Annals of Botany **104**: 417–430, 2009
doi:10.1093/aob/mcp101, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

A phylogenetic study of Laeliinae (Orchidaceae) based on combined nuclear and plastid DNA sequences

Cássio van den Berg[1,*], Wesley E. Higgins[2], Robert L. Dressler[3], W. Mark Whitten[3], Miguel A. Soto-Arenas[4] and Mark W. Chase[5]

[1]*Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Av. Transnordestina s/n, 44036-900, Feira de Santana, Bahia, Brazil,* [2]*Marie Selby Botanical Gardens, 811 South Palm Avenue, Sarasota, FL 34236, USA,* [3]*Florida Museum of Natural History, University of Florida, P.O. Box 110670, Gainesville, FL 32611-0670, USA,* [4]*Instituto de Ecología, UNAM, Apartado Postal 70-275, México D.F. 04510, Mexico and* [5]*Molecular Systematics Section, Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK*

Received: 4 August 2008 Returned for revision: 25 November 2008 Accepted: 24 March 2009 Published electronically: 7 May 2009

• *Background and Aims* Laeliinae are a neotropical orchid subtribe with approx. 1500 species in 50 genera. In this study, an attempt is made to assess generic alliances based on molecular phylogenetic analysis of DNA sequence data.
• *Methods* Six DNA datasets were gathered: plastid *trnL* intron, *trnL-F* spacer, *matK* gene and *trnK* introns upstream and dowstream from *matK* and nuclear ITS rDNA. Data were analysed with maximum parsimony (MP) and Bayesian analysis with mixed models (BA).
• *Key Results* Although relationships between Laeliinae and outgroups are well supported, within the subtribe sequence variation is low considering the broad taxonomic range covered. Localized incongruence between the ITS and plastid trees was found. A combined tree followed the ITS trees more closely, but the levels of support obtained with MP were low. The Bayesian analysis recovered more well-supported nodes. The trees from combined MP and BA allowed eight generic alliances to be recognized within Laeliinae, all of which show trends in morphological characters but lack unambiguous synapomorphies.
• *Conclusions* By using combined plastid and nuclear DNA data in conjunction with mixed-models Bayesian inference, it is possible to delimit smaller groups within Laeliinae and discuss general patterns of pollination and hybridization compatibility. Furthermore, these small groups can now be used for further detailed studies to explain morphological evolution and diversification patterns within the subtribe.

Key words: Laeliinae, Orchidaceae, ITS, *trnL* intron, *trnL-F* spacer, *matK*.

INTRODUCTION

Many phylogenetic studies based on molecular data have been carried out in different groups of Orchidaceae. With the advent of DNA sequencing, it was possible to begin disentangling the relationships between orchids and other families of monocotyledons and the internal structure of Orchidaceae (Cameron *et al.*, 1999; Freudenstein *et al.*, 2000, 2004). An analysis sampling 171 orchid taxa for *rbcL* gave a good idea of the relationships among subfamilies of orchids (Cameron *et al.*, 1999), although the level of variation was not enough to assist in delimitation of tribes and subtribes.

Laeliinae are strictly neotropical and comprise about 50 genera and 1500 species (Dressler, 1981, 1993), being the third largest subtribe in the family after Pleurothallidinae and Oncidiinae. Some genera such as *Cattleya*, *Guarianthe* and *Rhyncholaelia* are of outstanding horticultural value, and others such as *Encyclia*, *Epidendrum* and *Prosthechea* are common floristic elements in the neotropics. Morphological diversity is extremely high, probably due to specialization for particular pollinators coupled with adaptation to a broad range of habitats (van der Pijl and Dodson, 1969). Chromosome numbers are nearly constant within the subtribe (Tanaka and Kamemoto, 1984). The production of artificial hybrids for horticulture is possible for nearly any combination of genera, and many natural intergeneric and interspecific hybrids also occur (Adams and Anderson, 1958; Pabst and Dungs, 1975, 1977; Borba and Semir, 1998; Azevedo *et al.*, 2006).

Subtribe Laeliinae was established by Bentham (1881). Pfitzer (1889) divided the subtribe into two series: *Ponereae* and *Cattleyeae*, based on the presence of a column foot in the former. This concept was followed by Schlechter (1926) and most subsequent systems until Dressler removed *Meiracyllium* (Dressler, 1960) and *Arpophyllum* (Dressler, 1990) to their own monogeneric subtribes, based on pollinarium structure. More recent treatments included Baker (1972), based on leaf anatomic data, Brieger (1976), who divided the subtribe into four alliances (as 'Gattungsreihen'), mostly based on column-foot presence and habit, Dressler (1981), who proposed six alliances based on Baker (1972), presence of column-foot and habit; and Szlachetko (1995) who split the genera into three subtribes: Laeliinae, Epidendrinae and Ponerinae (based on column structure, pollinium number and habit). These systems differ in which genera are placed in alliances and subtribes because of the different intuitive emphasis on morphological characters by each author; all of them have

* For correspondence. E-mail vcassio@gmx.de

© The Author 2009. Published by Oxford University Press on behalf of the Annals of Botany Company. All rights reserved.
For Permissions, please email: journals.permissions@oxfordjournals.org

some aspects that appear to be highly artificial (e.g. emphasis on pollinium number, column-foot, and reed-stem habit).

Baker (1972) used leaf anatomy data for inferring relationships within Laeliinae and between Laeliinae and related subtribes. However, he did not carry out an explicit phylogenetic analysis, and many characters were polymorphic even within genera. He suggested alliances based on his results as a reticulate diagram. His results did not show *Meiracyllium* as distinct from Laeliinae. However, he found distinct features in *Arpophyllum*. His diagram was adapted and used by Dressler (1981) to propose generic alliances. The broad DNA studies published to date on orchids (Neyland and Urbatsch, 1996; Cameron *et al.*, 1999; Freudenstein *et al.*, 2000, 2004) did not have enough sampling to address most questions regarding circumscription of Laeliinae in relation to Epidendreae or to provide a clear picture of whether *Arpophyllum* and *Meiracyllium* were sister or embedded in Laeliinae. However, both Cameron *et al.* (1999) and Freudenstein *et al.* (2000) have shown *Dilomilis* to be sister to Pleurothallidinae rather than Laeliinae. A study centred on Epidendroideae and Epidendreae (van den Berg *et al.*, 2005) circumscribed Epidendreae as an exclusively neotropical tribe, and also showed that Laeliinae should should include *Arpophyllum* and *Meiracyllium*. It also showed that *Helleriella*, *Isochilus*, *Ponera* and *Nemaconia* should be a separate subtribe (nomenclatural changes published by Soto-Arenas *et al.*, 2007) and confirmed that *Dilomilis* and *Neocogniauxia* are part of Pleurothallidinae as shown by Pridgeon *et al.* (2001).

The only broad phylogenetic analysis within Laeliinae was performed using internal transcribed spacer (ITS) data for 295 taxa (van den Berg *et al.*, 2000). They found little resolution and support along the spine of the tree, but relationships were clear enough to show that some groups were polyphyletic, which led to the transfer of many species from *Laelia* to *Sophronitis* (van den Berg and Chase, 2000, 2001, 2005). The other study available emphasized *Encyclia* and relatives (Higgins *et al.*, 2003) and showed that there are distinctions between *Encyclia* and many genera previously included there, such as *Artorima*, *Dinema*, *Prosthechea* and *Psychilis*, as well and provided support for re-establishing *Microepidendrum* and describing *Oestlundia*. A detailed chronology of the taxonomic history and changing generic circumscriptions within Laeliinae can be found in van den Berg and Chase (2004).

In this study, a broad analysis of Laeliinae and putative outgroups is performed, based on six DNA regions: plastid *trnL* intron and *trnL-F* spacer (Taberlet *et al.*, 1991), *matK* gene, *trnK* introns up and downstream from *matK* (Johnson and Soltis, 1994, 1995) and the ITS data of van den Berg *et al.* (2000). In this paper, the main goals were to clarify the internal topology within Laeliinae, to assess how reliable previous ITS topologies are and to increase resolution in order to establish generic alliances for further investigation better.

MATERIALS AND METHODS

Plant material and voucher information for this analysis are given in the Appendix, for which a dataset of DNA sequences was assembled from 125 terminals. Distant outgroups *Earina valida* and *Polystachya galeata* were chosen based on the analysis of Epidendroideae (van den Berg *et al.*, 2005) and Cameron *et al.* (1999). Representatives of all other main clades of Epidendreae as defined by van den Berg *et al.* (2000, 2005) were included in the ingroup. Within Laeliinae, the aim to include all genera that have been listed in recent systems (Brieger, 1976; Dressler, 1981, 1993; Szlachetko, 1995), most infrageneric subgroups from the taxonomic literature and those that emerged from the ITS analysis of van den Berg *et al.* (2000). It was not possible to obtain material of *Pygmaeorchis* and *Pinelianthe sensu stricto* (this genus is now included in *Homalopetalum* by Soto-Arenas *et al.* 2007), and did not include *Basiphyllaea* due to technical difficulties in sequencing all five regions. However, Goldman *et al.* (2001) and van den Berg *et al.* (2005) clearly showed that *Basyphyllaea* is related to Bletiinae rather than Laeliinae.

DNA was extracted from fresh leaves, fresh flowers and silica gel-dried leaves and flowers (Chase and Hills, 1991), using in most cases the 2× CTAB protocol of Doyle and Doyle (1987). For samples that presented difficulties due to the presence of polysaccharides, DNA was extracted using the Nucleon Phytopure Kit (Amersham Plc., Little Chalfont, Bucks, UK). Total DNAs were purified either by caesium chloride/ethidium bromide gradient or on silica columns (QIAGEN, Ltd) and sometimes by a combination of both methods. The ITS was amplified following van den Berg *et al.* (2000). For the *trnL-F* region, the four universal primers (c, d, e and f) of Taberlet *et al.* (1991) were used and a programme 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 primers d and e, which are reverse complements. The *trnK/matK* region was amplified by using the primers –19F (Molvray *et al.*, 2000) and trnK-2R (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 2·5 min 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, an extra wash with 35 % guanidinium chloride solution was added to help to remove primer dimers. PCR products were sequenced in both directions using the Big Dye system and an ABI 377 automated sequencer following the manufacturer protocols (PE Applied Biosystems Inc., Warrington, Cheshire, UK). The following additional primers were employed for sequencing the *matK* gene: *matK*163F (Molvray *et al.*, 2000), *matK*458F (Molvray *et al.*, 2000), *matK*556R (Molvray *et al.*, 2000), *matK*731F (Pridgeon *et al.*, 2001), *matK*881R (Pridgeon *et al.*, 2001), *matK*877F (Molvray *et al.*, 2000), *matK*1155F (5′ TTC ACT TTT GGT YTC ACC CT 3′) and *matK*1592R (Goldman *et al.*, 2001). Electropherograms were assembled and edited using Sequencher 3·0 and 3·1 (Genecodes Inc., Ann Arbor, MI, USA). All sequences were aligned by eye using a coloured font in PAUP 4.0 (Swofford, 1998). 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. Gaps in the *trnK* intron were not coded,

because this part of the intron was not sequenced by some collaborators, thus precluding sensible gap coding.

Maximum parsimony (MP) analyses were performed using PAUP 4.0, with Fitch parsimony (equal weights, unordered; Fitch, 1971). Four separate searches were performed: the first with plastid data only, the second with ITS data only, the third with all data combined and the fourth with the combined data but deleting four ITS sequences suspected of being paralogues. These were all *Cattleya* species (*C. lawrenceana*, *C. lueddemanniana*, *C. maxima* and *C. wallisii*). Each search consisted of 1000 random taxa-addition replicates, with the tree-bisection-reconnection (TBR) swapping limited to 15 trees per replicate to eliminate extensive swapping on a single replicate. The resulting trees were then used as starting trees for TBR swapping with an upper limit of 50 000 trees. Internal support for groups was evaluated using 1000 replicates of character bootstrapping (Felsenstein, 1985), with simple taxon addition and TBR, saving 15 trees per replicate. The separate analyses were compared to assess phylogenetic incongruence, by comparing disagreement in moderate to well-supported clades among analyses. For bootstrap support, bootstrap percentages (BP) of 50–70 were considered as weak, 71–85 as moderate and >85 as strong (Kress *et al.*, 2002).

As an alternative phylogenetic reconstruction method, a Bayesian analysis of the combined dataset under mixed models was performed by using MRBAYES 3·1·2 (Ronquist and Huelsenbeck, 2003). Models for individual regions were selected by using hierachical likelihood ratio tests in MRMODELTEST 2 (Nylander, 2004) for four data partitions: ITS, *trnL-F*, *matK* gene and the two *trnK* introns. A fifth data partition was composed of indel characters, which were treated under the restriction site (binary) model assuming that characters that are constant in all taxa cannot be observed, as recommended by Ronquist *et al.* (2005). The values for all parameters in the data partitions were unlinked in MRBAYES, to allow more independent evolutionary models among DNA regions. For analysis, two simultaneous runs of four chains (one 'cold' and three 'heated') each were carried out with the MCMC algorithm, for 3000 000 generations, sampling one tree for each 100, until the average standard deviation of split ranges was smaller than 0·01, as recommended in the manual. Likelihoods of the trees produced were analysed graphically and, after discarding the initial 450 trees of each chain as burn-in, a majority-rule consensus was generated for the remaining trees in PAUP 4.0 to assess topology and clade posteriors.

RESULTS

General features of the datasets

General characteristics of the DNA datasets in relation to the combined trees are given in Table 1. A region of 480 bp in the *trnL* intron was of ambiguous alignment and was therefore excluded from the analyses. The non-coding portions of the *trnK* intron (upstream and downstream of the *matK* gene) were considered together. The most variable dataset was ITS (as a whole), followed by the *trnK* introns. The *trnL-F* (intron + exon + spacer) and *matK* gene had similar levels of variation. In terms of informativeness as measured by the retention index (RI) of each dataset, the *matK* gene and the *trnL-F* (intron + exon + spacer) performed similarly and slightly better than ITS. The indel matrix was composed of 26 indels from *trnL-F*, 23 from ITS and only three from *matK*. This matrix was used only in the combined parsimony and Bayesian analyses.

Individual analyses

Many possible trees were found for the plastid and ITS analyses (limited to 50 000 in the search). Because the trees do not show any major differences, and are largely unresolved along the spine of the tree, they are not shown here – only the overall patterns and discrepancies found are mentioned. In the plastid analysis, relationships are well resolved only in the outgroup and some of the terminal clades within the subtribe. Branch

TABLE 1. *Features of DNA datasets used in this study*

DNA region	Aligned length	No. variable sites	No. potentially parsimony informative	No. of changes/ variable site	Fitch tree length	CI	RI	ts : tv
ITS region	789	461 (58·43 %)	339 (42·97 %)	5·05	2326	0·35	0·52	2·20
ITS1	306	227 (74·18 %)	169 (55·23 %)	5·23	1188	0·35	0·51	2·15
5·8S	158	23 (14·56 %)	10 (6·33 %)	1·87	43	0·65	0·58	2·31
ITS2	325	211 (64·92 %)	160 (49·23 %)	5·19	1095	0·34	0·53	2·26
trnL-F region	1350	495 (36·66 %)	223 (16·5 %)	1·97	974	0·63	0·64	0·95
trnL-F intron	723	251 (34·72 %)	104 (14·38 %)	1·97	495	0·62	0·65	1·09
trnL-F exon	50	9 (18 %)	2 (4 %)	2·33	21	0·52	0·29	0·17
trnL-F interg. spacer	596	250 (41·95 %)	124 (20·8 %)	2·01	502	0·64	0·61	0·74
trnK introns	600	297 (49·5 %)	118 (19·67 %)	1·92	571	0·68	0·56	0·85
matK gene	1347	551 (40·91 %)	259 (19·23 %)	2·12	1167	0·58	0·64	1·03
matK (1st positions)					331 (28·36 %)	0·67	0·63	
matK (2nd positions)					357 (30·59 %)	0·59	0·69	
matK (3rd positions)					479 (41·04 %)	0·52	0·59	
All plastid data (except excluded bases)					2739	0·63	0·64	
All data (except excluded bases)					5154	0·49	0·58	

CI, consistency index; RI, retention index; ts:tv, transition/transversion ratio.

lengths along the spine of the tree are short. Many nodes collapse in the strict consensus, and BP are generally low in the spine of the tree but increase towards the terminal nodes. Within Laeliinae there are few groups having 50 BP or more. The relationships between the main clades within the subtribe do not appear consistently in all trees, and the strict consensus is largely unresolved. In the ITS analysis, several subtribes of Epidendreae are monophyletic, but their relationships are not clear, with most branches collapsing in the strict consensus. The separation of Ponerinae from Laeliinae (94 BP) and *Dilomilis* and *Neocogniauxia* from Pleurothallidinae (93 BP) supports the results of van den Berg *et al.* (2000, 2005). The only differences noteworthy between the ITS and plastid analyses are the position of some species within the *Cattleya* alliance. In the plastid trees, *Cattleya maxima* and *C. araguaiensis* form a clade that is sister to the whole of the alliance. In the ITS tree, *C. maxima* is sister to *Cattleya* section *Cattleyodes* and *C. araguaiensis* is sister to *Guarianthe*. Also in plastid trees, *C. lueddemanniana*, *C. percivaliana* and *C. wallisii* are collectively sister to the other species of unifoliate *Cattleya*, but in the ITS analysis they are sister to *Cattleya* section *Hadrolaelia* and *C.* section *Cattleyodes*. For this reason two combined analyses were run for MP: the first with a complete dataset and the second excluding the ITS sequences of *C. lueddemanniana*, *C. maxima*, *C. percivaliana* and *C. wallisii*.

Combined parsimony analyses

The analysis with all sequences recovered 360 trees (Figs 1 and 2) with tree length of 5154, consistency index (CI) = 0·49 and RI = 0·58. The strict consensus is much more resolved than the individual plastid or ITS analyses.

The outgroup relationships were nearly the same as in the plastid trees, with *Arpophyllum* sister to the rest of Laeliinae (100 BP). The immediate sister group of Laeliinae was Pleurothallidinae (69 BP), followed successively by Ponerinae (73 BP), Bletiinae (68 BP), Chysiinae (<50 BP) and finally Coeliinae (92 BP). Even though these relationships did not have high support, the monophyly of each subtribe did have high support: Bletiinae (100 BP), Ponerinae (100 BP), Pleurothallidinae including *Dilomilis* and *Neocogniauxia* (96 BP) and Laeliinae (including *Arpophyllum* and *Meiracyllium*; 100 BP).

Within Laeliinae most nodes of the spine are resolved in the strict consensus tree. The only branch with some (weak) internal support is the one leading to the *Cattleya* alliance (59 BP; Fig. 2). *Hagsatera* is placed between *Arpophyllum* and the rest of Laeliinae with <50 BP. The main groups with internal support above 50 BP in the combined trees were: *Dinema*/*Nidema* (99 BP), the *Scaphyglottis* alliance (85 BP), *Domingoa* with *Homalopetalum* (74 BP), *Laelia sensu stricto* and *Schomburgkia* (96 BP), the *Epidendrum* alliance (63 BP), *Encyclia* (100 BP), *Prosthechea* (91 BP), the *Broughtonia* alliance (100 BP), *Brassavola* (96 BP), a subclade of *Cattleya* including the type (*C. labiata*, 59 BP) and a group including three unifoliate *Cattleya* species (*C. lawrenceana*, *C. lueddemanniana* and *C. wallisii*) and the species formerly attributed to *Laelia* and *Sophronitis* (52 BP). It should be noted, however, that many of the groups follow previous taxonomic categories based on morphology, both at the generic and infrageneric levels. These previously recognized suites of characters increase our confidence in the tree, despite the low BPs.

The second analysis, excluding the ITS sequences of four *Cattleya* species, was identical to the complete dataset in the topology of all outgroup and major clades within the subtribe, except for relationships within the *Cattleya* alliance. Therefore, only this portion of the tree is shown (in Fig. 3). With the exclusion of the ITS sequences of *C. lawrenceana*, *C. lueddemaniana* and *C. wallisii* (our 'unifoliate *Cattleya* II', Fig. 3), this group is no longer sister to the species previously attributed to *Sophronitis* (*Cattleya* sections *Cattleyodes* and *Hadrolaelia* in Fig. 3). However, it is not sister to our 'unifoliate *Cattleya* I' as would be expected (these species were once considered subspecies of *C. labiata*). Rather it is sister to the remaining members of *Cattleya* (with less than 50 BP). When the ITS sequence of *C. maxima* (which we expected to cluster with other unifoliate species of *Cattleya*) is excluded, it moves to be sister to *C. araguaiensis*, and these two are sister to *Guarianthe* (<50 BP).

Bayesian analysis

The models selected by successive hierachical likelihood ratio tests were GTR + G for all three plastid non-coding data partitions (*trnL-F*, *trnK* introns up and downstream from *matK*), and GTR + I for ITS and the coding region of *matK*. The combined tree obtained from a majority-rule consensus of 59 100 trees produced by the two runs of MCMC in the mixed model context is presented in Fig. 4. Most relationships were similar to the combined MP tree with all sequences (Figs. 1 and 2). Because posterior probabilities (PP) from Bayesian analysis (BA) can be considered inflated in relation to the conservative values obtained for parsimony BP (Erixon *et al.*, 2003) those below 95 PP are considered as weakly supported. Even under this criterion, a much greater number of nodes in the BA tree attained high support compared with MP (often 100 PP). The outgroup relationships were essentially the same, with most relationships being strongly supported, except for Pleurothallidinae as sister to Laeliinae (86 PP). The position of *Arpophyllum* was identical (100 PP), as was that of *Hagsatera* (88 PP). There is a large polytomy within Laeliinae, which is equivalent to the many clades with <50 BP in MP. Some novel well-supported relationships that did not appear in the parsimony tree were: (*a*) the *Scaphyglottis* alliance including *Dinema* and *Nidema* (98 PP); (*b*) the *Epidendrum* and *Laelia* alliances as sister (96 PP); (*c*) the *Broughtonia*, *Cattleya*, *Epidendrum* and *Laelia* alliances forming a clade (95 PP). Relationships of the *Cattleya* alliance did not differ from the parsimony results, but many with <50 BP in the MP trees were well supported in BA, such as *Cattleya s.l.* (98 PP) and *Guarianthe*/*Rhyncholaelia* (96 PP). However, relationships among the *Brassavola*, *Cattleya* and *Guarianthe*/*Rhyncholaelia* clades remained unresolved.

DISCUSSION

Molecular evolution

The variation of the ITS dataset was similar to that in van den Berg *et al.* (2000). However, the performance (in terms of RI)

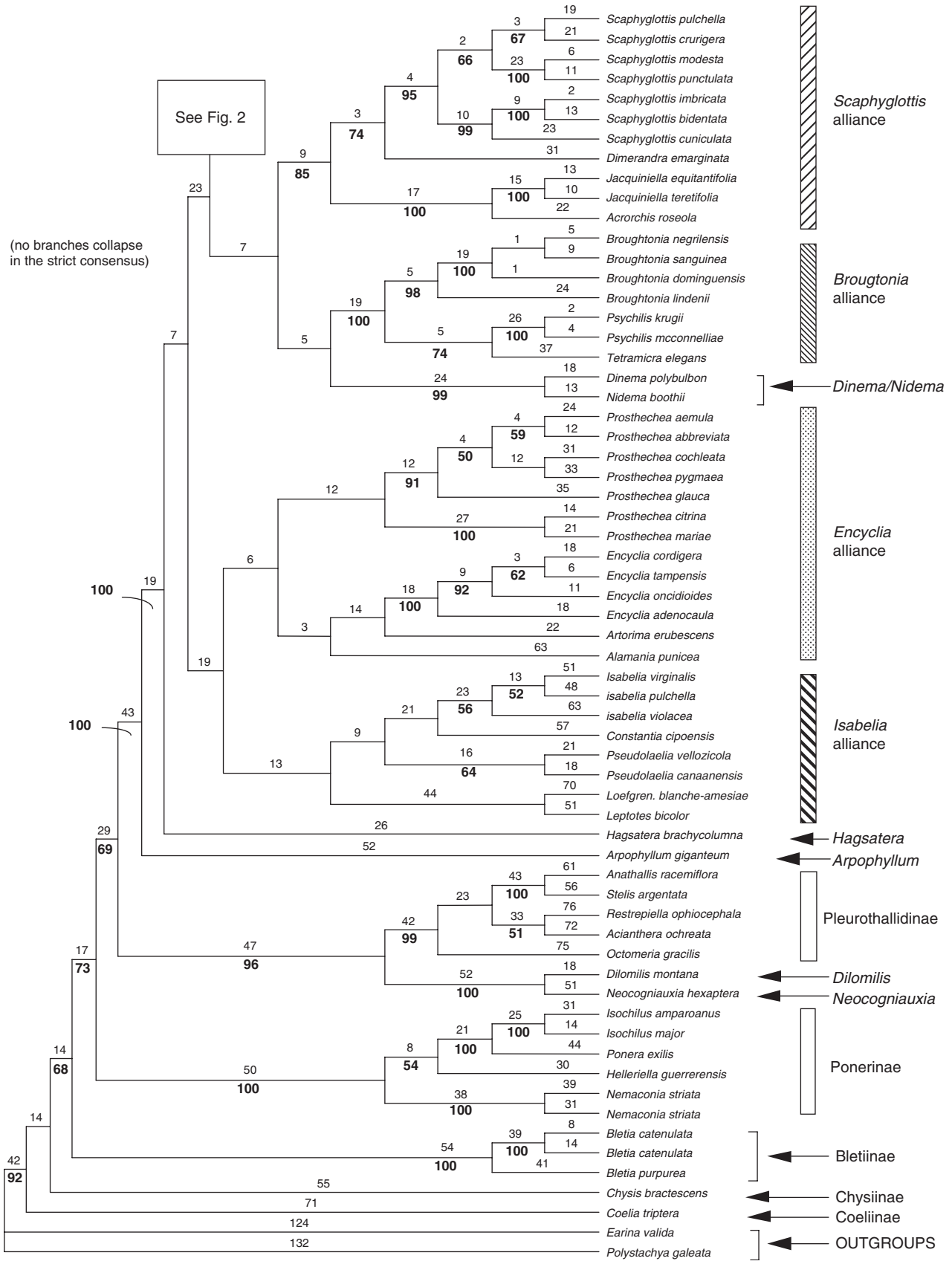

FIG. 1. First part of one of the 360 most-parsimonious trees for the combined analysis of six DNA regions in Laeliinae. L = 5154, CI = 0·49, RI = 0·58. Numbers above the branches are Fitch lengths and numbers below the branches are bootstrap percentages (branches without numbers received <50 % bootstrap support).

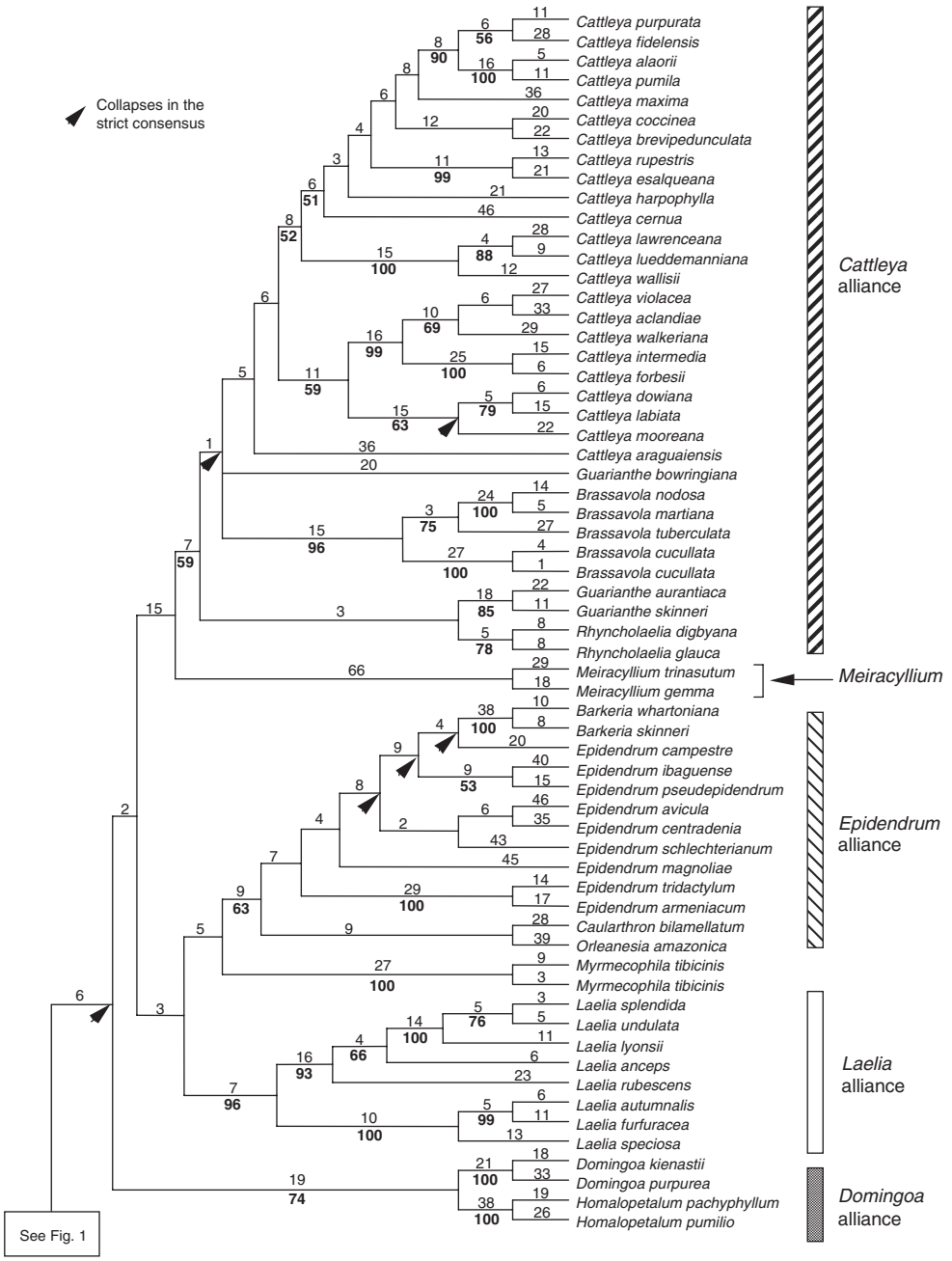

FIG. 2. Second part of the tree in Fig. 1. Numbers above the branches are Fitch lengths and numbers below the branches are bootstrap percentages (branches without numbers received <50 % bootstrap support).

of this region was worse than the plastid datasets. This could be explained by the fact that the ITS dataset has a higher number of changes per variable position than the plastid loci, and is therefore more likely to be affected by taxon-sampling (incomplete taxon sampling could preclude the reconstruction of multiple changes at a given position). In general, the levels of variation found in different regions in the present study were lower than those found by van den Berg *et al.* (2005), whereas CI and RI were higher, as would be expected when dealing with more closely related taxa. This effect is less obvious for *matK*, reinforcing the fact that this gene is often useful at all taxonomic levels.

Outgroup relationships

Arpophyllum is always sister to a strongly supported clade of the remaining members of Laeliinae (ITS and plastids, 98 BP; combined MP and BA, 98 BP and 98 PP). The sister group of Laeliinae is probably Pleurothallidinae (including *Dilomilis* and *Neocogniauxia*), which had already been seen in the previous ITS analysis (van den Berg *et al.*, 2000; <50 BP). This sister relationship is not supported in the plastid consensus tree but has weak (69 BP, 86 PP) support in the MP and BA combined. The relationship between Bletiinae and Ponerinae also remains ambiguous. In the plastid trees there is a polytomy

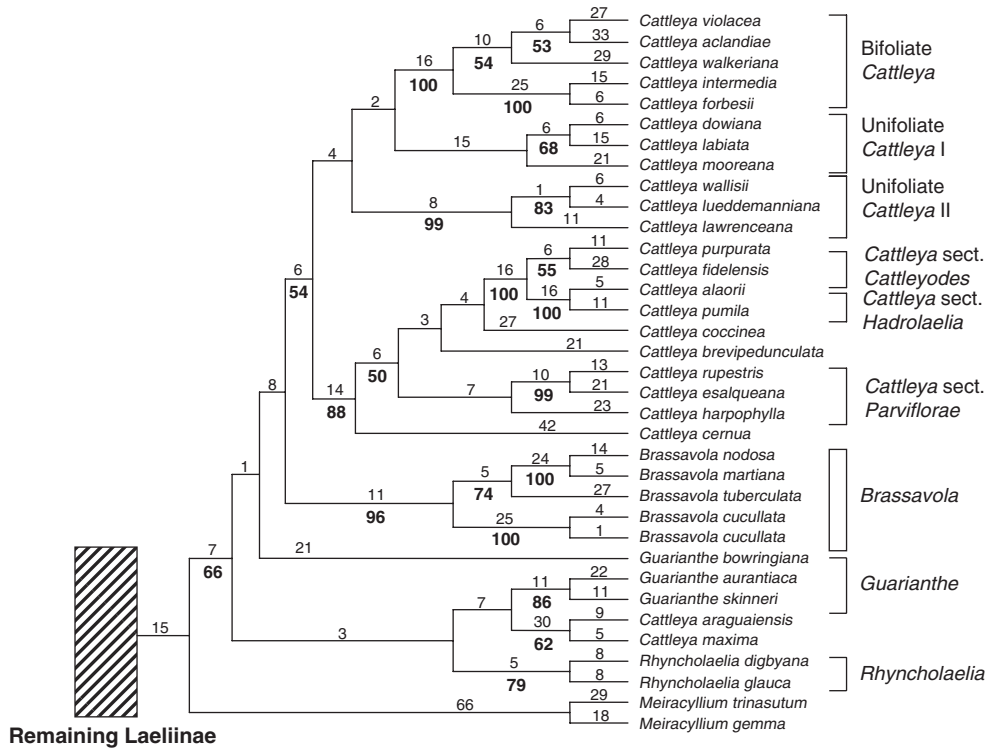

FIG. 3. *Cattleya* alliance portion of the combined analysis of six DNA regions in Laeliinae, excluding ITS sequences of four *Cattleya* species with incongruent placement in individual analyses. Numbers above the branches are Fitch lengths and numbers below the branches are bootstrap percentages (branches without values received <50 % bootstrap support).

among Pleurothallidinae, Ponerinae and Bletiinae, and in the combined analysis they are successive sister groups as in the ITS tree of van den Berg *et al.* (2000). However, in an analysis of all Epidendreae based on the same DNA regions as in this paper (van den Berg *et al.*, 2005), Bletiinae and Ponerinae were sister to each other with 90 BP and strongly supported in the BA tree. This pattern is different here due to the less extensive outgroup sampling in the present study. The position of *Meiracyllium*, deeply embedded in Laeliinae, appears in all analyses (and also in Cameron *et al.*, 1999; van den Berg *et al.*, 2000, 2005; Goldman *et al.*, 2001). Finally, the position of *Chysis* and *Coelia* is the same in all analyses, which agrees with ITS alone (van den Berg *et al.*, 2000). One important point to mention is the embedded placement of *Meiracyllium* in Laeliinae. Plastid analysis places it as sister to the *Cattleya* alliance (<50 BP), whereas in the ITS analysis of van den Berg *et al.* (2000) it was sister to some species of *Prosthechea* (*P. mariae* and *P.* citrina; 61 BP). The plastid placement reappeared in the combined analysis (but with <50 BP). The long branch-length leading to this genus, although correlated with the striking morphological peculiarities, could indicate long-branch attraction. This relationship was also found with BA (Fig. 4), but this method is also not immune against long-branch attraction artefacts.

Generic alliances within Laeliinae

When considering the topologies obtained within Laeliinae in this study one first important detail is that, although there is no conflict between our ITS trees and the larger analysis (295 taxa) used by van den Berg *et al.* (2000), the latter had many fewer branches collapsing in the strict consensus and stronger bootstrap support for relationships. This is probably the effect of taxon sampling since the alignment of both matrices was the same (the ITS dataset used here was produced by deleting taxa from the larger one without alteration of gaps). In this study, weak incongruence was found between the topologies resulting from the plastid and ITS analyses, but none of these relationships has BP >50, suggesting that most of the incongruence could be due to character sampling error. Results of the combined analyses are in agreement with the ITS data alone, at least for the few areas where ITS had internal support in van den Berg *et al.* (2000). To a much more limited extent there is a similarity between the DNA trees in the present study and the alliances proposed by Dressler (1981) based on the leaf anatomical characters of Baker (1972). The main weaknesses of his alliances were the inability to detect polyphyletic genera such as *Laelia* and *Cattleya* and also that the genera of Ponerinae (*Helleriella*, *Isochilus, Nemaconia*, *Ponera*), *Dilomilis* and *Neocogniauxia* did not belong to Laeliinae. All alliances proposed by Dressler (1981) each appear to include a few unrelated genera, and a system of generic alliances based on the results of the present study would need a larger number of smaller alliances, although this may be reduced as more data are collected, and the relationships among larger clades within Laeliinae are resolved. The generic alliances presented in this discussion are based on highly supported clades in BA

FIG. 4. Bayesian tree of 59 100 trees obtained from two runs 3000 000 chains of MCMC. Numbers above branches represent the posterior probabilities (PP). Nodes with PPs below 50 % have been collapsed.

(Fig. 4), which do not contradict any well-supported clades in the combined parsimony analysis.

Isabelia *alliance.* This is a small group (five genera and approx. 28 species) mainly from south-eastern Brazil, a few species extending north to Bahia State (Brazil) and south-west to Paraguay and northern Argentina. Many species grow exclusively as epiphytes on *Vellozia* (Velloziaceae) or lithophytes. They are generally small- (<5 cm) to medium-flowered (5–10 cm) for the subtribe, and have a short column in relation

to the lip and a stigma whch is much wider than long, adnation between the base of the column and the lip, and lateral lobes reduced to spreading auricles. These flower characters resemble *Hagsatera* (unresolved within the subtribe) and *Dilomilis* (sister to Pleurothallidinae) and might represent an ancestral suite of characters in this group.

Domingoa *alliance.* This is a clade with approx. 12 species and only *Domingoa* and *Homalopetalum*. These are small plants (<10 cm), mostly Caribbean, which often have a well-developed column-foot. Based on van den Berg *et al.* (2000) and preliminary plastid data, Soto-Arenas *et al.* (2007) synonymized *Nageliella* with *Domingoa*. In the same work, *Pinelianthe* was lumped in *Homalopetalum*, but no material of *Pinelianthe* has been obtained for DNA sequencing so far, and therefore their decision was based solely on similarities in flower structure and small habit, pending confirmation by molecular data.

Encyclia *alliance.* This alliance comprises some 250 species in four genera, and has been the most difficult group to circumscribe in the current study, with weak support in both the combined MP and BA. The main genera here are *Encyclia* and *Prosthechea* (the latter a segregate of the former; Higgins, 1997), which are only weakly supported as distinct; this pattern, however, was confirmed by the leaf anatomical data of Pires *et al.* (2003) despite the incomplete taxon sampling of the latter. Most genera and species have ovoid to clavate heteroblastic pseudobulbs and partial fusion between the column and lip, with only rare exceptions. Relationships between them were not included in this paper, but have been extensively discussed by Higgins *et al.* (2003).

Scaphyglottis *alliance.* This group includes four genera and approx. 50 species, most of which have a conspicuous column-foot (not as well developed in *Jacquiniella*). Despite the vegetative resemblance to *Epidendrum*, *Dimerandra* fits here, which is supported by floral morphology. Although unresolved in the present ITS trees, *Dimerandra* was related to *Dinema* and *Nidema* in the *Encyclia* clade in van den Berg *et al.* (2000). The internal topology of the *Scaphyglottis* alliance was studied in detail by Dressler *et al.* (2004), who synonymized *Hexadesmia*, *Hexisea*, *Reichenbachanthus* and *Platyglottis* with *Scaphyglottis*. The highly supported association of *Dinema* and *Nidema* with this clade in the present study is useful for understanding this group and is likely to indicate a Caribbean origin for the whole alliance. The *Scaphyglottis* alliance with *Jacquiniella* was present in the ITS results of van den Berg *et al.* (2000) with BP <50. The plastid and combined datasets show it as a moderately to well-supported clade (81 BP and 85, 98 PP, respectively)

Broughtonia *alliance.* This is a small group (approx. 30 species) of exclusively Caribbean genera including *Broughtonia*, *Psychilis* and *Tetramicra*. Despite the fact that species of *Psychilis* were considered members of *Encyclia* for a long time, there are many floral similarities between the latter and *Tetramicra*. Although *Quisqueya* was not sampled in the current study, it was shown to be closely related to *Tetramicra* by the combined molecular and plastid analyses of Higgins et al. (2003).

Laelia *and* Epidendrum *alliances.* The close relationship of these two alliances is an important result of this study. The *Epidendrum* alliance is the largest in number of species (*Epidendrum* alone has >1500 species) and was weakly supported in van den Berg *et al.* (2000) and the *Laelia* alliance (approx. 20 species) was mixed with the *Domingoa* alliance (<50 BP for both relationships). The *Laelia* alliance does seems to be characterized by plants with heteroblastic pseudobulbs, eight pollinia and large gullet-flowers which are not very different from those on the *Cattleya* alliance, which explains their placement previous to the first DNA analyses. The strongly supported paraphyly of *Laelia* in relation to *Schomburgkia* led to the synonymization of these genera by van den Berg and Chase (2005) and Soto-Arenas (2005). The *Epidendrum* alliance is composed largely of *Epidendrum* species, and can, in general terms, be characterized by a lip united with the column, four pollinia and a reed-stem habit. The free lip of *Caularthron* appears to be a plesiomophic character state in this clade, but within *Epidendrum* there are many reversals in character states, resulting in free lips, different pollinia numbers and *Cattleya*/*Encyclia*-like habit. A detailed analysis of *Epidendrum* was presented by Hágsater and Soto-Arenas (2005), which led to the inclusion of *Amblostoma*, *Lanium*, *Nanodes* and *Oerstedella* and 33 other genera in *Epidendrum*. The placement of *Myrmecophila* in this alliance is also noteworthy. ITS data placed this genus sister to the rest of the *Cattleya* alliance in van den Berg *et al.* (2000) and sister to *Guarianthe aurantiaca* in the ITS analysis of this study. However, plastid data place it in an unresolved clade above *Meiracyllium*. The MP combined analysis here places this genus in the *Epidendrum* clade (BP <50), but in BA it received 95 PP. This new placement is closer to *Caularthron*, and both genera are alone in Laeliinae in having hollow pseudobulbs that hold ant nests. It is also reasonably close to *Laelia* (including *Schomburgkia*, in which *Myrmecophila* was included). This could imply an ancestor with long stems and similar flower morphology.

Cattleya *alliance.* This alliance includes approx. 130 species in four genera. Relationships within this alliance remain confused due to several problems. Four *Cattleya* species together occupied an unexpected position (based on previous classifications and similar morphology to *C. labiata*) in the ITS analysis of this study and in van den Berg *et al.* (2000), but fell in a more intuitive position in the plastid trees. However, in the combined MP analysis, three of them were still grouped together, and *C. maxima* was sister to *C. araguaiensis*. These patterns could suggest reticulation events involving some members of this group. Due to the overall level of variation, the ITS data seem to override plastid patterns in the combined analyses. In fact, the plastid analysis produced a topology that is more in agreement with our understanding of the group from a morphological viewpoint: in the plastid analysis, the *Cattleya* species that were formerly *Sophronitis sensu stricto* and *Brassavola* form monophyletic groups, and *Cattleya harpophylla* clusters with two species of section *Parviflorae*, in agreement with the system of Withner (1990). The plastid analysis has fewer groups with internal support due to the lower levels of variation. The combined analysis followed

more closely the ITS data. The four troublesome *Cattleya* species (*C. maxima*, *C. lawrenceana*, *C. lueddemanniana* and *C. wallisii*) still occupied the same positions as in the ITS dataset, but the topology for *C. cernua*, *C. coccinea* and *C. brevipedunculata* is remarkably different. The dominance of the ITS dataset is still clear even after the four troublesome *Cattleya* sequences are removed (Fig. 3). Although the position of the four species changed, the rest of the tree remained nearly the same as in the ITS trees. Because of the discrepancies between the ITS and plastid trees, the adequacy of ITS for resolving the overall phylogeny of the *Cattleya* alliance is questionable, and probably the best strategy would be to collect more plastid data or look for an appropriate single-copy nuclear gene to strengthen support for the plastid topologies. On the other hand, contrasting alternative topologies of the plastid and ITS results emphasize the need for detailed studies of hybridization in this group. Several natural interspecific and intergeneric natural hybrids have been reported (Adams and Anderson, 1958), and for this reason hybridization could have played a significant role in the evolution of the *Cattleya* alliance before their early diversification. Adding plastid data to the original ITS dataset was a great improvement in the bootstrap support within *Cattleya*. Also, *Brassavola* was monophyletic as in the plastid analysis with good internal support. The paraphyly of *Brassavola* in relation to *Cattleya* in the ITS trees of van den Berg *et al.* (2000) might have been due to character sampling effects. An increase in characters solved this problem, as in Sheahan and Chase (2000).

Inferences about the evolution of Laeliinae

Hybridization in relation to phylogeny. The ability to produce artificial interspecific and intergeneric crosses of Laeliinae and outgroups and within the subtribe is often a reflection of the phylogenetic relationships. Although there are thousands of hybrids in *Cattleya* (including species formerly placed in *Sophronitis* and *Laelia*; International Orchid Register at http://www.rhs.org.uk/plants/registration_orchids.asp), and across the subtribe with genera from different alliances (e.g. *Sophronitis* × *Constantia* and *Scaphyglottis* × *Epidendrum*), there are no hybrids between *Arpophyllum* and other Laeliinae. Genera previously considered to be members of Laeliinae and found in Pridgeon *et al.* (2001) and van den Berg *et al.* (2005) and this study to be part of other subtribes (*Dilomilis*, *Helleriella*, *Isochilus*, *Neocogniauxia* and *Ponera*) have not produced 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 have probably been made at least with *Arpophyllum* and *Isochilus*, which are common in cultivation, and *Neocogniauxia*, which is showy. Even within generic alliances, the degree of fertility seems to be reduced (e.g. many hybrids of *Cattleya* with *Brassavola* and *Rhyncholaelia* have low seed viability). At the same time F_1 hybrids between *Cattleya* and *Epidendrum* are generally sterile, despite the fact most species have the same chromosome number ($2n = 40$). In light of the newly clarified phylogenetic relationships, there is a framework in which new artificial crosses for evaluating hybridization potential should be attempted and recorded systematically.

Pollination systems. Bee pollination is the plesiomorphic state in Laeliinae, as stated by Borba and Braga (2003). It occurs in all alliances, despite the small number and lack of detail of early studies (e.g. Dodson and Frymire, 1961; Dodson, 1965). The most common bees are *Bombus* spp. reported in *Cattleya* and *Pseudolaelia* (Borba and Braga, 2003; Smidt *et al.*, 2006) and *Xylocopa* spp. in *Constantia* (Matias *et al.*, 1996). A critical taxon for which pollination data are still needed is *Arpophyllum*, although flower colour, fragrance and appearance would suggest small bees. In smaller subclades within the alliances, such as the rupicoulous species of *Cattleya* section *Parviflorae*, shifts to smaller bees associated with polyploidy may be the driving force of radiation (Blumenschein, 1961; Verola, 2008). All other types of specialized pollination occur in well-defined subclades within alliances, such as bird pollination in some *Cattleya* and *Alamania* and butterfly and moth polination in *Epidendrum*, *Rhyncholaelia* and *Brassavola* (van der Pijl and Dodson, 1969), which might be key innovations that led to speciation. This is the most likely explanation for the huge number (>1000) of species in *Epidendrum*.

The main factors of diversification within Laeliinae remain a rich area for research involving pollination mechanisms, habitat preferences and biogeographical patterns, due to the great variation of morphological features and the large number of species and generic and infrageneric groupings. The results of this study improve our understanding of the overall phylogeny within the subtribe and should help in delimiting smaller sets of taxa for more detailed studies which can isolate the different mechanisms responsible for the the richness of species in the subtribe.

ACKNOWLEDGEMENTS

We would like to thank A. Ando, F. Barros, S. Bell, E. L. Borba, D. Hayes, N. B. Machado, W. Foster and S. Beckendorf for plant material and A. de Bruijn, R. Cowan, J. Joseph, C. A. Oliveira and M. Powell for help in the laboratory. This work was supported by the American Orchid Society (USA), Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazil) and the Royal Botanic Gardens, Kew (UK).

LITERATURE CITED

Adams H, Anderson E. 1958. A conspectus of hybridization in the Orchidaceae. *Evolution* **12**: 512–518.

Azevedo CO, Borba EL, van den Berg C. 2006. Evidence of natural hybridization and introgression in *Bulbophyllum involutum* Borba, Semir & Barros and *B. weddellii* (Lindl.) Rchb.f. (Orchidaceae) im the Chapada Diamantina, Brazil, by using allozyme markers. *Revista Brasileira de Botânica* **29**: 415–421.

Baker RK. 1972. *Foliar anatomy of the Laeliinae (Orchidaceae)*. PhD Thesis, Washington University, St Louis.

Bentham G. 1881. Notes on Orchideae. *Journal of the Linnaean Society* **18**: 281–360.

van den Berg C, Chase MW. 2000. Nomenclatural notes on Laeliinae-I. *Lindleyana* **15**: 115–119.

van den Berg C, Chase MW. 2001. Nomenclatural notes on Laeliinae. II. Additional combinations and notes. *Lindleyana* **16**: 109–112.

van den Berg C, Chase MW. 2004. A chronological view of Laeliinae taxonomical history. *Orchid Digest* **68**: 226–254.

van den Berg C, Chase MW. 2005. Nomenclatural notes on Laeliinae. IV. New combinations in *Laelia* and *Sophronitis*. *Kew Bulletin* **59**: 565–567.

van den Berg C, Higgins WE, Dressler RL, *et al*. 2000. A phylogenetic analysis of Laeliinae (Orchidaceae) based on sequence data from internal transcribed spacers (ITS) of nuclear ribosomal DNA. *Lindleyana* **15**: 96–114.

van den Berg C, Goldman DH, Freudenstein JV, Pridgeon AM, Cameron KM, Chase MW. 2005. An overview of the phylogenetic relationships within Epidendroideae (Orchidaceae) inferred from multiple DNA regions and recircumscription of Epidendreae and Arethuseae (Orchidaceae). *American Journal of Botany* **92**: 613–624.

Blumenschein A. 1961. Estudos citológicos nas orquídeas. In: Brieger FG. ed. *Atas do Primeiro Congresso Sul-Americano de Genética*. Piracicaba: Cadeira de Citologia e Genética Gerald a Escola Superior de Agricultura 'Luiz de Queiroz', 161–163.

Borba EL, Braga PIS. 2003. Biologia reprodutiva de *Pseudolaelia corcovadensis* (Orchidaceae): melitofilia e autoimcompatibilidade em uma Laeliinae basal. *Revista Brasileira de Botânica* **26**: 541–549.

Borba EL, Semir J. 1998. *Bulbophyllum* × *cipoense* (Orchidaceae), a new natural hybrid from the Brazilian 'campos rupestres': description and biology. *Lindleyana* **13**: 113–120.

Brieger FG. 1976. Subtribus Epidendrinae. In: Brieger FG, Maatsch R, Senghas K eds. *Schlechter's Die Orchideen*, 3rd edn. Berlin: Paul Parey, 460–635.

Cameron KM, Chase MW, Whitten WM, *et al*. 1999. A phylogenetic analysis of the Orchidaceae: evidence from *rbcL* nucleotide sequences. *American Journal of Botany* **86**: 208–224.

Chase MW, Hills HG. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* **40**: 215–220.

Dodson CH. 1965. *Agentes de polinización e su influencia sobre la evolución en la família Orchidaceae*. Iquitos: Universidad Nacional de la Amazonia Peruana, Instituto General de Investigaciones.

Dodson CH, Frymire GP. 1961. Natural pollination of orchids. *Missouri Botanical Garden Bulletin* **49**: 133–152.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin of the Botanical Society of America* **19**: 11–15.

Dressler RL. 1960. Nomenclatural notes on Orchidaceae I. *Taxon* **9**: 213–214.

Dressler RL. 1981. *The orchids: natural history and classification*. Cambridge, MA: Harvard University Press.

Dressler RL. 1990. The major clades of the Orchidaceae-Epidendroideae. *Lindleyana* **5**: 117–125.

Dressler RL. 1993. *Phylogeny and classification of the orchid family*. Portland, OR: Dioscorides Press.

Dressler RL, Whitten WM, Williams NH. 2004. Phylogenetic relationships of *Scaphyglottis* and related genera (Laeliinae: Orchidaceae) based on nrDNA ITS sequence data. *Brittonia* **56**: 58–66.

Erixon P, Svennblad B, Britton T, Oxelman B. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology* **52**: 665–673.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Fitch WM. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* **20**: 406–416.

Freudenstein JV, Senyo DM, Chase MW. 2000. Mitochondrial DNA and relationships in the Orchidaceae. In: Wilson KL, Morrison DA . eds. *Monocots: systematics and evolution*. Melbourne: CSIRO Publishing, 421–429.

Freudenstein JV, van den Berg C, Goldman DH, Kores PJ, Molvray M, Chase MW. 2004. An expanded plastid DNA phylogeny of the Orchidaceae and analysis of jackknife branch support strategy. *American Journal of Botany* **91**: 149–157.

Goldman DH, Freudenstein JV, Kores PJ, *et al*. 2001. Phylogenetics of Arethuseae (Orchidaceae) based on plastid *matK* and *rbcL* sequences. *Systematic Botany* **26**: 670–695.

Hágsater E, Soto-Arena MA. 2005. *Epidendrum* In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN eds. *Genera orchidacearum*, Vol. 4. Oxford: Oxford University Press, 236–251.

Higgins WE. 1997. A reconsideration of the genus *Prosthechea* (Orchidaceae). *Phytologia* **82**: 370–383.

Higgins WE, van den Berg C, Whitten WM. 2003. A combined molecular phylogeny of *Encyclia* (Orchidaceae) and relationships within Laeliinae. *Selbyana* **24**: 165–179.

Johnson LA, Soltis DE. 1994. *matK* DNA sequences and phylogenetic reconstruction in Saxifragaceae *s.s*. *Systematic Botany* **19**: 143–156.

Johnson LA, Soltis DE. 1995. Phylogenetic inference in Saxifragaceae *sensu stricto* and *Gilia* (Polemoniaceae) using *matK* sequences. *Annals of the Missouri Botanical Garden* **82**: 149–175.

Kress WJ, Prince LM, Williams KJ. 2002. The phylogeny and a new classification of the gingers (Zigiberaceae): evidence from molecular data. *American Journal of Botany* **89**: 1682–1696.

Matias LQ, Braga PIS, Freire AG. 1996. Biologia reprodutiva de *Constantia cipoensis* Porto & Brade, endêmica da Serra do Cipó. *Revista Brasileira de Botânica* **19**: 119–225.

Molvray M, Kores PJ, Chase MW. 2000. Polyphyly of mycoheterotrophic orchids and functional influences on floral and molecular characters. In: Wilson KL, Morrison DA. eds. *Monocots: systematics and evolution*. Melbourne: CSIRO Publishing, 441–448.

Neyland R, Urbatsch LE. 1996. Phylogeny of subfamily Epidendroideae (Orchidaceae) inferred from *ndhF* chloroplast gene sequences. *American Journal of Botany* **83**: 1195–1206.

Nylander JAA. 2004. *MRMODELTEST v2*. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.

Pabst GFJ, Dungs F. 1975. *Orchidaceae Brasilienses. V. 1*. Hildesheim: Brücke-Verlag Kurt Schmersow.

Pabst GFJ, Dungs F. 1977. *Orchidaceae Brasilienses V. 2*. Hildesheim: Brücke-Verlag Kurt Schmersow.

Pfitzer E. 1889. Orchidaceae. II.B.13. Monandrae-Laeliinae, II.B.13.a. Monandrae-Laeliinae-Ponereae. In: Engler A, Prantl K eds. *Die Natürlichen Pflanzenfamilien Ergaenzungsheft*. Leipzig: Engelmann, 52–222.

van der Pijl L, Dodson CH. 1969. *Orchid flowers: their pollination and evolution*. Coral Gables, FL: University of Miami Press.

Pires MFO, Semir J, Pinna GFAM, Felix LP. 2003. Taxonomic separation of the genera *Prosthechea* and *Encyclia* (Laeliinae: Orchidaceae) using leaf and root anatomical features. *Botanical Journal of the Linnean Society* **143**: 293–303.

Pridgeon AM, Solano R, Chase MW. 2001. Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. *American Journal of Botany* **88**: 2286–2308.

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Ronquist F, Huelsenbeck JP, van der Mark P. 2005. *MRBAYES 3.1. Manual*. Distributed with the program by the authors.

Schlechter R. 1926. Das System der Orchidaceae. *Notizblatt des Botanischen Gartens und Museums zu Berlin-Dahlem* **9**: 563–591.

Sheahan MC, Chase MW. 2000. Phylogenetic relationships within Zygophyllaceae based on DNA sequences of three plastid regions, with special emphasis on Zygophylloideae. *Systematic Botany* **25**: 371–384.

Smidt EC, Silva-Pereira V, Borba EL. 2006. Reproductive biology of two *Cattleya* (Orchidaceae) species endemic to northeastern Brazil. *Plant Species Biology* **21**: 85–91.

Soto-Arenas MA. 2005. *Laelia*. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN. eds. *Genera Orchidacearum*, Vol. 4. Oxford: Oxford University Press, 265–271.

Soto-Arenas MA, Salazar G, van den Berg C. 2007. New combinations in *Domingoa*, *Homalopetalum* (Orchidaceae: Laeliinae) and *Nemaconia* (Orchidaceae: Ponerinae). *Neodiversity* **2**: 7–9.

Swofford DL. 1998. *PAUP* – Phylogenetic analysis using parsimony (*and other methods) version 4.0*. Sunderland, MA: Sinauer Associates.

Szlachetko DL. 1995. Systema orchidalium. *Fragmenta Floristica et Geobotanica Supplementum* **3**: 1–152.

Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105–1109.

Tanaka R, Kamemoto H. 1984. Chromosomes in orchids: counting and numbers. In: Arditti J ed. *Orchid biology: reviews and perspectives*, Vol. 3. Ithaca, NY: Comstock Publishing Associates, 323–410.

Verola CF. 2008. *Estudos biossistemáticos em espécies de* Hoffmannseggella *H.G. Jones (Orchidaceae:Laeliinae) ocorrentes nos complexos rupestres de altitude*. PhD Thesis, Universidade Estadual de Campinas, Brazil.

Withner CL. 1990. *The cattleyas and their relatives*. Vol. 2. *The laelias*. Portland, OR: Timber Press.

APPENDIX

Voucher information and Genbank accession numbers for the samples used in this study.

Species name	Voucher	ITS	*trnL-F*	*matK*
Acianthera ochreata (Lindl.) Pridgeon & M.W.Chase	Harley 15636 (K spirit)	AF262858	AY008446–7	AY008458
Acrorchis roseola Dressler	W.M. Whitten 399 (FLAS)	AY008521	AY422389	AY397086
Alamania punicea La Llave & Lex.	van den Berg C184 (ESA)	AF260177	AF267005	AF263783
Anathallis racemiflora (Lindl. ex Lodd.) Pridgeon & M.W.Chase	W.E. Higgins 140 (FLAS 198267)	AY008477	AY422379	AY396076
Arpophyllum giganteum Hartw. ex Lindl.	Chase O-586 (K)	AF266742	AF266975	AF265485
Artorima erubescens (Lindl.) Dressler & G.E.Pollard	Chase O-6412 (K)	AF260178	AF267006–7	AF263756
Barkeria skinneri (Batem. ex Lindl.) Lindl. ex Paxton	van den Berg C250 (K spirit)	AF260171	AF266066–7	AF263750
Barkeria whartoniana (C.Schweinf.) Soto Arenas	van den Berg C249 (K spirit)	AF260170	AF266999	AF263754
Bletia catenulata Ruiz & Pav.	E.L. Borba 590 (UEC)	AY008462	AF008449–50	AY121720
Bletia catenulata Ruiz & Pav.	W. Forster 10 (ESA)	AY008461	AF219024–5	AY121718
Bletia purpurea DC.	van den Berg C342 (K spirit)	AY008463	AY008451–2	AF518022–3
Brassavola cucullata (L.) R.Br.	W.E. Higgins 130 (FLAS 198290)	AY008589	AF263819	AY396097
Brassavola martiana Lindl.	Unvouchered (Kew 1995-2685)	AF260220	AF267060–1	AF263821
Brassavola nodosa (L.) Lindl.	Chase O-339 (K)	AF260219	AF267059	AF263820
Brassavola tuberculata Hook.	Brieger Coll. 3497 (ESA)	AF260217	AF267057	AF263818
Broughtonia dominguensis (Lindl.) Rolfe	W.E. Higgins 1039 (FLAS)	AF260187	AF267016–7	AF263791
Broughtonia lindenii (Lindl.) Dressler	W.E. Higgins 251 (FLAS 198289)	AY008570	AY422399	AY396096
Broughtonia negrilensis Fowlie	W.E. Higgins 152 (FLAS 198288)	AF008569	AY422396	AY396093
Broughtonia sanguinea (Sw.) R.Br.	Brieger Coll. 14440 (ESA)	AF260186	AF267015	AF263790
Cattleya aclandiae Lindl.	Brieger Coll. 32982 (ESA)	AF260207	AF267040	AF263810
Cattleya alaorii Brieger & Bicalho	Brieger Coll. 19179 (ESA)	AF260195	AF267026	AF263799
Cattleya araguaiensis Pabst	Unvouchered (Kew 1999-1443)	AF260215	AF267054	AF263817
Cattleya brevipedunculata (Cogn.) Van den Berg	São Paulo B.G. s.n. IBDF (SP)	AF260202	AF267034	AF263805
Cattleya cernua (Lindl.) Van den Berg	Brieger Coll. 15737 (ESA)	AF260200	AF267032	AF263803
Cattleya coccinea (Lindl.) Rchb.f.	São Paulo B.G. 9577 (SP)	AF260201	AF267033	AF263804
Cattleya dowiana Batem.	Chase O-282 (K)	AF260210	AF267045	AF263638
Cattleya esalqueana (Blumensch. ex Pabst) Van den Berg	Brieger Coll. 4980 (ESA)	AF260198	AF267029	AF263751
Cattleya fidelensis (Pabst) Van den Berg	C225-Machado s.n. (ESA)	AF260194	AF267025	AF263028
Cattleya forbesii Lindl.	Brieger Coll. 2448 (ESA), W.E. Higgins 59 (FLAS 200709)	AY008617 (Brieger)	AY422405 (Higgins)	AY396102 (Higgins)
Cattleya harpophylla (Rchb.f.) Van den Berg	Brieger Coll. 6687 (ESA)	AF260199	AF267030–1	AF263802
Cattleya intermedia Graham ex Hook.	Brieger Coll. 4095 (ESA)	AF260204	AF267036	AF263807
Cattleya labiata Lindl.	Brieger Coll. 5487 (ESA)	AF008594	AF267051	AF263759
Cattleya lawrenceana Rchb.f.	Brieger Coll. 3802 (ESA)	AF260208	AF267041–2	AF263811
Cattleya lueddemanniana Rchb.f.	Brieger Coll. 3759 (ESA)	AF266744	AF267052–3	AF263816
Cattleya maxima Lindl.	Unvouchered (Kew 1983-4362)	AY008631	AY008456	AY008460
Cattleya mooreana Withner, D.Alison & Guenard	Unvouchered (Kew 1999-1599)	AF260216	AF267055–6	AF263760
Cattleya pumila Hook.	Brieger Coll. 7794 (ESA)	AF260196	AF267027	AF263800
Cattleya purpurata (Lindl. & Paxton) Van den Berg	Chase O-997 (K)	AY008641	AF267024	AF263797
Cattleya rupestris (Lindl.) Van den Berg	van den Berg C33 (ESA)	AF260197	AF267028	AF263801
Cattleya violacea (Kunth) Rolfe	Brieger Coll. 28495 (ESA)	AF260206	AF267039	AF263709
Cattleya walkeriana Gardner	Brieger Coll. 1627 (ESA)	AF260205	AF267037–8	AF263808
Cattleya wallisii (Linden ex Rchb.f.) Rchb.f.	Brieger Coll. 28787 (ESA)	AF260213	AF267050	AF263815
Caularthron bilamellatum (Rchb.f.) R.E.Schultes	Brieger Coll. 3690 (ESA)	AF260173	AF267001	AF263780
Chysis bractescens Lindl.	Chase O-436 (K)	AF260150	AF266971	AF263640
Coelia triptera (Smith) G.Don ex Steud.	Chase O-324 (K)	AF260151	AF266972	AF263643
Constantia cipoensis Pôrto & Brade	São Paulo B.G. s.n. (SP)	AF260193	AF267023	AF263796
Dilomilis montana (Sw.) Summerh.	Chase O-206 (K)	AF260147	AF266967	AF263765
Dimerandra emarginata (G.Mey.) Hoehne	Chase O-335 (K)	AF260179	AF267008	AF263784

Continued

APPENDIX *Continued*

Species name	Voucher	ITS	*trnL-F*	*matK*
Dinema polybulbon (Sw.) Lindl.	Brieger Coll. 6052 (ESA)	AF260154	AF266976–7	AF263769
Domingoa kienastii (Rchb.f.) Dressler	W.E. Higgins 225 (FLAS 198291)	AY008564	AY422398	AY396095
Domingoa purpurea (Lindl.) Van den Berg & Soto Arenas	van den Berg C260 (K spirit)	AF266743	AF266980	AF263771
Earina valida Rchb.f.	van den Berg C296 (Leiden 950080)	AF521077	AY008448	AY121741
Encyclia adenocaula (La Llave & Lex.) Schltr.	W.E. Higgins 12 (FLAS 198274)	AY008526	AY422414	AY396111
Encyclia cordigera (Kunth) Dressler	W.E. Higgins 24 (FLAS 198276)	AY008528	AY422417	AY396114
Encyclia oncidioides (Lindl.) Schltr.	Brieger Coll. 5420 (ESA)	AF260184	AF267013	AF263788
Encyclia tampensis (Lindl.) Small	W.E. Higgins 27 (FLAS 198277)	AY008529	AY422418	AY396115
Epidendrum armeniacum (Lindl.)	Brieger Coll. 33081 (ESA)	AF260165	AF266993	AF263748
Epidendrum schlechterianum Ames	Chase O-301 (K)	AF260172	AF267000	AF263779
Epidendrum avicula (Lindl.) Dressler	Brieger Coll. 23319 (ESA)	AF260169	AF266998	AF263778
Epidendrum campestre Lindl.	E.L. Borba 553 (UEC)	AF260174	AF267002	AF263781
Epidendrum centradenia Rchb.f.	van den Berg C169 (K spirit)	AF260175	AF267003	AF263782
Epidendrum ibaguense Lindl.	W.E. Higgins 60 (FLAS 198270)	AY008505	AY422382	AY396079
Epidendrum magnoliae Muhl.	W.E. Higgins 244 (FLAS 198271)	AY008506	AY422383	AY396080
Epidendrum pseudepidendrum Rchb.f.	van den Berg C4 (ESA)	AF260160	AF266986	AF263753
Epidendrum tridactylum Lindl.	Brieger Coll. 15628 (ESA)	AF260164	AF266692	AF263775
Guarianthe aurantiaca (Batem. ex Lindl.) Dressler & W.E.Higgins	Brieger Coll. 124 (ESA)	AF260209	AF267043–4	AF263812
Guarianthe bowringiana (J.H.Veitch) Dressler & W.E.Higgins	Chase O-1174 (K)	AF260212	AF267048–9	AF263814
Guarianthe skinneri (Batem.) Dressler & W.E.Higgins	Kew DNA bank MWC 6497*	AF260211	AF267046–7	AF263813
Hagsatera brachycolumna (L.O.Williams) R.González	W.E. Higgins 229 (FLAS 198272)	AY008515	AY422391	AY396088
Helleriella guerrerensis Dressler & Hágsater	van den Berg C172 (K spirit)	AF260142	AF266961	AF518029
Homalopetalum pachyphyllum (L.O.Williams) Dressler	M. Soto 7640 (AMO)	AF260155	AF266978–9	AF263770
Homalopetalum pumilio (Rchb.f.) Schltr.	W.E. Higgins 234 (FLAS 200730)	AY429389	AY422392	AY396089
Isabelia pulchella (Kraenzl.) Van den Berg & M.W.Chase	Brieger Coll. 6367 (ESA)	AF260163	AF266990–1	AF263745
Isabelia violacea (Lindl.) Schltr.	van den Berg C127 (ESA)	AF260168	AF266997	AF263777
Isabelia virginalis Barb.Rodr.	Brieger Coll. 30243 (ESA)	AF260161	AF266987	AF263747
Isochilus amparoanus Schltr.	Chase O-204 (K)	AF260143	AF266962	AF263762
Isochilus major Cham. & Schltdl.	W.M. Whitten 91348 (FLAS)	AY008481	AY422381	AY396078
Jacquiniella teretifolia Britton & P.Wilson	W.M. Whitten 97026 (FLAS)	AY008519	AY422390	AY396087
Jaquiniella equitantifolia (Ames) Dressler	van den Berg C171 (K spirit)	AF260158	AF266982–3	AF263773
Laelia anceps Lindl.	Chase O-998 (K)	AF260191	AF267021	AF263794
Laelia autumnalis (La Llave & Lex.) Lindl.	Chase O-1314 (K)	AF260189	AF267019	AF263759
Laelia furfuracea Lindl.	Chase 6410 (K)	AF260190	AF267020	AF263793
Laelia lyonsii (Lindl.) L.O.Williams	Brieger Coll. 16846 (ESA)	AF260222	AF267063	AF263823
Laelia rubescens Lindl.	Chase O-284 (K)	AY008575	AY422401	AY396098
Laelia speciosa (Kunth) Schltr.	Chase O-6088 (K)	AF260188	AF267018	AF263792
Laelia splendida (Schltr.) L.O.Williams	W.M. Whitten 93026 (FLAS)	AY008573	AY422408	AY396105
Laelia undulata (Lindl.) L.O.Williams	Chase O-1251 (K)	AF260223	AF267064–5	AF263749
Leptotes bicolor Lindl.	Brieger Coll. 1068 (ESA)	AF260185	AF267014	AF263789
Loefgrenianthus blanche-amesiae (Loefgr.) Hoehne	São Paulo B.G. s.n. (SP)	AF260183	AF267012	AF263787
Meiracyllium gemma Rchb.f.	M. Soto 8731 (AMO)	AF260153	AF266974	AF263767
Meiracyllium trinasutum Rchb.f.	Chase O-202 (K)	AF260152	AF266973	AF263670
Myrmecophila tibicinis (Batem.) Rolfe	Brieger Coll. 6128 (ESA)	AF260203	AF267035	AF263806
Myrmecophila tibicinis (Batem.) Rolfe	Chase O-281 (K)	AF008581	AY422402	AY396099
Nemaconia striata Lindl.	Chase 6178 (K)	AF260145	AF266965	AY121728
Nemaconia striata Lindl.	W.E. Higgins 197 (FLAS 198268)	AY008484	AY422380	AY396077
Neocogniauxia hexaptera (Cogn.) Schltr.	van den Berg C244 (K)	AF260148	AF266968–9	AF263766
Nidema boothii (Lindl.) Schltr.	W.E. Higgins 192 (FLAS 198273)	AY008522	AY422384	AY396081
Octomeria gracilis Lodd. ex Lindl.	Chase O-977 (K)	AF262911	AF265526	AF265484
Orleanesia amazonica Barb.Rodr.	São Paulo B.G. 15936 (SP)	AF260176	AF267004	AF263755

Continued

APPENDIX *Continued*

Species name	Voucher	ITS	*trnL-F*	*matK*
Polystachya galeata Rchb.f.	van den Berg C283 (K spirit)	AY008470	AY008453	AY008464
Ponera exilis Dressler	M. Soto s.n. Paracho, Michoacán (AMO)	AF260144	AF266963–4	AF263763
Prosthechea abbreviata (Schltr.) W.E.Higgins	Brieger Coll. 10092 (ESA)	AF260181	AF267010	AF263757
Prosthechea aemula (Lindl.) W.E.Higgins	W.E. Higgins 17 (FLAS 198279)	AY008544	AY422428	AY396125
Prosthechea citrina (La Llave & Lex.) W.E.Higgins	W.E. Higgins 54 (FLAS 198269)	AY008501	AY422409	AY396106
Prosthechea cochleata (L.) W.E.Higgins	MBG 75-0658 (FLAS 198280)	AY008545	AY422429	AY396126
Prosthechea glauca Knowles & Westc.	W.E. Higgins 176 (FLAS 200722)	AY429410	AY422433	AY396130
Prosthechea mariae (Ames) W.E.Higgins	Chase O-158 (K)	AF260192	AF267022	AF263795
Pseudolaelia canaanensis Ruschi	Brieger Coll. 16205 (ESA)	AF260167	AF266995–6	AF263746
Pseudolaelia vellozicolla (Hoehne) Pôrto & Brade	São Paulo B.G. 13362 (SP)	AF260166	AF266994	AY121748
Psychilis krugii (Bello) Sauleda	Chase O-1062 (K)	AF260157	AF266891	AF263772
Psychilis macconnelliae Sauleda	W.E. Higgins 53 (FLAS 198287)	AY008568	AY422394	AY396091
Restrepiella ophiocephala (Lindl.) Garay & Dunst.	Chase O-291 (K)	AF262909	AF265523	AF265482
Rhyncholaelia digbyana (Lindl.) Schltr.	Chase O-331 (K)	AF260221	AF267062	AF263822
Rhyncholaelia glauca (Lindl.) Schltr.	van den Berg C30 (ESA), W.E. Higgins 134 (FLAS)	AY008584 (van den Berg)	AY422404 (Higgins)	AY396101 (Higgins)
Scaphyglottis bidentata (Lindl.) Dressler	Brieger Coll. 1253 (ESA)	AF260162	AF266988–9	AF263774
Scaphyglottis crurigera (Lindl.) Ames & Correll	Chase O-336 (K)	AF260180	AF267009	AF263785
Scaphyglottis cuniculata (Schltr.) Dressler	W.M. Whitten 96051 (FLAS)	AY008551	AY422387	AY396084
Scaphyglottis imbricata (Lindl.) Dressler	W.M. Whitten (FLAS 97039)	AY429388	AY422386	AY396083
Scaphyglottis modesta (Rchb.f.) Schltr.	Brieger Coll. 2756 (ESA)	AF260159	AF266984–5	AF263752
Scaphyglottis pulchella (Schltr.) L.O.Williams	W.M. Whitten 208 (FLAS)	AY174740	AY422385	AY396082
Scaphyglottis punctulata (Rchb.f.) C.Schweinf.	Chase O-299 (K)	AF260182	AF267011	AF263786
Stelis argentata Lindl.	Kew 1984-7410 (K spirit 60886)	AF262878	AF265503	AF265464
Tetramicra elegans (Ham.) Cogn.	W.E. Higgins 160 (FLAS 198285)	AY008566	AY422397	AY396094

* This sample was originally taken from a plant in the living collection (Kew 1986-04870).