

# MOLECULAR SYSTEMATICS OF THE LAELIINAE

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**L**aeliinae is one of the largest subtribes in the Orchidaceae (1466 species; Dressler, 1993), surpassed only by Pleurothallidinae (3021 species; Dressler, 1993). Pleurothallidinae might be the sister group of Laeliinae but the boundaries between these subtribes are not clear. In addition there are two other closely related subtribes, Meiracylliinae and Arpophyllinae, established for *Meiracyllium* Reichenbach (Dressler, 1960) and *Arpophyllum* Llave & Lex. (Dressler, 1990a), both former genera of Laeliinae.

In the early classification of Laeliinae by Bentham and Hooker (1883), there were two subtribes, Stenoglossae and Laeliae, based on putative differences in the caudicles that later were found to be inaccurate. Pfitzer (1889) gathered all these genera under the subtribe Laeliinae, with two new series: *Ponereae* for the genera bearing a column foot and *Cattleyeae* for genera without this character. This idea was followed by Schlechter (1926), who changed the names to *Ponereae* and *Laelieae*.

Dressler (1961) presented a proposal for the generic relationships of *Epidendrum* L. alliance that suggested three or four basic complexes centered in *Cattleya* Lindl. and *Laelia* Lindl., *Epidendrum*, *Scaphyglottis* Poepp. & Endl. and perhaps *Sophranitis* Lindl. The genera around *Scaphyglottis* are rather similar to Schlechter's *Ponereae*, but the distinction between *Cattleya-Laelia-Encyclia* Hooker complex and the *Epidendrum* group is evident. An anatomical approach led Baker (unpublished PhD Thesis, 1972) to propose several generic relationships. He recognised a *Cattleya-Laelia* complex as well as complexes based

on *Epidendrum*, *Scaphyglottis*, *Domingoa* Schltr. and *Broughtonia* R. Br. He also concluded that *Meiracyllium* should be part of the Laeliinae but *Arpophyllum* had distinct features that excluded it from both Laeliinae and Pleurothallidinae.

Several recent systems have been proposed for the classification of Laeliinae, some keeping the group as a single subtribe (Dressler, 1993), with generic alliances (Dressler, 1990b), or splitting in more subtribes (Brieger, 1977; Szlachetko, 1995). The latter two systems were based on the choice of traditional floral characters such as presence of column foot and fusion of the column with the labellum to define the major groups. The system presented by Dressler (1990b) was largely based on the relationships suggested by Baker (unpublished thesis, 1972). However, Baker's study lacks an explicit way of analysing his data and the alliances were finally abandoned by Dressler (1993).

Finally, there has been controversy in the definition of genera in the *Cattleya* alliance. *Cattleya* and *Laelia* were traditionally kept as separate genera based only on the number of pollinia. Dressler (1993) said that this distinction is quite improbable. *Laelia* as defined now has a disjunct distribution (Mexico and eastern Brazil). Its Brazilian section *Cattleyodes* Schlechter seems to be more similar to the unifoliate *Cattleyas* rather than to rest of the genus, whereas Jones (1968) split *Laelia* section *Parviflorae* Lindley to make the genus *Hoffmansegella* H. G. Jones. Halbinger and Soto (1997), after a parsimony analysis based on morphological characters, concluded that the Brazilian sections of *Laelia* are closer to *Cattleya* than to the Mexican species, and say that the number of pollinia used for the distinction between *Laelia* and *Cattleya* causes *Laelia* to be an artificial genus. The number of pollinia has been shown not to be a good character in other orchid tribes in which it was emphasized (Chase, pers. comm.). Vegetative characters that were frequently neglected showed good correlation with the phylogenetic patterns found in Pridgeon et al. (1997).

Molecular techniques based on DNA sequencing are becoming increasingly popular to study the phylogenetic relationships in all biological groups, especially due to explicit methodology used, the possibility of studying a broad taxonomic range and the large number of characters obtained. The use of plastid DNA for systematic purposes goes back to the early work of Vedel (1976), and there are good reviews of the results in Palmer et al. (1986) and Clegg & Zurawsky (1993).

The most commonly used gene has been *rbcL* and the amount of data available culminated with a broad assessment of the angiosperm families' phylogeny in Chase et al. (1993). For the study of closely related genera, on the other hand, it is often necessary to use more variable regions, such as *matK* (Johnson & Soltis, 1995) and the *trnL-F* intron and intergenic spacer (Taberlet et al., 1991). On the species level, it is often necessary to use even more variable genes, and the ITS region (Internal Transcribed Spacers of nuclear ribosomal DNA) has been widely used in plants (Baldwin, 1992; for orchids see Cox, et al., 1997; Pridgeon, et al., 1997).

## MATERIAL AND METHODS

We conducted a very broad sampling of the subtribe, covering most known genera except *Pygmaeorchis* Brade and *Quisqueya* Dod, for which we did not manage to obtain any material. In *Cattleya*, *Laelia*, *Sophronitis*, *Rhyncholaelia* Schltr., most species known were sampled and in *Myrmecophila* Rolfe and *Brassavola* R. Br. we sampled more than 70% of the species.

We extracted DNA using a methodology modified from Doyle and Doyle (1987), mostly from fresh material but also from silica-gel dried material. The ITS region was amplified by PCR using the primers ITS4, ITS5 (Baldwin, 1992) and also AB101 and AB102 (unpublished sources). The amplified products were sequenced in an ABI 377 automated sequencer with the same primers. Sequences were edited using "Sequencher 3.0" and aligned using "Clustal W" and also manually by eye.

We performed Parsimony using PAUP version 4.0d.65 with a multiple outgroup with members of Bletiinae, Chysiinae, Coeliinae and Pleurothallidinae. The matrices were analysed using heuristic search with 500 replicates and algorithm TBR, and then swapping the trees to an upper limit of 5000. These trees were used then for successive weighting until the branch length achieved a stable number. We used 1000 bootstraps replicates to test the support of the groups found (Felsenstein, 1985). Although we are also sequencing all the genera for the plastid regions *matK* and *trnL-F*, the matrices have not been completed yet and the results will be presented elsewhere (van den Berg et al., 2000).

## RESULTS

In the ITS study, we produced >5000 equally parsimonious trees after successive weighting with 3095 steps, consistency index (CI) of 0.318 and retention index (RI) of 0.691. The consensus of these trees showing the major groups of Laeliinae is shown in Fig. 1, and the outgroup relationships in Fig 2. Although most of the nodes were present in all trees and the strict consensus is well resolved, very few nodes were well supported in the bootstrap consensus tree (not presented). However, most of the patterns found in the ITS consensus tree agree with the incomplete plastid gene phylogenies (van den Berg et al., 2000).

## DISCUSSION

The overall variation of the ITS in the Laeliinae is much lower than that found in other orchid subtribes (Cox et al., 1997; Pridgeon et al., 1997; Williams et al., unpublished data). This explains the low bootstrap values for most groups, as most internodes of the tree have very frequently only 1–2 substitutions (bootstrap values are correlated with these branch lengths). The sequencing of additional plastid genes to be combined with ITS should improve the resolution. Some patterns in the ITS dataset are very clear and also appear in the plastid phylogenies: *Arpophyllum* is the sister group of the rest of Laeliinae and the most reasonable solution is including it in the subtribe. *Meiracyllium* is also part of the Laeliinae and is closely related to *Euchile* (Dressler & Pollard) Withner. On the other hand *Isochilus* R. Br., *Ponera* Lindl. and *Helleriella* A. D. Hawkes (restricted here to *H. guerrerensis* Dressler & Hágsater and *H. nicaraguensis* A. D. Hawkes) form a distinct subtribe outside the Laeliinae (Fig. 2), that should have the name. This agrees with the anatomical work of Baker (unpublished thesis, 1972) that found *Ponera* very distinct of *Scaphyglottis* and close to *Isochilus*, that itself was put into a separate subtribe by Szlachetko (1991). The remaining species *Helleriella punctulata* (Rchb.f) Garay & H. R. Sweet belongs to *Scaphyglottis*. The genera *Dilomilis* Raf. and *Neocogniauxia* Schltr. (and presumably also *Tomzanonia* Nir) are also outside the Laeliinae as a sister group to the Pleurothallidinae, but we need more data to decide whether they belong to the latter or deserve a new subtribe.

*Encyclia* is polyphyletic, and the genera *Prosthechea* Knowles & Wesc., with new combinations

in Higgins (1997), *Dinema* Lindl. and *Euchile* (see Withner, 1998) need to be considered distinct. *Laelia* is also polyphyletic, with the Mexican species (which retain the name) very distinct from the Brazilian species, that form a clade sister to *Cattleya*.

Additionally the species of *Sophronitis* are embedded in the basis of section Parviflorae. *Myrmecophila* is related to *Cattleya* whereas *Schomburgkia* Lindl. is related to the Mexican *Laelia*. The Brazilian species of *Laelia* are imbedded in *Sophronitis* and new combinations for these species were made by van den Berg and Chase (2000). There are some nomenclatural changes in this group that should be noticed. The name *Soph. crispata* (Thunb.) van den Berg & M. W. Chase is the correct name for *L. flava* Lindl. (as shown by Miranda, 1993) and not *L. rupestris* Lindl. The name *Sophronitis mirandae* van den Berg & M. W. Chase should replace *L. rupestris* in *Sophronitis* because *Soph. rupestris* is already taken for the species known as *Constantia rupestris* (Barb. Rodr.) Cogn.

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**Table 1.** Combinations under *Sophronitis* for the species previously in *Laelia* section *Parviflorae* and *Microlaelia*

- Sophronitis* Lindley Bot. Reg. sub t. 1147 (1828)  
 Synonym: *Hoffmannseggella* H. G. Jones in *Acta Bot. Acad. Sci. Hung.* 14: 69 (1968)
- Sophronitis angereri* (Pabst) C. van den Berg  
 Basionym: *Laelia angereri* G. F. J. Pabst in *Orchid Dig.* 39(4): 153 (1975)
- Sophronitis bahiensis* (Schlechter) C. van den Berg  
 Basionym: *Laelia bahiensis* Schlechter in *Fedde, Repert.* 17: 272 (1921)
- Sophronitis blumenscheini* (Pabst) C. van den Berg  
 Basionym: *Laelia blumenscheinii* Pabst in *Bradea* 1(50): 487 (1975)
- Sophronitis bradei* (Pabst) C. van den Berg  
 Basionym: *Laelia bradei* Pabst in *Bradea* 1(31): 332 (1973)
- Sophronitis briegeri* (Blumenschein ex Pabst) C. van den Berg  
 Basionym: *Laelia briegeri* Blum. ex Pabst in *Bradea* 1 (36): 367 (1973)
- Sophronitis caulescens* (Lindley) C. van den Berg  
 Basionym: *Laelia caulescens* Lindl. *Bot. Reg.* (1841) sub t. 1. see Miranda (1994, 1998)
- Sophronitis cinnabarina* (Bateman) C. van den Berg  
 Basionym: *Laelia cinnabarina* Batem. ex Lindl. *Sert. Orch.*, t. 28 (1839)
- Sophronitis crispata* (Thunb.) C. van den Berg  
 Basionym: *Cymbidium crispatum* Thunb. *Plantarum Brasiliensium* 2: 18 (1818), see Miranda (1993)
- Sophronitis duveenii* (Fowlie) C. van den Berg  
 Basionym: *Laelia duveenii* J. A. Fowlie in *Orchid Dig.* 52(4): 180 (1988)
- Sophronitis endsfeldzii* (Pabst) C. van den Berg  
 Basionym: *Laelia endsfeldzii* Pabst in *Bradea*, 2(10): 51 (1975)
- Sophronitis esalqueana* (Blum. ex Pabst) C. van den Berg  
 Basionym: *Laelia esalqueana* Blum. ex Pabst in *Bradea* 1(36): 367 (1973)
- Sophronitis ghillanyi* (Pabst) C. van den Berg  
 Basionym: *Laelia ghillanyi* Pabst in *Bradea* 1(31): 332 (1973)
- Sophronitis gloedeniana* (Hoehne) C. van den Berg  
 Basionym: *Laelia gloedeniana* Hoehne in *Bol. Agric.*, S. Paulo, 1933, 34: 624 (1934)
- Sophronitis gracilis* (Pabst) C. van den Berg  
 Basionym: *Laelia gracilis* G. F. J. Pabst in *Bradea* 2(48): 314 (1979)
- Sophronitis harpophylla* (Reichb. f.) C. van den Berg  
 Basionym: *Laelia harpophylla* Reichb. f. in *Gard. Chron.* : 542 (1873)
- Sophronitis hispidula* (Pabst & Mello) C. van den Berg  
 Basionym: *Laelia hispidula* G. F. J. Pabst & A. F. Mello in *Bradea* 2(33): 227 (1978)
- Sophronitis itambana* (Pabst) C. van den Berg  
 Basionym: *Laelia itambana* Pabst in *Bradea* 1(31): 333 (1973)
- Sophronitis kautskyi* (Pabst) C. van den Berg  
 Basionym: *Laelia kautskyi* Pabst in *Orch. Rev.* 78 : 321 (1970) see also Zappi (1995)
- Sophronitis kettieana* (Pabst) C. van den Berg  
 Basionym: *Laelia kettieana* G. F. J. Pabst in *Bradea* 2(22): 1 (1976)
- Sophronitis liliputiana* (Pabst) C. van den Berg  
 Basionym: *Laelia liliputana* Pabst in *Bradea* 1(31): 334 (1973)
- Sophronitis longipes* (Reichb. f.) C. van den Berg  
 Basionym: *Bletia longipes* Reichb. f. *Xenia Orch.* 2: 59
- Sophronitis lundii* (Reichb. f.) C. van den Berg  
 Basionym: *Bletia lundii* Reichb. f. in *Otia Bot. Hamb.* 92
- Sophronitis milleri* (Blum ex Pabst) C. van den Berg  
 Basionym: *Laelia milleri* Blum. ex Pabst in *Bradea* 1 (36): 367 (1973)
- Sophronitis mixta* (Hoehne) C. van den Berg  
 Basionym: *Laelia mixta* Hoehne in *Arquiv. Bot. Estad. S. Paulo* 1(1): 20 (1938)
- Sophronitis pfisteri* (Pabst & Senghas) C. van den Berg  
 Basionym: *Laelia pfisteri* Pabst & Senghas in *Orchidee* 26(6): 254 (1975)
- Sophronitis reginae* (Pabst) C. van den Berg  
 Basionym: *Laelia reginae* Pabst in *Bradea* 2(6): 24 (1975)
- Sophronitis mirandai* (Lindl.) C. van den Berg (replaced homonym)  
 Basionym: *Laelia rupestris* Lindl. *Bot. Reg.* 28 (1842) sub t. 62, see Miranda (1991, 1998)
- Sophronitis sanguiloba* (C. L. Withner) C. van den Berg  
 Basionym: *Laelia sanguiloba* C. L. Withner, *Cattleyas & Relatives*, Vol. 2: 122 (1990)
- Sophronitis tereticaulis* (Hoehne) C. van den Berg  
 Basionym: *Laelia tereticaulis* Hoehne in *Arquiv. Bot. Estad. S. Paulo* 2: 163 (1952)
- Sophronitis verboonenii* (F. E. Miranda) C. van den Berg  
 Basionym: *Laelia verboonenii* F. E. Miranda in *Bradea* 6(18): 157 (1993)

Fig. 1. Consensus tree with successive weighting of a parsimony analysis using ITS sequences of Laeliinae.

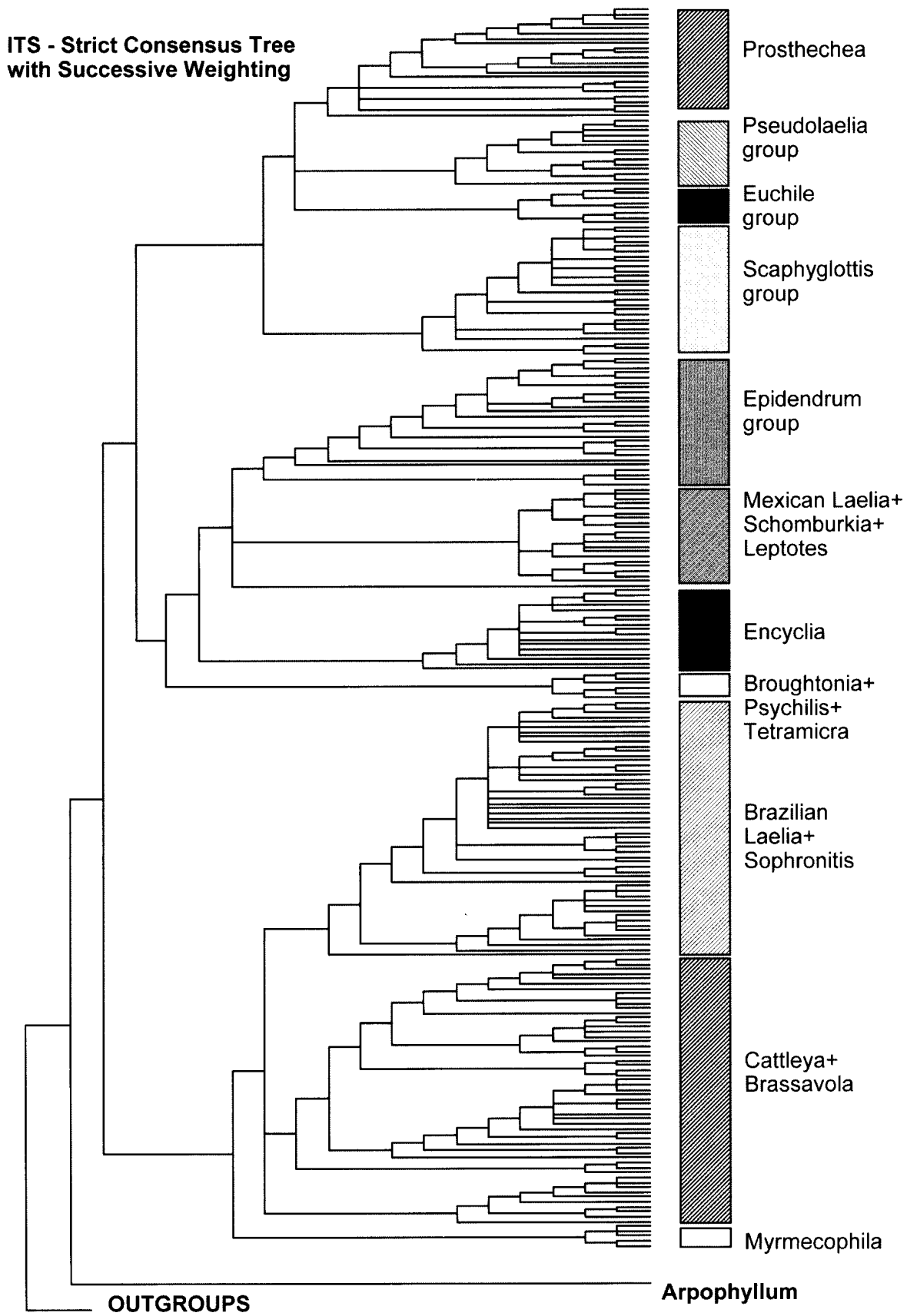


Fig 2. Outgroup relationships between Laeliinae and related subtribes in the tree shown on Fig. 1.

