

# A PHYLOGENETIC ANALYSIS OF LAELIINAE (ORCHIDACEAE) BASED ON SEQUENCE DATA FROM INTERNAL TRANSCRIBED SPACERS (ITS) OF NUCLEAR RIBOSOMAL DNA<sup>1</sup>

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**ABSTRACT:** Nuclear ribosomal ITS1 and ITS2 DNA sequences were used in a phylogenetic analysis for 295 accessions representing most genera of subtribe Laeliinae (Orchidaceae), as well as select members of Pleurothallidinae, Coeliinae, Meiracylliinae, Bletinae, and other potential outgroups from Epidendroideae. The level of ITS variation was low, and most of the clades have low bootstrap support. Despite the large number of trees found (>10,000), the groups identified correspond in part to previous taxonomic groups, at both the generic and infrageneric levels, and also correlate with geographic distribution. *Arpophyllum* was identified as sister to the rest of Laeliinae, and *Meiracyllium* (Meiracylliinae) was embedded in a position close to *Euchile*, rather than in a distinct subtribe. On the other hand, *Ponera*, *Isochilus*, and *Helleriella* would best be classified in a distinct subtribe (Ponerinae), and *Dilomilis* and *Neocogniauxia* are sister to Pleurothallidinae. *Cattleya*, *Encyclia*, *Epidendrum*, and *Laelia* are clearly polyphyletic.

THE Neotropical orchid subtribe Laeliinae comprises 43 genera and 1466 species (Dressler, 1993), among them some of the most important horticultural genera in Orchidaceae, such as *Cattleya* and *Laelia*, and also some genera such as *Epidendrum*, *Encyclia*, and *Prosthechea* that make up a large part of the orchid flora of the Neotropics. Most species are epiphytic or rupicolous and have thickened leaves and pseudobulbs as an adaptation for xeric habitats. Many species of *Cattleya*, *Laelia*, *Brassavola*, and *Rhyncholaelia* have tubular nectaries partially embedded in the ovary and advertise nectar for attracting pol-

linators. *Cattleya*, *Laelia*, *Pseudolaelia*, and *Encyclia* are pollinated by bees and birds, *Brassavola* and *Rhyncholaelia* by moths, and *Epidendrum* by moths, butterflies, and birds (Dodson and Frymire, 1965; van der Pijl and Dodson, 1966). The chromosome number varies from  $2n = 24$  to  $2n = 56$  but is most commonly  $2n = 40$  (Tanaka and Kamemoto, 1984). Most of this chromosome number variation appears within species rather than characterizing genera or groups of genera. Hybridization in nature has been documented by a few intra- and intergeneric hybrids, especially involving *Cattleya* and related genera (Adams and Anderson, 1958), and there are thousands of interspecific and intergeneric artificial hybrids made for horticultural purposes.

In both vegetative and floral characters, Laeliinae are exceedingly diverse. Some genera such as *Epidendrum*, *Isochilus*, *Jacquinella*, and *Ponera* have a reed-stem habit, although most have thickened pseudobulbs with one to many terminal leaves (e.g., *Encyclia*, *Prosthechea*, and *Cattleya*). The number of pollinia varies from 2–12

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(most commonly eight) and has been emphasized for the separation of some pairs of genera such as *Cattleya* (four) and *Laelia* (eight), although the same character has been accepted as polymorphic in *Encyclia*, *Broughtonia*, and *Homalopetalum* (Baker, 1972).

Dressler (1993) grouped Laeliinae with Coeliinae, Pleurothallidinae, Arpophyllinae, Meiracylliinae, and Sobraliinae in what he called 'New World Epidendreae.' The most distinctive character separating Laeliinae from the other subtribes is lateral flattening of the pollinia. Consequently, because of their different types of pollinia *Arpophyllum* and *Meiracyllium* were previously removed from Laeliinae to the monogeneric subtribes Arpophyllinae (Dressler, 1990) and Meiracylliinae (Dressler, 1960). Coeliinae can be distinguished from Laeliinae by their lateral inflorescences and from Pleurothallidinae by lacking a joint between the ovary and pedicel. Sobraliinae have been shown recently to be only distantly related to these subtribes in an analysis of Orchidaceae based on *rbcL* sequence data (Cameron et al., 1999).

Several different classifications have been proposed to divide Laeliinae into generic series (Schlechter, 1926; Brieger, 1976), generic alliances (Dressler, 1981), and even into three related subtribes (Szlachetko, 1995). A separate subtribe, Ponerinae, has been used for the genera with a column foot, including *Helleriella*, *Hexadesmia*, *Ponera*, *Scaphyglottis*, *Isochilus*, *Domingoa*, *Jacquinella*, and *Orleanesia* (Schlechter, 1926), but in the system of Dressler (1993) Laeliinae included all these genera. The only large-scale study of generic relationships used foliar anatomy (Baker, 1972). Among other results he found *Arpophyllum*, but not *Meiracyllium*, to be reasonably distinct from other Laeliinae. He proposed a reticulate graph depicting the relationships among genera that was later transformed into six generic alliances by Dressler (1981). However, Baker (1972) did not use an explicit method of analysis to convert his results into a phylogenetic tree, and a large number of genera were polymorphic for many of the characters surveyed, leading Dressler (1993) later to abandon the alliances completely.

Many authors have suggested the artificiality of some genera; this is especially true for *Laelia* (Dressler 1981, 1993), which has a disjunct distribution between Mexico and northern Central

America and southeast Brazil. A recent morphological analysis of the Mexican *Laelia* species indicated no relationship to Brazilian groups at all (Halbinger and Soto, 1997). A similar analysis (Higgins, 1997) of the genus *Encyclia* was used to separate the genus *Prosthechea* from *Encyclia*, but Higgins also transferred to *Prosthechea* species later moved into *Euchile* (e.g., *E. mariae* and *E. citrina*) by Withner (1998). There are many small or monospecific genera with uncertain affinities and unusual vegetative and floral characters, such as *Isabelia*, *Sophronitella*, *Neolauchea*, *Pseudolaelia*, *Leptotes*, *Loefgrenianthus*, *Constantia*, *Hagsatera*, *Artorima*, and *Alamania*, and some putatively related sets of genera such as *Broughtonia*, *Cattleyopsis*, *Laeliopsis* (Saulea, 1989; Díaz Dumas, 1998), and *Psychilis*, *Tetramicra*, and *Quisqueya*, that are morphologically so similar to each other as to make generic boundaries unclear. The phylogeny of none of the genera has been studied except for the Mexican species of *Laelia* (Halbinger and Soto, 1997). Nevertheless, there have been many competing systems for infrageneric classification of *Cattleya* and *Laelia* (Schlechter, 1917; Pabst, 1975; Brieger, 1976; Fowlie, 1977; Braem, 1984, 1986; Withner, 1988, 1990).

Many studies using DNA sequence data have been performed to resolve phylogeny of animals and plants at different levels. In Orchidaceae, plastid regions have been used for higher level phylogeny (Chase et al., 1994; Neyland and Urbatsch, 1996; Yukawa, Cameron, and Chase, 1996; Kores et al., 1997; Cameron et al., 1999), as well as nuclear ribosomal internal transcribed spacers (ITS) for lower taxonomic levels (Cox et al., 1997; Pridgeon et al., 1997; Pridgeon and Chase, 1998; Douzery et al., 1999; Cameron and Chase, 1999; Ryan et al., 2000; Whitten et al., in press). ITS was useful in most of these studies, although the level of variation is neither consistent nor predictable in different subtribes. In this work we use ITS nrDNA sequences of Laeliinae and putatively related subtribes to study relationships of genera within the subtribe as well as the species phylogeny of *Cattleya* and related genera.

## MATERIALS AND METHODS

Material from most genera of Laeliinae and nearly all species in the *Cattleya* alliance was sampled (Table 1). We were unable to obtain sam-

TABLE 1. Plant material and voucher information in this study.

Species	Voucher
<i>Acrorchis roseola</i> Dressler	unvouchered (coll. W.M. Whitten)
<i>Alamania punicea</i> La Llave & Lex.	<i>van den Berg C184</i> (ESA)
<i>Amblostoma armeniacum</i> (Lindl.) Brieger ex Pabst	<i>van den Berg C2</i> (ESA)
<i>Amblostoma cernuum</i> Scheidw.	<i>Brieger Coll. 15628</i> (ESA)
<i>Aplectrum hyemale</i> Torr.	<i>Chase O-104</i> (K)
<i>Arpophyllum giganteum</i> Hartw. ex Lindl.	<i>Chase O-586</i> (K)
<i>Arpophyllum spicatum</i> La Llave & Lex.	<i>Soto MAS 7814</i> (AMO)
<i>Artorima erubescens</i> (Lindl.) Dressler & G.E. Pollard	unvouchered (coll. S. Beckendorf)
<i>Barkeria skinneri</i> (Batem. ex Lindl.) Lindl. ex Paxton	<i>van den Berg C250</i> (K spirit)
<i>Barkeria whartoniana</i> (C. Schweinf.) Soto Arenas	<i>van den Berg C163</i> (K spirit)
<i>Barkeria whartoniana</i> (C. Schweinf.) Soto Arenas	<i>van den Berg C249</i> (K spirit)
<i>Bletia parkinsonii</i> Hook.	<i>Chase O-1215</i> (K)
<i>Brassavola acaulis</i> Lindl. & Paxton	<i>W. M. Whitten 99218</i> (FLAS)
<i>Brassavola cucullata</i> (L.) R.Br.	<i>W.E. Higgins 130</i> (FLAS 198290)
<i>Brassavola cucullata</i> (L.) R.Br.	<i>van den Berg C174</i> (K spirit)
<i>Brassavola grandiflora</i> Lindl.	<i>W. M. Whitten 99216</i> (FLAS)
<i>Brassavola martiana</i> Lindl.	unvouchered (Kew 1995–2685)
<i>Brassavola nodosa</i> (L.) Lindl.	<i>Chase O-339</i> (K)
<i>Brassavola subulifolia</i> Lindl.	<i>W. M. Whitten 99217</i> (FLAS)
<i>Brassavola tuberculata</i> Hook.	<i>Brieger Coll. 3497</i> (ESA)
<i>Briegeria equitantifolia</i> (Ames) Senghas	<i>van den Berg C171</i> (K spirit)
<i>Broughtonia negrilensis</i> Fowlie	<i>W.E. Higgins 152</i> (FLAS 198288)
<i>Broughtonia sanguinea</i> (Sw.) R.Br.	<i>Brieger Coll. 14440</i> (ESA)
<i>Calanthe tricarinata</i> Lindl.	<i>Chase O-820</i> (K)
<i>Cattleya aelandiae</i> Lindl.	<i>Brieger Coll. 32982</i> (ESA)
<i>Cattleya amethystoglossa</i> Linden & Rchb.f. ex Warner	<i>Brieger Coll. 8272</i> (ESA)
<i>Cattleya araguaiensis</i> Pabst	unvouchered (Kew 1999–1443)
<i>Cattleya aurantiaca</i> (Batem. ex Lindl.) P.N. Don	<i>Brieger Coll. 124</i> (ESA)
<i>Cattleya aurea</i> Linden	<i>Brieger Coll. 2589</i> (ESA)
<i>Cattleya bicolor</i> Lindl. (Brasília)	<i>Brieger Coll. 22574</i> (ESA)
<i>Cattleya bicolor</i> Lindl. (Diamantina)	<i>Brieger Coll. 30656</i> (ESA)
<i>Cattleya bicolor</i> Lindl. (Formiga)	<i>Brieger Coll. 4336</i> (ESA)
<i>Cattleya bicolor</i> Lindl. (Itatiaia)	<i>Brieger Coll. 891</i> (ESA)
<i>Cattleya bowringiana</i> Veitch	<i>Brieger Coll. 96</i> (ESA)
<i>Cattleya bowringiana</i> Veitch	<i>van den Berg C284</i> (K)
<i>Cattleya candida</i> (Kunth) Lehm.	<i>Brieger Coll. 748</i> (ESA)
<i>Cattleya dormaniana</i> (Rchb.f.) Rchb.f.	<i>Brieger Coll. 23977</i> (ESA)
<i>Cattleya dowiana</i> Batem.	<i>Chase O-282</i> (K)
<i>Cattleya elongata</i> Lindl.	<i>Brieger Coll. 8078</i> (ESA)
<i>Cattleya forbesii</i> Lindl.	<i>Brieger Coll. 5358</i> (ESA)
<i>Cattleya gaskelliana</i> Braem	<i>Brieger Coll. 6253</i> (ESA)
<i>Cattleya granulosa</i> Lindl. (Bahia State-BA)	<i>Brieger Coll. 19216</i> (ESA)
<i>Cattleya granulosa</i> Lindl. (Pernambuco state-PE)	<i>Brieger Coll. 22482</i> (ESA)
<i>Cattleya guttata</i> Lindl.	<i>Brieger Coll. 11299</i> (ESA)
<i>Cattleya harrisoniana</i> Batem. ex Lindl.	<i>Brieger Coll. 16036</i> (ESA)
<i>Cattleya intermedia</i> Graham ex Hook.	<i>Brieger Coll. 4095</i> (ESA)
<i>Cattleya iricolor</i> Rchb.f.	unvouchered (Kew 1999–1502)
<i>Cattleya jenmanii</i> Rolfe	unvouchered (coll. C. van den Berg)
<i>Cattleya kerrii</i> Brieger & Bicalho	<i>Brieger Coll. 18765</i> (Holotype-HB)
<i>Cattleya labiata</i> Lindl. (Pernambuco State)	<i>Brieger Coll. 5487</i> (ESA)
<i>Cattleya labiata</i> Lindl. (Ceará State-CE)	<i>Brieger Coll. 20545</i> (ESA)
<i>Cattleya lawrenceana</i> Rchb.f.	<i>Brieger Coll. 3802</i> (ESA)
<i>Cattleya loddigesii</i> Lindl.	<i>Brieger Coll. 2483</i> (ESA)
<i>Cattleya lueddemanniana</i> Rchb.f.	<i>Brieger Coll. 755</i> (ESA)
<i>Cattleya lueddemanniana</i> Rchb.f.	<i>Brieger Coll. 3759</i> (ESA)
<i>Cattleya luteola</i> Lindl.	<i>Brieger Coll. 32187</i> (ESA)
<i>Cattleya maxima</i> Lindl.	<i>Brieger Coll. 2986-32</i> (ESA)
<i>Cattleya maxima</i> Lindl.	unvouchered (Kew 1983–4362)
<i>Cattleya mendelii</i> Backh.f.	<i>Brieger Coll. 2418</i> (ESA)
<i>Cattleya mooreana</i> Withner, D. Allison & Guenard	unvouchered (Kew 1999–1569)
<i>Cattleya mossiae</i> Hook.	<i>Brieger Coll. 6219</i> (ESA)
<i>Cattleya nobilior</i> Rchb.f.	<i>Brieger Coll. 30978</i> (ESA)
<i>Cattleya patinii</i> Cogn.	<i>Brieger Coll. 4138</i> (ESA)
<i>Cattleya percivaliana</i> O'Brien	<i>van den Berg C279</i> (ESA)

TABLE 1. Continued.

Species	Voucher
<i>Cattleya porphyroglossa</i> Linden & Rchb.f.	unvouchered (Kew 1986–2034)
<i>Cattleya schilleriana</i> Rchb.f.	Brieger Coll. 6640 (ESA)
<i>Cattleya schofieldiana</i> Rchb.f.	Brieger Coll. 6656 (ESA)
<i>Cattleya schroderae</i> Rchb.f.	Brieger Coll. 94 (ESA)
<i>Cattleya skinneri</i> Batem.	Brieger Coll. 10103 (ESA)
<i>Cattleya skinneri</i> Batem.	unvouchered (Kew 1986–4870)
<i>Cattleya skinneri</i> Batem.	Brieger Coll. 708 (ESA)
<i>Cattleya tenuis</i> Campacci & Vedovello	C211-Machado s.n. (ESA)
<i>Cattleya tigrina</i> A.Rich. (syn <i>C. leopoldii</i> Verschaff.)	van den Berg C186 (K spirit)
<i>Cattleya trianaei</i> Linden & Rchb.f.	Brieger Coll. 2608 (ESA)
<i>Cattleya trichopiliochila</i> Barb.Rodr. (syn. <i>C. eldorado</i> Linden)	Brieger Coll. 28787 (ESA)
<i>Cattleya velutina</i> Rchb.f.	Brieger Coll. 7843 (ESA)
<i>Cattleya violacea</i> (Kunth) Rolfe	Brieger Coll. 28495 (ESA)
<i>Cattleya walkeriana</i> Gardner	Brieger Coll. 1627 (ESA)
<i>Cattleya warneri</i> T.Moore	Brieger Coll. 6605 (ESA)
<i>Cattleya warszewiczii</i> Rchb.f.	Brieger Coll. 754 (ESA)
<i>Cattleyopsis lindenii</i> (Lindl.) Cogn.	W.E. Higgins 251 (FLAS 198289)
<i>Caularthron bicornutum</i> (Hook.) Raf.	Brieger Coll. 7959 (ESA)
<i>Caularthron bilamellatum</i> Rchb.f. (R.E.Schultes)	Brieger Coll. 3690 (ESA)
<i>Chysis bractescens</i> Lindl.	Chase O-436 (K)
<i>Coelia guatemalensis</i> Rchb.f.	M.Soto 7973 (AMO)
<i>Coelia macrostachya</i> Lindl.	Chase O-817 (K)
<i>Coelia triptera</i> G.Don	Chase O-324 (K)
<i>Constantia cipoensis</i> Porto & Brade	São Paulo B.G. s.n. (SP)
<i>Constantia microscopica</i> F.E.L.Miranda	E.L.Borba 515 & J.M.Felix (UEC)
<i>Dilomilis montana</i> (Sw.) Summerh.	Chase O-206 (K)
<i>Dimerandra emarginata</i> (G.Mey.) Hoehne	Chase O-335 (K)
<i>Dinema polybulbon</i> (Sw.) Lindl.	Brieger Coll. 6052 (ESA 35552)
<i>Domingoa kienastii</i> (Rchb.f.) Dressler	W. E. Higgins 225 (FLAS 198291)
<i>Domingoa nodosa</i> (Cogn.) Schltr.	W. E. Higgins 1034 (FLAS 198284)
<i>Dracula chimaera</i> (Rchb.f.) Luer	Chase O-967 (K)
<i>Earina autumnalis</i> Hook.	Chase O-298 (K)
<i>Encyclia adenocaula</i> (La Llave & Lex.) Schltr.	W.E. Higgins 12 (FLAS 198274)
<i>Encyclia bractescens</i> (Lindl.) Hoehne	W.E. Higgins 21 (FLAS 198275)
<i>Encyclia cordigera</i> (Kunth) Dressler	W.E. Higgins 24 (FLAS 198276)
<i>Encyclia cyperifolia</i> (C.Schweinf.) Carnevali & I.Ramírez	Brieger Coll. 5758 (ESA)
<i>Encyclia dichroma</i> (Lindl.) Schltr.	Selby BG.88-0310 (FLAS 198278)
<i>Encyclia granitica</i> (Lindl.) Schltr.	Brieger Coll. 21371 (ESA)
<i>Encyclia maderoi</i> Schltr.	Brieger Coll. 2619 (ESA)
<i>Encyclia oncidoides</i> (Lindl.) Schltr.	Brieger Coll. 5420 (ESA)
<i>Encyclia</i> sp.	Brieger Coll. 11024 (ESA)
<i>Encyclia tampensis</i> (Lindl.) Small	W.E. Higgins 27 (FLAS 198277)
<i>Epidendrum campestre</i> Lindl.	E.L. Borba 553 (UEC)
<i>Epidendrum capricornu</i> Kraenzl.	van den Berg C251 (K spirit)
<i>Epidendrum ciliare</i> L.	Brieger Coll. 1024 (ESA)
<i>Epidendrum cinnabarinum</i> Salzm. ex Lindl.	van den Berg C277 (K spirit)
<i>Epidendrum conopseum</i> R.Br.	W. E. Higgins 244 (FLAS 198271)
<i>Epidendrum criniferum</i> Rchb.f.	van den Berg C252 (K spirit)
<i>Epidendrum ibaguense</i> Lindl.	W. E. Higgins 60 (FLAS 198270)
<i>Epidendrum latifolium</i> (Lindl.) Garay & H.R.Sweet	van den Berg C254 (K spirit)
<i>Epidendrum nocturnum</i> Jacq.	Chalets s.n. (AMO)
<i>Epidendrum pseudepidendrum</i> Rchb.f.	van den Berg C4 (ESA)
<i>Epidendrum radioferens</i> (Ames, F.T.Hubb. & C.Schweinf.) Hágsater	Chase O-300 (K)
<i>Epidendrum secundum</i> Jacq.	E.L.Borba 552 (UEC)
<i>Epidendrum stamfordianum</i> Bateman	Brieger Coll. 1200 (ESA)
<i>Epidendrum veroscriptum</i> Hágsater	van den Berg C247 (K spirit)
<i>Euchile 'sinaloensis'</i> (ined.)	unvouchered (Kew 1999–1710)
<i>Euchile citrina</i> (La Llave & Lex.) Withner	W.E. Higgins 54 (FLAS 198269)
<i>Euchile mariae</i> (Ames) Withner	Chase O-158 (K)
<i>Hagsatera brachycolumna</i> (L.O.Williams) R.González	W. E. Higgins 229 (FLAS 198272)
<i>Helleriella guerrerensis</i> Dressler & Hágsater	van den Berg C172 (K spirit)
<i>Helleriella punctulata</i> (Rchb.f.) Garay & H.R.Sweet	Chase O-299 (K)
<i>Hexadesmia cruriger</i> Lindl.	Chase O-336 (K)
<i>Hexadesmia micrantha</i> Lindl.	unvouchered (coll. R.L.Dressler)

TABLE 1. Continued.

Species	Voucher
<i>Hexalectris revoluta</i> Correll	<i>D. Goldman 1364</i> (TEX)
<i>Hexisea bidenata</i> Lindl.	<i>Brieger Coll. 1253</i> (ESA)
<i>Hexisea imbricata</i> (Lindl.) Rchb.f.	<i>W.M. Whitten 97039</i> (FLAS)
<i>Homalopetalum pachyphyllum</i> (L.O.Williams) Dressler	<i>M.Soto 7640</i> (AMO)
<i>Homalopetalum pumilio</i> (Rchb.f.) Schltr.	<i>M.Soto 7443</i> (AMO)
<i>Homalopetalum pumilum</i> (Ames) Dressler	<i>M.Soto 8950</i> (AMO)
<i>Isabelia virginialis</i> Barb.Rodr.	<i>Brieger Coll. 17289</i> (ESA)
<i>Isabelia virginialis</i> Barb. Rodr.	<i>Brieger Coll. 30243</i> (ESA)
<i>Isochilus alatus</i> Schltr.	<i>M. Soto 7190</i> (AMO)
<i>Isochilus amparoanus</i> Schltr.	<i>Chase O-204</i> (K)
<i>Isochilus brasiliensis</i> Schltr.	<i>Brieger Coll. 33696</i> (ESA 35553)
<i>Isochilus langlassei</i> Schltr.	<i>M.Soto 7808</i> (AMO)
<i>Isochilus major</i> Cham. & Schldtl.	<i>W. M. Whitten 91348</i> (FLAS)
<i>Jacquiella globosa</i> Schltr.	<i>W. M. Whitten 97064</i> (FLAS)
<i>Jacquiella teretifolia</i> Britton & P.Wilson	<i>W. M. Whitten 97026</i> (FLAS)
<i>Laelia alaoorii</i> Brieger & Bicalho	<i>Brieger Coll. 19179</i> (ESA)
<i>Laelia albida</i> Batem. ex Lindl.	unvouchered (coll. S. Beckendorf)
<i>Laelia alvaroana</i> F.E.L.Miranda	<i>van den Berg C227</i> (ESA)
<i>Laelia alvaroana</i> F.E.L.Miranda	<i>C207-Machado s.n.</i> (ESA)
<i>Laelia anceps</i> Lindl.	<i>Chase O-998</i> (K)
<i>Laelia anceps</i> Lindl.	<i>Brieger Coll. 3811</i> (ESA)
<i>Laelia angereri</i> Pabst	<i>C223-Machado s.n.</i> (ESA)
<i>Laelia autumnalis</i> (La Llave & Lex.) Lindl.	unvouchered (coll. S. Beckendorf)
<i>Laelia bahiensis</i> Schltr.	<i>C221-Machado s.n.</i> (ESA)
<i>Laelia blumenscheinii</i> Pabst	<i>C209-Machado s.n.</i> (ESA)
<i>Laelia bradei</i> Pabst	<i>C215-Machado s.n.</i> (ESA)
<i>Laelia brevicaulis</i> (H.G.Jones) Withner	<i>C208-Machado s.n.</i> (ESA)
<i>Laelia briegeri</i> Blumensch. ex Pabst	<i>Brieger Coll. 4612</i> (ESA)
<i>Laelia cardimii</i> Pabst & A.F.Mello	<i>C205-Machado s.n.</i> (ESA)
<i>Laelia caulescens</i> Lindl.	<i>Brieger Coll. 1916</i> (ESA)
<i>Laelia cinnabarina</i> Batem. ex Lindl.	<i>Brieger Coll. 1395</i> (ESA)
<i>Laelia crispa</i> Rchb.f.	<i>Brieger Coll. 3914</i> (ESA)
<i>Laelia crispata</i> Thunb. (Garay) (syn. <i>L. flava</i> Lindl.)	<i>van den Berg C32</i> (ESA)
<i>Laelia crispilabia</i> (A.Rich. ex Rchb.f.) Warner	<i>Brieger Coll. 4837</i> (ESA)
<i>Laelia dayana</i> Rchb.f.	<i>Brieger Coll. 15795</i> (ESA)
<i>Laelia duveenii</i> Fowlie	<i>C213-Machado s.n.</i> (ESA)
<i>Laelia esalqueana</i> Blumensch. ex Pabst	<i>Brieger Coll. 4980</i> (ESA)
<i>Laelia fidelensis</i> Pabst	<i>C225-Machado s.n.</i> (ESA)
<i>Laelia furfuracea</i> Lindl.	unvouchered (coll. S. Beckendorf)
<i>Laelia ghillanyi</i> Pabst	<i>C214-Machado s.n.</i> (ESA)
<i>Laelia gloedeniana</i> Hoehne	<i>van den Berg C35</i> (ESA)
<i>Laelia gouldiana</i> Rchb.f.	unvouchered (coll. S. Beckendorf)
<i>Laelia grandis</i> Lindl. & Paxton	<i>Brieger Coll. 19209</i> (ESA)
<i>Laelia harpophylla</i> Rchb.f.	<i>Brieger Coll. 6687</i> (ESA)
<i>Laelia itambana</i> Pabst	<i>C212-Machado s.n.</i> (ESA)
<i>Laelia jongheana</i> Rchb.f.	<i>Brieger Coll. 31534</i> (ESA)
<i>Laelia kautskyi</i> Pabst	<i>van den Berg C286</i> (K spirit)
<i>Laelia kettieana</i> Pabst	<i>C210-Machado s.n.</i> (ESA)
<i>Laelia liliputiana</i> Pabst	<i>C206-Machado s.n.</i> (ESA)
<i>Laelia lobata</i> (Lindl.) Veitch	<i>Brieger Coll. 3557</i> (ESA)
<i>Laelia longipes</i> Rchb.f.	<i>Brieger Coll. 5183</i> (ESA)
<i>Laelia lundii</i> (Rchb.f.) Withner	<i>Brieger Coll. 30692</i> (ESA)
<i>Laelia mantiqueirae</i> Pabst ex D.C.Zappi	<i>van den Berg C224</i> (ESA)
<i>Laelia milleri</i> Blumensch. ex Pabst	<i>Brieger Coll. 5070</i> (ESA)
<i>Laelia mixta</i> Hoehne ex Ruschi	<i>C220-Machado s.n.</i> (ESA)
<i>Laelia perrinii</i> Batem.	<i>Brieger Coll. 652</i> (ESA)
<i>Laelia pfisteri</i> Pabst & Senghas	<i>van den Berg C226</i> (ESA)
<i>Laelia praestans</i> Linden & Rchb.f.	<i>C217-Machado s.n.</i> (ESA)
<i>Laelia pumila</i> (Hook.) Rchb.f.	<i>Brieger Coll. 7794</i> (ESA)
<i>Laelia purpurata</i> Lindl. & Paxton	Selby B.G. 84-0459 (SEL)
<i>Laelia reginae</i> Pabst	<i>C218-Machado s.n.</i> (ESA)
<i>Laelia rubescens</i> Lindl.	<i>Chase O-1205</i> (K)
<i>Laelia rupestris</i> Lindl.	<i>van den Berg C33</i> (ESA)
<i>Laelia sanguiloba</i> Withner	<i>C216-Machado s.n.</i> (ESA)

TABLE 1. Continued.

Species	Voucher
<i>Laelia sincorana</i> Schltr.	van den Berg C263 (K spirit)
<i>Laelia speciosa</i> (Kunth) Schltr.	Chase O-6088 (unvouchered)
<i>Laelia speciosa</i> (Kunth) Schltr.	Chase O-6411 (unvouchered)
<i>Laelia tenebrosa</i> (Rölfe) Rölfe	van den Berg C279 (K spirit)
<i>Laelia tereticaulis</i> Hoehne	van den Berg C222 (ESA)
<i>Laelia virens</i> Lindl.	van den Berg C18 (ESA)
<i>Laelia xanthina</i> Lindl. ex Hook.	Brieger Coll. 6662 (ESA)
<i>Laelia xanthina</i> Lindl. ex Hook.	Brieger Coll. 6635 (ESA)
<i>Laeliopsis dominguensis</i> (Lindl.) Lindl. & Paxton	unvouchered (coll. W.E. Higgins)
<i>Lanium avicula</i> (Lindl.) Benth.	Brieger Coll. 23319 (ESA)
<i>Leptotes bicolor</i> Lindl.	Brieger Coll. 1068 (ESA)
<i>Leptotes</i> cf. <i>tenuis</i> Rchb.f.	São Paulo B.G. 16809 (SP)
<i>Leptotes</i> cf. <i>unicolor</i> Barb.Rodr.	São Paulo B.G. 13534 (SP)
<i>Leptotes</i> cf. <i>unicolor</i> Barb.Rodr.	C204-Machado s.n. (ESA)
<i>Loefgrenianthus blanche-amesiae</i> (Loefgr.) Hoehne	São Paulo B.G. s.n. (SP)
<i>Masdevallia floribunda</i> Lindl.	Chase O-296 (K)
<i>Meiracyllium gemma</i> Rchb.f.	M.Soto 8731 (AMO)
<i>Meiracyllium trinasutum</i> Rchb.f.	Chase O-202 (K)
<i>Meiracyllium trinasutum</i> Rchb.f.	van den Berg C7 (ESA)
<i>Myrmecophila galeottiana</i> (A.Rich.) Rölfe	unvouchered (Kew 1982–3743)
<i>Myrmecophila</i> sp.	Chase O-281 (K)
<i>Myrmecophila thomsoniana</i> (Rchb.f.) Rölfe	van den Berg C167 (K spirit)
<i>Myrmecophila tibicinis</i> (Batem.) Rölfe	van den Berg C81 (ESA)
<i>Myrmecophila wendlandii</i> (Rchb.f.) G.C.Kenn	van den Berg C165 (K spirit)
<i>Nageliella angustifolia</i> (Booth ex Lindl.) Ames & Correll	W. Bussey s.n. Guatemala (AMO)
<i>Nageliella purpurea</i> (Lindl.) L.O.Williams	van den Berg C260 (K spirit)
<i>Nanodes mathewsii</i> (Rchb.f.) Rölfe	Brieger Coll. 16746 (ESA)
<i>Nanodes schlechterianum</i> (Ames) Brieger	Chase O-301 (K)
<i>Neocogniauxia hexaptera</i> (Cogn.) Schltr.	van den Berg C244 (K)
<i>Neocogniauxia monophylla</i> (Griseb.) Schltr.	van den Berg C245 (K)
<i>Neolauchea pulchella</i> Kraenzl.	Brieger Coll. 11737 (ESA)
<i>Neolauchea pulchella</i> Kraenzl.	Brieger Coll. 6367 (ESA)
<i>Nidema boothii</i> (Lindl.) Schltr.	W. E. Higgins 192 (FLAS 198273)
<i>Oerstedella centradenia</i> Rchb.f.	van den Berg C169 (K spirit)
<i>Orleanesia amazonica</i> Barb.Rodr.	São Paulo B.G. 15936 (SP)
<i>Orleanesia pleurostachys</i> (Linden & Rchb.f.) Garay & Dunst.	J.T. Atwood et al. 5614 (FLAS)
<i>Platyglottis coriacea</i> L.O.Williams	unvouchered (coll. R.L. Dressler)
<i>Pleione chunii</i> C.L.Tso	van den Berg C290 (K spirit)
<i>Pleurothallis racemiflora</i> Lindl.	W. E. Higgins 140 (FLAS 198267)
<i>Polystachya galeata</i> Rchb.f.	van den Berg C283 (K spirit)
<i>Ponera australis</i> Cogn.	Brieger Coll. 33642 (ESA 35548)
<i>Ponera exilis</i> Dressler	M.Soto s.n., Paracho, Michoacan (AMO)
<i>Ponera glomerata</i> Correll	M.Soto 8224 (AMO)
<i>Ponera striata</i> Lindl.	W. E. Higgins 197 (FLAS 198268)
<i>Ponera striata</i> Lindl.	Chase O-6178 (K spirit)
<i>Prosthechea abbreviata</i> (Schltr.) W.E.Higgins	Brieger Coll. 10092 (ESA)
<i>Prosthechea aemula</i> (Lindl.) W.E.Higgins	W. E. Higgins 17 (FLAS 198279)
<i>Prosthechea allemanii</i> (Barb.Rodr.) W.E.Higgins	Brieger Coll. 5940 (ESA)
<i>Prosthechea calamaria</i> (Lindl.) W.E.Higgins	Brieger Coll. 10368 (ESA)
<i>Prosthechea</i> cf. <i>moojenii</i> (Pabst) W.E.Higgins	Brieger Coll. 8118 (ESA)
<i>Prosthechea cochleata</i> (L.) W.E.Higgins	MBG 75-0658 (FLAS 198280)
<i>Prosthechea fausta</i> (Rchb.f. ex Cogn.) W.E.Higgins	van den Berg C95 (ESA)
<i>Prosthechea lambda</i> (Linden & Rchb.f.) W.E.Higgins	Brieger Coll. 6032 (ESA)
<i>Prosthechea linkiana</i> (Klotzsch) W.E.Higgins	Brieger Coll. 3879 (ESA)
<i>Prosthechea prismatocarpa</i> (Rchb.f.) W.E.Higgins	W. E. Higgins 19 (FLAS 198283)
<i>Prosthechea pygmaea</i> (Hook.) W.E.Higgins	Selby B.G. 92-0206 (FLAS 198281)
<i>Prosthechea suzanensis</i> (Hoehne) W.E.Higgins	van den Berg C119 (K spirit)
<i>Prosthechea venezuelana</i> (Schltr.) W.E.Higgins	Brieger Coll. 2543 (ESA)
<i>Prosthechea vitellina</i> (Lindl.) W.E.Higgins	W. E. Higgins 57 (FLAS 198282)
<i>Prosthechea widgrenii</i> (Lindl.) W.E.Higgins	Brieger Coll. 30565 (ESA)
<i>Pseudolaelia</i> cf. <i>cipoensis</i> Pabst	São Paulo B.G. 12759 (SP)
<i>Pseudolaelia</i> cf. <i>cipoensis</i> Pabst	São Paulo B.G. 12406 (SP)
<i>Pseudolaelia</i> cf. <i>citrina</i> Pabst	São Paulo B.G. 12323 (SP)
<i>Pseudolaelia</i> cf. <i>dutraei</i> Ruschi	São Paulo B.G. 12243 (SP)

TABLE 1. Continued.

Species	Voucher
<i>Pseudolaelia</i> cf. <i>geraensis</i> Pabst	<i>E.L.Borba</i> 554 (UEC)
<i>Pseudolaelia</i> cf. <i>vellozicola</i> (Hoehne) Porto & Brade	São Paulo B.G. 13358 (SP)
<i>Pseudolaelia</i> cf. <i>vellozicola</i> (Hoehne) Porto & Brade	São Paulo B.G. 13362 (SP)
<i>Pseudolaelia vellozicola</i> (Hoehne) Porto & Brade	<i>Brieger Coll.</i> 6736, <i>Chase O-1200</i> (ESA)
<i>Pseudolaelia vellozicola</i> (Hoehne) Porto & Brade	<i>Brieger Coll.</i> 6736 (ESA)—C201
<i>Psychilis krugii</i> (Bello) Sauleda	<i>Chase O-1062</i> (K)
<i>Psychillis macconnelliae</i> Sauleda	<i>W. E. Higgins</i> 53 (FLAS 198287)
<i>Quisqueya ekmanii</i> Dod	<i>W. E. Higgins</i> 1043 (FLAS 198286)
<i>Reichenbachanthus cuniculatus</i> (Schltr.) Pabst	<i>W. M. Whitten</i> 96051 (FLAS)
<i>Renata canaanensis</i> Ruschi	<i>Brieger Coll.</i> 16205 (ESA) C150
<i>Renata canaanensis</i> Ruschi	<i>Brieger Coll.</i> 16205 (ESA) C188
<i>Rhyncholaelia digbyana</i> (Lindl.) Schltr.	<i>Chase O-331</i> (K)
<i>Rhyncholaelia digbyana</i> (Lindl.) Schltr.	<i>van den Berg</i> C73 (ESA)
<i>Rhyncholaelia glauca</i> (Lindl.) Schltr.	<i>van den Berg</i> C30 (ESA)
<i>Scaphyglottis bilineata</i> Schltr.	<i>W. M. Whitten</i> 96054 (FLAS)
<i>Scaphyglottis boliviensis</i> (Rolfe) B.R.Adams	<i>W. M. Whitten</i> 97006 (SEL)
<i>Scaphyglottis geminata</i> Dressler & Mora Retana	<i>W. M. Whitten</i> 96050 (FLAS)
<i>Scaphyglottis gentryi</i> Dodson & Monsalve	<i>W. M. Whitten</i> 97007 (FLAS)
<i>Scaphyglottis graminifolia</i> Poepp. & Endl.	<i>W. M. Whitten</i> 97012 (FLAS)
<i>Scaphyglottis lindeniana</i> (A.Rich & Galeotti) L.O.Williams	<i>W. M. Whitten</i> 96051 (FLAS)
<i>Scaphyglottis pulchella</i> (Schltr.) L.O.Williams	unvouchered (coll. W.M. Whitten)
<i>Schomburgkia crispa</i> Lindl.	<i>van den Berg</i> C154 (ESA 35551)
<i>Schomburgkia lyonsii</i> Lindl.	<i>Brieger Coll.</i> 16846 (ESA)
<i>Schomburgkia splendida</i> Schltr.	<i>Whitten</i> 93026 (FLAS)
<i>Schomburgkia superbiens</i> (Lindl.) Rolfe	<i>van den Berg</i> C164 (K spirit)
<i>Schomburgkia undulata</i> Lindl.	<i>van den Berg</i> C29 (ESA)
<i>Sophranitella violacea</i> (Lindl.) Schltr.	<i>van den Berg</i> C127 (ESA)
<i>Sophronitis brevipedunculata</i> (Cogn.) Fowlie	<i>C129-Machado</i> s.n. (ESA)
<i>Sophronitis brevipedunculata</i> (Cogn.) Fowlie	São Paulo B.G. s.n. IBDF (SP)
<i>Sophronitis cernua</i> Lindl.	<i>Brieger Coll.</i> 15737 (ESA)
<i>Sophronitis cernua</i> Lindl.	<i>van den Berg</i> C246 (K spirit)
<i>Sophronitis coccinea</i> (Lindl.) Rchb.f.	<i>van den Berg</i> C173 (K spirit)
<i>Sophronitis coccinea</i> (Lindl.) Rchb.f.	São Paulo B.G. 9577 (SP)
<i>Sophronitis mantiqueirae</i> (Fowlie) Fowlie	São Paulo B.G. 12195 (SP)
<i>Sophronitis wittigiana</i> Barb.Rodr.	São Paulo B.G. 8961 (SP)
<i>Tetragamestus modestus</i> Rchb.f.	<i>Brieger Coll.</i> 2756 (ESA)
<i>Tetramicra elegans</i> (Ham.) Cogn.	<i>W. E. Higgins</i> 160 (FLAS 198285)
<i>Thunia alba</i> Rchb.f.	<i>Chase O-589</i> (K)

ples of *Pinelia*, *Pygmaeorchis*, and *Basiphyllaea*. The latter, however, was found to be a member of Blettiinae in analyses of *matK* (D. Goldman, pers. comm.) and ITS (V. Sosa, pers. comm.). We also sampled multiple taxa representing Chysiinae, Coeliinae, Blettiinae, 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 on unpublished data of ITS, *trnL-F*, and *matK* (van den Berg et al., unpubl.) and D. Goldman (pers. comm.). *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), which included purification through a cesium chloride/ethidium bromide gradient (1.55 g ml<sup>-1</sup>). 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 with the same primers and also ITS5 and ITS4 (White et al., 1990; Baldwin, 1992), using an ABI 377 automated sequencer following manufacturer's proto-

cols (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, 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 we performed 1000 random taxon-addition replicates to look for multiple optimal-tree islands (Maddison, 1991). The search was performed with the subtree pruning-regrafting (SPR) algorithm, but we 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) until we obtained a set limit of 10,000 trees. We 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, 1995) of 1000 replicates with simple taxon addition and TBR branch swapping, but saving only 15 trees per replicate. All sequences have been submitted to GenBank.

## 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, we decided to use the analysis including gaps as a basis for the present discussion. The aligned ITS sequence matrix had 851 positions, to which we added 198 gap characters (coded as plus/minus). The gap positions themselves were coded as missing characters. In the complete matrix, 535 of the 1049 characters were potentially parsimony informative. In the heuristic search, we found more than 10,000 trees (the limit we enforced) of 3958 steps, with the consistency index (CI, including autapomorphies) = 0.26 and the retention index (RI) = 0.71. One of these trees is presented in summary in Figure 1 and as a series of detailed subclades in Figure 6, with the Fitch lengths above and the bootstrap percentages below each branch. An arrowhead indicates a node 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.

On the basis of ITS data, Laeliinae are monophyletic provided that 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. 2), an assemblage of small Brazilian genera that are either epiphytic on *Vellozia* (Velloziaceae) or found in rather dry habitats in savanna 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. 2), all from the Caribbean. In Figure 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 in a separate subclade from *L. anceps* and *L. rubescens*, which in turn go with *Schomburgkia*. It is important to no-



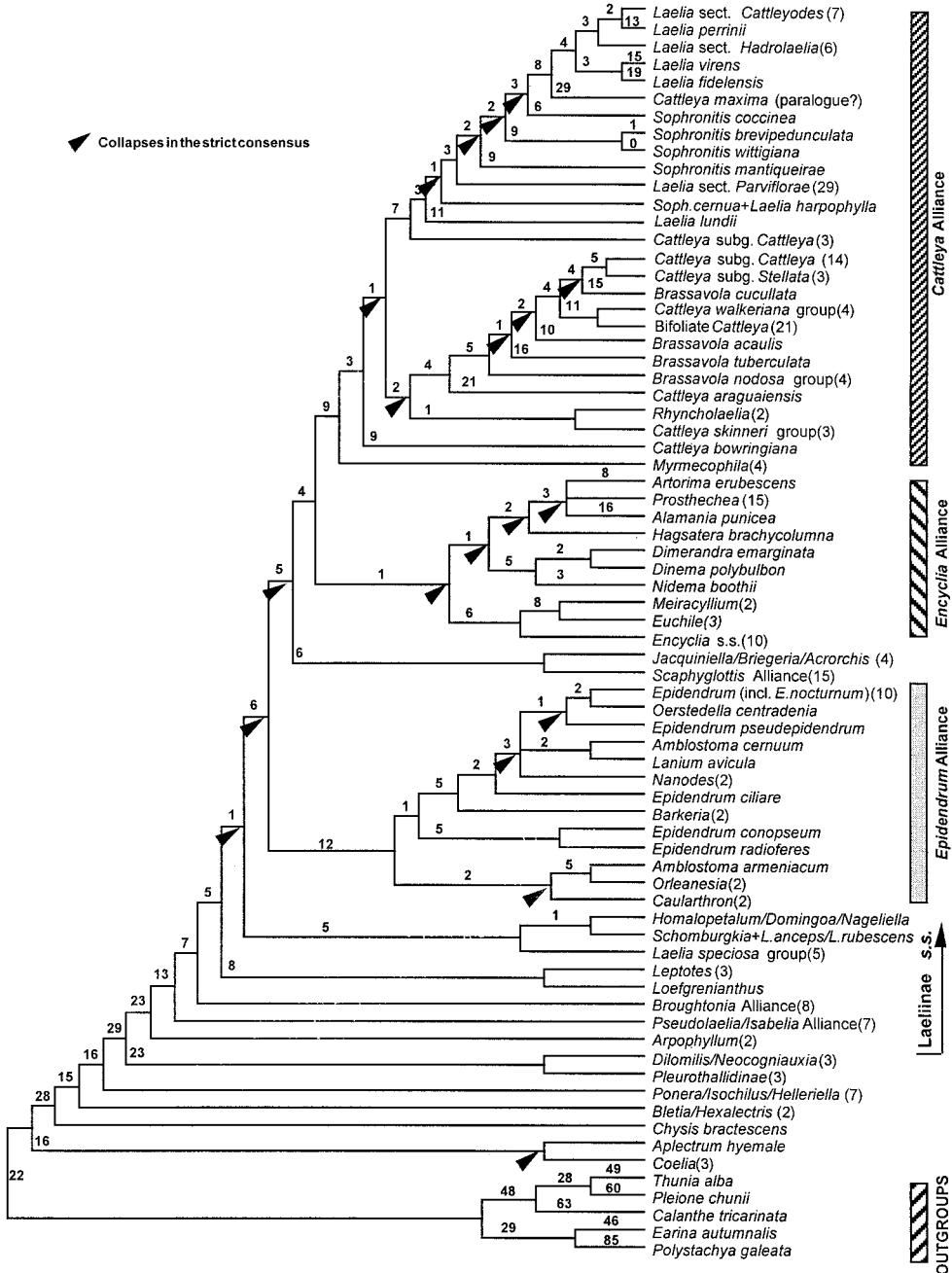


Fig 1. A summary of the relationships of one of 10,000 most parsimonious trees of the combined ITS and gap coding matrix.

tice that all these species of *Laelia sensu stricto* are distantly placed from the Brazilian species of *Laelia*, which belong to the ‘*Cattleya* alliance’ (Fig. 6). Another clade in Figure 3 contains the genera with a column foot: *Scaphyglottis*, *Reichenbachanthus*, *Hexisea*, and *Platyglottis*. This also shows clearly the positions of *Hexadesmia* and *Te-*

*tragamestus* embedded in *Scaphyglottis*. The species known as ‘*Helleriella punctulata*’ is in fact also a *Scaphyglottis* and has no relationship to *H. nicaraguensis* and *H. guerrerensis* of Ponerinae (Fig. 2). The ‘*Epidendrum* alliance’ appears as a clade (Fig. 3) and includes *Epidendrum*, *Orleanesia*, *Amblostoma*, *Barkeria*, *Lanium*, *Nanodes*, and

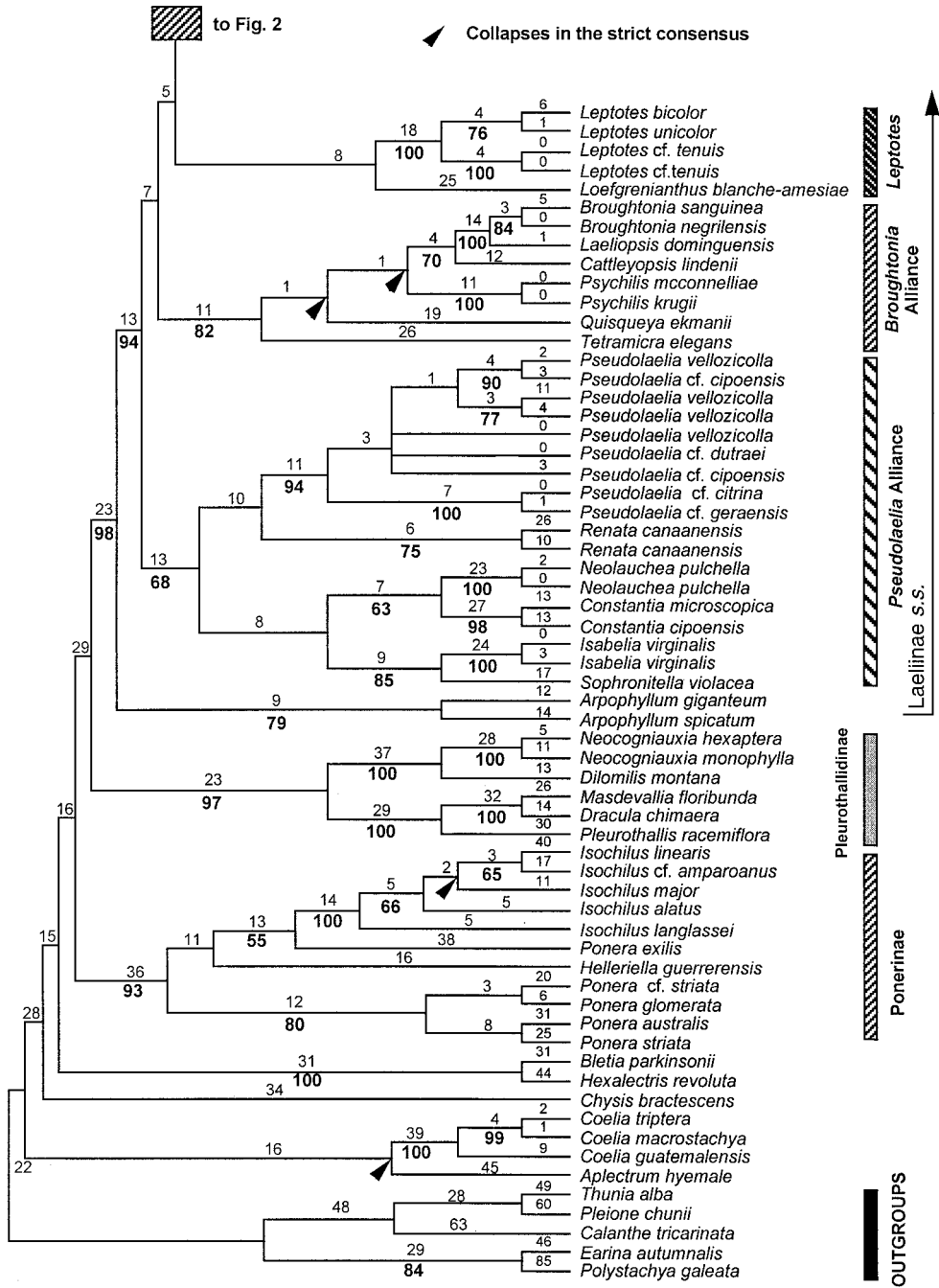


Fig. 2. A portion of one of 10,000 most parsimonious trees of the combined ITS and gap coding matrix, CI = 0.26 (excluding non-informative characters), RI = 0.71, Fitch tree length = 3958. Fitch branch lengths are above branches, and bootstrap percentages (50% or more) are below. Arrows indicate branches not present in the strict consensus.

*Caularthron*. Although there is a clade with all genera once considered to be part of *Encyclia* (excluding *Psychilis*; Fig. 4), it appeared in only 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 *Hag-*

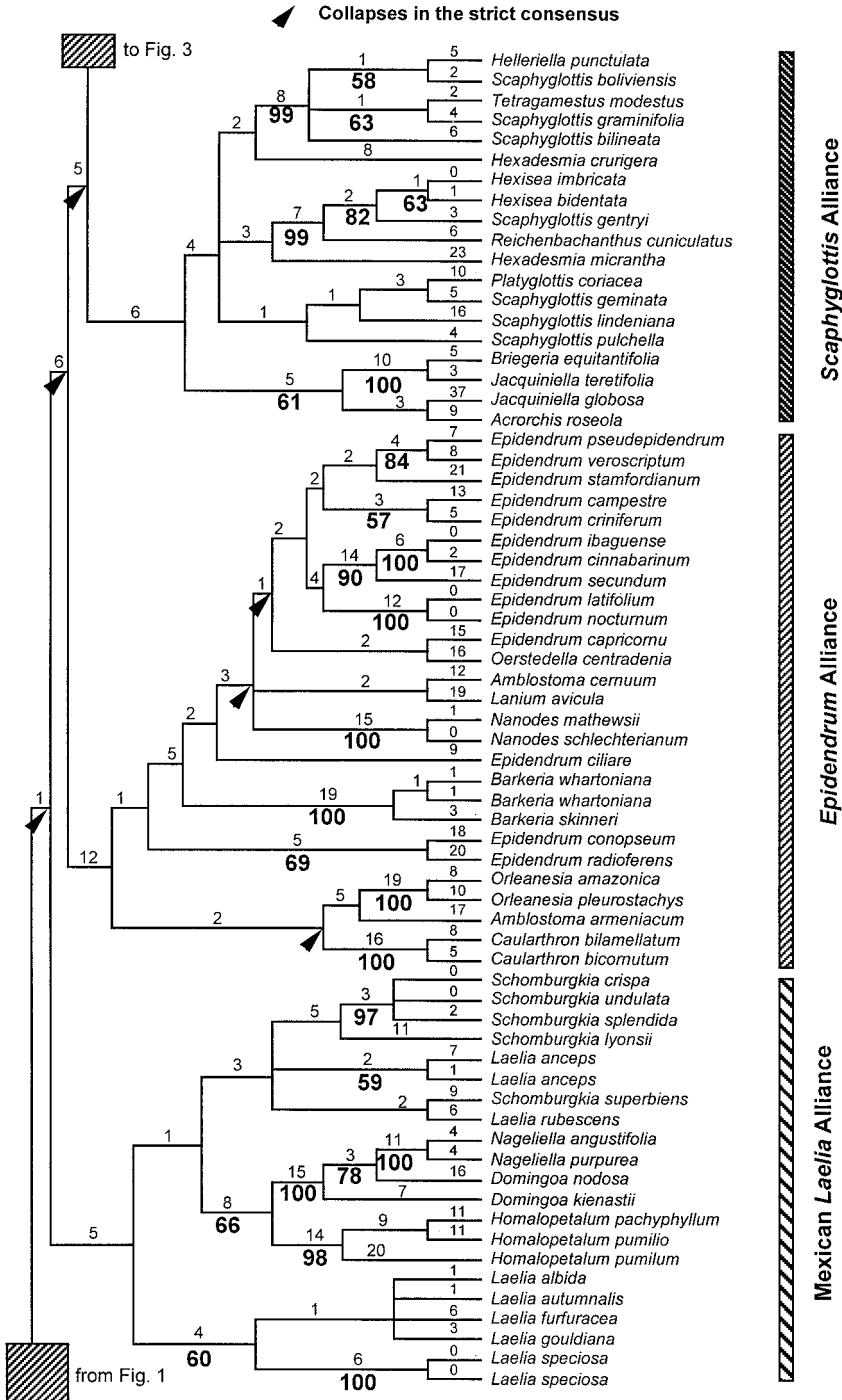


Fig. 3. *Laelia* s.s., *Epidendrum*, and *Scaphyglottis* alliances in the same most parsimonious tree as Figure 2.

*satera* 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

we will refer to here as the ‘*Cattleya* alliance’ (Figs. 5, 6), which includes *Cattleya*, *Brassavola*, *Myrmecophila*, *Sophronitis*, and the Brazilian species of *Laelia*. Although we sampled most of

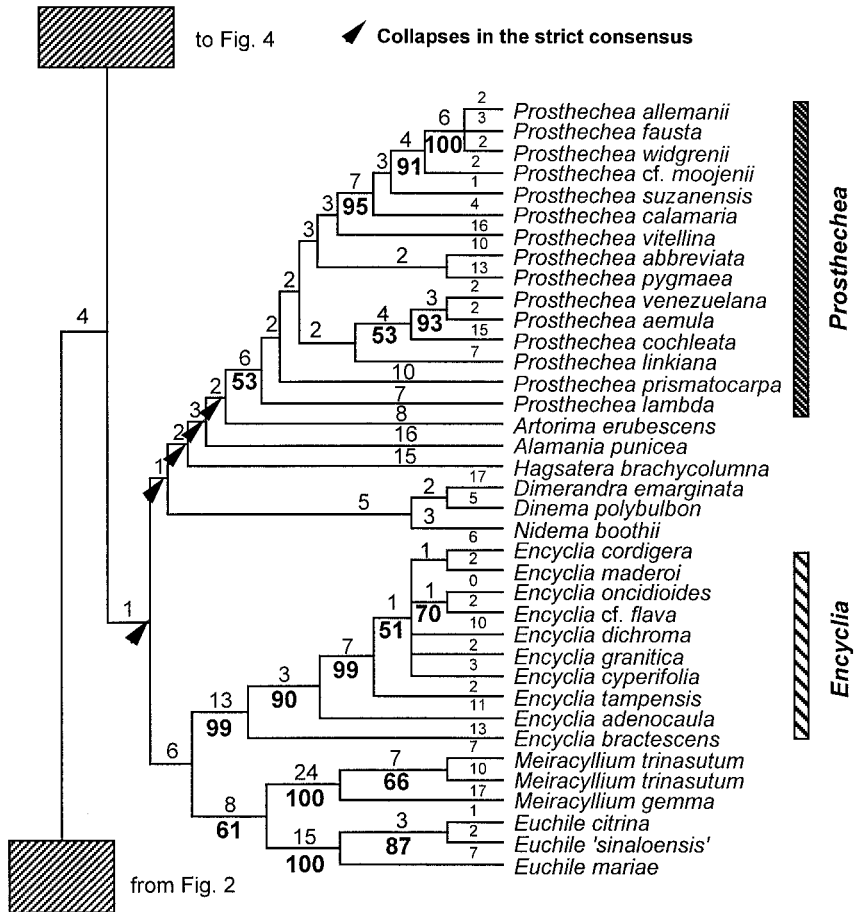


Fig. 4. *Encyclia* and related genera in the same most parsimonious tree as Figure 2.

the species in these genera, phylogeny reconstruction was made difficult by the low level of variation among species complexes, for example in *Laelia* section *Parviflorae* (Fig. 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 including the unifoliate species in one and the other composed of the Brazilian bifoliate species. However, the group of *Cattleya skinneri* (*C. skinneri*, *C. patinii*, *C. aurantiaca*) is closer to *Rhyncholaelia*, whereas *C. bowringiana* and *C. araguaiensis* occur in isolated positions. There was also an unpredicted group of unifoliate *Cattleya* species (*C. trichopiliochila*, *C. lawrenceana*, *C. lueddemanniana*) that are sister to the Brazilian species of *Laelia*, which includes also *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. 5). However, these relationships received less than 50% bootstrap support and collapse in the strict consensus.

### 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, Mishler, and Chase 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 in-

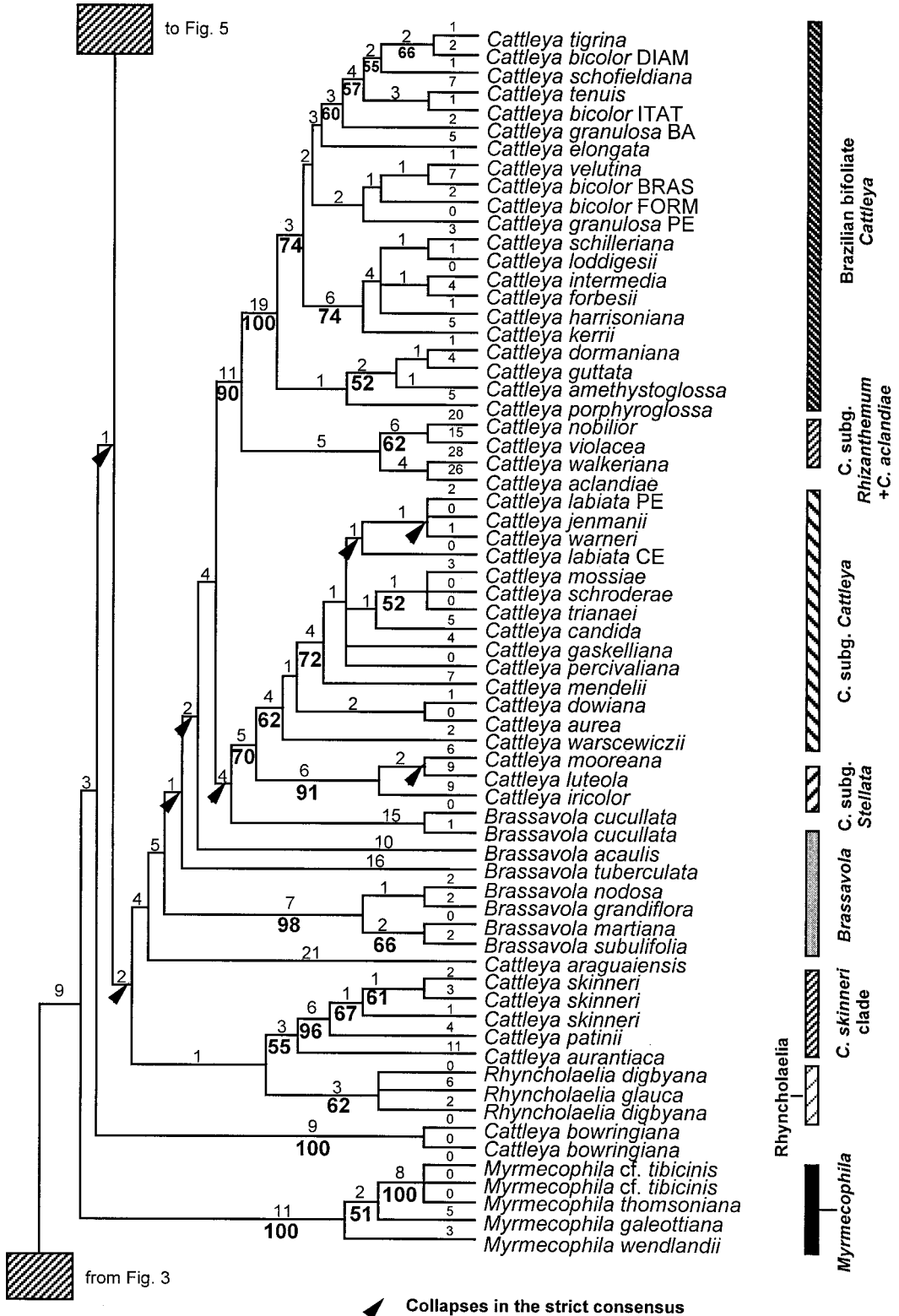


Fig. 5. *Cattleya*, *Brassavola*, *Myrmecophila*, and *Rhyncholaelia* in the same most parsimonious tree as Figure 2.

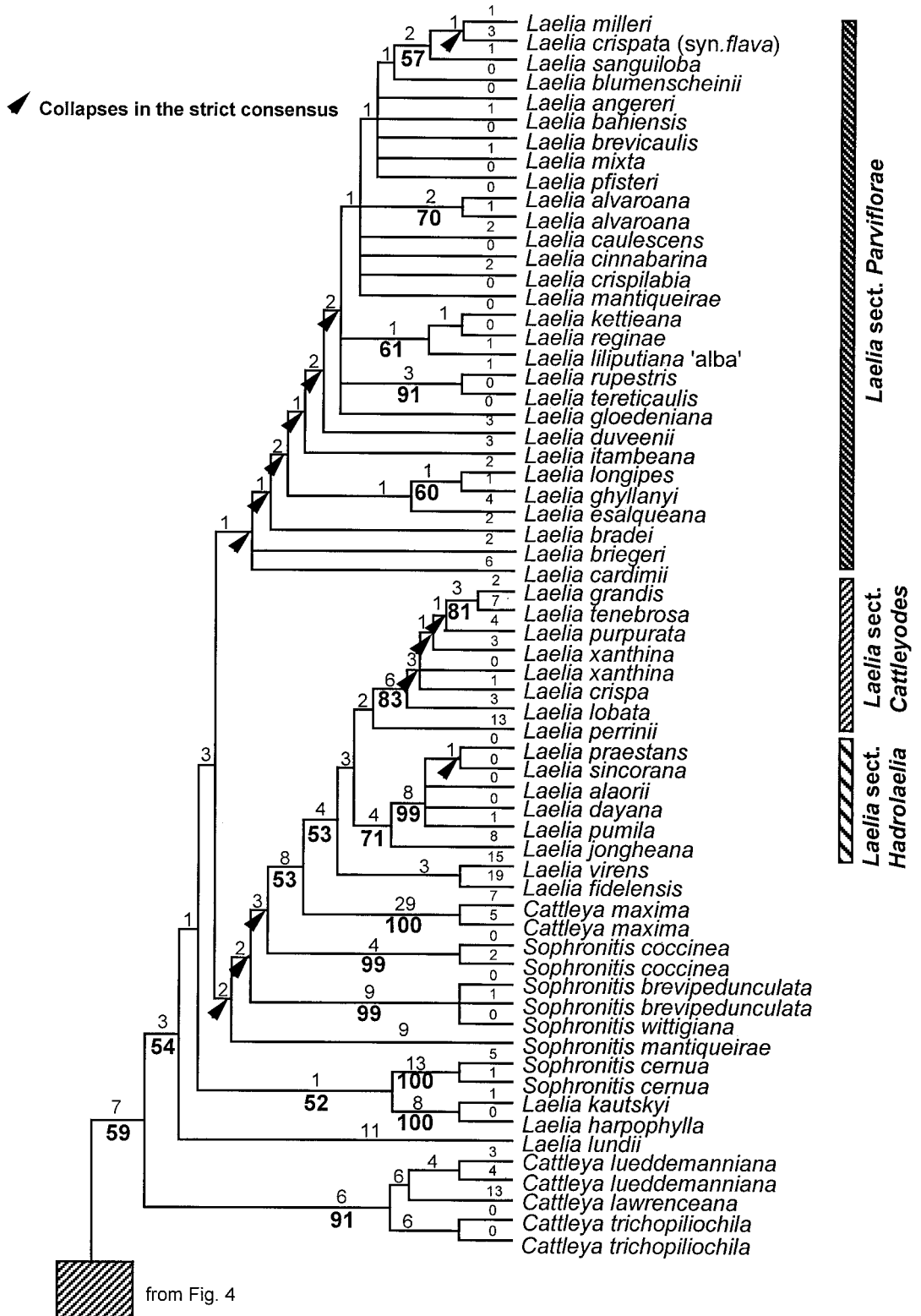


Fig. 6. *Sophronitis* and the Brazilian *Laelia* in the same most parsimonious tree as Figure 2.

cludes *Tomzania*, 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 articulation 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 must 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* disagree 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 disagree 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 (1960) and Dressler (1990) to place these genera in their own monogeneric subtribes (i.e. ovoid and clavate pollinia, respectively). 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 in *Arpophyllum*. However, it lacks as well the helical thickenings of the internal foliar tissues typical for Pleurothallidinae.

*Laelia*, *Cattleya*, *Encyclia s.l.*, and *Epidendrum* are clearly shown to be polyphyletic here. *Laelia* was suggested to be artificial by Dressler (1981,

1993) and more recently by Halbinger and Soto (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* seem to be plesiomorphies, such as the presence of eight pollinia. The same 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 like the hollow pseudobulbs of *Caularthron* and *Myrmecophila*.

It is still unclear if the montane species of *Laelia s.s.* (*L. albida*, *L. autumnalis*, *L. furfuracea*, *L. gouldiana*, and *L. speciosa*) are reasonably distinct from *L. anceps* and *L. rubescens*, but obviously the Brazilian species have to be reclassified. Because *Sophronitis* is polyphyletic and clearly embedded in them, the reasonable 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 *Hoffmansegella* (Jones, 1968), 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 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 *Hoffmansegella*. We prefer instead to incorporate all of these species in *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 are proposed in the accompanying paper by van den Berg and Chase (2000).

The placement of *C. trichopiliochila*/*C. lueddemanniana*/*C. lawrenceana* in the Brazilian *Lae-*

*lia* 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. 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 we were unable to obtain other ITS copies that would provide a more reasonable placement of these members of *Cattleya* subgenus *Cattleya*. Past hybridization events and gene conversion could be alternative explanations. Hopefully, analysis of plastid DNA sequences (now in progress) should aid in assessing the position of these species of *Cattleya*.

In a similar manner, it is clear that *Schomburgkia* and *Myrmecophila* belong to distinct clades (Figs. 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 we 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 characterized 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 sampling error as the cause of these unexpected placements. Although *C. araguaiensis* is morphologically distinct from all other species of *Cattleya*, the only 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 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* (van den Berg et al., unpubl.) *Brassavola* becomes monophyletic. With low levels of divergence, a set of species forms a grade,

whereas with more data these same taxa form a well supported clade (Sheahan and Chase, in press).

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, however, was extremely limited, and a larger study is needed to clarify the relationships. The small clade with *Orleanesia*, *Caularthron*, and *Amblostoma armeniacum* (Fig. 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 lip unfused to the column (at least *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 that we did not confirm here. Increased sampling in *Encyclia* and related genera is required, due to the large number of species (Higgins et al., unpubl.).

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.

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 by 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 sisters to *Prosthechea*, and two Mexican species of *Encyclia* (*E. bractescens*, *E. adenocaula*) are sisters to the rest of that genus. When we move to the most derived members of the subtribe, in the *Cattleya* alliance, species diversity is centered in southeastern Brazil, but always with 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 colonized twice by taxa coming from 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.

*Assessment of selected taxonomic characters in Laeliinae*—Some of the morphological characters previously emphasized 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 are *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 Bletinae, 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 *Jacquiiniella* 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 if this would be a fourth evolutionary event.

Pollinium number also shows this same sort of multiple parallelism. 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 used by ants as nesting sites. This sort of specialized 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 and Thien, 1989), but in *Caularthron* the association seems to have a protective function only (Fisher and Zimmermann, 1988).

The reed-stem habit is likely to be plesiomorphic. In many cases, it could reflect a primary primitive state: *Ponera/Isochilus/Helleriella* (Ponerinae); *Dilomilis/Neocogniauxia*, and *Jacquiiniella*. 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 (1976) to segregate *Auliza*. However, the vegetative diversity in this clade is extremely high (Pérez-García, 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 con-

stitute the only alternative large-scale study of Laeliinae. Most of the characters he studied are polymorphic in the generic groupings he proposed, and an attempt to produce a cladogram by coding these characters in addition to other morphological characters produced an unresolved polytomy (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 show extreme plasticity. The generic relationships he traced based on trends rather than a 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 *Schomburgkia 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 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.*, 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. Therefore, hybridization cannot be disregarded as a mode of speciation and a cause of conflict when trying to reconstruct phylogenies.

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