	Blumenschein, 1960
Sophronitis cernua (Lindl.) W.J.Hook.		40	Blumenschein, 1960

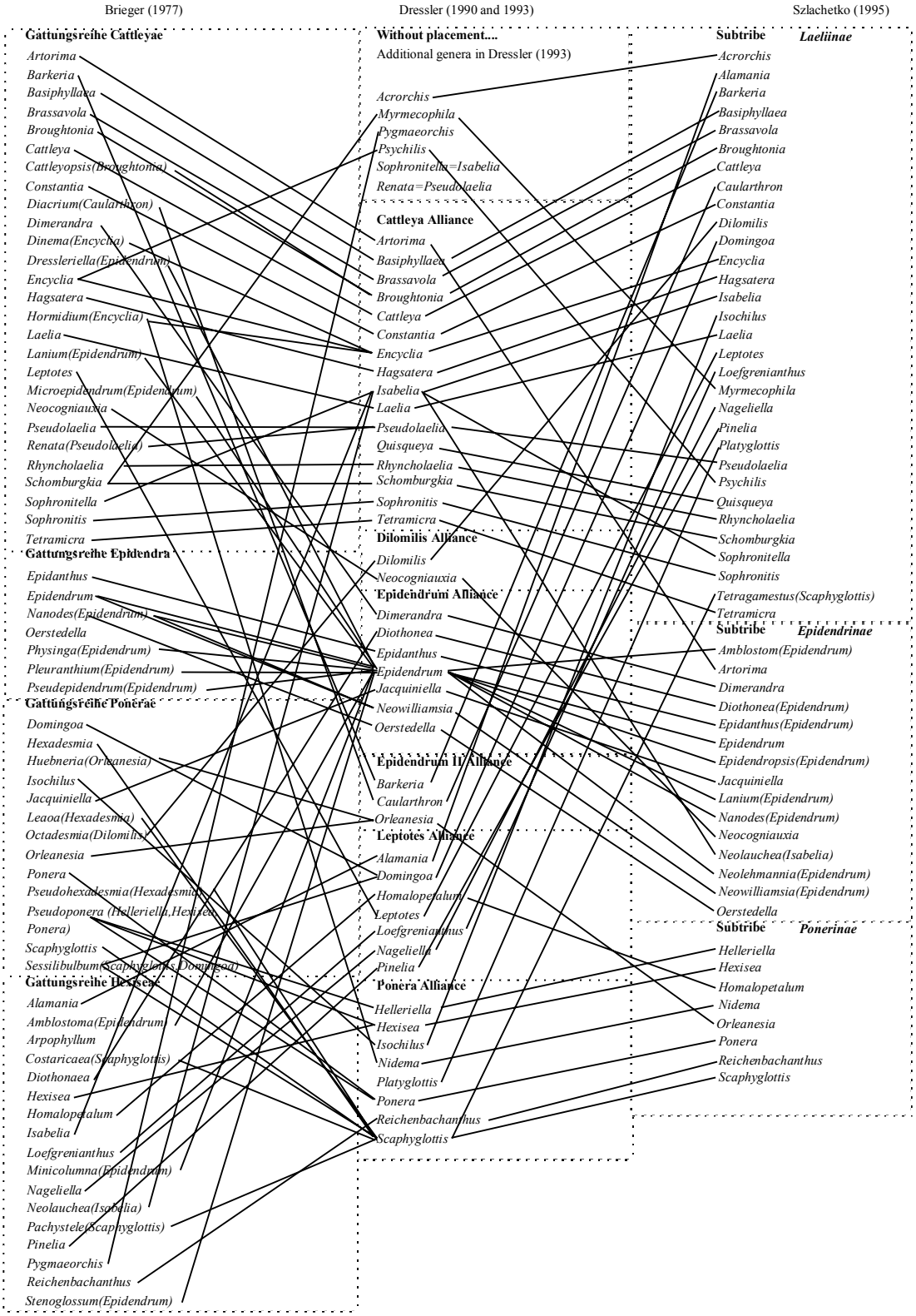


Fig. 2.1. Three recent systems of classification for Laeliinae. The lines show equivalent concepts in each system and the boxes outline proposed generic alliances.

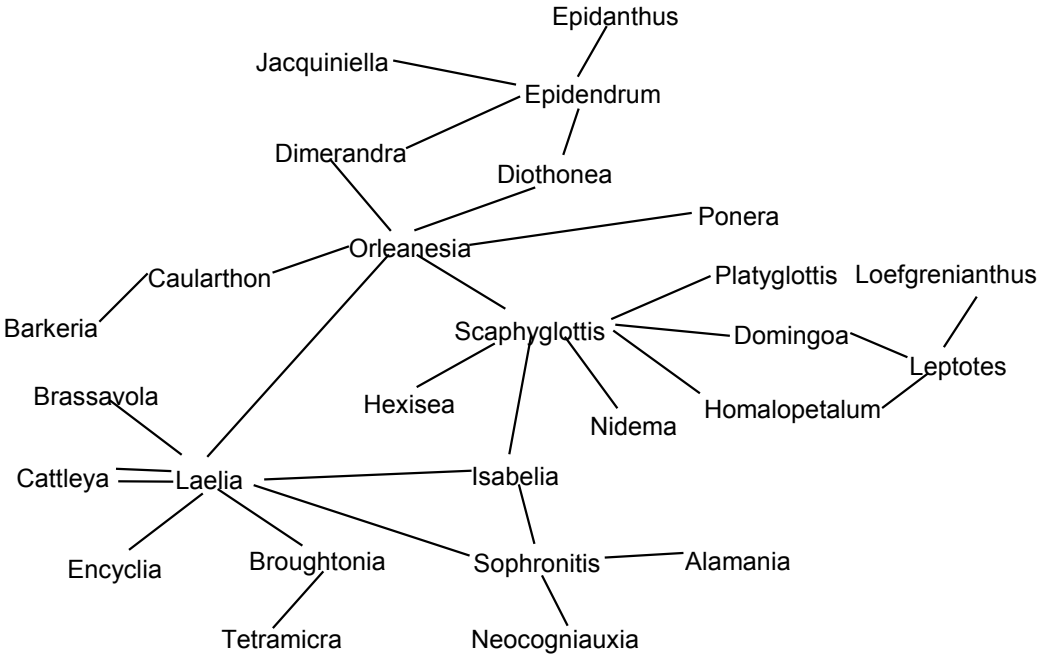


Fig 2.2. Generic relationships proposed by Dressler (1961). Note that *Cattleya* and *Laelia* are considered congeneric and many genera are not taken into account.

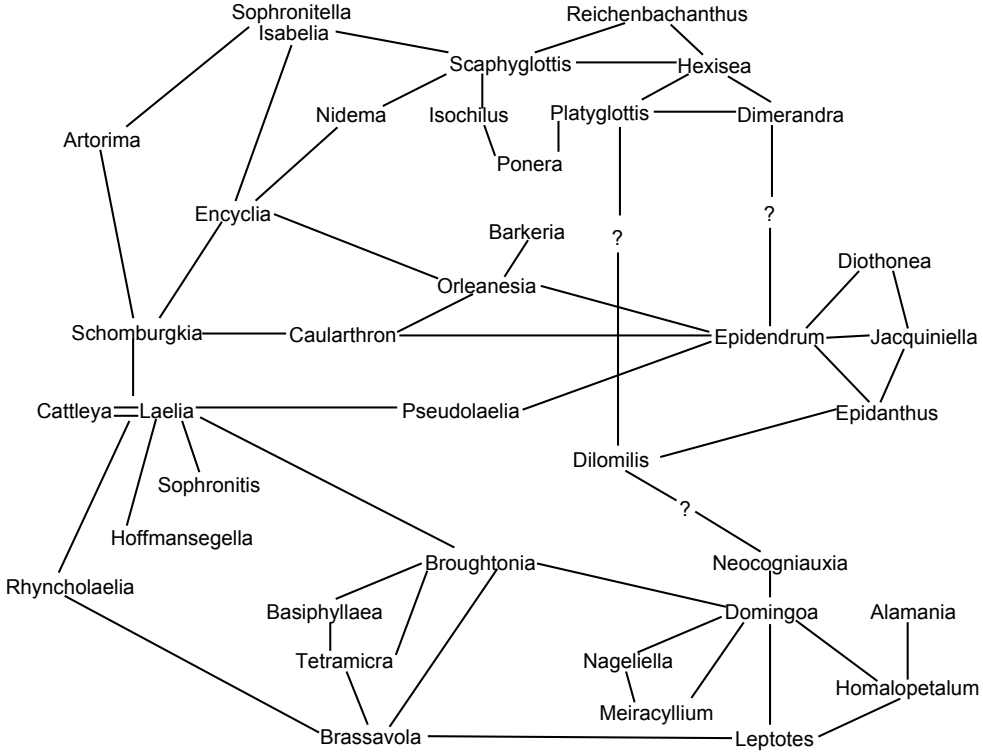


Fig 2.3. Relationships in the Laeliinae based on anatomical characters (after Baker, 1972).

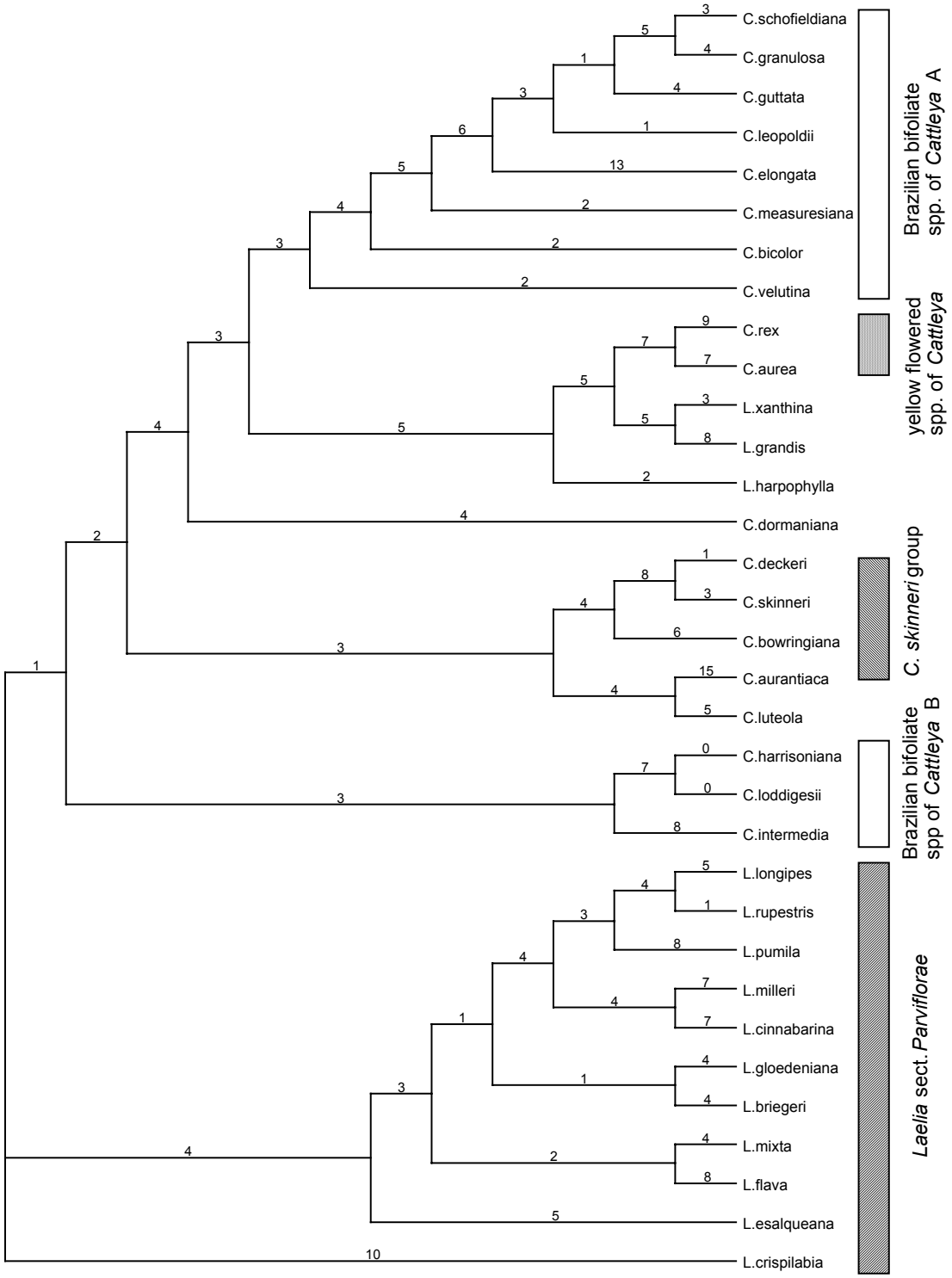


Fig. 2.4. A flavonoid phylogeny of *Cattleya* and *Laelia* constructed through a parsimony analysis of the data of Tosello (1969). One of 16 most parsimonious trees, rooted with all species of *Laelia* as outgroup.

Chapter 3 – A phylogenetic analysis of Laeliinae (Orchidaceae) based on sequence data from nuclear internal transcribed spacers (ITS) of ribosomal DNA

3.1. INTRODUCTION

In this chapter, I am particularly concerned with the delimitation of genera in Laeliinae. With a large number of samples (295 accessions) it is feasible to sequence only one DNA region, and I chose ITS to maximise the number of variable sites. All genera of Laeliinae are included, with a very thorough sampling of the *Cattleya* alliance, whereas only a limited sampling of *Epidendrum* and related genera is taken. A second aim was to infer species phylogenies and infrageneric groups within some genera of the *Cattleya* alliance. One possible disadvantage of the use of ITS without comparison with data from other regions (e.g. plastid) is that topologies could differ if past hybridisation is involved in past speciation events. For this reason, a complementary approach with several regions is used in Chapter 4.

3.2. MATERIALS AND METHODS

Material from most genera of Laeliinae and nearly all species in the *Cattleya* alliance was sampled (Table 3.1). I was unable to obtain samples of *Pinelia, Pygmaeorchis* and *Basiphyllaea*. The latter, however, was found to be a member of Bletiinae in recent analyses of *matK* (D. Goldman, pers. comm., 1999) and ITS (V. Sosa, pers. comm., 1999). I also sampled multiple taxa representing Chysiinae, Coeliinae, Bletiinae, Pleurothallidinae, Arpophyllinae and Meiracylliinae. An assemblage of Old World Epidendroideae was used as multiple outgroups: *Thunia alba, Pleione chunii, Calanthe tricarinata, Earina autumnalis* and *Polystachya galeata.* These were chosen based unpublished data of ITS, *trnL-F* and *matK* (Chapter 4) and D. Goldman (pers. comm., 1999). *Polystachya* was included because it was placed near Laeliinae by Cameron et al. (1999). Despite being putatively related to Laeliinae in the classification of Dressler (1993), members of Sobraliinae were not included because of their excessively divergent sequences as well as their distant position in Cameron et al. (1999).

DNA was extracted mostly from fresh leaves or flowers using a method based on Doyle and Doyle (1987), modified by inclusion of purification through caesium

chloride/ethidium bromide gradient (1.55 g ml^{-1}). The ITS region including the 5.8S gene was then amplified with the primers 17SE and 26SE of Sun et al. (1994). PCR products were cleaned with QIAquick silica columns (QIAGEN Ltd.), adding guanidinium chloride (35%) to remove primer dimers. PCR products were sequenced in both directions using the same primers and also ITS5 and ITS4 (White et al., 1990; Baldwin, 1992), using the Big Dye kit in an ABI 377 automated sequencer following manufacturer's protocols (PE Applied Biosystems Inc., Warrington, Cheshire, UK). Electropherograms were superposed and edited using Sequencher 3.0 (Genecodes Inc., Ann Arbor, Michigan), and the resultant sequences were first aligned using Clustal W (Thompson et al., 1995) and then further adjusted by eye. Phylogenetic analysis was performed with PAUP 4.0b2 (Swofford, 1998) with Fitch parsimony (equal weights, unordered; Fitch, 1971). Initially 1000 random taxon-addition replicates were performed to look for multiple optimal tree islands (Maddison, 1991). The search was performed with the subtree pruning-regrafting (SPR) algorithm, but I limited swapping to only 15 trees per replicate to prevent extensive swapping on suboptimal islands. The resulting shortest trees were then used as starting trees using the tree bisection-reconnection (TBR) algorithm until I obtained a set limit of 10,000 trees. I used both a matrix with the sequences alone as well as another including binary gap coding of all gaps of three base pairs (bp) or more. This was constructed with PAUPGAP v. 1.1.2 (Cox, 1997) but then limited to only gaps of three bp or more. Support was evaluated through bootstrapping (Felsenstein, 1985) of 1000 replicates with simple taxon addition and TBR branch swapping, but saving only 15 trees per replicate.

3.3. RESULTS

The results including the gaps did not conflict with the original matrix, and because the trees were much more resolved due to the extra information contained in the gaps, I decided to use the analysis including gaps as a basis for the present discussion. The aligned ITS sequence matrix had 851 positions, to which I added 198 gap characters (coded as plus/minus). The gap positions themselves were coded as missing information. In the complete matrix, 535 of the 1049 characters were potentially parsimony informative. In the heuristic search, I found more than 10,000 trees (the limit I enforced) of 3958 steps, with consistency index (CI, including autapomorphies) = 0.26 and retention index (RI) = 0.71. One of these trees is

presented in summary in Figure 3.1 and as a series of detailed subclades in Figures 3.2-3.6, with the Fitch length above and the bootstrap percentages below each branch. An arrowhead indicates a group collapsing in the strict consensus of the 10,000 trees. The CI/RI for transitions (ts) and transversions (tv) were 0.25/0.71 and 0.30/0.69, respectively, and the ts/tv ratio was 2.08. The CI excluding uninformative characters and RI from the DNA sequences and gap-coding characters were 0.28/0.71 and 0.19/0.76, respectively.

Based on ITS data, Laeliinae are monophyletic if some genera are removed to other subtribes. One such case is *Dilomilis* and *Neocogniauxia*, which are sister to Pleurothallidinae with high bootstrap support (97%). The other is a group of genera with a column foot, namely *Ponera, Helleriella,* and *Isochilus*, which form an independent clade sister to both Laeliinae and Pleurothallidinae/*Dilomilis*/ *Neocogniauxia*. However, additional genera with a column foot, such as *Scaphyglottis*, *Hexisea, Reichenbachanthus*, *Domingoa* and *Homalopetalum,* are members of Laeliinae. The ITS data place *Arpophyllum* as sister to Laeliinae with high bootstrap support (98%), but place *Meiracyllium* within the subtribe, close to *Euchile* (the former *Encyclia mariae/E. citrina* group).

There are several distinct generic clusters in Laeliinae, although only few of them have high bootstrap support, which is due to the overall low variability of ITS, especially in the spine of the tree. Despite the low support, most of these clusters appear consistently in 10,000 shortest trees and are consistent with previous taxonomy, whereas others represent assemblages of genera from distinct floristic regions.

One of these clades (68%) is composed of *Pseudolaelia, Renata, Isabelia, Neolauchea, Sophronitella* and *Constantia* (Fig. 3.2), an assemblage of small Brazilian genera that are either epiphytic on *Vellozia* (Velloziaceae) or found in rather dry habitats in savannah vegetation. They also share peculiar similar short side lobes of the lip and short columns. Another such group (82%) is *Broughtonia, Laeliopsis, Cattleyopsis, Psychilis, Quisqueya* and *Tetramicra* (Fig. 3.2), all from the Caribbean. In Figure 3.3, the clade of Mexican *Laelia/Schomburgkia* and *Domingoa, Nageliella* and *Homalopetalum* does not appear in the strict consensus, although all of its members are also principally Mexican. The montane species of *Laelia* (containing the type species *L. speciosa*) fall into a separate subclade from *L. anceps*

and *L. rubescens,* which in turn cluster with *Schomburgkia*. It is important to notice that all these species of *Laelia sensu stricto* are distantly placed from the Brazilian species of *Laelia*, which belong to the '*Cattleya* alliance' (Fig. 3.6). Another clade in Figure 3.3 contains the genera with a column foot: *Scaphyglottis, Reichenbachanthus, Hexisea* and *Platyglottis*. This also shows clearly the position of *Hexadesmia* and *Tetragamestus* embedded in *Scaphyglottis*. The species known as *'Helleriella' punctulata* is in fact also a *Scaphyglottis* and has no close relationship to *H. nicaraguensis* and *H. guerrerensis* of Ponerinae (Fig. 3.2). The '*Epidendrum* alliance' appears as a clade (Fig. 3.3) and includes *Epidendrum*, *Orleanesia, Amblostoma, Barkeria, Lanium, Nanodes* and *Caularthron*. Although there is a clade with all genera once considered to be part of *Encyclia* (excluding *Psychilis;* Fig. 3.4), it appeared only in 98% of the trees and therefore collapses in the strict consensus. One of its subclades has *Encyclia sensu stricto* plus *Meiracyllium* and *Euchile* (the latter segregated by Withner, 1998), and a second has *Prosthechea*, with *Alamania, Artorima* and *Hagsatera* as consecutive sister taxa, which is in turn sister to a small clade containing *Dinema, Nidema* and *Dimerandra*.

Finally, there is a large assemblage of taxa that I will refer to here as the '*Cattleya* alliance' (Figs 3.5, 3.6), which includes *Cattleya, Brassavola, Myrmecophila, Sophronitis* and the Brazilian species of *Laelia*. Although I sampled most of the species in these genera, the low level of variation among species complexes made phylogeny reconstruction difficult, for example in *Laelia* section *Parviflorae* (Fig. 3.6). It is quite clear that *Sophronitis* and *Laelia* are closely related, and most of the sections proposed by Schlechter (1917) and Withner (1990) are present. *Cattleya* is polyphyletic, but there are two main sister clades, one including the unifoliate species and the other composed of the Brazilian bifoliate species. However, the group of *Cattleya skinneri* (*C. skinneri, C. patinii* and *C. aurantiaca*) is closer to *Rhyncholaelia,* whereas *C. bowringiana* and *C. araguaiensis* occur in isolated positions. There was also a previously unrecognised group of unifoliate *Cattleya* species (*C. trichopiliochila, C. lawrenceana* and *C. luedemmaniana*) that are sister to the Brazilian species of *Laelia*, which also includes *C. maxima*. *Brassavola* has one group of species with high (98%) bootstrap support but is paraphyletic to *Cattleya* due to the position of three species that fall outside this group (*B. acaulis, B. tuberculata* and *B. cucullata*; Fig. 3.5). However, these

relationships received less than 50% bootstrap support and collapse in the strict consensus.

3.4. DISCUSSION

Despite the large number of informative characters in the matrix, most groups exhibited low levels of sequence divergence. There was a significant bias toward transitions, but both transitions and transversions had nearly identical RIs and therefore performed equally well in providing phylogenetic patterns. As a consequence there is no reason to apply differential weights to each category (e.g. Albert et al. 1993).

The placement of *Dilomilis* and *Neocogniauxia* as sister to Pleurothallidinae agrees with the *rbcL* results of Cameron et al. (1999), which included only *Dilomilis*. This group presumably also includes *Tomzanonia*, which was not available for this study. Dressler (1993) mentioned that *Dilomilis scirpoidea* has seed-coat characters between the *Pleurothallis* and *Elleanthus* seed types. However, *Dilomilis* and *Neocogniauxia* both lack the articulated joint that is a synapomorphy for Pleurothallidinae and also have a reed-stem habit (although reduced in *Neocogniauxia monophylla*), which is absent in that subtribe. The placement of this group should be confirmed with additional genes before a taxonomic decision to include them in Pleurothallidinae or treat them as a separate subtribe is made.

In the morphological analysis of Freudenstein and Rasmussen (1999), *Isochilus* also fell outside Laeliinae, but Cameron et al. (1999) did not sample *Ponera, Helleriella* and *Isochilus*. Therefore, the fact that *Ponera* and *Helleriella* belong in a separate clade with *Isochilus* is new to these results. The subtribal name, Ponerinae, has been used by Schlechter (1926), Szlachetko (1995), and Brieger (as a 'Gattungsreihe'; 1976), for all the members of Laeliinae *sensu* Dressler (1993) possessing a column foot and hinged lip. Based on the ITS results, Ponerinae need to be used in a more restricted sense, including only *Ponera, Isochilus* and *Helleriella* (excluding *H. punctulata*).

The positions of *Arpophyllum* and *Meiracyllium* conflict with the topology of Cameron et al. (1999), but their sampling was limited and bootstrap support in the *rbcL* trees was low for these taxa. These also conflict with the placement of *Arpophyllum* and *Meiracyllium* as sister to each other and sister to the rest of Laeliinae in Freudenstein and Rasmussen (1999), which was likely due to the same

characters of the pollinaria used by Dressler (1971, 1990b) to place these genera in their own monogeneric subtribes (i.e. ovoid and clavate pollinia). It was unexpected that *Arpophyllum* would be sister to Laeliinae because this genus seems to have an overall morphological similarity with Pleurothallidinae. Baker (1972) found that many of the characteristic anatomical features of Laeliinae are absent from *Arpophyllum.* However, *Arpophyllum* also lacks the helical thickenings of the internal foliar tissues typical for Pleurothallidinae.

In this study *Laelia*, *Cattleya*, *Encyclia s.l.* and *Epidendrum* are clearly indicated to be polyphyletic. *Laelia* was suggested to be artificial by Dressler (1981, 1993) and more recently by Halbinger and Soto Arenas (1997). In the morphological cladistic analysis of Halbinger and Soto (1997) the several clades of *Laelia* formed an unresolved polytomy with different sections of *Cattleya*, *Brassavola* and *Sophronitis*, but *L. anceps* (Mexican) was sister to *Schomburgkia*. The polyphyly of *Laelia* can be explained by the fact that the diagnostic characters for *Laelia*, such as the presence of eight pollinia, seem to be plesiomorphies. The same interpretation applies to the simple, large and showy bee-pollinated flowers that differ little from *Cattleya.* Other unrelated orchid genera with such bee flowers include *Bletia, Epistephium, Sobralia* and *Trichopilia,* which are undoubtedly the result of convergent evolution. *Laelia* has also been defined by the absence of all characters used to segregate other genera in Laeliinae, such as hinged lips, reed-stem habit, fusion of the column with the lip, or particular vegetative adaptations such as the hollow pseudobulbs of *Caularthron* and *Myrmecophila*.

It is still unclear whether the montane species of *Laelia s.s.* (*L. albida, L. autumnalis, L. furfuracea, L. gouldiana* and *L. speciosa*) are in a clade distinct from *L. anceps* and *L. rubescens*, but the Brazilian species of *Laelia* have to be reclassified. Because *Sophronitis* is polyphyletic and clearly embedded within them, the best solution is to transfer all the Brazilian *Laelia* species into *Sophronitis*. It could be argued that *Sophronitis* should be maintained distinct and instead that resurrection of *Hoffmannseggella* (Jones, 1968b), which had been proposed for *Laelia* sect. *Parviflorae*, would be more appropriate. However, the type species of *Sophronitis* is *S. cernua,* and the only way to keep *Sophronitis* as a distinct, monophyletic genus would be by restricting it to *S. cernua* plus *L. harpophylla* and *L. kautskyi*. In that case, *L. lundii* would need to be a monotypic genus, and all the

other species of *Sophronitis* would have to be placed in *Hoffmannseggella.* I prefer instead to incorporate all of these species into *Sophronitis s.l.* because there are no greater morphological differences between *Sophronitis* and the *Parviflorae, Hadrolaelia* and *Cattleyodes* than among these subgroups themselves. The new combinations were proposed by van den Berg and Chase (2000).

The placement of *C. trichopiliochila/C. lueddemanniana/C. lawrenceana* in the Brazilian *Laelia* clade, and especially *C. maxima*, is unexpected, because they always have been considered part of the *C. labiata* complex. The high level of divergence for the latter (29 steps; Fig. 3.6) in comparison with the overall low variation in this part of the tree could mean that these are paralogous copies of ITS. However, by cloning these species I was unable to obtain other ITS copies that would provide a more reasonable placement of these members of *Cattleya* subgenus *Cattleya*. Past hybridisation events and gene conversion could be alternative explanations for these observations.

In a similar manner, it is clear that *Schomburgkia* and *Myrmecophila* belong to distinct clades (Figs 3.3, 3.5), the first close to *Laelia s.s.* and the second in the *Cattleya* alliance. However, the position of *Schomburgkia* in relation to *Laelia s.s.* needs to be clarified. In *Cattleya,* there is a clear distinction between bifoliate and unifoliate clades, but for nomenclatural stability I recommend keeping them all as a single genus. However, a new genus would be needed for *C. skinneri*, *C. aurantiaca* and *C. patinii* unless they are transferred to *Rhyncholaelia*. These bifoliate species of *Cattleya* are characterised by a mosaic of characters present in the uni- and bifoliate species, such as an entire lip and fusiform pseudobulbs typical of the former but the leaf number of the latter (two to three). If it is accurate, the position of *C. araguaiensis* and *C. bowringiana* would also require them each to be made monotypic genera, but the low levels of divergence detected could implicate character sampling error as the cause of these unexpected placements. Although *C. araguaiensis* is morphologically distinct from all other species of *Cattleya*, the only morphological difference between *C. bowringiana* and the group of *C. skinneri* is the dilated discoid base of the pseudobulbs. Due to the lack of bootstrap support, it appears more appropriate to postpone these taxonomic decisions until additional regions of DNA are sequenced to confirm these placements. The paraphyly of *Brassavola* in relation to *Cattleya* might serve as a model for this sampling error

phenomenon because in a combined analysis of ITS, *matK* and *trnL-F* (Chapter 4) *Brassavola* becomes monophyletic. One of the effects of taxon sampling in low levels of divergence is that a set of species can form a grade, whereas with more data these same taxa form a well-supported clade (Sheahan and Chase, 2000).

In the *Epidendrum* alliance, it appears also that *Epidendrum* would need further segregation of genera to be able to maintain groups such as *Barkeria* and *Oerstedella*. The sampling of species in these genera was, however, extremely limited, and a larger study is needed to clarify the relationships. The small clade with *Orleanesia, Caularthron* and *Amblostoma armeniacum* (Fig. 3.3) appears to be related to *Epidendrum* (although with bootstrap support <50%). At least *Caularthron* has anatomical affinities to *Epidendrum* according to Baker (1972). Unlike the other genera in this group, *Caularthron* has a sessile lip (at least in *C. bicornutum*), but the hollow stems seem to be just a thicker version of the typical reed-stem habit of *Epidendrum*.

In *Encyclia s.l.,* segregated genera formerly included in this genus (e.g. *Euchile, Prosthechea* and *Dinema,* but not *Psychilis*) did not form a clade in all shortest trees. Several monospecific genera (e.g. *Hagsatera, Artorima* and *Alamania*) were located near *Prosthechea*, and *Meiracyllium* near *Euchile. Meiracyllium* should be included in the Laeliinae, rather than in its own subtribe. In agreement with this placement, Baker (1972) did not find any differences in the foliar anatomy between *Meiracyllium* and the rest of Laeliinae and suggested that it is close to *Domingoa* and *Nageliella*, a placement not confirmed here. Increased sampling in *Encyclia* and related genera is required, due to the large number of species (W. E. Higgins et al., in prep.).

An interesting pattern found here is the placement of most monotypic genera or species with unusual/unique morphology as sister to large clades rather than being embedded in them (i.e. they are not derived from their more species-rich sister taxa). Examples of these are *Loefgrenianthus, Hagsatera, Alamania, Artorima, Laelia lundii, Laelia perrinii, Laelia virens, Laelia fidelensis, Cattleya aurantiaca, Cattleya araguaiensis, Cattleya bowringiana* and *Myrmecophila wendlandii.* Such species in Laeliinae therefore often represent relic lineages that never speciated and occupy habitats atypical for the subtribe. An alternative explanation based on extinction does not seem likely given the low molecular divergence in these groups.

On biogeographic grounds, it appears that Laeliinae and perhaps Pleurothallidinae originated in Mesoamerica and the Caribbean. This is clearer from the outgroup relationships; for example *Arpophyllum, Ponera* and *Isochilus* have representatives extending to Colombia, or even southern Brazil, but these genera are far more diverse in Mexico and Guatemala. *Bletia*, *Hexalectris, Chysis* and *Coelia* follow the same pattern. Similarly, *Dilomilis/Neocogniauxia* are exclusively Caribbean. The *Epidendrum* and *Encyclia* clades have their diversity more or less evenly spread through the Neotropics, but northern elements are sister to the rest of the more derived groups. For example *Artorima, Alamania* and *Hagsatera* are sister to *Prosthechea*, and two Mexican species of *Encyclia* (*E. bractescens, E. adenocaula*) are sisters to the rest of that genus. Examining the most derived members of the subtribe such as the *Cattleya* alliance, species diversity is centred in southeastern Brazil, but always retaining Caribbean/Mexican elements as sisters (e.g. *Myrmecophila*, *Brassavola* and the *Cattleya skinneri* group). However, this pattern is difficult to assess among the main groups of the subtribe because the group containing *Pseudolaelia* and relatives is exclusively Brazilian and sister to the rest of Laeliinae. There is no bootstrap support for the main spine on the tree, but if the position of this group is maintained in further studies it would indicate that South America was colonised twice by taxa originating in the north. The other explanation for the pattern of Mexican/Caribbean taxa being sister to more widespread clades is that the former are relics of lineages that have died out in South America.

3.4.1. ASSESSMENT OF SELECTED TAXONOMIC CHARACTERS IN LAELIINAE

Some of the morphological characters previously emphasised in the taxonomy of Laeliinae appear to be especially homoplastic. Overall flower morphology seems to be susceptible to rapid change, driven by pollinator selection. A clear case of this is *Rhyncholaelia* and *Brassavola*, which were formerly considered a single genus and are both pollinated by sphingid moths, but which appear to be independently derived here.

Possession of a column foot is another such case. This character appears to be widespread in many different groups in Epidendroideae, including Bletiinae, Chysiinae, Cyrtopodiinae, Dendrobiinae, Eriinae, Pleurothallidinae and many

Maxillarieae. In Laeliinae, it seems to have evolved independently in *Scaphyglottis* and its relatives and in *Domingoa/Nageliella/Homalopetalum*. If it is not a plesiomorphy, the column foot in *Ponera*, *Isochilus,* and *Helleriella* could be the result of a third separate evolutionary event. In *Jacquiniella* the column foot is a saccate nectary (Dressler, 1981), and based on the ITS topology this genus might be sister to the *Scaphyglottis* clade, so it is unclear whether this would be a fourth evolutionary event.

Pollinium number also shows multiple parallelisms. The primitive number would appear to be eight, present also in the sister group of Laeliinae, *Arpophyllum*. Reduction to four pollinia therefore occurred independently in *Isochilus, Reichenbachanthus, Hexisea*, *Nageliella* and some subgroups within *Encyclia, Epidendrum* and *Cattleya*.

In vegetative characters, there are also clear examples of multiple origins. The most striking are the hollow stems of *Caularthron* and *Myrmecophila,* which are regularly used by ants as nesting sites. This sort of specialised morphological adaptation is relatively rare in terrestrial angiosperms, although repeatedly evolved in different families of epiphytes (Benzing, 1990). In *Myrmecophila,* this phenomenon appears to include absorption of nutrients (Rico-Gray et al., 1989), but in *Caularthron* the association seems to have a protective function only (Fisher and Zimmermann, 1988).

The reed-stem habit seems to be so common in Epidendroideae that it is likely to be plesiomorphic. In many cases, it could reflect a primary primitive state: *Ponera/Isochilus/Helleriella* (Ponerinae); *Dilomilis/Neocogniauxia* and *Jacquiniella.* This character was the primary reason that *Scaphyglottis punctulata* was transferred by Garay and Sweet (1974) to *Helleriella*. In the *Epidendrum* clade, which typically have reed-stems, there are also obvious reversals to the typical pseudobulbs, and species such as *E. ciliare* and *E. oerstedii,* which are vegetatively similar to *Cattleya*, led Brieger (1976b) to segregate *Auliza*. However, the vegetative diversity in this clade is unusually high (Pérez Garcia, 1993), and plants with similar flowers can have strikingly different habits (e.g. *E. ciliare, E. oerstedii, E. nocturnum, E. falcatum*, *E. parkinsonianum* and *E. viviparum*). The widespread nature of the reed-stem habit and the many apparent reversals leads us to conclude that its taxonomic importance is limited.

It is important to compare our results with the foliar anatomy data of Baker (1972), which constitute the only alternative large-scale study of Laeliinae. Most of the characters he studied are polymorphic in the generic grouping he proposed, and an attempt to produce a cladogram by coding these characters in addition to other morphological characters produced an unresolved polytomy (C. van den Berg, unpubl.). This could be explained by the fact that many vegetative characters are adaptations to specific climatic conditions and therefore likely to change according to habitat selective pressures. The generic relationships he traced based on trends rather than any strict character coding (reproduced in Dressler, 1981) coincide with some of the groups present in the ITS tree, but most of these have at least one genus misplaced. Notably, Baker (1972) failed to report any differences between *L. anceps* (Mexico) and *L. purpurata* and *L. pumila* (both Brazilian). Similarly, he found no differences between *Myrmecophila wendlandii* and *Schomburkgia splendida*, which he treated under *Schomburgkia*. He reported, however, the distinctness of *Ponera* from *Scaphyglottis* but mentioned that *Isochilus* is related to both. The main difficulty in using Baker's data is the subjective manner in which the characters were assessed.

Further work is needed to clarify the relationships of Laeliinae both at the generic and species levels, although most of the outgroup relationships have been well resolved with ITS data alone. In groups for which the species sampling is nearly complete (e.g. the *Cattleya* alliance), the use of additional DNA regions should lead to increased support of some clades and resolution of polytomies. In other groups, such as the *Epidendrum* alliance and *Encyclia s.l.,* a much more thorough taxonomic sampling is required. The use of regions with different patterns of molecular evolution, such as nuclear protein-coding genes and plastid genes and spacers, should also clarify how much of the organismal phylogeny is recovered by ITS data. This is an especially important issue in groups such as Laeliinae in which only ecological and limited physiological incompatibility barriers exist (Stort, 1984; van den Berg, 1998). Therefore, hybridisation cannot be disregarded as a mode of speciation and a cause of conflict in trying to reconstruct phylogenies.

Table 3.1. Plant material and voucher information in this study.

Species	Voucher
Acrorchis roseola Dressler	unvouchered (coll. M.W. Whitten)
Alamania punicea La Llave & Lex.	van den Berg C184 (ESA)
Amblostoma armeniacum (Lindl.) Brieger ex Pabst	van den Berg C2 (ESA)
Amblostoma cernuum Scheidw.	Brieger Coll. 15628 (ESA)
Aplectrum hyemale Torr.	Chase O-104 (K)
Arpophyllum giganteum Hartw. ex Lindl.	Chase O-586 (K)
Arpophyllum spicatum La Llave & Lex.	M. Soto 7814 (AMO)
Artorima erubescens (Lindl.) Dressler & G.E.Pollard	unvouchered (coll. S. Beckendorf)
Barkeria skinneri (Batem. ex Lindl.) Lindl. ex Paxton	van den Berg C250 (K spirit)
Barkeria whartoniana (C.Schweinf.) Soto Arenas	van den Berg C163 (K spirit)
Barkeria whartoniana (C.Schweinf.) Soto Arenas	van den Berg C249 (K spirit)
Bletia parkinsonii Hook.	Chase O-1215 (K)
Brassavola acaulis Lindl. & Paxton	W. M. Whitten 99218 (FLAS)
Brassavola cucullata (L.) R.Br.	W. E. Higgins 130 (FLAS 198290)
Brassavola cucullata (L.) R.Br.	van den Berg C174 (K spirit)
Brassavola grandiflora Lindl.	W. M. Whitten 99216 (FLAS)
Brassavola martiana Lindl.	unvouchered (Kew 1995-2685)
Brassavola nodosa (L.) Lindl.	Chase O-339 (K)
Brassavola subulifolia Lindl.	W. M. Whitten 99217 (FLAS)
Brassavola tuberculata Hook.	Brieger Coll. 3497 (ESA)
Briegeria equitantifolia (Ames) Senghas	van den Berg C171 (K spirit)
Broughtonia negrilensis Fowlie	W.E. Higgins 152 (FLAS 198288)
Broughtonia sanguinea (Sw.) R.Br.	Brieger Coll. 14440 (ESA)
Calanthe tricarinata Lindl.	Chase O-820 (K)
Cattleya aclandiae Lindl.	Brieger Coll. 32982 (ESA)
Cattleya amethystoglossa Linden & Rchb.f. ex Warner	Brieger Coll. 8272 (ESA)
Cattleya araguaiensis Pabst	unvouchered (Kew 1999-1443)
Cattleya aurantiaca (Batem. ex Lindl.) P.N. Don	Brieger Coll. 124 (ESA)
Cattleya aurea Linden	Brieger Coll. 2589 (ESA)
Cattleya bicolor Lindl. (Brasília)	Brieger Coll. 22574 (ESA)
Cattleya bicolor Lindl. (Diamantina)	Brieger Coll. 30656 (ESA)
Cattleya bicolor Lindl. (Formiga)	Brieger Coll. 4336 (ESA)
Cattleya bicolor Lindl. (Itatiaia)	Brieger Coll. 891 (ESA)
Cattleya bowringiana Veitch	Brieger Coll. 96 (ESA)
Cattleya bowringiana Veitch	van den Berg C284 (K)
Cattleya candida (Kunth) Lehm.	Brieger Coll. 748 (ESA)
Cattleya dormaniana (Rchb.f.) Rchb.f.	Brieger Coll. 23977 (ESA)

Species	Voucher
Cattleya dowiana Batem.	Chase O-282 (K)
Cattleya elongata Lindl.	Brieger Coll. 8078 (ESA)
Cattleya forbesii Lindl.	Brieger Coll. 5358 (ESA)
Cattleya gaskelliana Braem	Brieger Coll. 6253 (ESA)
Cattleya granulosa Lindl. (Bahia State-BA)	Brieger Coll. 19216 (ESA)
Cattleya granulosa Lindl. (Pernambuco state-PE)	Brieger Coll. 22482 (ESA)
Cattleya guttata Lindl.	Brieger Coll 11299 (ESA)
Cattleya harrisoniana Batem. ex Lindl.	Brieger Coll. 16036 (ESA)
Cattleya intermedia Graham ex Hook.	Brieger Coll. 4095 (ESA)
Cattleya iricolor Rchb.f.	unvouchered (Kew 1999-1502)
Cattleya jenmanii Rolfe	unvouchered (coll. C. van den Berg)
Cattleya kerrii Brieger & Bicalho	Brieger Coll 18765 (Holotype-HB)
Cattleya labiata Lindl. (Pernambuco State)	Brieger Coll. 5487 (ESA)
Cattleya labiata Lindl. (Ceará State-CE)	Brieger Coll. 20545 (ESA)
Cattleya lawrenceana Rchb.f.	Brieger Coll. 3802 (ESA)
Cattleya loddigesii Lindl.	Brieger Coll. 2483 (ESA)
Cattleya lueddemanniana Rchb.f.	Brieger Coll. 755 (ESA)
Cattleya lueddemanniana Rchb.f.	Brieger Coll. 3759 (ESA)
Cattleya luteola Lindl.	Brieger Coll. 32187 (ESA)
Cattleya maxima Lindl.	Brieger Coll. 2986-32 (ESA)
Cattleya maxima Lindl.	unvouchered (Kew 1983-4362)
Cattleya mendelii Backh.f.	Brieger Coll. 2418 (ESA)
Cattleya mooreana Withner, D.Allison & Guenard	unvouchered (Kew 1999-1569)
Cattleya mossiae Hook.	Brieger Coll. 6219 (ESA)
Cattleya nobilior Rchb.f.	Brieger Coll. 30978 (ESA)
Cattleya patinii Cogn.	Brieger Coll. 4138 (ESA)
Cattleya percivaliana O'Brien	van den Berg C279 (ESA)
Cattleya porphyroglossa Linden & Rchb.f.	unvouchered (Kew 1986-2034)
Cattleya schilleriana Rchb.f.	Brieger Coll. 6640 (ESA)
Cattleya schofieldiana Rchb.f.	Brieger Coll. 6656 (ESA)
Cattleya schroderae Rchb.f.	Brieger Coll. 94 (ESA)
Cattleya skinneri Batem.	Brieger Coll. 10103 (ESA)
Cattleya skinneri Batem.	unvouchered (Kew 1986-4870)
Cattleya skinneri Batem.	Brieger Coll. 708 (ESA)
Cattleya tenuis Campacci & Vedovello	C211-Machado s.n. (ESA)
Cattleya tigrina A.Rich. (syn. *C. leopoldii* Verschaff.)	van den Berg C186 (K spirit)
Cattleya trianaei Linden & Rchb.f.	Brieger Coll. 2608 (ESA)

Species	Voucher
Cattleya trichopiliochila Barb.Rodr. (syn. *C. eldorado* Linden)	Brieger Coll. 28797 (ESA)
Cattleya velutina Rchb.f.	Brieger Coll. 7043 (ESA)
Cattleya violacea (Kunth) Rolfe	Brieger Coll. 28495 (ESA)
Cattleya walkeriana Gardner	Brieger Coll. 1627 (ESA)
Cattleya warneri T.Moore	Brieger Coll. 6605 (ESA)
Cattleya warscewiczii Rchb.f.	Brieger Coll. 754 (ESA)
Cattleyopsis lindenii (Lindl.) Cogn.	W. E. Higgins 251 (FLAS 198289)
Caularthron bicornutum (Hook.) Raf.	Brieger Coll. 7959 (ESA)
Caularthron billamellatum Rchb.f. (R.E.Schultes)	Brieger Coll. 3690 (ESA)
Chysis bractescens Lindl.	Chase O-436 (K)
Coelia guatemalensis Rchb.f.	M. Soto 7973 (AMO)
Coelia macrostachya Lindl.	Chase O- 817 (K)
Coelia triptera G.Don	Chase O-324 (K)
Constantia cipoensis Porto & Brade	São Paulo B.G. s.n. (SP)
Constantia microscopica F.E.L.Miranda	E. L. Borba 515 & J. M. Felix (UEC)
Dilomilis montana (Sw.) Summerh.	Chase O-206 (K)
Dimerandra emarginata (G.Mey.) Hoehne	Chase O-335 (K)
Dinema polybulbon (Sw.) Lindl.	Brieger Coll. 6052 (ESA 35552)
Domingoa kienastii (Rchb.f.) Dressler	W. E. Higgins 225 (FLAS 198291)
Domingoa nodosa (Cogn.) Schltr.	W. E. Higgins 1034 (FLAS 198284)
Dracula chimaera (Rchb.f.) Luer	Chase O-967 (K)
Earina autumnalis Hook.	Chase O-298 (K)
Encyclia adenocaula (La Llave & Lex.) Schltr.	W. E. Higgins 12 (FLAS 198274)
Encyclia bractescens (Lindl.) Hoehne	W. E. Higgins 21 (FLAS 198275)
Encyclia cordigera (Kunth) Dressler	W. E. Higgins 24 (FLAS 198276)
Encyclia cyperifolia (C.Schweinf.) Carnevali & I.Ramírez	Brieger Coll. 5758 (ESA)
Encyclia dichroma (Lindl.) Schltr.	Selby B.G. 88-0310, FLAS 198278
Encyclia granitica (Lindl.) Schltr.	Brieger Coll. 21371 (ESA)
Encyclia maderoi Schltr.	Brieger Coll. 2619 (ESA)
Encyclia oncidioides (Lindl.) Schltr.	Brieger Coll. 5420 (ESA)
Encyclia sp.	Brieger Coll. 11024 (ESA)
Encyclia tampensis (Lindl.) Small	W. E. Higgins 27 (FLAS 198277)
Epidendrum campestre Lindl.	E. L. Borba 553 (UEC)
Epidendrum capricornu Kraenzl.	van den Berg C251 (K spirit)
Epidendrum ciliare L.	Brieger Coll 1024 (ESA)
Epidendrum cinnabarinum Salzm. ex Lindl.	van den Berg 277 (K spirit)

Species	Voucher
Epidendrum conopseum R.Br.	W. E. Higgins 244 (FLAS 198271)
Epidendrum criniferum Rchb.f	van den Berg C252 (K spirit)
Epidendrum ibaguense Lindl.	W. E. Higgins 60 (FLAS 198270)
Epidendrum latifolium (Lindl.) Garay & H.R.Sweet	van den Berg 254 (K spirit)
Epidendrum nocturnum Jacq.	Chalets s.n. (AMO)
Epidendrum pseudepidendrum Rchb.f.	van den Berg C4 (ESA)
Epidendrum radioferens (Ames, F.T.Hubb. & C.Schweinf.) Hágsater	Chase O-300 (K)
Epidendrum secundum Jacq.	E. L. Borba 552 (UEC)
Epidendrum stamfordianum Batem.	Brieger Coll. 1200 (ESA)
Epidendrum veroscriptum Hágsater	van den Berg C247 (K spirit)
Euchile sinaloensis ined.	unvouchered (Kew 1999-1710)
Euchile citrina (La Llave & Lex.) Withner	W. E. Higgins 54 (FLAS 198269)
Euchile mariae (Ames) Withner	Chase O-158 (K)
Hagsatera brachycolumna (L.O.Williams) R.González	W. E. Higgins 229 (FLAS 198272)
Helleriella guerrerensis Dressler & Hágsater	van den Berg C172 (K spirit)
Helleriella punctulata (Rchb.f.) Garay & H.R.Sweet	Chase O-299 (K)
Hexadesmia crurigera Lindl.	Chase O-336 (K)
Hexadesmia micrantha Lindl.	unvouchered (coll. R.L. Dressler)
Hexalectris revoluta Correll	D. Goldman 1364 (TEX)
Hexisea bidenata Lindl.	Brieger Coll. 1253 (ESA)
Hexisea imbricata (Lindl.) Rchb.f.	W. M. Whitten 97039 (FLAS)
Homalopetalum pachyphyllum (L.O.Williams) Dressler	M. Soto 7640 (AMO)
Homalopetalum pumilio (Rchb.f.) Schltr.	M. Soto 7443 (AMO)
Homalopetalum pumilum (Ames) Dressler	M. Soto 8950 (AMO)
Isabelia virginalis Barb.Rodr.	Brieger Coll. 17289 (ESA)
Isabelia virginalis Barb.Rodr.	Coll. Brieger 30243 (ESA)
Isochilus alatus Schltr.	M. Soto 7190 (AMO)
Isochilus amparoanus Schltr.	Chase O-204 (K)
Isochilus brasiliensis Schltr.	Brieger Coll. 33696 (ESA 35553)
Isochilus langlassei Schltr.	M.Soto 7808 (AMO)
Isochilus major Cham. & Schltdl.	W. M. Whitten 91348 (FLAS)
Jacquiniella globosa Schltr.	W. M. Whitten 97064 (FLAS)
Jacquiniella teretifolia Britton & P.Wilson	W. M. Whitten 97026 (FLAS)
Laelia alaorii Brieger & Bicalho	Brieger Coll. 19179 (ESA)
Laelia albida Batem. ex Lindl.	unvouchered (coll. S. Beckendorf)
Laelia alvaroana F.E.L.Miranda	van den Berg C227 (ESA)

Species	Voucher
Laelia alvaroana F.E.L.Miranda	C207-Machado s.n. (ESA)
Laelia anceps Lindl.	Chase O-998 (K)
Laelia anceps Lindl.	Brieger Coll. 3811 (ESA)
Laelia angereri Pabst	C223-Machado s.n. (ESA)
Laelia autumnalis (La Llave & Lex.) Lindl.	unvouchered (coll. S. Beckendorf)
Laelia bahiensis Schltr.	C221-Machado s.n. (ESA)
Laelia blumenscheinii Pabst	C209-Machado s.n. (ESA)
Laelia bradei Pabst	C215-Machado s.n. (ESA)
Laelia brevicaulis (H.G.Jones) Withner	C208-Machado s.n. (ESA)
Laelia briegeri Blumensch. ex Pabst	Brieger Coll. 4612 (ESA)
Laelia cardimii Pabst & A.F.Mello	C205-Machado s.n. (ESA)
Laelia caulescens Lindl.	Brieger Coll. 1916 (ESA)
Laelia cinnabarina Batem. ex Lindl.	Brieger Coll. 1395 (ESA)
Laelia crispa Rchb.f.	Brieger Coll. 3914 (ESA)
Laelia crispata Thunb. (Garay) (syn. *flava* Lindl.)	van den Berg C32 (ESA)
Laelia crispilabia (A.Rich. ex Rchb.f) Warner	Brieger Coll. 4837 (ESA)
Laelia dayana Rchb.f.	Brieger Coll. 15795 (ESA)
Laelia duveenii Fowlie	C213-Machado s.n. (ESA)
Laelia esalqueana Blumensch. ex Pabst	Brieger Coll. 4980 (ESA)
Laelia fidelensis Pabst	C225-Machado s.n. (ESA)
Laelia furfuracea Lindl.	unvouchered (coll. S. Beckendorf)
Laelia ghillanyi Pabst	C214-Machado s.n. (ESA)
Laelia gloedeniana Hoehne	van den Berg C35 (ESA)
Laelia gouldiana Rchb.f.	unvouchered (coll. S. Beckendorf)
Laelia grandis Lindl. & Paxton	Brieger Coll. 19209 (ESA)
Laelia harpophylla Rchb.f	Brieger Coll. 6687 (ESA)
Laelia itambana Pabst	C212-Machado s.n. (ESA)
Laelia jongheana Rchb.f.	Brieger Coll. 31534 (ESA)
Laelia kautskyi Pabst	van den Berg C286 (K spirit)
Laelia kettieana Pabst	C210-Machado s.n. (ESA)
Laelia liliputiana Pabst	C206-Machado s.n. (ESA)
Laelia lobata (Lindl.) Veitch	Brieger Coll 3557 (ESA)
Laelia longipes Rchb.f.	Brieger Coll. 5183 (ESA)
Laelia lundii (Rchb.f.) Withner	Brieger Coll. 30692 (ESA)
Laelia mantiqueirae Pabst ex D.C.Zappi	van den Berg C224 (ESA)
Laelia milleri Blumensch. ex Pabst	Brieger Coll. 5070 (ESA)
Laelia mixta Hoehne ex Ruschi	C220-Machado s.n. (ESA)
Laelia perrinii Batem.	Brieger Coll. 652 (ESA)

Species	Voucher
Laelia pfisteri Pabst & Senghas	van den Berg C226 (ESA)
Laelia praestans Lindl. & Rchb.f.	C217-Machado s.n. (ESA)
Laelia pumila (Hook.) Rchb.f.	Brieger Coll. 7794 (ESA)
Laelia purpurata Lindl. & Paxton	Selby B.G. 84-0459 (SEL)
Laelia reginae Pabst	C218-Machado s.n. (ESA)
Laelia rubescens Lindl.	Chase O-1205 (K)
Laelia rupestris Lindl.	van den Berg C33 (ESA)
Laelia sanguiloba Withner	C216-Machado s.n. (ESA)
Laelia sincorana Schltr.	van den Berg C263 (K spirit)
Laelia speciosa (Kunth) Schltr.	Chase O-6088 (unvouchered)
Laelia speciosa (Kunth) Schltr.	Chase O-6411 (unvouchered)
Laelia tenebrosa (Rolfe) Rolfe	van den Berg C279 (K spirit)
Laelia tereticaulis Hoehne	van den Berg C222 (ESA)
Laelia virens Lindl.	van den Berg C18 (ESA)
Laelia xanthina Lindl. ex Hook.	Brieger Coll 6662 (ESA)
Laelia xanthina Lindl. ex Hook.	Brieger Coll. 6635 (ESA)
Laeliopsis dominguensis (Lindl.) Lindl. & Paxton	unvouchered (coll. W.E. Higgins)
Lanium avicula (Lindl.) Benth.	Brieger Coll. 23319 (ESA)
Leptotes bicolor Lindl.	Brieger Coll. 1068 (ESA)
Leptotes cf. *tenuis* Rchb.f.	São Paulo B.G. 16809 (SP)
Leptotes cf. *unicolor* Barb.Rodr.	São Paulo B.G. 13534 (SP)
Leptotes cf. *unicolor* Barb.Rodr.	C204-Machado s.n. (ESA)
Loefgrenianthus blanche-amesiae (Loefgr.) Hoehne	São Paulo B.G. s.n. (SP)
Masdevallia floribunda Lindl.	Chase O-296 (K)
Meiracyllium gemma Rchb.f.	M.Soto 8731 (AMO)
Meiracyllium trinasutum Rchb.f.	Chase O-202 (K)
Meiracyllium trinasutum Rchb.f.	van den Berg C7 (ESA)
Myrmecophila galeottiana (A.Rich.) Rolfe	unvouchered (Kew 1982-3743)
Myrmecophila sp.	Chase O-281 (K)
Myrmecophila thomsoniana (Rchb.f.) Rolfe	van den Berg 167 (K spirit)
Myrmecophila tibicinis (Batem.) Rolfe	van den Berg C81 (ESA)
Myrmecophila wendlandii (Rchb.f.) G.C.Kenn	van den Berg C165 (K spirit)
Nageliella angustifolia (Booth ex Lindl.) Ames & Correll	W. Bussey s.n., Guatemala (AMO)
Nageliella purpurea (Lindl.) L.O.Williams	van den Berg C260 (K spirit)
Nanodes mathewsii (Rchb.f) Rolfe	Brieger Coll. 16746 (ESA)
Nanodes schlechterianum (Ames) Brieger	Chase O-301 (K)
Neocogniauxia hexaptera (Cogn.) Schltr.	van den Berg C244 (K)

Species	Voucher
Neocogniauxia monophylla (Griseb.) Schltr.	van den Berg C245(K)
Neolauchea pulchella Kraenzl.	Coll. Brieger 11737 (ESA)
Neolauchea pulchella Kraenzl.	Coll. Brieger 6367 (ESA)
Nidema boothii (Lindl.) Schltr.	W. E. Higgins 192 (FLAS 198273)
Oerstedella centradenia Rchb.f.	van den Berg C169 (K spirit)
Orleanesia amazonica Barb.Rodr.	São Paulo B.G. 15936 (SP)
Orleanesia pleurostachys (Linden & Rchb.f) Garay & Dunst.	J. T. Atwood et al. 5614 (FLAS)
Platyglottis coriacea L.O.Williams	unvouchered (coll. R.L. Dressler)
Pleione chunii C.L.Tso	van den Berg C290 (K spirit)
Pleurothallis racemiflora Lindl.	W. E. Higgins 140 (FLAS 198267)
Polystachya galeata Rchb.f.	van den Berg C283 (K spirit)
Ponera australis Cogn.	Brieger Coll. 33642 (ESA 35548)
Ponera exilis Dressler	M. Soto s.n., Paracho, Michoacan (AMO)
Ponera glomerata Correll	M. Soto 8224 (AMO)
Ponera striata Lindl.	W. E. Higgins 197 (FLAS 198268)
Ponera striata Lindl.	Chase O-6178 (K spirit)
Prosthechea abbreviata (Schltr.) W.E.Higgins	Brieger Coll. 10092 (ESA)
Prosthechea aemula (Lindl.) W.E.Higgins	W. E. Higgins 17 (FLAS 198279)
Prosthechea allemanii (Barb.Rodr.)W.E.Higgins	Brieger Coll. 5940 (ESA)
Prosthechea calamaria (Lindl.) W.E.Higgins	Brieger Coll. 10368 (ESA)
Prosthechea cf. *moojenii* (Pabst) W.E.Higgins	Brieger Coll. 8118 (ESA)
Prosthechea cochleata (L.) W.E.Higgins	MBG 75-0658 (FLAS 198280)
Prosthechea fausta (Rchb.f. ex Cogn.) W.E.Higgins	van den Berg C95 (ESA)
Prosthechea lambda (Linden & Rchb.f) W.E.Higgins	Brieger Coll. 6032 (ESA)
Prosthechea linkiana (Klotzsch) W.E.Higgins	Brieger Coll. 3879 (ESA)
Prosthechea prismatocarpa (Rchb.f) W.E.Higgins	W. E. Higgins 19 (FLAS 198283)
Prosthechea pygmaea (Hook.) W.E.Higgins	Selby B.G. 92-0206 (FLAS 198281)
Prosthechea suzanensis (Hoehne) W.E.Higgins	van den Berg 119 (K spirit)
Prosthechea venezuelana (Schltr.) W.E.Higgins	Brieger Coll. 2543 (ESA)
Prosthechea vitellina (Lindl.) W.E.Higgins	W. E. Higgins 57 (FLAS 198282)
Prosthechea widgrenii (Lindl.) W.E.Higgins	Brieger Coll. 30565 (ESA)
Pseudolaelia cf. *cipoensis* Pabst	São Paulo B.G. 12759 (SP)
Pseudolaelia cf. *cipoensis* Pabst	São Paulo B.G. 12406 (SP)
Pseudolaelia cf. *citrina* Pabst	São Paulo B.G. 12323 (SP)
Pseudolaelia cf. *dutraei* Ruschi	São Paulo B.G. 12243 (SP)
Pseudolaelia cf. *geraensis* Pabst	E. L. Borba 554 (UEC)
Pseudolaelia cf. *vellozicola* (Hoehne) Porto & Brade	São Paulo B.G. 13358 (SP)

Species	Voucher
Pseudolaelia cf. *vellozicola* (Hoehne) Porto & Brade	São Paulo B.G. 13362 (SP)
Pseudolaelia vellozicola (Hoehne) Porto & Brade	Brieger Coll. 6736 (sample O-1200) (ESA)
Pseudolaelia vellozicola (Hoehne) Porto & Brade	Brieger Coll. 6736 (ESA) (sample C201)
Psychilis krugii (Bello) Sauleda	Chase O-1062 (K)
Psychilis macconnelliae Sauleda	W. E. Higgins 53 (FLAS 198287)
Quisqueya ekmanii Dod	W. E. Higgins 1043 (FLAS 198286)
Reichenbachanthus cuniculatus (Schltr.) Pabst	W. M. Whitten 96051 (FLAS)
Renata canaanensis Ruschi	Brieger Coll.16205 (ESA) C150
Renata canaanensis Ruschi	Brieger Coll.16205 (ESA) C188
Rhyncholaelia digbyana (Lindl.) Schltr.	Chase O-331 (K)
Rhyncholaelia digbyana (Lindl.) Schltr.	van den Berg C73 (ESA)
Rhyncholaelia glauca (Lindl.) Schltr.	van den Berg C30 (ESA)
Scaphyglottis bilineata Schltr.	W. M. Whitten 96054 (FLAS)
Scaphyglottis boliviensis (Rolfe) B.R.Adams	W. M. Whitten 97006 (SEL)
Scaphyglottis geminata Dressler & Mora Retana	W. M. Whitten 96050 (FLAS)
Scaphyglottis gentryi Dodson & Monsalve	W. M. Whitten 97007 (FLAS)
Scaphyglottis graminifolia Poepp. & Endl.	W. M. Whitten 97012 (FLAS)
Scaphyglottis lindeniana (A.Rich. & Galeotti) L.O.Williams	W. M. Whitten 96051 (FLAS)
Scaphyglottis pulchella (Schltr.) L.O.Williams	unvouchered (coll. W.M. Whitten)
Schomburgkia crispa Lindl.	van den Berg C154 (ESA 35551)
Schomburgkia lyonsii Lindl.	Brieger Coll. 16846 (ESA)
Schomburgkia splendida Schltr.	Whitten 93026 (FLAS)
Schomburgkia superbiens (Lindl.) Rolfe	van den Berg C164 (K spirit)
Schomburgkia undulata Lindl.	van den Berg C29 (ESA)
Sophronitella violacea (Lindl.) Schltr.	van den Berg C127 (ESA)
Sophronitis brevipedunculata (Cogn.) Fowlie	C219-Machado s.n. (ESA)
Sophronitis brevipedunculata (Cogn.) Fowlie	São Paulo B.G. s.n. IBDF (SP)
Sophronitis cernua (Lindl.) Hook.	Brieger Coll. 15737 (ESA)
Sophronitis cernua (Lindl.) Hook.	van den Berg C246 (K spirit)
Sophronitis coccinea (Lindl.) Rchb.f.	van den Berg C173 (K spirit)
Sophronitis coccinea (Lindl.) Rchb.f.	São Paulo B.G. 9577 (SP)
Sophronitis mantiqueirae (Fowlie) Fowlie	São Paulo B.G. 12195 (SP)
Sophronitis wittigiana Barb.Rodr.	São Paulo B.G. 8961 (SP)
Tetragamestus modestus Rchb.f.	Brieger Coll. 2756 (ESA)
Tetramicra elegans (Ham.) Cogn.	W. E. Higgins 160 (FLAS 198285)
Thunia alba Rchb.f.	Chase O-589 (K)

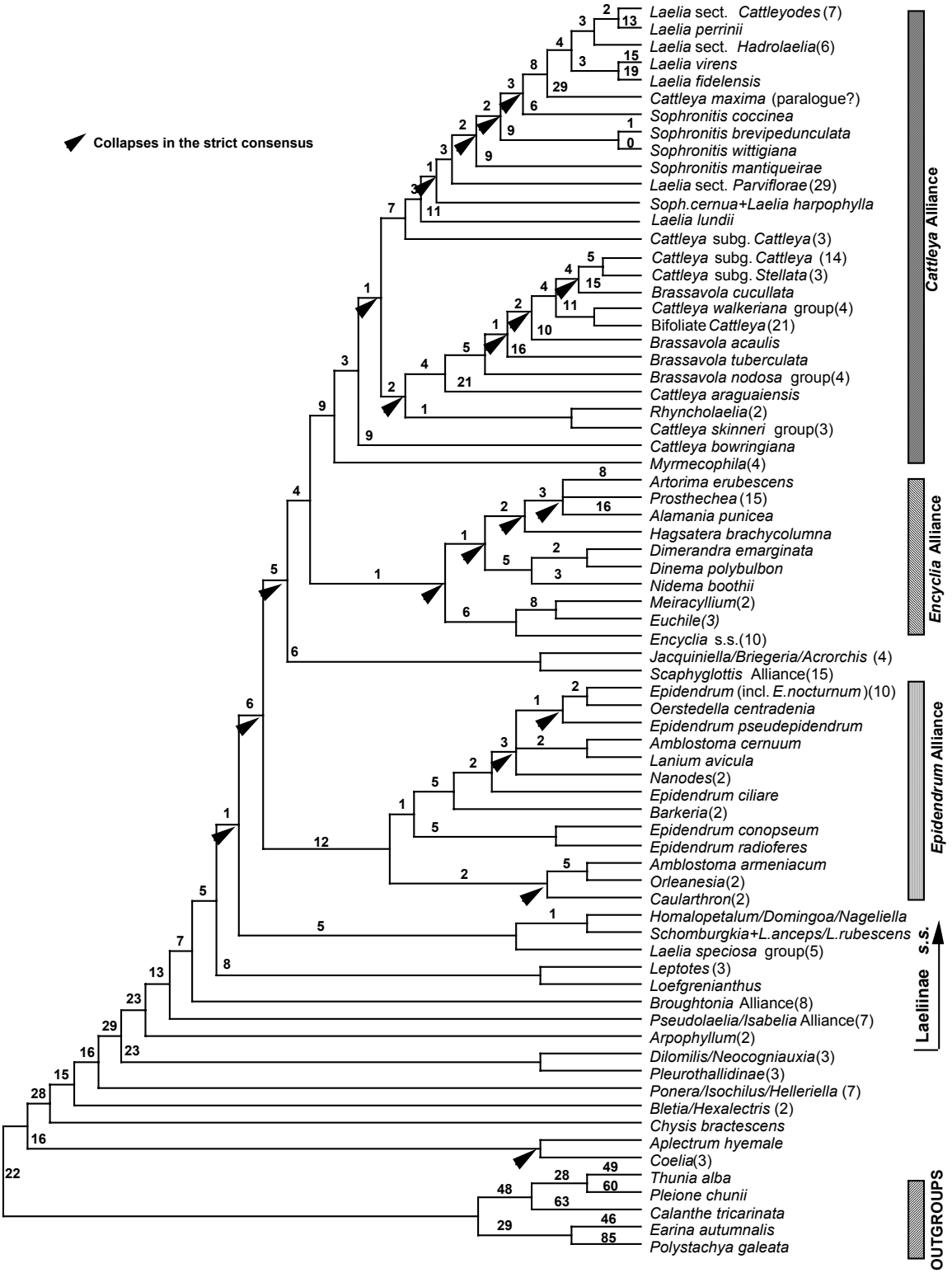


Fig 3.1. A summary of the relationships of one of 10,000 most parsimonious trees of the combined ITS and gap coding matrix.

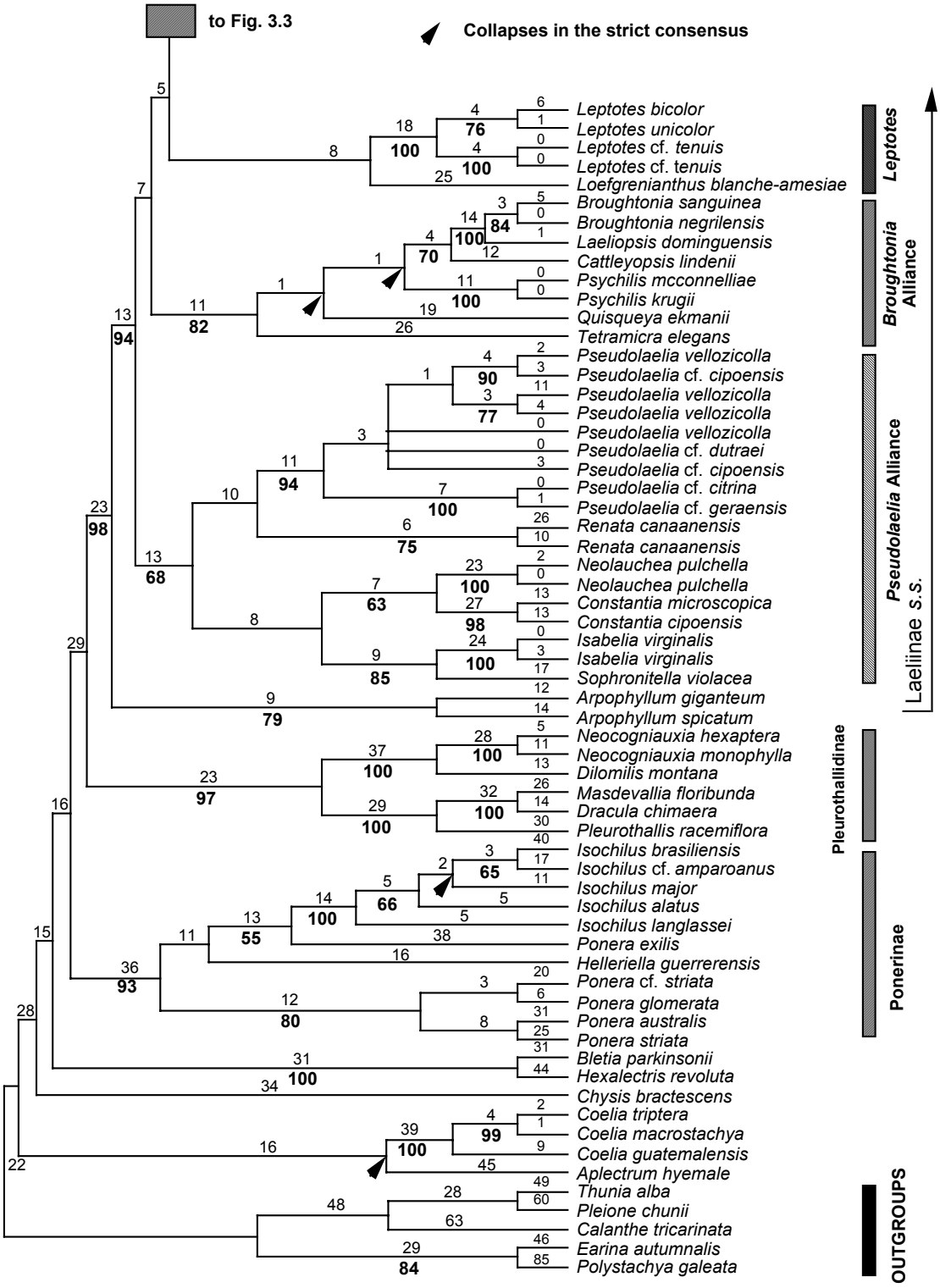


Fig. 3.2. A portion of one of 10,000 most parsimonious trees of the combined ITS and gap coding matrix, CI=0.26 (excluding non-informative characters), RI=0.71, Fitch tree length=3958. Fitch branch lengths are above branches, and bootstrap support (50% or more) is below. Arrows indicate branches not present in the strict consensus.

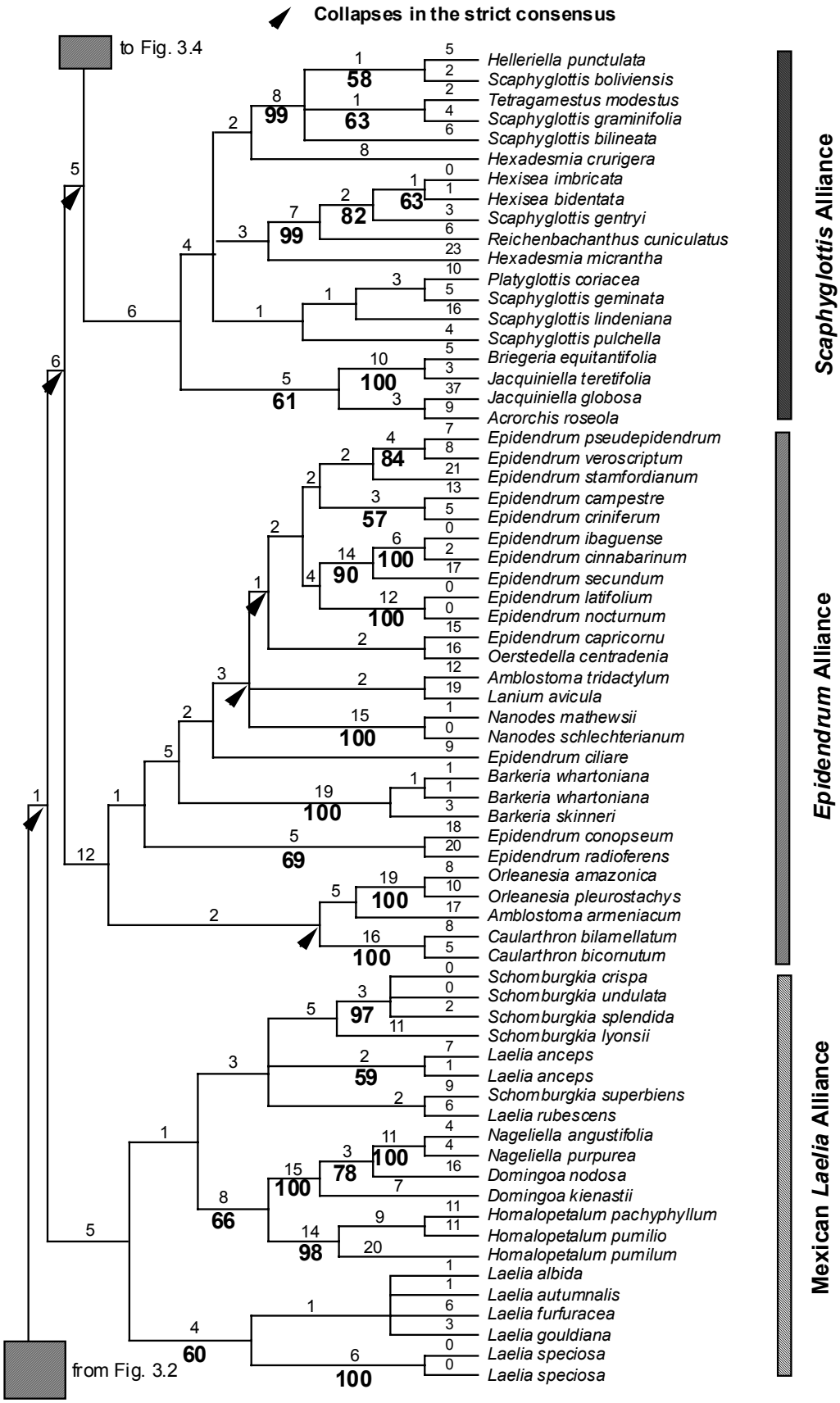


Fig. 3.3. *Laelia s.s.*, *Epidendrum* and *Scaphyglottis* alliances in the same most parsimonious tree as Fig. 3.2.

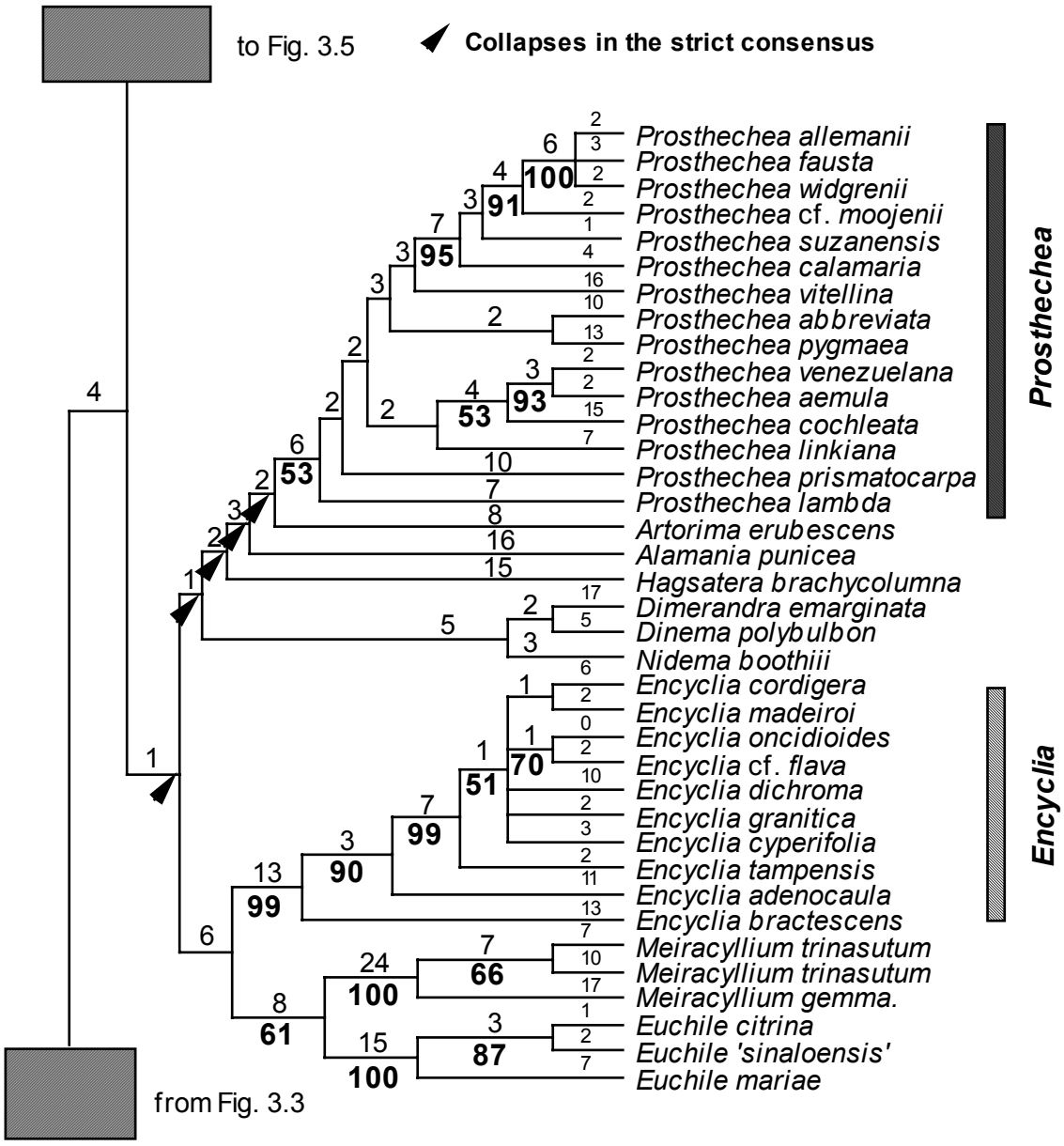


Fig. 3.4. *Encyclia* and related genera in the same most parsimonious tree as Fig. 3.2.

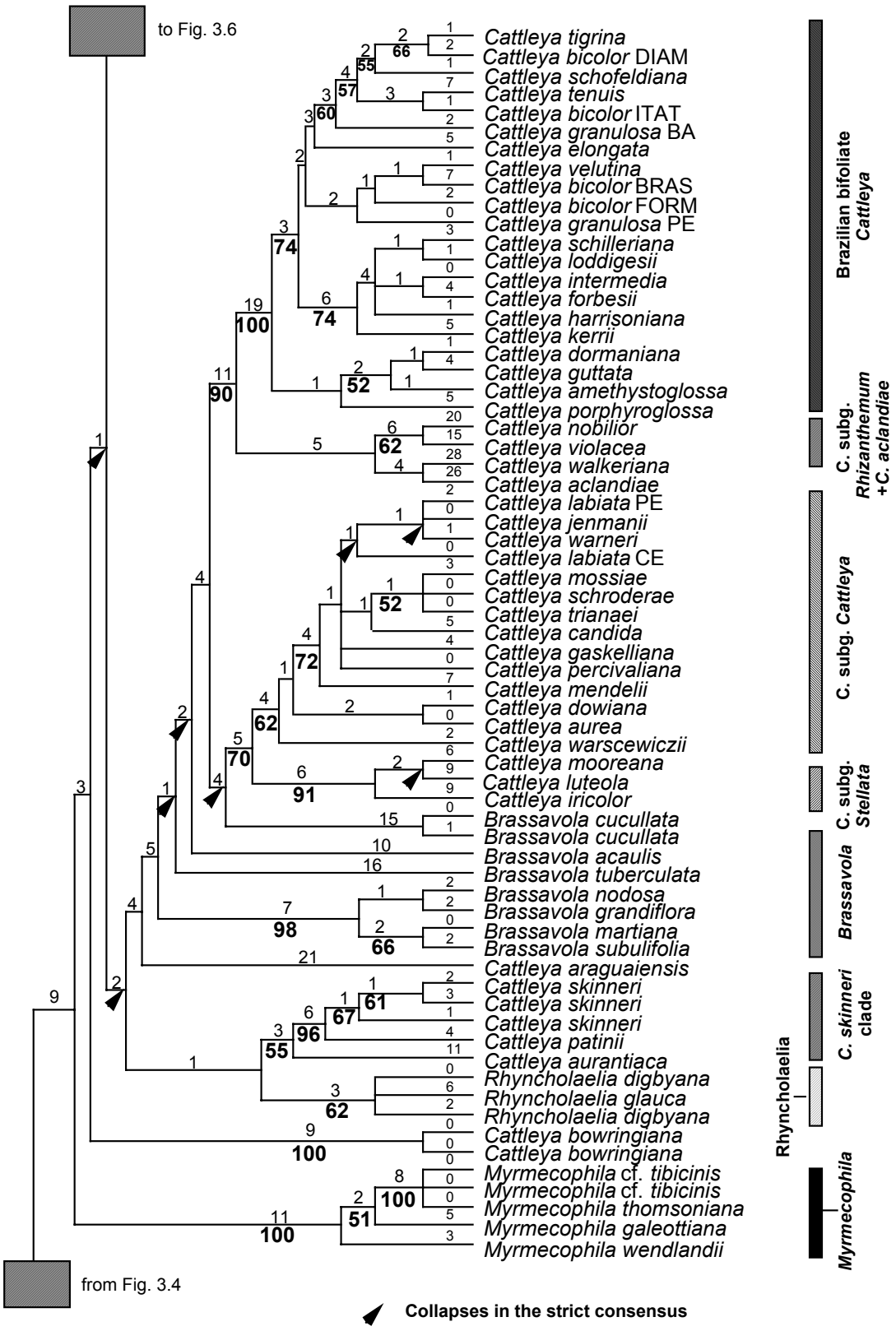


Fig. 3.5. *Cattleya, Brassavola, Myrmecophila,* and *Rhyncholaelia* in the same most parsimonious tree as Fig. 3.2.

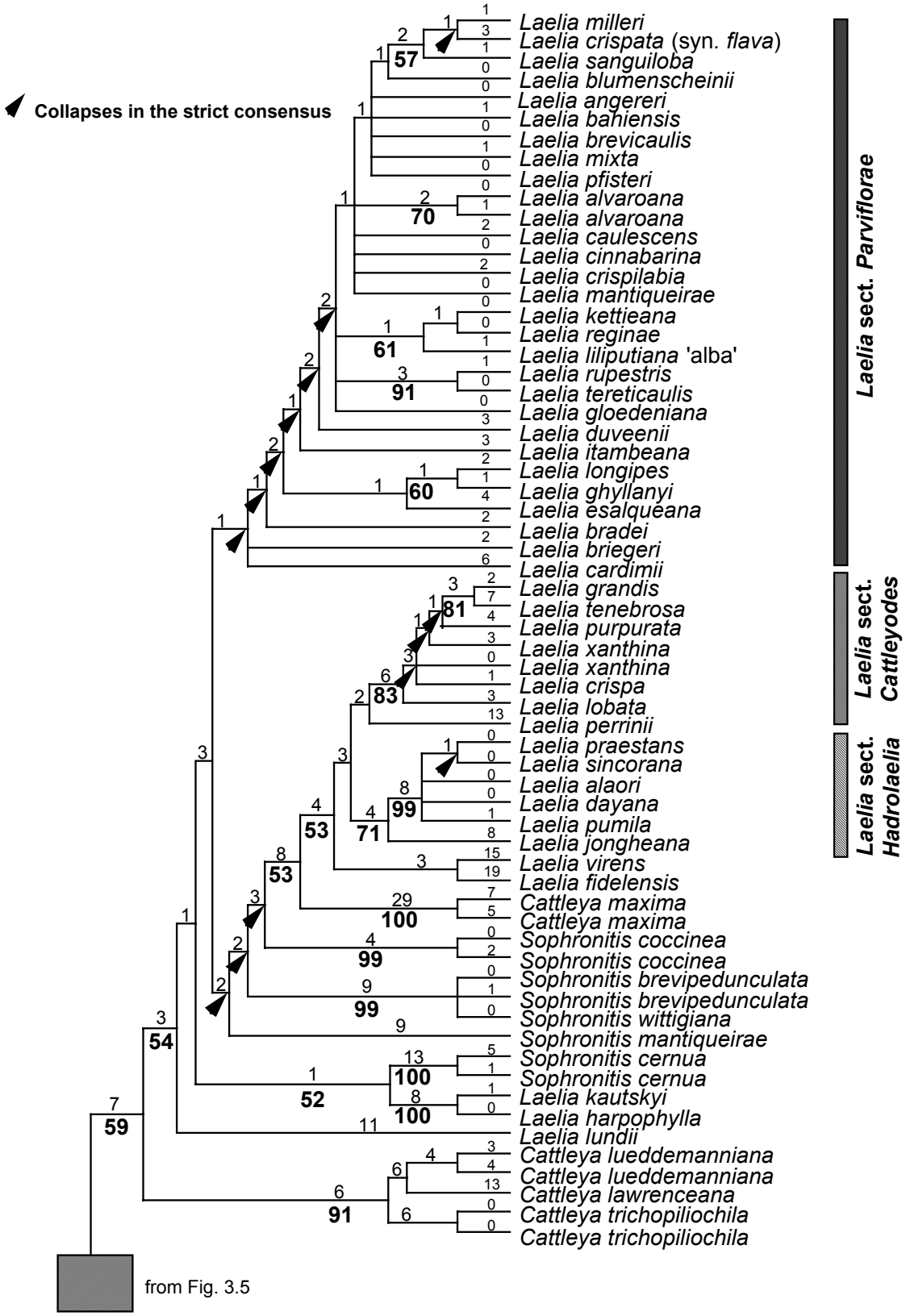


Fig. 3.6. *Sophronitis* and the Brazilian *Laelia* in the same most parsimonious tree as Fig. 3.2.

Chapter 4 – A phylogenetic study of Laeliinae based on combined nuclear and plastid DNA sequences

4.1. INTRODUCTION

In this chapter, my main aim is to obtain better-supported overall topologies within Laeliinae. Whereas most outgroup relationships have been clarified in Chapter 1, many issues have been raised in Chapter 3 which needed additional research. Among these, there is the suspicion that some ITS topologies could be caused by paralogous copies. In addition, some genera were paraphyletic (e.g. *Brassavola* to *Cattleya* and subgroups of Brazilian species of *Laelia*). Increasing the amount of data could potentially make these groups monophyletic and sister, in a similar manner as in Sheahan and Chase (2000). The additional data consisted of two plastid regions (*trnL-F* intron, exon and spacers) and *matK* (spacers and gene) in addition to the ITS from the previous chapter in a subset of taxa representing most clades from the ITS analysis.

4.2. MATERIAL AND METHODS

Plant material and voucher information for this analysis is given in Table 4.1. Distant outgroups *Earina valida* Hook. and *Polystachya galeata* Rchb.f. were chosen based on the analysis of Chapter 1, and also because *Polystachya* was placed near Laeliinae in Cameron et al. (1999). Representatives of all other main clades of Epidendreae as defined in Chapter 1 were included in the ingroup. Within Laeliinae, sampling aimed to include all genera that have been listed in recent systems (Brieger, 1977; Dressler, 1981, 1993; Szlachetko, 1995) and also taxonomic subgroups from the literature as well as those that emerged from the ITS analysis. I was unable to obtain material of *Pygmaeorchis* and *Pinelia*, and did not include *Basiphyllaea* due to technical difficulties in sequencing all three regions. However, according to Goldman (2000), V. Sosa (pers. comm., 1999) and the analysis in Chapter 1, this taxon is related to Bletiinae rather than Laeliinae.

DNA was extracted from fresh leaves, fresh flowers and silica gel-dried leaves and flowers, using in most cases a modified version of the CTAB procedure of Doyle and Doyle (1987). For samples that presented difficulties due to the presence

of polysaccharides, DNA was extracted using the Nucleon Phytopure Kit (Kit (Amersham Plc., Little Chalfont, UK). DNAs were purified either by caesium chloride/ethidium bromide gradient, or in silica columns (QIAGEN, Ltd.) and sometimes by a combination of both methods. Methods for amplification and sequencing of ITS were described in Chapter 3. For *trnL-F*, we used the four universal primers (c, d, e, f) of Taberlet et al. (1991) and a program consisting of 28-30 cycles of 94 C denaturation for 1 min, 50 C annealing for 30 s and 72 C of extension for 1 min. Most species were amplified with primers c and f, but difficult samples had to be amplified in two halves with the consequent insertion of missing characters in the area corresponding to the primers d and e, which are reverse complements. The *matK* region was amplified as using the primers -19F (CGT TCT GAC CAT ATT GCA CTA TG; Molvray et al., 2000) and trnK-2R (AAC TAG TCG GAT GGA GTA; Johnson and Soltis, 1994). PCR conditions were a hot start with 2 min of initial denaturation at 94C, followed by 28-30 cycles of 94 C denaturation, 52 C annealing for 45 s and 72 C for an initial time of 2 min. 30 s with auto-extension of 8 s per cycle. Purification of PCR products was performed with QIAquick (QIAGEN Ltd.) and Concert (Gibco BRL Ltd.) silica columns. For ITS only, we added an extra wash with 35% guanidinium chloride solution to help to remove primer dimers. PCR products were sequenced in both directions using Big Dye system and an ABI 377 automated sequencer following protocols of the manufacturer (PE Applied Biosystems Inc., Warrington, Cheshire, UK). We employed the same primers used in PCR and also matK-163F (AGT TTA GTR CTT GTG AAA CG; Molvray et al., 2000), matK-458F (CTA CTA ATA CCC YAT CCC ATC; Molvray et al. 2000), matK-556R (GAA GRA ACA TCT TTK ATC CA; Molvray et al., 2000), matK- TCT GGA GTC TTT CTT GAG CGA; new), matK-881R (TTM TCA TCA GAA TAA GAG T; new), matK-877F (AGG AAC TCT TAT TCT GAT; Molvray et al. 2000), matK-1155F (TTC ACT TTT GGT YTC ACC CT; new) and matK-1592R (TCA TGA ATG ATC CAC CAG A; Goldman, 2000). Electropherograms were assembled and edited using Sequencher 3.0 and 3.1 (Genecodes Inc., Ann Arbor, Michigan). All sequences were aligned by eye. Gaps were treated as missing characters, but were translated into a manually coded binary gap-matrix (presence/absence) with all non-autapomorphic, unambiguous indels in the *trnL-F*, ITS and *matK* gene datasets. In the upstream and downstream spacers of

matK 30-40% of the sequences were missing, precluding sensible gap coding in these regions.

Analyses were performed using PAUP 4.0 (Swofford, 1998), with Fitch parsimony (equal weights, unordered; Fitch, 1971). Three separate searches were performed: the first with plastid data only, the second with all data combined, and the third with the combined data but replacing four ITS sequences suspected of being paralogues with missing data. These were all *Cattleya* species (*C. lawrenceana, C. lueddemanniana, C. maxima* and *C. trichopiliochila*). Because the analysis from Chapter 3 contained a much larger number of taxa (295) and therefore is expected to be a better estimate of the ITS topologies, searches using the reduced ITS data alone were not performed; rather, the ITS trees from Chapter 3 are used in this discussion. Each search consisted of 1000 random taxa-addition replicates, with the tree-bisection-reconnection (TBR) swapping limited to 15 trees per replicate to prevent extensive swapping on a single replicate. The resulting trees were then used as starting trees for TBR swapping with an upper limit of 2000 trees. Internal support for groups was evaluated using 1000 replicates of character bootstrapping (Felsenstein, 1985), with simple taxon-addition and TBR algorithm, saving 15 trees per replicate.

4.3. RESULTS

4.3.1. GENERAL FEATURES OF THE DATASETS

General characteristics of the three DNA datasets in relation to the combined trees are given in Table 4.2. A region of 480bp in the *trnL* intron was of ambiguous alignment and therefore was excluded from the analyses. The *matK* upstream and downstream spacers were considered as a single region. The most variable dataset was ITS, followed by the *matK* spacers. The *trnL-F* region and *matK* gene had similar level of variation. In terms of informativeness as measured by the ensemble RI of each dataset, *matK* gene and the *trnL-F* region performed similarly and slightly better than the ITS dataset. The indel matrix was composed by 26 indels from *trnL-F*, 23 from ITS and only three from *matK*.

4.3.2. PLASTID ANALYSIS

A large number of possible trees (2000; limited in the search; tree length (L)=2739; CI=0.63; RI=0.64) was found. Figs 4.1 and 4.2 show one of the trees, indicating the branches that collapse in the strict consensus; bootstrap percentages are indicated below the branches.

The topologies in the plastid trees (Fig. 4.1) are well resolved only in the outgroup relationships and in some of the lower clusters of taxa within the subtribe and have short branch lengths along the spine of the tree. Many nodes collapse in the strict consensus and bootstrap support is generally low in the spine of the tree but increases towards the terminal nodes. *Arpophyllum* is supported as sister to all Laeliinae with 100% and 98% of internal support. Pleurothallidinae (Fig 4.1) and Ponerinae (Fig. 4.1) are both well-supported groups, but they are in an unresolved trichotomy with the group of Laeliinae (including *Arpophyllum*). The next subtribe is Bletiinae, followed by Chysiinae (in a polytomy with Bletiinae), and finally Coeliinae appears as the sister to the rest of Epidendreae. Within the subtribe there are fewer groups with 50% support or more. In the group of *Isabelia* (Fig 4.1) there is only low support (63%) for the relationship between *Isabelia* and *Neolauchea*, and then to *Sophronitella* (51%), whereas most nodes above these two collapse in the strict consensus. Similarly, the relationships between the main clusters of genera do not appear consistently in all trees. However, several smaller clusters of taxa are supported: *Encyclia* (87%), *Prosthechea* with *Euchile* (73%), *Prosthechea* (95%), *Homalopetalum* (100%), *Domingoa* with *Nageliella* (100%), *Dinema* with *Nidema* (97%), and the *Broughtonia* (97%) and *Scaphyglottis* (81%) alliances. From this polytomy there is one group (Fig. 4.2) that does not collapse in the strict consensus (BS less than 50%). It contains two alliances that are also not well-supported: the first contains *Laelia sensu stricto*, *Schomburgkia* and *Myrmecophila* with *Epidendrum* and its segregates, and the second includes *Meiracyllium* and *Cattleya*, *Sophronitis s.l.* (including the Brazilian species of *Laelia*), *Rhyncholaelia* and *Brassavola* (i.e. the 'Cattleya alliance'). Nearly all branches collapse in the strict consensus within the *Epidendrum* clade, and the only weakly supported group is *Oerstedella centradenia* with *Epidendrum conopseum.* In the *Cattleya* alliance there are several groups that do not collapse: *Brassavola* (100%), *Cattleya aurantiaca* with *C. skinneri* (84%), *Sophronitis s.l.* (<50%), *Sophronitis* sect. *Hadrolaelia* with sect.

Cattleyodes (94%), sect. *Parviflorae* (99%), bifoliate species of *Cattleya* (77%) with two subgroups (98% and 79%), unifoliate species of *Cattleya* (<50%) with two subgroups (99% and 68%).

4.3.3. COMBINED ANALYSIS

This analysis found 360 trees with tree length of 5154 steps, CI=0.49, RI=0.58. The strict consensus is much more resolved than the plastid (Figs 4.1 and 4.2) or ITS (Figs 3.1 to 3.6) analyses. I present here one of the trees (Figs 4.3, 4.4) with the Fitch branch lengths above and bootstrap percentages below each branch. An arrow indicates nodes that are not present in the strict consensus tree.

The outgroup relationships are nearly the same as in the plastid trees, with *Arpophyllum* having 100% support as the sister group of the rest of Laeliinae. However, the trichotomy in the plastid trees appears resolved in the combined analysis, and the immediate sister group to Laeliinae appears to be Pleurothallidinae (69%), followed successively by Ponerinae (73%), Bletiinae (68%), Chysiinae (<50%) and finally Coeliinae (92%).

The monophyly of each of the subtribes has high internal support: Bletiinae (100%), Ponerinae (100%), Pleurothallidinae including *Dilomilis* and *Neocogniauxia* (96%), and finally Laeliinae including *Arpophyllum* and *Meiracyllium* (100%).

Within Laeliinae most branches of the spine are resolved in the strict consensus tree. On the other hand, the only branch with some internal support is the one leading to the *Cattleya* alliance (59%; Fig. 4.4). *Hagsatera* is placed between *Arpophyllum* and the rest of Laeliinae. The main groups with internal support above 50% in the combined trees were: *Dinema* with *Nidema* (99%), the *Scaphyglottis* alliance (85%), *Domingoa* with *Nageliella* and *Homalopetalum* (74%), *Laelia sensu stricto* and *Schomburgkia* (96%), the *Epidendrum* alliance (63%), *Encyclia* (100%), *Euchile* (100%), *Prosthechea* (91%), the *Broughtonia* alliance (100%), *Brassavola* (96%), the main part of *Cattleya* including the type (59%) and a group including some *Cattleya*, Brazilian species of *Laelia* and *Sophronitis* (52%), among others. It should be noted, however, that these groups follow previous taxonomic categories both at the generic and infrageneric levels. This increases our confidence on the tree, in spite of the low bootstrap percentages

4.3.4. Analysis excluding possible paralogues in the *Cattleya* Alliance

Figure 4.5 shows only the portion of the tree in the *Cattleya* alliance that is affected by possible ITS paralogues (*C. lawrenceana, C. lueddemanniana, C. maxima* and *C. trichopiliochila*; see Chapter 3). After these ITS sequences are excluded, the group we call here "Unifoliate *Cattleya* II" (Fig. 4.5) is no longer sister to *Sophronitis s.l.* On the other hand, it does not go with "Unifoliate *Cattleya* I" as we would expect, but rather as sister to the remaining members of *Cattleya* (excluding the *C. skinneri* group) with less than 50% bootstrap support. When the ITS sequence of *C. maxima* (which I would also expect to cluster with other unifoliate species of *Cattleya*) is excluded, it moves to a more basal position, sister to *C. araguaiensis*, and these two are sister to the group of *Cattleya skinneri*, although again with less than 50% of bootstrap.

4.4. Discussion

4.4.1. Molecular evolution

DNA regions sampled in this Chapter within Laeliinae behaved similarly to the analysis in Chapter 1 (see section 1.4.1), although at a lower taxonomic level the variation in ITS was lower. The performance (in terms of RI) of this region did not improve. This could be explained by the fact that ITS has a higher number of changes per variable position than the platid genes, and is therefore more likely to be affected by taxon sampling effects (missing taxa could preclude the reconstruction of multiple changes in a given position).

4.4.2. Outgroup relationships

The outgroup relationships of Laeliinae are stable and differ little between datasets (e.g. plastid versus nuclear). *Arpophyllum* is always sister to the rest of the subtribe with high internal support (ITS, chapter 3, 98%; plastids 100%, combined 100%). The next sister group is probably a clade with Pleurothallidinae (including *Dilomilis* and *Neocogniauxia*), which was already present in the ITS strict consensus tree (chapter 3, <50% support). This relationship collapses in the plastid consensus tree, but has 69% of support in the combined analysis, although it had lower bootstrap percentages (54%) in Chapter 1. The relationship between Ponerinae and

Bletiinae also remains ambiguous. In the plastid trees there is a polytomy among Pleurothallidinae/Dilomilidinae, Ponerinae and Bletiinae, and in the combined analysis they are successive sister groups as in the ITS phylogeny of Chapter 3. However, in the Epidendreae analysis of Chapter 1, Ponerinae and Bletiinae were sister to each other with 90% bootstrap. Probably this pattern is different here due to the less extensive outgroup used for rooting the trees in the current analysis. The position of *Meiracyllium*, deeply embedded in Laeliinae, appeared in both analyses (and also in ITS; Chapter 1; Cameron et al., 1999; Goldman et al., in press). Finally, the position of *Chysis* and *Coelia* is the same in both analyses, in agreement with ITS alone (Chapter 3, Fig. 3.1).

One interesting aspect of this study in the Laeliinae concerns the ability to produce artificial interspecific and intergeneric crosses in relation to phylogeny. Although there are thousands of hybrids between *Cattleya, Laelia* and *Sophronitis* (Royal Horticultural Society, 1997), and right across most of the subtribe (e.g. *Sophronitis* x *Constantia* and *Scaphyglottis* x *Epidendrum*) there are no hybrids between *Arpophyllum* and other Laeliinae. Genera previously considered to be Laeliinae and found in this study as being part of other subtribes (*Isochilus, Ponera, Helleriella, Dilomilis* and *Neocogniauxia*) have not engendered any registered hybrids. It could be argued there might have been no attempt to produce such hybrids because these genera are not showy. However, such attempts probably have been made at least with *Arpophyllum* and *Isochilus*, which are very common in cultivation, and *Neocogniauxia,* which is showy. There is a registered hybrid between *Chysis* and *Bletia*, which increases confidence that they have a close relationship.

4.4.3. Internal topologies and taxonomic groups in the Laeliinae

Although there is some incongruence between the topologies resulting from the plastid analysis and ITS data (from Chapter 3), none of these relationships has internal support greater then 50%, suggesting that most of the incongruence could be due to character sampling error. One important point to mention is the placement of *Meiracyllium*; plastid data place it as sister to the *Cattleya* alliance with support <50%, while ITS places is sister to *Euchile* with 61%. The plastid placement remains in the combined analysis, although with support still lower than 50%. The long

branch length leading to this genus, although correlated with the striking morphological peculiarities, could produce spurious attraction. Another such incongruence is the position of *Myrmecophila*. ITS data placed this genus as sister to the rest of the *Cattleya* alliance, whereas plastid data place it unresolved in the node above *Meiracyllium*. The consensus of the combined analysis places this genus in the *Epidendrum* clade, but still with bootstrap value lower than 50%. This new placement is close to *Caularthron*, and both genera have uniquely hollow pseudobulbs that hold ant nests. It is also reasonably close to *Schomburgkia*, which previously included *Myrmecophila*; this might explain the long stems and similar flower morphology as a plesiomorphic character suite. However, *Myrmecophila* and *Schomburgkia* clearly should be kept as separate due the well-supported relationship of the latter to *Laelia s.s.* Many clades can be defined based on well-supported relationships. The *Scaphyglottis* alliance with *Jacquiniella* was present in the strict consensus of ITS (Chapter 3), however with no meaningful support. The plastid and combined datasets show a well-supported clade (81% and 85%, respectively), which now also includes *Dimerandra.* This latter genus has generally been considered related to *Epidendrum* due to the reed-stem habit. In the ITS dataset is was related to *Dinema* and *Nidema* in the *Encyclia* clade. In the combined analysis the latter two genera move to a position sister to the *Broughtonia* alliance, and then sister to the *Scaphyglottis* alliance. The *Broughtonia* alliance (excluding *Dinema/Nidema*) is well-supported (100%) and shows two alliances of genera, one with *Broughtonia/Cattleyopsis* and *Laeliopsis* (98%) and the other with *Psychilis* and *Tetramicra* (74%).

The clade including *Laelia s.s.* and *Schomburgkia* also significantly improved in resolution and support. In the ITS topologies (Chapter 3, Fig. 3.3) a clade with *Nageliella/Domingoa/Homalopetalum* was embedded here, splitting the *Laelia/Schomburgkia* clade in two. Although plastid data make it monophyletic (Fig. 4.2), this whole clade is in a polytomy with the *Cattleya* alliance and *Epidendrum* alliance. The combined dataset indicates a closer affinity to the *Epidendrum* alliance, and although there was less than 50% support for this, the *Laelia s.s.* clade is well-supported (96%), whereas the *Nageliella* group moved to an unresolved position in the spine of the tree. The existence of an *Encyclia* alliance, possibly sister to the *Isabelia* alliance, is emerging. This pattern started to appear in most of the trees in

ITS and plastid data, but collapsed in a strict consensus. In the combined analysis they are consistently resolved, but still do not receive bootstrap support above 50%. From a morphological point of view there are interesting floral and vegetative similarities between *Artorima* and *Pseudolaelia*, which appear to share plesiomorphic traits in relation to other members of this group. In the *Encyclia* alliance all the basal members are montane Mexican taxa, and in the *Isabelia* clade all members are from unusual habitats in the Brazilian Plateau. Since these two clades are at the base of Laeliinae after *Hagsatera*, it seems there was an early dispersal through the Neotropics. (see Fig. 4.6 for a tree with geographic information on each clade). Compared with the overall patterns within Laeliinae the Brazilian group is probably relictual. As for the whole subtribe, most clades have basal nodes separating Mexican or Caribbean taxa. *Hagsatera,* a small Mexican genus, is separated by the basalmost node within Laeliinae, while *Arpophyllum* has all of its species in Mexico (only one species extends southwards to Colombia). The distribution of other clades in the Epidendreae (Fig. 4.6) and also these two first nodes seem to indicate the primary origin of Laeliinae is Mexico/North America. In the *Cattleya* alliance the pattern is similar, with *Meiracyllium* restricted to Mexico, and then some basal taxa (*Rhyncholaelia* and *Cattleya skinneri* group) in Central America. More derived taxa are present only in South America, and have higher species numbers and diversity (*Cattleya, Sophronitis s.l.* and to some extent *Brassavola*).

Phylogeny within the *Cattleya* alliance remains slightly confused due to several problems. Four *Cattleya* species together occupied an unexpected position in the ITS analysis (Chapter 3), but were more reasonably placed in the plastid trees. However, three of them still grouped together, and one moved to be sister of the peculiar *C. araguaiensis*. These patterns could suggest reticulation events involving some members of this group. Due to the overall amount of variation, the ITS data seems to override plastid patterns in the combined analysis. In fact, the plastid analysis (Fig. 4.2) produced a topology that is more in agreement with our understanding of this group from a morphological viewpoint. In the plastid analysis, *Sophronitis* and *Brassavola* appeared monophyletic, and *Laelia harpophylla* clusters with two species of section *Parviflorae*, in agreement with the system of Withner (1990). However, the plastid analysis has few groups with any internal support due to

the low levels of variation. The combined analysis followed more closely the ITS-only analysis of Chapter 3 (Fig. 3.6). The four suspected paralogues occupied the same position as with ITS only, and *Sophronitis* was no longer monophyletic, with *S. cernua* separated from the other two species (*S. coccinea* and *S. brevipedunculata*). However, there was great improvement on the bootstrap support in *Cattleya*, and *Brassavola* had the monophyletic topology of the plastid analysis with good internal support. The paraphyletic position of *Brassavola* in relation to *Cattleya* in ITS data might have been due to character sampling effects. An increase in characters solved this problem, as in Sheahan and Chase (2000). This empirical observation could be explained by the fact that with a low number of characters there is a larger probability that characters supporting a given branch will be missing entirely by chance. The dominance of the ITS dataset is still clear even after the four problematic sequences were removed (Fig. 4.5). Although the position of the four species with ITS removed improved, the rest of the tree remained exactly nearly the same, following closely the relationships suggested by ITS only.

However, the change in topology at least suggests that these four species of *Cattleya* are not related to *Laelia*, as it was indicated by the ITS dataset. Nevertheless, *C. trichopiliochila, C. lawrenceana* and *C. lueddemanniana* still group together with high support, and *C. maxima* is sister to *C. araguaiensis* rather than embedded on the middle of the Brazilian *Laelia.* Because this result is rather unexpected from a morphological point of view, it could mean there are evolutionary events not accounted for, which made their phylogenetic affinities obscure. The adequacy of ITS for resolving the overall phylogeny of the *Cattleya* alliance is questionable, and probably the best strategy would be to collect an increasing amount of plastid data to strengthen support for the plastid topologies. On the other hand, the existence of such constrasting alternative topologies between plastid data and ITS suggest detailed studies should be conducted to investigate effects of hybridisation in this group, and then any confirmed hybrids should be excluded from the analysis. We should stress the high genetic compatibility in artificial crosses involving these species, accounting for more the 35,000 hybrids produced among only around 150 natural species. There are also a few interspecific and intergeneric natural hybrids reported (Adams and Anderson, 1958), and for this reason hybridisation could have

played a significant role in the evolution of genera and species of Laeliinae before the early diversification within each lineage.

The overall results of the combined analysis are in agreement with the ITS data alone, at least for the few areas where ITS had internal support. To a much more limited extent there is a correlation between the DNA phylogenies and the alliances proposed by Dressler (1981) based on Baker (1972). As previously discussed in Chapter 3, the main weakness of those alliances was the inability of detecting polyphyletic genera such as *Laelia* and *Schomburgkia* and also of detecting the fact that Ponerinae and the genera related to *Dilomilis* did not belong in Laeliinae. All the alliances proposed by Dressler (1981) appear to be too large and include unrelated genera, and a system of generic alliances based on my results would need a larger number of smaller alliances. Finally, it seems that we are in a situation where the collection of a lot more DNA data should be enough to resolve the phylogeny of Laeliinae with improved bootstrap support.

Table 4.1. Voucher information for the taxa used in this study.

Species Name	**Voucher**
Acrorchis roseola Dressler	unvouchered (coll. W.M.Whitten)
Alamania punicea La Llave & Lex.	van den Berg C184 (ESA)
Amblostoma armeniacum (Lindl.) Brieger ex Pabst	Brieger Coll. 33081 (ESA)
Amblostoma cernuum Scheidw.	Brieger Coll. 15628 (ESA)
Arpophyllum giganteum Hartw. ex Lindl.	Chase O-586 (K)
Artorima erubescens (Lindl.) Dressler & G.E.Pollard	unvouchered (coll. S.Beckendorf)
Barkeria skinneri (Batem. ex Lindl.) Lindl. ex Paxton	van den Berg C250 (K spirit)
Barkeria whartoniana (C.Schweinf.) Soto Arenas	van den Berg C249 (K spirit)
Bletia catenulata Ruiz & Pav.	E. L. Borba 590 (UEC)
Bletia catenulata Ruiz & Pav.	W. Forster 10 (ESA)
Bletia purpurea DC.	van den Berg C342 (K spirit)
Brassavola cucullata (L.) R.Br.	W. E. Higgins 130 (FLAS 198290)
Brassavola martiana Lindl.	unvouchered (Kew 1995-2685)
Brassavola nodosa (L.) Lindl.	Chase O-336 (K)
Brassavola tuberculata Hook.	Brieger Coll. 3497 (ESA)
Briegeria equitantifolia (Ames) Senghas	van den Berg C171 (K spirit)
Broughtonia negrilensis Fowlie	W. E. Higgins 152 (FLAS 198288)
Broughtonia sanguinea (Sw.) R.Br.	Brieger Coll. 14440 (ESA)
Cattleya aclandiae Lindl.	Brieger Coll. 32982 (ESA)
Cattleya araguaiensis Pabst	unvouchered (Kew 1999-1443)
Cattleya aurantiaca (Batem. ex Lindl.) P.N.Don	Brieger Coll. 124 (ESA)
Cattleya dowiana Batem.	Chase O-282 (K)
Cattleya forbesii Lindl.	Brieger Coll. 2448 (ESA)
Cattleya intermedia Graham ex Hook.	Brieger Coll. 4095 (ESA)
Cattleya labiata Lindl.	Brieger Coll. 5487 (ESA)
Cattleya lawrenceana Rchb.f.	Brieger Coll. 3802 (ESA)
Cattleya lueddemanniana Rchb.f.	Brieger Coll. 3759 (ESA)
Cattleya maxima Lindl.	unvouchered (Kew 1983-4362)
Cattleya mooreana Withner, D.Alison & Guenard	unvouchered (Kew 1999-1599)
Cattleya skinneri Batem.	unvouchered (Kew 1986-4870)
Cattleya trichopiliochila Barb.Rodr.	Brieger Coll. 28787 (ESA)
Cattleya violacea (Kunth) Rolfe	Brieger Coll. 28495 (ESA)
Cattleya walkeriana Gardner	Brieger Coll. 1627 (ESA)
Cattleyopsis lindenii (Lindl.) Cogn.	W. E. Higgins 251 (FLAS 198289)
Caularthron bilamellatum (Rchb.f.) R.E.Schultes	Brieger Coll. 3690 (ESA)
Chysis bractescens Lindl.	Chase O-436 (K)
Coelia triptera (Smith) G.Don ex Steud.	Chase O-324 (K)

Species Name	Voucher
Constantia cipoensis Pôrto & Brade	São Paulo B.G. s.n. (SP)
Dilomilis montana (Sw.) Summerh.	Chase O-206 (K)
Dimerandra emarginata (G.Mey.) Hoehne	Chase O-335 (K)
Dinema polybulbon (Sw.) Lindl.	Brieger Coll. 6052 (ESA)
Domingoa kienastii (Rchb.f.) Dressler	W. E. Higgins 225 (FLAS 198291)
Earina valida Rchb.f.	van den Berg C296 (Leiden 950080)
Encyclia adenocaula (La Llave & Lex.) Schltr.	W. E. Higgins 12 (FLAS 198274)
Encyclia cordigera (Kunth) Dressler	W. E. Higgins 24 (FLAS 198276)
Encyclia oncidioides (Lindl.) Schltr.	Brieger Coll. 5420 (ESA)
Encyclia tampensis (Lindl.) Small	W. E. Higgins 27 (FLAS 198277)
Epidendrum campestre Lindl.	E. L. Borba 553 (UEC)
Epidendrum conopseum R.Br.	W. E. Higgins 244 (FLAS 198271)
Epidendrum ibaguense Lindl.	W.E. Higgins 60 (FLAS 198270)
Epidendrum pseudepidendrum Rchb.f.	van den Berg C4 (ESA)
Euchile citrina (La Llave & Lex.) Withner	W. E. Higgins 54 (FLAS 198269)
Euchile mariae (Ames) Withner	Chase O-158 (K)
Hagsatera brachycolumna (L.O.Williams) R.González	W. E. Higgins 229 (FLAS 198272)
Helleriella guerrerensis Dressler & Hágsater	van den Berg C172 (K spirit)
Helleriella punctulata (Rchb.f.) Garay & Dunst.	Chase O-299 (K)
Hexadesmia crurigera Lindl.	Chase O-336 (K)
Hexisea bidentata Lindl.	Brieger Coll. 1253 (ESA)
Hexisea imbricata (Lindl.) Rchb.f.	W. M. Whitten 97039 (FLAS)
Homalopetalum pachyphyllum (L.O.Williams) Dressler	M. Soto 7640 (AMO)
Homalopetalum pumilio (Rchb.f.) Schltr.	W. E. Higgins 234 (FLAS 200730)
Isabelia virginalis Barb.Rodr.	Brieger Coll. 30243 (ESA)
Isochilus amparoanus Schltr.	Chase O-204 (K)
Isochilus major Cham. & Schltdl.	W. M. Whitten 91348 (FLAS)
Jacquiniella teretifolia Britton & P.Wilson	W. M. Whitten 97026 (FLAS)
Laelia alaorii Brieger & Bicalho	Brieger Coll. 19179 (ESA)
Laelia anceps Lindl.	Chase O-998 (K)
Laelia autumnalis (La Llave & Lex.) Lindl.	unvouchered (coll. S. Beckendorf)
Laelia esalqueana Blumensch. ex Pabst	Brieger Coll. 4980 (ESA)
Laelia fidelensis Pabst	C225-Machado s.n. (ESA)
Laelia furfuracea Lindl.	unvouchered (coll. S. Beckendorf)
Laelia harpophylla Rchb.f.	Brieger Coll. 6687 (ESA)
Laelia pumila (Hook.) Rchb.f.	Brieger Coll. 7794 (ESA)
Laelia purpurata Lindl. & Paxton	Chase O-997 (K)
Laelia rubescens Lindl.	Chase O-1205 (K)

Species Name	Voucher
Laelia rupestris Lindl.	van den Berg C33 (ESA)
Laelia speciosa (Kunth) Schltr.	unvouchered Chase O-6088
Laeliopsis dominguensis (Lindl.) Lindl. & Paxton	unvouchered (coll. W.E.Higgins)
Lanium avicula (Lindl.) Benth.	Brieger Coll. 23319 (ESA)
Leptotes bicolor Lindl.	Brieger Coll. 1068 (ESA)
Loefgrenianthus blanche-amesiae (Loefgr.) Hoehne	São Paulo B.G. s.n. (SP)
Meiracyllium gemma Rchb.f.	M. Soto 8731 (AMO)
Meiracyllium trinasutum Rchb.f.	Chase O-202 (K)
Myrmecophila aff. *tibicinis* (Batem.) Rolfe	van den Berg C81 (ESA)
Myrmecophila tibicinis (Batem.) Rolfe	Chase O-281 (K)
Nageliella purpurea (Lindl.) L.O.Williams	van den Berg C260 (K spirit)
Nanodes schlechterianum (Ames) Brieger	Chase O-301 (K)
Neocogniauxia hexaptera (Cogn.) Schltr.	van den Berg C244 (K)
Neolauchea pulchella Kraenzl.	Brieger Coll. 6367 (ESA)
Nidema boothii (Lindl.) Schltr.	W. E. Higgins 192 (FLAS 198273)
Octomeria gracilis Lodd. ex Lindl.	Chase O-977 (K)
Oerstedella centradenia Rchb.f.	van den Berg C169 (K spirit)
Orleanesia amazonica Barb.Rodr.	São Paulo B.G. 15936 (SP)
Pleurothallis ochreata Lindl.	van den Berg C (K spirit)
Pleurothallis racemiflora Lindl. ex Hook.	W. E. Higgins 140 (FLAS 198267)
Polystachya galeata Rchb.f.	van den Berg C283 (K spirit)
Ponera exilis Dressler	M. Soto s.n. Paracho, Michoacán (AMO)
Ponera striata Lindl.	van den Berg C175 (K)
Ponera striata Lindl.	W. E. Higgins 197 (FLAS 198268)
Prosthechea abbreviata (Schltr.) W.E.Higgins	Brieger Coll. 10092 (ESA)
Prosthechea aemula (Lindl.) W.E.Higgins	W. E. Higgins 17 (FLAS 198279)
Prosthechea cochleata (L.) W.E.Higgins	MBG 75-0658 (FLAS 198280)
Prosthechea glauca Knowles & Westc.	W. E. Higgins 176 (FLAS 200722)
Pseudolaelia vellozicolla (Hoehne) Pôrto & Brade	São Paulo B.G. 13362 (SP)
Psychilis krugii (Bello) Sauleda	Chase O-1062 (K)
Psychilis macconnelliae Sauleda	W. E. Higgins 53 (FLAS 198287)
Reichenbachanthus cuniculatus (Schltr.) Pabst	W. M. Whitten 96051 (FLAS)
Renata canaanensis Ruschi	Brieger Coll. 16205 (ESA)
Restrepiella ophiocephala (Lindl.) Garay & Dunst.	Chase O-291 (K)
Rhyncholaelia digbyana (Lindl.) Schltr.	Chase O-331 (K)
Rhyncholaelia glauca (Lindl.) Schltr.	van den Berg C30 (ESA)
Scaphyglottis pulchella (Schltr.) L.O.Williams	unvouchered (coll. W. M. Whitten)

Species Name	Voucher
Schomburgkia lyonsii Lindl.	Brieger Coll. 16846 (ESA)
Schomburgkia splendida Schltr.	W. M. Whitten 93026 (FLAS)
Schomburgkia undulata Lindl.	van den Berg C29 (ESA)
Sophronitella violacea (Lindl.) Schltr.	van den Berg C127 (ESA)
Sophronitis brevipedunculata (Cogn.) Fowlie	São Paulo B.G. s.n. IBDF (SP)
Sophronitis cernua (Lindl.) Hook.	Brieger Coll. 15737 (ESA)
Sophronitis coccinea (Lindl.) Rchb.f.	São Paulo B.G. 9577 (SP)
Stelis argentata Lindl.	Kew 1984-7410 (K spirit 60886)
Tetragamestus modestus Rchb.f.	Brieger Coll. 2756 (ESA)
Tetramicra elegans (Ham.) Cogn.	W. E. Higgins 160 (FLAS 198285)

Table 4.2. Features of DNA datasets used in this study.

DNA Region	**aligned length**	**# variable sites**	**# potentially parsimony informative**	**number of changes/variable site**	**Fitch Tree length**	**CI**	**RI**	**ts:tv**
***trnL-F* region**	1350	495 (36.66%)	223 (16.5%)	1.97	974	0.6294	0.6426	0.95
trnL-F intron	723	251 (34.72%)	104 (14.38%)	1.97	495	0.6162	0.6520	1.09
trnL-F exon	50	9 (18%)	2 (4%)	2.33	21	0.5238	0.2857	0.17
trnL-F interg. spacer	596	250 (41.95%)	124 (20.8%)	2.01	502	0.6375	0.6136	0.74
ITS region	789	461 (58.43%)	339 (42.97%)	5.05	2326	0.3469	0.5208	2.20
ITS1	306	227 (74.18%)	169 (55.23%)	5.23	1188	0.3451	0.5054	2.15
5.8S	158	23 (14.56%)	10 (6.33%)	1.87	43	0.6512	0.5833	2.31
ITS2	325	211 (64.92%)	160 (49.23%)	5.19	1095	0.3370	0.5349	2.26
***matK-trnK* spacers**	600	297 (49.5%)	118 (19.67%)	1.92	571	0.6830	0.5628	0.85
***matK* gene**	1347	551 (40.91%)	259 (19.23%)	2.12	1167	0.5835	0.6358	1.03
matK (1st positions)					331 (28.36%)	0.6707	0.6293	
matK (2nd positions)					357 (30.59%)	0.5938	0.6934	
matK (3rd positions)					479 (41.04%)	0.5177	0.5904	
All plastid data (except excluded)					2739	0.6301	0.6408	
All data (except excluded)					5154	0.4902	0.5806	

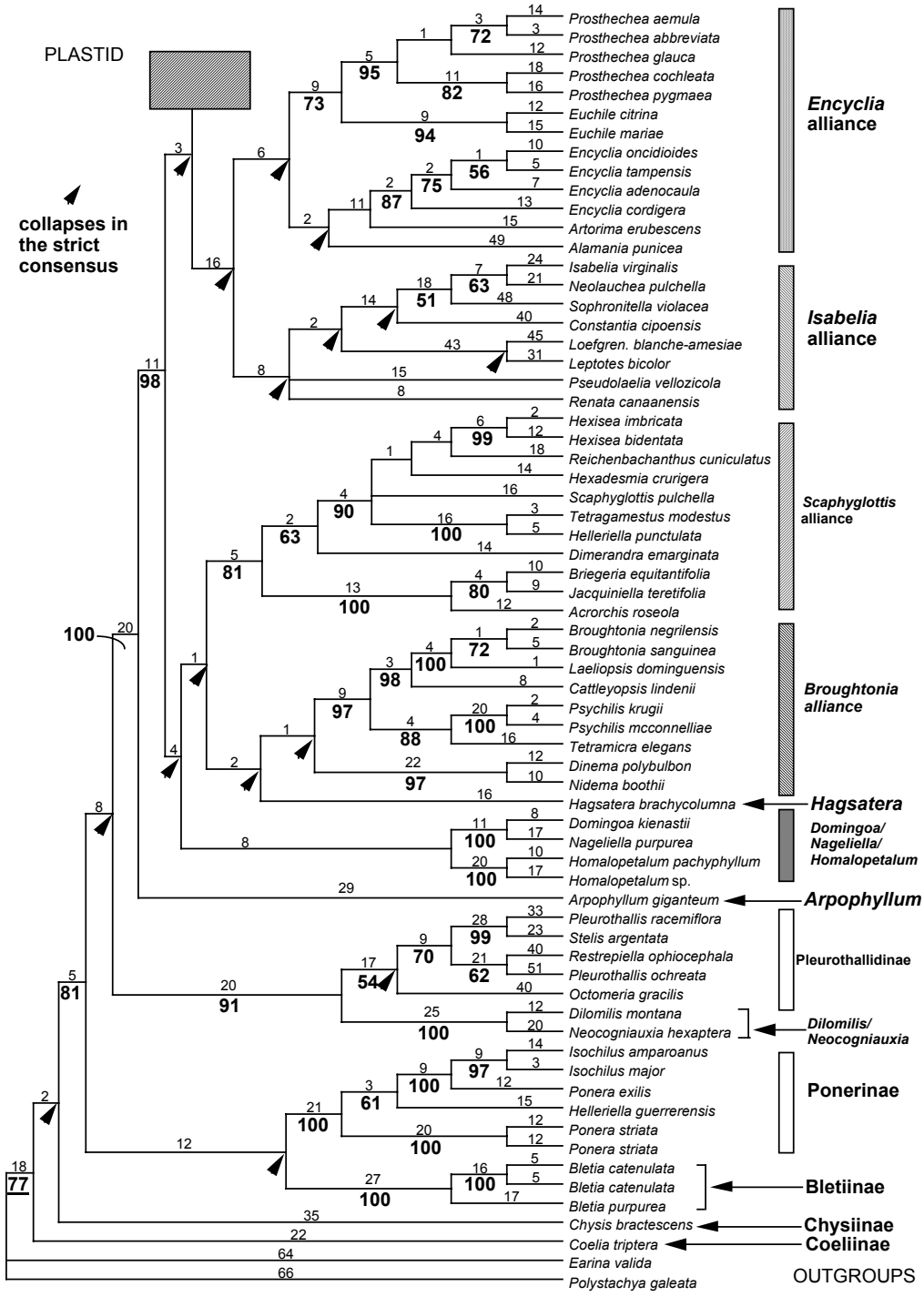


Fig. 4.1. First part of one of the most parsimonious trees for the analysis including plastid data only. L=2739, CI=0.63, RI=0.64. Numbers above the branches are Fitch tree-lengths and numbers below the branches are bootstrap percentages (branches without values received less than 50% support).

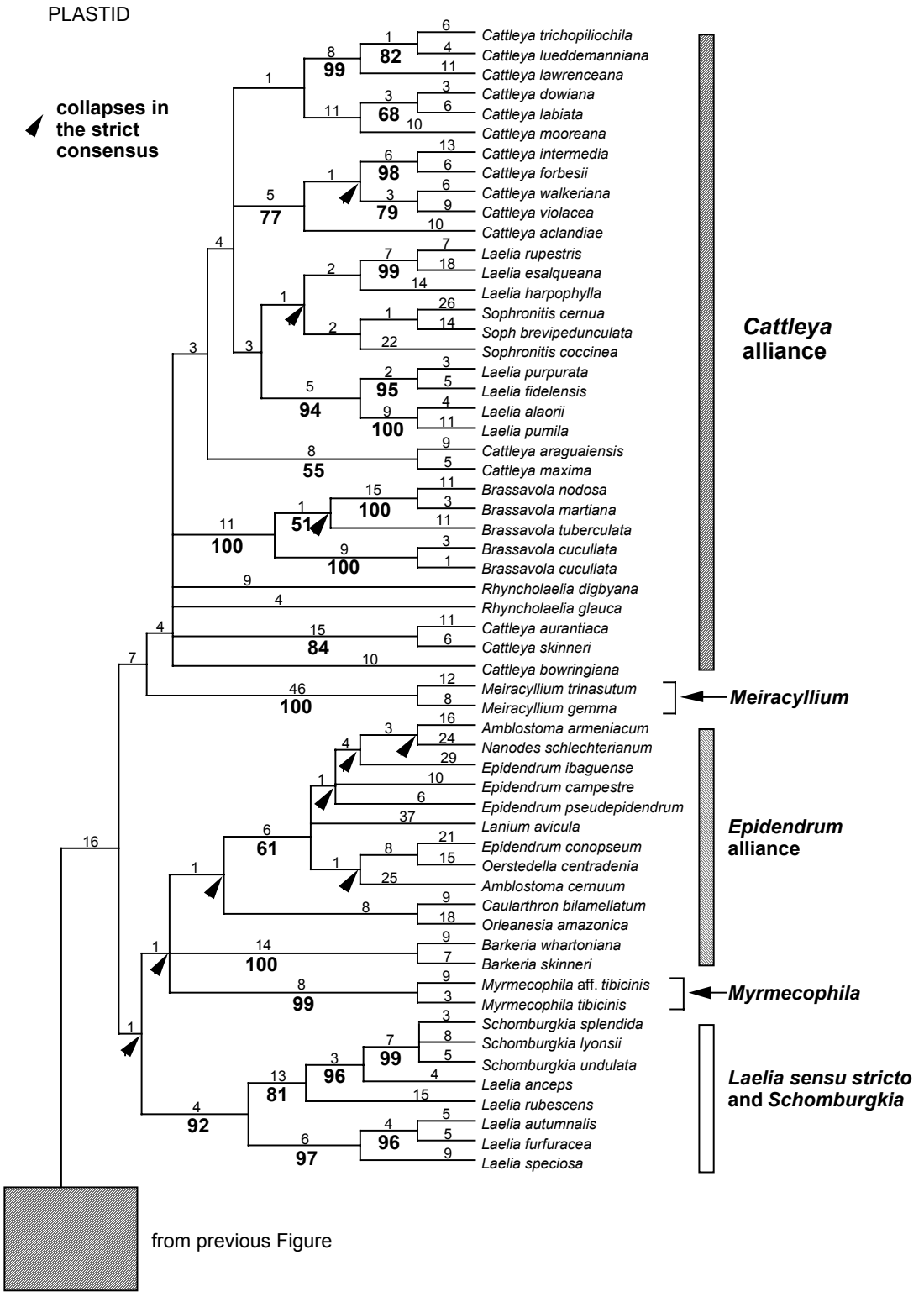


Fig 4.2. Second part of the tree in Fig. 4.1, including plastid data only. Numbers above the branches are Fitch tree-lengths and numbers below the branches are bootstrap percentages (branches without values received less than 50% support).

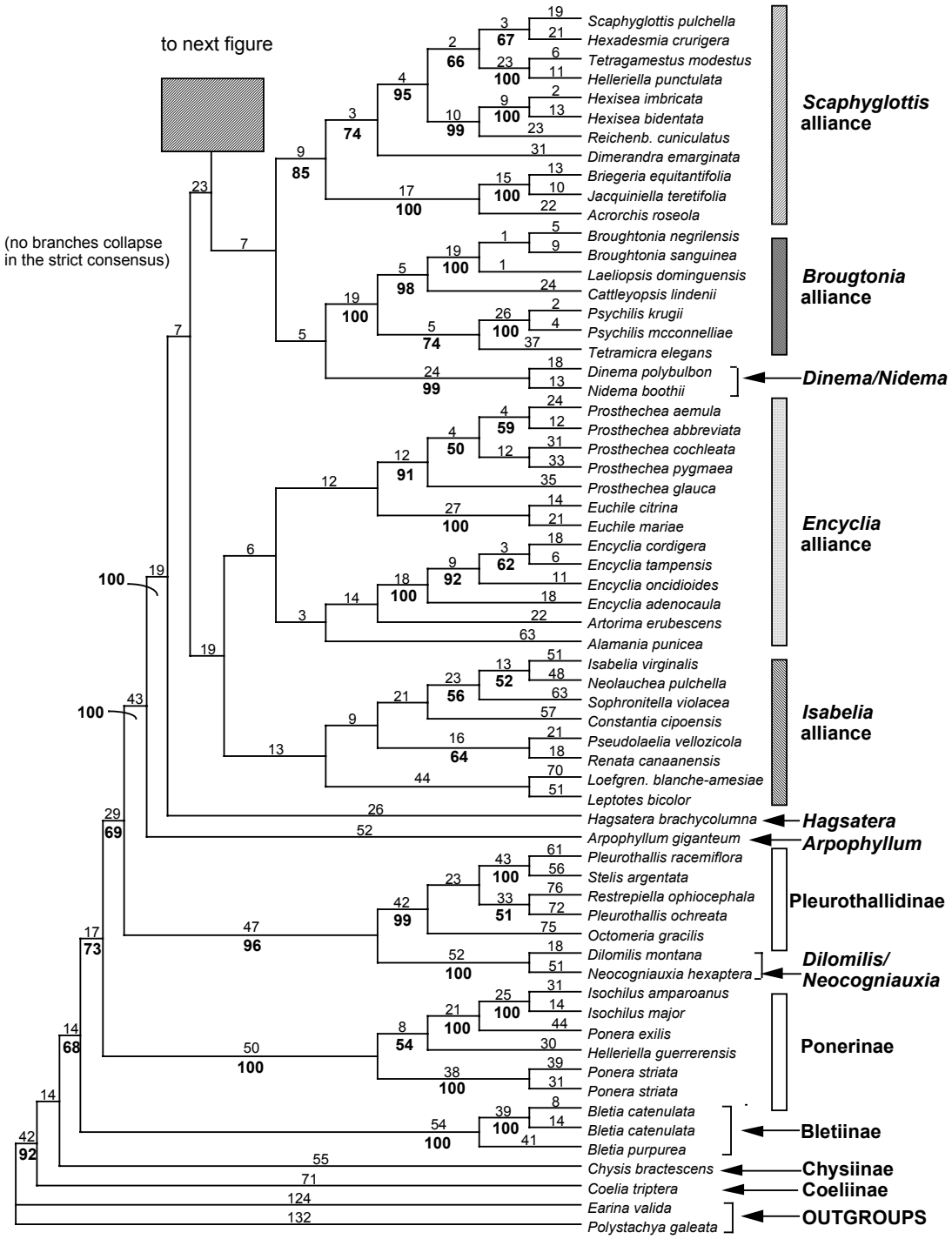


Fig. 4.3. First part of one of the most parsimonious trees for the combined analysis. L=5154, CI=0.49, RI=0.58. Numbers above the branches are Fitch tree-lengths and numbers below the branches are bootstrap percentages (branches without values received less than 50% support).

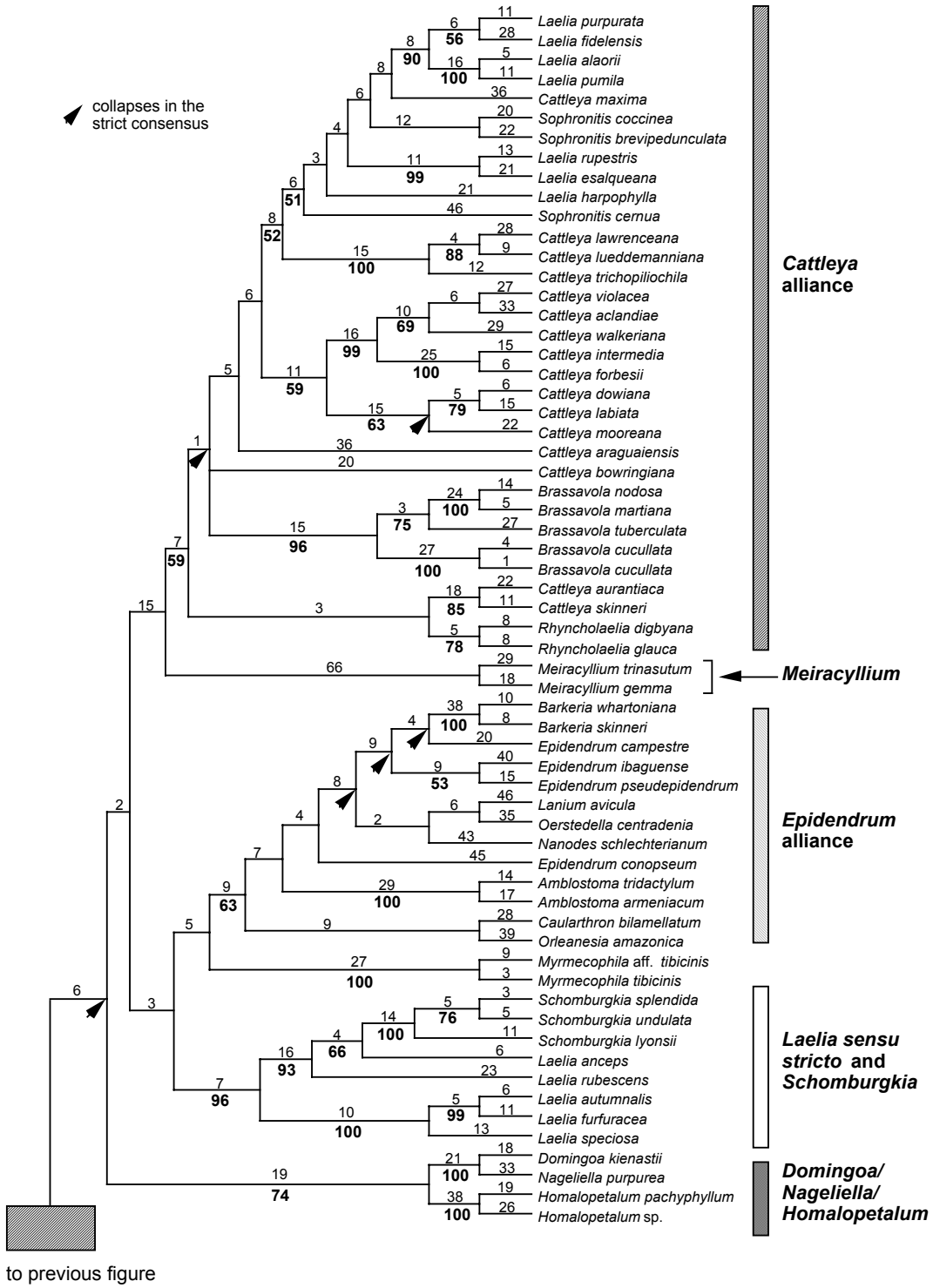


Fig. 4.4. Second part of the tree in Fig. 4.3. Numbers above the branches are Fitch tree-lengths and numbers below the branches are bootstrap percentages (branches without values received less than 50% support).

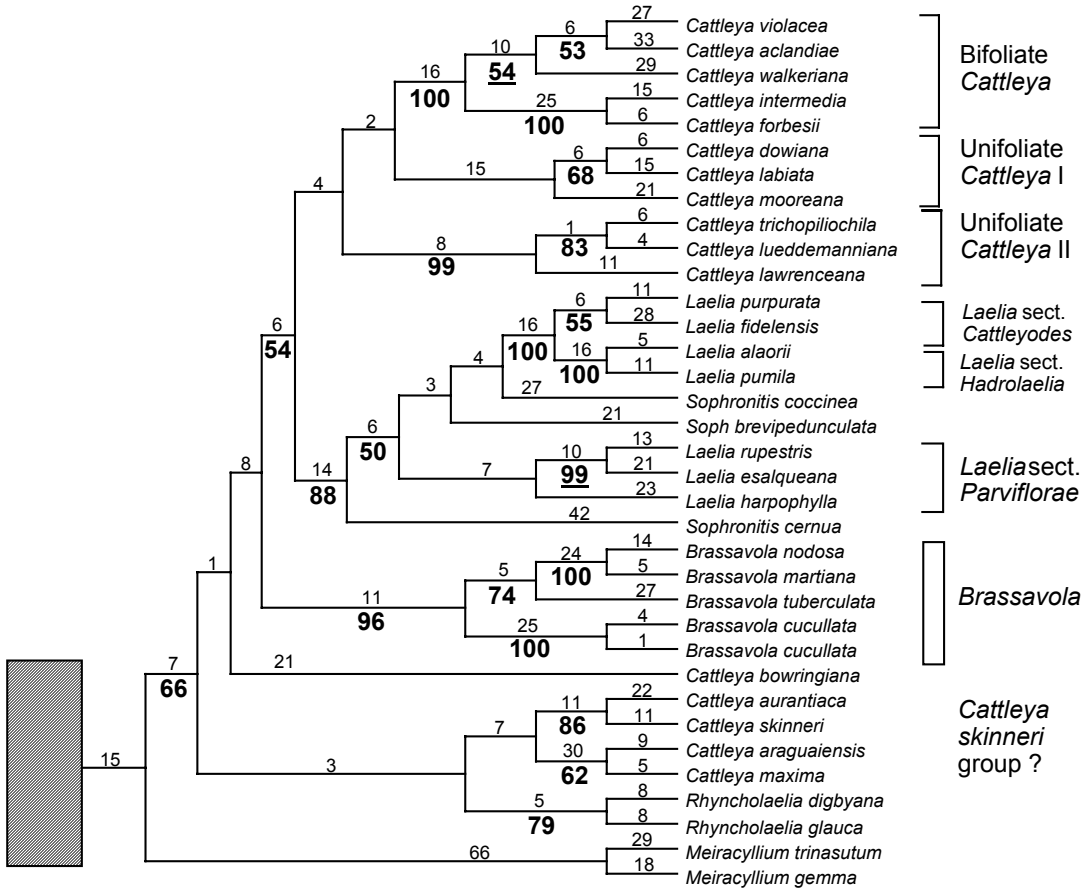


Fig 4.5. A portion of one tree (*Cattleya* alliance) of the analysis excluding putative paralogue ITS sequences of *Cattleya*. Numbers above the branches are Fitch tree-lengths and numbers below the branches are bootstrap percentages (branches without values received less than 50% support).

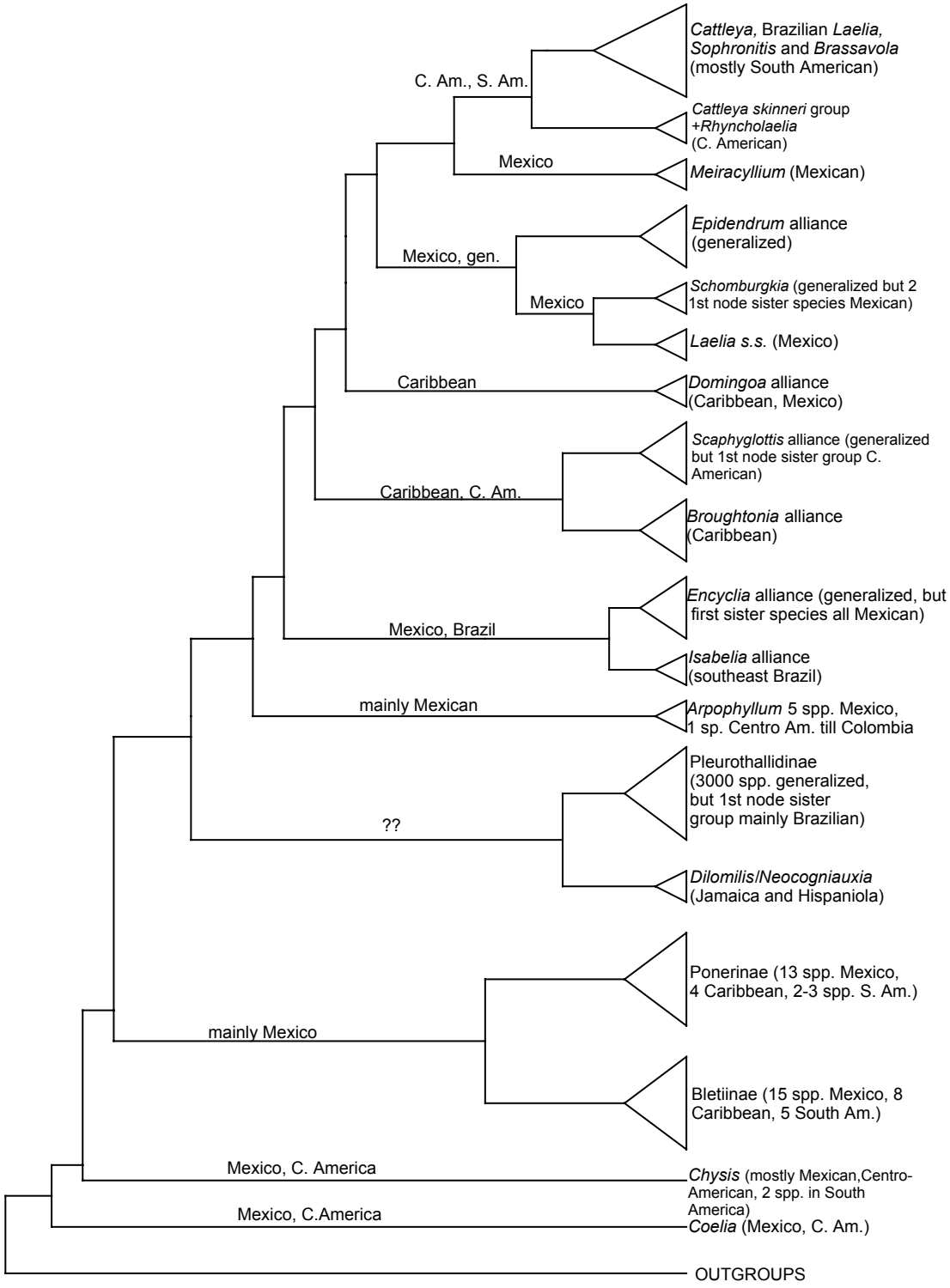


Fig. 4.6. A summary of the tree obtained in the combined analysis, with geographic information on each clade and a manual optimization for the main branches.

Chapter 5 – General conclusions

In the Epidendreae study (Chapter 1) I learned that this tribe is in reality smaller than most previously used concepts (Schlechter, 1926; Mansfeld, 1937; Dressler and Dodson, 1960; Dressler, 1993). In fact, the circumscription I obtained is unique. It includes all the subtribes of Dressler (1993) plus a very restricted version of Bletiinae, which were always placed in a different subtribe. This shows clearly that the strong emphasis that has been given to soft versus hard pollinia as decisive characters for tribal and subtribal delimitation was unfounded. This study also demonstrated several switches between reed-stem (Ponerinae, *Dilomilis/Neocogniaxia*, *Epidendrum* alliance), and pseudobulbs (part of Laeliinae) plus cormous (Bletiinae) habit. Coupled with many other occurrences of these habits in other Epidendroideae, it is now clear that reed-stem condition cannot be used as a defining habit of any tribe (as attempted by Dressler, 1981). However, it can probably be useful to make keys at lower levels.

In the large ITS study (Chapter 3; van den Berg et al., 2000) I was able to detect most species-groups in Laeliinae. Although these corresponded in part to currently used genera, several horticulturally important genera were polyphyletic, including *Laelia* and *Encyclia*. The solution for these was sometimes to break them up in smaller genera (e.g. *Encyclia*, *Prosthechea, Dinema* and *Euchile*) or to transfer part of the species to other genera, as was done for the Brazilian species of *Laelia* to *Sophronitis* (van den Berg and Chase, 2000). However, the pattern along the spine of the ITS-only tree was not sufficiently clear enough to create reliable groups of genera (many branches collapsing in the strict consensus tree). Nevertheless, some alliances (*Cattleya*, *Epidendrum* and *Scaphyglottis* alliances) that were present but not strongly supported with ITS-only analysis were confirmed in the combined analysis of Chapter 4. Another failure of this study is that one of my main personal objectives when I started was not achieved, namely a well-resolved species phylogeny of *Cattleya* and *Laelia*. This was due to two main drawbacks. The first one was the low level of variation among the species of these two genera. This seems to be a common problem for species in recently radiating genera of Epidendroideae (Pridgeon et al., 1998, unpublished data; Ryan et al., 2000; Whitten et al., in press; Williams et al., unpublished data). A second problem was some unclear patterns of ITS in the *Cattleya* alliance, with some sequences that I suspected were

paralogues. These obscured the relationships among some species of *Cattleya*, so that their placement is still unclear. Significantly more work will be necessary to clarify species phylogenies in the genera by finding suitably variable DNA regions and DNA fingerprinting could be used in related species-complexes. At present, there are few alternatives in the monocots, although many low-copy nuclear genes are appearing in the literature for more distantly related angiosperms (e.g. *ncpGS*; Emshwiler and Doyle, 1999; *adh*; Gaut and Clegg, 1991, 1993). Another possibility would be ETS (Baldwin and Markos, 1998; Linder et al., 2000), but this would be likely to suffer the same problems of paralogy as ITS. Such regions would not only be useful for resolving species phylogenies, but also in increasing support for the overall topologies.

The combined analysis of three DNA regions (ITS, *trnL-F*, *matK*) clarified partially the results of Chapter 3. Although the strict consensus is much more resolved, the spine of the tree is far from ideal, and bootstrap percentages are low. Some noteworthy changes in topology occurred, such as the position of *Meiracyllium*, *Myrmecophila* and *Dimerandra*. These changes were not unexpected, given the resolution in the ITS tree. The separate analysis for plastid DNA in the *Cattleya* clade confirmed my initial suspicion for the presence of ITS paralogues. The exclusion of the ITS sequences for the taxa in question did not resolve the problem, mainly because there is insufficient variation in the plastid datasets. The collection of two or three more plastid datasets will be necessary before we can compare the plastid topologies with ITS, but this will be relatively easy to achieve due the smaller number of taxa in this clade.

In general, from the results of the molecular studies performed in Epidendreae and Laeliinae, it is clear that incomplete taxon or character sampling has pronounced effects in phylogenetic reconstruction. The importance of taxon sampling was indicated when the better-sampled portions of Epidendreae were much better resolved and supported than the remaining Epidendroideae with more limited sampling. It was also clear in the high retention index achieved in the ITS-only study. The latter indicated that most homoplasious characters in a general context were reconstructed as being good synapomorphies in localised areas of the tree, and clear phylogenetic pattern was obtained from them. The main problem of the ITS study was character sampling by the inclusion of a single DNA region, as well as the risk of constrasting gene and organismal phylogenies. The combined study for Laeliinae (Chapter 4) was in a way the

converse of this, as it suffered primarily from taxon-sampling problems, indicated by lower RIs in all gene regions included. The best example of this was ITS, with an RI of 0.52 in this analysis against 0.71 in Chapter 3. Given that the alignment was identical (ITS dataset of Chapter 4 was a simple subset from that in Chapter 3) this change in RI has to be attributed to taxon-sampling only. It is clear that future studies in Laeliinae will need a combined strategy. A first step will be to collect more DNA regions to increase the number of variable positions. That could be achieved more simply with additional variable plastid spacers. Once the gain from adding positions stabilises, the strategy should be switched to increasing the number of taxa. In the Epidendreae study the latter strategy seems more appropriate because there was more variation that in Laeliinae. In that study, better sampling in Epidendroideae should improve the contribution of ITS, both in alignment and by recovering phylogenetic pattern from homoplasious characters.

The use of molecular data in Laeliinae and Epidendreae is quite promising, and the results I obtained in this study contributed more to the understanding of the phylogeny of these two groups (especially of Laeliinae) more than any single previous work. However, before a stable, well-supported phylogenetic hypothesis can be achieved in various levels, it will be necessary to collect an equal or greater amount of data to that already collected. This task will be simplified because of the comprehensive DNA collection that was put together for this thesis. Many satellite projects will be necessary to resolve species phylogenies in each genus. Also, a large study is in progress for the *Epidendrum* alliance (Soto Arenas, van den Berg and others).

Finally, I will present a short summary of the conclusions from this thesis:

(a) Epidendreae needs to be delimited as an exclusively Neotropical tribe, including Laeliinae, Pleurothallidinae, Ponerinae and Bletiinae.
(b) Subtribe Bletiinae should be restricted to *Bletia, Basiphyllaea* and *Hexalectris*.
(c) Ponerinae consists of *Ponera, Isochilus* and *Helleriella*. All other genera that possess a column-foot and were previously included in Laeliinae (e.g. *Scaphyglottis* alliance) should be kept in that subtribe.
(d) *Arpophyllum* and *Meiracyllium* should be included in Laeliinae.

(e) *Dilomilis* and *Neocogniauxia* should be included in Pleurothallidinae.

(f) Several genera within Laeliinae are polyphyletic, and subgroups of several large genera should be regarded as distinct, such as *Prosthechea* from *Encyclia* and *Euchile, Myrmecophila* from *Schomburgkia*, *Rhyncholaelia* from *Brassavola*. Brazilian species of *Laelia* are unrelated to the typical Mexican group, and should be moved to *Sophronitis*. Some subgroups of *Cattleya* are probably distinct, but the patterns I found were not sufficiently reliable to justify taxonomic change.

(g) Some genera could be combined under *Isabelia,* such as *Sophronitella* and *Neolauchea*, and possibly *Constantia* and *Pseudolaelia*.

(h) ITS was the most variable region in all studies in both the percentage of variable sites and the number of changes per site. The latter caused ITS to perform worse in terms of RI in both the Epidendreae (Chapter 1) and combined Laeliinae (Chapter 4) studies, on account of taxon sampling error. In the large ITS dataset of Laeliinae of Chapter 3, the RI was much higher because a much more dense taxon sampling compensates for the large number of changes per site.

(i) Both *matK* gene, spacers bordering *matK* and *trnL-F* presented similar levels of variation and informativeness (based on RI indexes). Compared with other plant groups, overall variation of plastid regions within Laeliinae was low, but this pattern is similar to other studies at similar taxonomic levels within Orchidaceae. This low variation results in low bootstrap support for plastid phylogenies and increased probability character sampling error.

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