

## A COMBINED MOLECULAR PHYLOGENY OF *ENCYCLIA* (ORCHIDACEAE) AND RELATIONSHIPS WITHIN LAELIINAE

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**ABSTRACT.** Nuclear (nrITS) and plastid (*matK* and *trnL-F*) DNA sequence data were used to estimate the phylogeny of Laeliinae and *Encyclia* sensu lato. The combined molecular plus indel matrix was analyzed with a successively weighted parsimony analysis. Bootstrap percentages were used to estimate support in the tree topology. The following relationships have strong bootstrap support: Meiracylliinae is shown to be embedded in Laeliinae; Ponerinae is supported as monophyletic; *Neocogniauxia* is sister to the Ponerinae plus Laeliinae clade; and *Encyclia* sensu stricto, *Euchile*, and *Dinema* are supported as monophyletic clades. The *Prosthechea* clade has strong bootstrap support. *Encyclia* sensu lato is not monophyletic. The taxonomic histories of *Anacheilium*, *Dinema*, *Encyclia*, *Epithecia*, *Euchile*, *Hormidium*, *Oestlundia*, *Osmophytum*, *Artorima*, *Hagsatera*, *Psychilis*, and *Prosthechea* are elucidated.

**Key words:** Systematics, taxonomy, nomenclature, classification, molecular, genomes

### INTRODUCTION

*Encyclia* is a neotropical genus of mostly epiphytic species. The genus has at least 421 published species names (The Plant Names Project 1999). *Encyclia* is included in the subtribe Laeliinae Benth., which consists of ca. 43 genera (Dressler 1993), although generic concepts are still in flux (van den Berg et al. 2000). Laeliinae are among the most commonly cultivated and frequently hybridized orchids (Withner 1988). The objectives of this research were to determine the position of *Encyclia* within the subtribe Laeliinae and to resolve the phylogeny of *Encyclia* sensu Dressler (1961) at the sectional level. The differences in floral morphology between the sections of *Encyclia* sensu Dressler exceed the differences between many genera in Laeliinae.

### TAXONOMIC HISTORY

*Encyclia* was described by Hooker (1828) based on a plant (*E. viridiflora* Hook.) collected by William Harrison near Rio de Janeiro, Brazil.

Taxonomic treatments of *Encyclia* represent a complex succession of invalid and misapplied nomenclature, involving the following ten generic names: *Epidendrum* L. (1763), *Encyclia* Hook. (1828), *Dichaea* Lindl. (1833), *Prosthechea* Knowles & Westc. (1838), *Psychilis* Raf. (1838), *Epithecia* Knowles & Westc. (1839), *Hormidium* Lindl. ex Heynh. (1841), *Anacheilium* Hoffmanns. (1842), *Artorima* Dressler & G.E. Pollard (1971), and *Hagsatera* R. González (1974). The result has been a problematic taxonomic classification of the genus *Encyclia* (TABLE 1).

***Encyclia*.** When Hooker (1828) described *Encyclia viridiflora*, he based the generic name on the circumstance of the column being enclosed by the lip (circumvolvo). He also stated that it would be unnatural to include this species in any of the existing genera of Epidendreae. Subsequently, Lindley sunk the genus into *Epidendrum* subgenus *Encyclium* in 1853. *Encyclia* was unused until Schlechter (1914b) revived it. Other taxonomists then started placing various taxa in the genus. Lemée (1955) inexplicably transferred five taxa from *Epidendrum* subgenus *Aulizeum* Lindl. to *Encyclia*, thereby enlarging

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TABLE 1. Comparison of recent *Encyclia* classifications.

Dressler 1961, 1971	Brieger 1977	Pabst 1981	Higgins 1997	Withner 1996, 1998
<i>Encyclia</i> subg. <i>Dinema</i>	<i>Dinema</i>	<i>Dinema</i>	<i>Dinema</i>	
<i>Encyclia</i> subg. <i>Encyclia</i>	<i>Encyclia</i>	<i>Encyclia</i>	<i>Encyclia</i>	<i>Encyclia</i>
<i>Encyclia</i> sect. <i>Encyclia</i>				
<i>Encyclia</i> sect. <i>Leptophyllum</i>				
<i>Encyclia</i> subg. <i>Osmophytum</i>			<i>Prosthechea</i>	
<i>Encyclia</i> sect. <i>Osmophytum</i>	<i>Hormidium</i>	<i>Anacheilium</i>		
<i>Encyclia</i> sect. <i>Hormidium</i>	<i>Hormidium</i>	<i>Hormidium</i>		
<i>Encyclia</i> sect. <i>Euchile</i>	<i>Hormidium</i> sect. <i>Euchile</i>	<i>Anacheilium</i> sect. <i>Euchile</i>		<i>Euchile</i>

the circumscription of *Encyclia*. Dressler (1961) circumscribed *Encyclia* describing two sections, *Encyclia* section *Encyclia* (capsule cylindrical in cross section) and *Encyclia* section *Osmophytum* (capsule 3-angled in cross section). Subsequently, Dressler (Dressler & Pollard 1971) revised the genus to include six sections and three subgenera. Pabst, Moutinho, and Pinto (1981) transferred the taxa in *Encyclia* section *Hormidium* Dressler to *Hormidium*, raising that group to generic level. Pabst, Moutinho, and Pinto (1981) also transferred some of the taxa in *Encyclia* section *Osmophytum* to *Anacheilium*.

***Prosthechea*.** Knowles and Westcott (1838) first published *Prosthechea* to describe the species *P. glauca*. In the following year, however, they changed the generic name to *Epithecia*, because they felt that *Prosthechea* was too similar to another unspecified generic name (Knowles & Westcott 1839). Examination of the generic names published in Index Kewensis revealed the very similar generic name *Prosthesis* (Violaceae) that Blume previously had published (Blume 1826, Royal Botanic Gardens Kew 1993). This may be the unspecified name that induced Knowles and Westcott to change the generic name from *Prosthechea* to *Epithecia*. Because *Prosthechea* is not a homonym of *Prosthesis*, the original publication remains valid according to the International Code of Botanical Nomenclature (Greuter et al. 2000). *Epithecia* was superfluous since *Prosthechea* had been validly published and should have been accepted by the authors. The derivation of the name *Prosthechea* is from the Greek word prostheke (an appendage), in reference to the appendage of tissue on the back of the column of *P. glauca*. *Prosthechea* has been resurrected by the author (Higgins 1997, 1999), and species in *Encyclia* subgenus *Osmophytum* have been renamed *Prosthechea*.

***Euchile*.** Taxa in this group have been assigned to *Sobralia*, *Cattleya*, *Epidendrum*, *Encyclia*, *Hormidium*, or *Prosthechea* at various

times. *Encyclia* section *Euchile* was described by Dressler and Pollard (1971) for the taxa with anomalous lip morphology in *Encyclia* subgenus *Osmophytum*. Withner (1998) raised the sectional name to generic status because of the atypical pattern of leaf epidermal cells. Higgins (1997) considered the two species in this group as members of *Prosthechea*.

***Oestlundia*.** The genus *Oestlundia* W.E. Higgins (2001) was established for four members of *Encyclia* subgenus *Encyclia* section *Leptophyllum* Dressler & G.E. Pollard. The combination of narrow grass-like leaves, four pollinia, a horizontal rostellum, and wing-like lateral teeth on the column, along with an unlobed lip markedly adnate to the column bearing fleshy ridges/papillae, is unique in the subtribe Laeliinae. Dressler included *Encyclia subulatifolia* (A. Rich. & Gal.) Dressler in this section, but *Microepidendrum subulatifolium* (A. Rich. & Gal.) W.E. Higgins is not a member of this genus.

***Microepidendrum*.** Higgins (2002) validated this Brieger genus for a diminutive species that appears to be a small reed-stem *Epidendrum* with terete leaves but with a flower unlike *Epidendrum* or *Encyclia*. This species, which is found growing in the dry oak forests of Mexico, has no close allies in subtribe Laeliinae; and the other species placed in *Microepidendrum* by Brieger are not closely related to *M. subulatifolium*.

***Psychilis*.** The genus *Psychilis* was described by Rafinesque (1838) based on *P. amena*, but the generic name was ignored; and most members of *Psychilis* have been considered *Encyclia*. Britton and Wilson (1930) transferred *Epidendrum krugii* Bello to *Encyclia*; Schrenk (1977) transferred *Epidendrum bifidum* Aubl. to *Encyclia*; Beckner (1970) transferred *Epidendrum olivaceum* Cogn. and *Epidendrum truncatum* Cogn. to *Encyclia*; Dod (1983) described *Encyclia vernicosa* and transferred *Epi-*

*dendrum cogniauxii* L.O. Williams, *Epidendrum buchii* Cogn., and *Epidendrum domingense* Cogn. to *Encyclia*; and Ackerman (1987) transferred *Epidendrum kraenzlinii* Bello to *Encyclia*. Sauleda (1988), however, reestablished the genus and transferred all the above to *Psychilis*.

**Dichaea.** When Swartz (1788) described the taxon *Epidendrum glaucum*, he placed it in *Epidendrum* sensu Linnaeus. Lindley (1831) transferred the taxon to *Dichaea glauca* (Sw.) Lindl., thus establishing the genus *Dichaea*. Inexplicably, Schlechter (1914a, 1915) attempted to revive *Epithecium* by transferring 20 *Dichaea* taxa to the genus *Epithecium*, which is a synonym of *Prosthechea* Knowles & Westc. Schlechter (1915) listed *Dichaea glauca* (Sw.) Lindl. as a synonym for *Epithecium glauca* of Knowles and Westcott. This was incorrect because *Dichaea* was based on *Epidendrum glaucum* Sw. and not on *Epidendrum glaucum* (Knowles & Westc.) Lindl. Schlechter probably was confused by Lindley's treatment of *Epidendrum glaucum* (Knowles & Westc.) Lindl. rather than by *Epidendrum glaucum* Sw. All of the species placed in *Epithecium* by Schlechter are placed correctly in the genus *Dichaea* (Zygopetalinae, Maxillarieae), which belongs to a completely different tribe. In any case, Schlechter's revision must be rejected because *Epithecium* is a superfluous name (Greuter et al. 2000).

**Artorima.** This genus was described by Dressler and Pollard (1971) for *Encyclia erubescens* (Lindl.) Schltr. (Schlechter 1914c), because the retrorse, hook-like callus is unlike that of any known genus. Brieger (1977b) invalidly transferred *Epidendrum kermesinum* Lindl. to *Artorima*. Pedro Ortiz (1995) then inexplicably transferred that species to *Encyclia kermesina* (Lindl.) Ortiz.

**Hagsatera.** Roberto González (1974) described this monotypic genus for *Epidendrum brachycolumna* L.O. Williams, which had been placed into *Encyclia* by Dressler (1961). *Hagsatera* has a short column and eight pollinia, atypical of either *Encyclia* or *Epidendrum*.

**Dinema.** The genus *Dinema* was established when Lindley (1831) made the combination *D. polybulbon* (Sw.) Lindl. The taxon had been described originally as *Epidendrum polybulbon* by Swartz (1788). Dressler (1961) transferred the taxon to *Encyclia*; and the *Encyclia* subgenus *Dinema* (Lindley) Dressler & Pollard was established because the taxon did not fit into the other subgenera (Dressler & Pollard 1974).

**Anacheilium.** Following the publication of *A. cochleatum* (L.) Hoffmanns. in 1842, the ge-

neric name had only been used for one other taxon, *A. fragrans* (Sw) Acuña (Acuña Galé 1938), until it was applied to the species of *Encyclia* section *Osmophytum* by Pabst, Moutinho, and Pinto (1981). *Anacheilium*, however, does not have priority for this group of taxa, because it includes *Prosthechea glauca* Knowles & Westc. Acuña Galé accepted the genus *Anacheilium* Rchb. ex Hoffmanns. placing *Epidendrum fragrans* Sw. in the genus along with *A. cochleatum* (L.) Hoffmanns. (Hoffmannsegg 1842).

**Hormidium.** After the description of *H. uniflorum* (Lindl.) Heynh. in 1841, the generic name was unused until revived by Cogniaux (1898). Schlechter (1914a, 1915), Brieger, and Hunt (Brieger & Hunt 1969, Brieger 1977a) already had begun transferring plants into the genus, when Pabst, Moutinho, and Pinto (1981) presented their revision of *Hormidium*. Although the genus *Hormidium* Lindl. ex Heynh. is validly published (Dressler 1970), this group of more than 100 species, treated as *Hormidium* by Brieger (Brieger & Hunt 1969), includes *Prosthechea glauca*; and therefore the generic name *Prosthechea* has priority over *Hormidium*. Additionally, *Hormidium* has been used as an algal name.

**Osmophytum.** *Epidendrum* section *Osmophytum* was established by Lindley (1839) for plants with usually scented flowers. Subsequently, Lindley (1840) transferred *Prosthechea glauca* to *Epidendrum*, making the combination *E. glaucum* (Knowles & Westc.) Lindl. Thus recognizing *Epithecium glauca* Knowles & Westc. as a synonym, he placed it in *Epidendrum* section *Osmophytum* (Lindley 1840). This combination is illegitimate, based on its being a later homonym of *E. glaucum* Sw., which was transferred to *Dichaea* by Lindley himself. *Prosthechea glauca* subsequently was transferred to *Encyclia* as *E. glauca* (Knowles & Westc.) Dressler & G.E. Pollard and assigned to *Encyclia* subgenus *Osmophytum* (Lindl.) Dressler (Dressler & Pollard 1971).

## APPROACH

The use of DNA sequencing for phylogenetic studies has become a mainstream tool for Orchidaceae. Current techniques with appropriate selection of DNA for the taxonomic level being studied have proven successful (Chase & Palmer 1989). For example, ITS sequences have been extremely valuable in evaluating monophyly at generic level and below in Cypripedioideae (Cox et al. 1997), Disinae (Douzery et al. 1999), and Pogoniinae (Cameron & Chase 1999). The

*matK* gene has been used to determine relationships in Gastrodieae and Neottieae (Kores et al. 2000). Phylogenetic relationships in *Disa* have been studied using *trnL-F* sequences (Bellstedt et al. 2001). Analyses that combine DNA sequences from different regions and genomes are giving better resolution than are single gene sets. Phylogenetic examples in Orchidaceae at the subtribal level and below include Oncidiinae (Williams et al. 2001), Stanhopeinae (Whitten et al. 2000), Catasetinae (Pridgeon & Chase 1998), Pleurothallidinae (Pridgeon et al. 2001), Diurideae (Kores et al. 2001), Arethuseae (Goldman et al. 2001), Coelogyne (Gravendeel et al. 2001), and Bifrenaria (Koehler et al. 2002). This study uses DNA sequences from both the nuclear and plastid genomes in a combined analysis.

### Nuclear Genome

Sequencing ITS regions has provided a good source of nuclear DNA characters for inferring intrageneric and intergeneric evolutionary relationships in many plant groups (Baldwin et al. 1995), and previous studies suggest it also will be useful in *Encyclia*. The study of intrageneric relationships requires DNA sequences of adequate size and fast evolutionary rate (nucleotide variation) (Nickrent et al. 1994). The ITS regions of rDNA have been shown to evolve at rates appropriate for examining diverging lineages (Baldwin 1992). The ubiquity of rDNA and available techniques for rapid determination of the nucleotide sequence make rDNA a good tool for inferring evolutionary relationships, except in cases of hybridization (Hamby & Zimmer 1992). In hybrids, the nuclear genome is a recombination of DNA from both parents. Thus hybrid ITS sequences can be polymorphic. The nuclear genes that code for ribosomal DNA are arranged in a tandemly repeated unit found in high and variable copy number (Rogers & Bendich 1987). The functioning regions are highly conserved because of selective pressures, while the spacer regions that fail to code for a functional RNA are not subject to the same selective pressures. The spacer regions are not highly conserved and contain species-specific variation (Hamby & Zimmer 1992). In these internal transcribed spacer (ITS 1 & 2) regions, the number of substitutions is typically twice as large between genera as within genera (Savard et al. 1993). Thus ITS regions are valuable for taxonomic studies at subgeneric levels in some taxa.

### Plastid Genome

Plastid DNA is a relatively abundant component of total plant DNA with a conservative rate

of nucleotide substitution (Palmer et al. 1988). The chloroplast genomes of photosynthetic land plants are circular DNA molecules ranging from 120 to 217 kilobase pairs. The genome contains two large inverted repeats that separate the large and small copy regions (Palmer 1986). Expansions or contractions of the inverted repeat regions are largely responsible for variations in the molecular size of the genome. Both strands of the chloroplast genome are actively expressed. Recombination does not play a major role in cpDNA evolution, where biparental transmission is rare, and intraspecific diversity is low. Chloroplast DNA provides uniparental (usually maternal) phylogenetic markers (Soltis et al. 1992). The types of mutations found in DNA include: nucleotide rearrangements, point mutation substitutions, insertions, and deletions. Studies of combined plastid DNA have been useful in cladistic analyses of other petaloid monocotyledons (Meerow et al. 1999). The *trnL-F* region and *matK* gene were chosen for this study because they have appropriate levels of variation (mutation).

### The *trnL-F* Region

The DNA that encodes for the transfer RNA for leucine is designated as *trnL*. The region of the chloroplast genome spanning the area from the *trnL* 5' exon to the *trnF* 5' exon is defined as the *trnL-F* (UAA) intron sequence (Taberlet et al. 1991). This non-coding region displays one of the highest frequencies of mutation in the chloroplast genome (Palmer et al. 1988). Additionally, length-mutation indels (insertions/deletions) provide parsimony-informative characters (McDade & Moody 1999). The *trnL-F* sequences have proven useful in the phylogenetic analysis at the generic level (Gielly et al. 1996). Researchers at the Jodrell Laboratory, Royal Botanic Gardens Kew, have found the *trnL-F* region to be useful in the resolution of intrageneric relationships (Molvray et al. 1999). This region provided an intermediate level of resolution within Laeliinae.

### The *matK* Gene

The *matK* gene encodes an RNA maturase involved in splicing introns from transcripts. This region is located between the 5' and 3' exons of the transfer RNA gene for lysine. The *matK* gene has proven useful in resolving relationships in Saxifragaceae (Johnson & Soltis 1995) and Ericaceae (Kron & Judd 1997, Kron et al. 1999). Indels in *matK* sequence data provide additional support for clades in *Saxifraga* (Soltis et al. 1996). This region provided limited deeper resolution within the Laeliinae phylogeny.

## MATERIALS AND METHODS

The selection of materials is as important as the methods used for the study. Taxa selection and gene selection can greatly affect resolution of the analysis. DNA and sequence processing describes the techniques used to obtain the phylogenetic tree.

### Taxa Selection

The ingroup taxa (35 specimens) were selected to represent all sections of *Encyclia* sensu Dressler (Dressler 1961, Dressler & Pollard 1971, see TABLE 1). The type species for each section was sequenced when possible; however, the type for the genus (*Encyclia* section *Encyclia*), *E. viridiflora*, has never been recollected and may be extinct. Specimens have been chosen to include as much geographic variation as possible from Florida, Mexico, Brazil, and the Caribbean. To account for variation in floral morphology and biology, we included species with resupinate and non-resupinate flowers, as well as wasp and bee pollinated species. Four species were sampled from two separate individuals to confirm their position in the topology: *Dinema polybulbon* (Sw.) Lindl., *Microepidendrum subulatifolium* (A.Rich & Gal.) W.E. Higgins, *Euchile mariae* (Ames) Withner, and *Oestlundia luteorosea* (A.Rich. & Gal.) W.E. Higgins.

A large outgroup was required because *Encyclia* sensu lato (s.l.) may not be monophyletic (van den Berg et al. 2000). The outgroup taxa (42 specimens) were selected from the subtribe Laeliinae and sister subtribes within Epidendreae based on the affinities proposed by Dressler (1993) and a plastid analysis of Orchidaceae (Cameron et al. 1999). Three taxa not in Laeliinae were used as an outgroup for the subtribe. *Meiracyllium trinasutum* Rchb.f. (subtribe Meiracylliinae) was chosen as an outgroup because of a velamen type that suggests a close alliance to the Laeliinae (Dressler 1993). *Pleurothallis racemiflora* Lindl. ex Lodd. and *Restrepiella ophioccephala* (Lindl.) Garay & Dunsterv. (subtribe Pleurothallidinae) were selected because the *Pleurothallis* seed type was present in *Ponera*, a member of Laeliinae (Dressler 1993). Outgroups (TABLE 2) were chosen to include species from several different clades of Laeliinae (van den Berg et al. 2000) to account for the variation within the subtribe.

### Gene Selection

We attempted to clarify these generic and subtribal relationships by performing parsimony

analyses of sequence data from three regions: *matK* (plastid); *trnL* intron and *trnL-F* spacer—hereafter treated as a single matrix designated *trnL-F* (plastid); and nuclear ribosomal DNA (nrDNA) ITS 1, 5.8S, and ITS 2—hereafter referred to as ITS. The *matK* gene codes for a maturase ~1550 bp in length and several times more variable than *rbcL* in most angiosperms (Soltis & Soltis 1998). The *trnL-F* region (Taberlet et al. 1991) is largely noncoding and consists of an intron in the *trnL* (UAA) gene and the *trnL-trnF* (GAA) intergene spacer. The ITS region (Baldwin et al. 1995) consists of two noncoding spacer regions flanking the 5.8 S gene and is widely used for phylogenetic studies. Recent studies have indicated that combined molecular data sets using regions with different levels of variation provide resolution at different areas of the cladogram. They also indicate that phylogenetic resolution and levels of support are improved by directly combining independent molecular data sets (Chase & Cox 1998, Soltis et al. 1998). Additionally, length-mutation indels can provide parsimony-informative characters (McDade & Moody 1999). Indels in the plastid sequences were coded by hand and included in the combined matrix.

### DNA Processing

Total DNA was extracted from fresh or silica-dried leaves using a modification of the Doyle and Doyle (1987) protocol, and stored at  $-20^{\circ}\text{C}$ . The DNA regions of interest were amplified using the polymerase chain reaction (PCR). The amplification annealing temperatures and primers for each region were: ITS  $72^{\circ}\text{C}$ , 17SE (Forward), and 26SE (Reverse) (Sun et al. 1994); *trnL-F*  $58^{\circ}\text{C}$ , C(F), and F(R) (Taberlet et al. 1991); and *matK*  $51^{\circ}\text{C}$ , 56(F) and 1520(R) (Whitten et al. 2000). Betaine (N,N,N-trimethylglycine) was added to the PCR reactions to relax DNA secondary structure and enhance amplification (Frackman et al. 1998). The Applied Biosystems, Inc. (ABI) Big Dye<sup>TM</sup> Terminator Kit was used for cycle sequencing, and products were run on an ABI 377 Sequencer. Detailed protocols can be found in Higgins (2000).

### Sequence Data Processing

The electropherograms were trimmed and edited using Sequence Navigator 1.01 (Applied Biosystems 1994a). Sequence fragments were then combined to produce a continuous sequence using AutoAssembler 1.30 (Applied Biosystems 1994b). The consensus sequence was exported as a text file and placed in a NEXUS matrix (Maddison et al. 1997). The sequences

TABLE 2. GenBank Accession Numbers.

Taxon	ITS	matK	trnL-F
<i>Acrorchis roseola</i> Dressler	AY008521	AY396086	AY422389
<i>Alamania punicea</i> La Llave & Lex.	AF260177	AY425801	AY422452
<i>Artorima erubescens</i> (Lindl.) Dressler & G.E. Pollard	AF260178	AY425798	AY422449
<i>Brassavola cucullata</i> (L.) R.Br.	AY008589	AY396097	AY422400
<i>Broughtonia negrilensis</i> Fowlie	AY008569	AY396093	AY422396
<i>Cattleya dowiana</i> Batem.	AF260210	AY396100	AY422403
<i>Cattleya forbesii</i> Lindl.	AY429394	AY396102	AY422405
<i>Cattleyopsis lindenii</i> Cogn.	AY008570	AY396096	AY422399
<i>Dimerandra emarginata</i> (G. Mey.) Hoehne	AF260179	AY425795	AY422446
<i>Dinema polybulbon</i> (Sw.) Dressler	AY429398	AY396109	AY422412
<i>Dinema polybulbon</i> (Sw.) Dressler	AY429399	AY396110	AY422413
<i>Domingoa kienastii</i> (Rchb.f.) Dressler	AY008564	AY396095	AY422398
<i>Domingoa nodosa</i> (Cogn.) Schltr.	AY008565	AY425794	AY422445
<i>Encyclia adenocaula</i> (Llav & Lex.) Schltr.	AY008526	AY396111	AY422414
<i>Encyclia asperula</i> Dressler & G.E. Pollard	AY429403	AY396119	AY422422
<i>Encyclia bractescens</i> (Lindl.) Hoehne	AY008527	AY396112	AY422415
<i>Encyclia candollei</i> (Lindl.) Schltr.	AY429404	AY396120	AY422423
<i>Encyclia cordigera</i> (Kunth) Dressler	AY008528	AY396114	AY422417
<i>Encyclia dichroma</i> (Lindl.) Schltr. in Schlechter	AY008530	AY396117	AY422420
<i>Encyclia diurna</i> Schltr. in Fedde	AY429402	AY396118	AY422421
<i>Encyclia incumbens</i> (Lindl.) D.J. Mabberley	AY429400	AY396113	AY422416
<i>Encyclia kienastii</i> (Rchb.f.) Dressler & G.E. Pollard	AY429406	AY396122	AY422425
<i>Encyclia</i> aff. <i>oncidioides</i> (Lindl.) Schltr.	AY429386	AY425802	AY422453
<i>Encyclia randii</i> (Barb. Rodr.) Pôrto & Brade	AY429405	AY396121	AY422424
<i>Encyclia tampensis</i> (Lindl.) Small	AY429401	AY396115	AY422418
<i>Epidendrum ibaguense</i> Pavon ex Lindl.	AY008505	AY396079	AY422382
<i>Epidendrum conopseum</i> R. Br. in Ait.	AY008506	AY396080	AY422383
<i>Euchile citrina</i> (La Llave & Lex.) Dressler	AY008501	AY396106	AY422409
<i>Euchile mariae</i> (Ames) Withner	AY429396	AY396107	AY422410
<i>Euchile mariae</i> (Ames) Withner	AY429397	AY396108	AY422411
<i>Hagsatera brachycolumna</i> (L.O. Williams) R. González	AY008515	AY396088	AY422391
<i>Hexadesmia crurigera</i> Lindl.	AF260180	AY396085	AY422388
<i>Hexisea imbricata</i> (Lindl.) Rchb.f.	AY429388	AY396083	AY422386
<i>Homalopetalum pumilio</i> (Rchb.f.) Schltr.	AY429389	AY396089	AY422392
<i>Isochilus major</i> Cham. & Schltdl.	AY008481	AY396078	AY422381
<i>Jacquinella teretifolia</i> (Sw.) Britton & P. Wilson	AY008519	AY396087	AY422390
<i>Laelia rubescens</i> Lindl.	AY429391	AY396098	AY422401
<i>Laeliopsis domingensis</i> (Lindl.) Lindl. & Paxton	AF260187	AY425797	AY422448
<i>Meiracyllium trinasutum</i> Rchb.f.	AY429390	AY396090	AY422393
<i>Microepidendrum subulatifolium</i> (A. Rich & Gal.) W.E. Higgins	AY429416	AY396137	AY422440
<i>Microepidendrum subulatifolium</i> (A. Rich & Gal.) W.E. Higgins	AY429417	AY396138	AY422441
<i>Myrmecophila tibicinis</i> (Batem.) Rolfe	AY429392	AY396099	AY422402
<i>Neocogniauxia hexaptera</i> (Cogn.) Schltr.	AY429385	AY425793	AY422444
<i>Nidema boothii</i> (Lindl.) Schltr.	AY008522	AY396081	AY422384
<i>Oestlundia cyanocolumna</i> (Ames, F.T. Hubb. & C. Schweinf.) W.E. Higgins	AY429418	AY396139	AY422442
<i>Oestlundia distantiflora</i> (Rich. & Gal.) W.E. Higgins	AY429387	AY425803	AY422454
<i>Oestlundia luteorosea</i> (Rich. & Gal.) W.E. Higgins	AY429414	AY396135	AY422438
<i>Oestlundia luteorosea</i> (Rich. & Gal.) W.E. Higgins	AY429415	AY396136	AY422439
<i>Oestlundia tenuissima</i> (Ames, Hubb. & Schweinf.) W.E. Higgins	AY429419	AY396140	AY422443
<i>Orleanesia amazonica</i> Barb. Rodr.	AF260176	AY425799	AY422450
<i>Orleanesia pleurostachys</i> (Linden & Rchb.f.) Garay & Dunst.	AY008525	AY425800	AY422451
<i>Pleurothallis racemiflora</i> Lindl. ex Lodd.	AY008477	AY396076	AY422379
<i>Prosthechea abbreviata</i> (Schltr.) W.E. Higgins	AF260181	AY425804	AY422455
<i>Prosthechea aemula</i> (Lindl.) W.E. Higgins	AY008544	AY396125	AY422428
<i>Prosthechea chimborazonensis</i> (Schltr.) W.E. Higgins	AY429407	AY396123	AY422426
<i>Prosthechea cochleata</i> (L.) W.E. Higgins	AY008545	AY396126	AY422429

TABLE 2. Continued.

Taxon	ITS	matK	trnL-F
<i>Prosthechea cretacea</i> (Dressler & G.E. Pollard) W.E. Higgins	AY429413	AY396134	AY422437
<i>Prosthechea fragrans</i> (Sw.) W.E. Higgins	AY429408	AY396124	AY422427
<i>Prosthechea glauca</i> Knowles & Westc.	AY429410	AY396130	AY422433
<i>Prosthechea ionocentra</i> (Dressler) W.E. Higgins	AY429411	AY396131	AY422434
<i>Prosthechea ochracea</i> (Lindl.) W.E. Higgins	AY429412	AY396133	AY422436
<i>Prosthechea prismatocarpa</i> (Rchb. f) W.E. Higgins	AY008548	AY396132	AY422435
<i>Prosthechea pseudopygmaea</i> (Finet) W.E. Higgins	AY429409	AY396128	AY422431
<i>Prosthechea pygmaea</i> (Hook.) W.E. Higgins	AY008546	AY396127	AY422430
<i>Prosthechea vitellina</i> (Lindl.) W.E. Higgins	AY008547	AY396129	AY422432
<i>Ponera striata</i> Lindl.	AY008484	AY396077	AY422380
<i>Psychilis mcconnelliae</i> Saulea	AY008568	AY396091	AY422394
<i>Psychilis krugii</i> (Bello) Saulea	AF260157	AY396092	AY422395
<i>Quisqueya ekmanii</i> Dod	AY008567	AY425796	AY422447
<i>Reichenbachanthus cuniculatus</i> (Schltr.) Pabst.	AY008551	AY396084	AY422387
<i>Restrepiella ophiocephala</i> (Lindl.) Garay & Dunsterv.	AF262909	AY396075	AY422378
<i>Rhyncholaelia glauca</i> (Lindl.) Schltr.	AY429393	AY396101	AY422404
<i>Scaphyglottis pulchella</i> (Schltr.) L.O. Williams	AY008559	AY396082	AY422385
<i>Schomburgkia splendida</i> Schltr.	AY008573	AY396105	AY422408
<i>Sophranitis cernua</i> Lindl.	AY429395	AY396103	AY422406
<i>Sophranitis purpurata</i> (Lindl. & Paxton) van den Berg & M.W. Chase	AY008641	AY396104	AY422407
<i>Tetramicra elegans</i> (Hamilt.) Cogn.	AY008566	AY396094	AY422397

were manually aligned in the matrix, and 16 indels in the *trnL-F* and *matK* regions were coded as present or absent. The matrix was analyzed using Fitch (1971) parsimony and ACCTRAN character optimization with the PAUP\* 4.0b3a software (Swofford 1998). An initial heuristic search of 1000 random addition replicates was used to identify islands of shortest trees. This search used equal-weighted, unordered characters, and the subtree pruning-regrafting (SPR) branching swapping algorithm, saving 10 trees per replicate. These trees were then used as initial trees and swapped to completion using the tree bisection-reconnection (TBR) algorithm to locate all possible phylogenetic trees, saving only the shortest length trees.

In the combined matrix only, the resulting set of trees was used to reweight the characters based on the Retention Index (RI) values for each character (Farris 1969) using a base weight of 1. The 1000 replicate heuristic search (SPR, 10 trees) was repeated, and the characters reweighted until the tree length stabilized. The trees identified in the reweighting procedure were swapped to completion using TBR. The support for the tree topology was evaluated with the full heuristic bootstrap algorithm of 1000 replicates using the equally weighted character values, 100 sub-replicates saving 10 trees per replicate, and nearest neighbor interchange (NNI) branch swapping (Felsenstein 1985). The bootstrap analysis was accomplished using equally weighted characters, because the use of fixed

weights derived by successive weighting leads to inflated bootstrap values (Tholleson 2000).

## RESULTS

The results of the individual gene region analyses provided limited resolution and thus are discussed but not presented. The combined analysis produced a well-supported topology.

### Individual Genomes

The statistics for the individual analyses are presented in TABLE 3. The bootstrap consensus trees for the ITS, *trnL-F*, and *matK* regions do not conflict, i.e., the supported clades do not have topologies that disagree between consensus trees. The three gene regions proved useful at different levels in the phylogeny: ITS being most variable was informative at the species level; *trnL-F* provided an intermediate level of resolution; and the *matK* region provided limited deeper resolution within the Laeliinae phylogeny. Several differences occur between the supported phylogenies of the nuclear and plastid genomes. *Encyclia* s.s. is supported by the ITS and *trnL-F* regions, *Prosthechea* is supported by the *trnL-F* region, *Dinema* is supported by the *trnL-F* region and *matK* gene, and *Euchile* is supported by the ITS and *matK* gene.

TABLE 3. Statistics from PAUP\* analyses of separate and combined data matrices for the combined data set of 77 taxa in the Laeliinae multi-sequence analysis. SW = successively weighted statistics.

	ITS	<i>trnL-F</i>	<i>matK</i>	Combined + indels	Combined SW
No. included positions in matrix	732	1284	1367	3741	3741
No. variable sites	352	369	364	1201	1201
No. phylogenetically informative sites	223	163	175	593	593
Sites weighted <1	0	0	0	0	404
No. of trees (Fitch)**	10000	10000	10000	3780	9
No. of steps	1175	601	647	2625	1581 (Fitch 2625)
CI	0.45	0.71	0.66	0.58	0.72
CI excluding non-informative sites	0.37	0.53	0.48	0.43	0.50
RI	0.55	0.68	0.66	0.59	0.71
RC	0.25	0.48	0.43	0.34	0.51
Ave. no. changes per variable site*** (no. steps/no. var. sites)	4.7	2.3	2.6	3.3	2.7

\* PAUP and Other Methods.

\*\* Search limited to 10,000 trees.

\*\*\* Calculated with one of the SW trees from the combined analysis.

### Combined Analysis

The aligned DNA regions plus indel matrix had 3741 total characters of which 68% (2540) characters were constant, 16% (608) variable characters were parsimony-uninformative, and 16% (593) were parsimony-informative characters. The following synapomorphic indels were found in the Laeliinae matrix: in the *trnL-F* region, there is a 324-base deletion in subtribe Laeliinae; a 17-base deletion in *Encyclia* s.s.; a 10-base insertion, and an 11-base deletion in *Encyclia* s.s., with the exception of *E. cordigera* (Kunth) Dressler; a 7-base insertion in *Hormidium*; a 7-base and a 10-base insertion in *Orleanesia*, and a 4-base insertion and 8-base deletion in *Psychilis*. In the *matK* gene, there is a 6-base insertion in Caribbean clade of *Psychilis*, *Tetramicra*, *Quisqueya*, *Broughtonia*, *Laeliopsis*, and *Cattleyopsis*. The initial heuristic search identified 1354 shortest trees with a length of 2625 steps. These trees were swapped to completion, and 3780 equally parsimonious trees were retained. The parsimony tree scores are presented in TABLE 3. Bootstrap percentages shown in FIGURES 1 and 2 were determined using an equally weighted analysis.

After three rounds of successive reweighting, nine trees with a weighted length of 1581 steps were identified and swapped to completion, in contrast to Fitch's 2625 steps. The parsimony tree scores are presented in TABLE 3. One of the nine trees was randomly selected as an example. The selected tree (FIGURES 1 & 2) has Fitch branch lengths in character steps above the line and bootstrap values below, and the nodes that collapse in the strict consensus are indicated

with an arrow (◄). Bootstrap indexes were limited to values greater than 50%.

The placement of several clades in the combined analysis is informative regarding the phylogeny of Laeliinae. Rooting the tree with Pleurothallidinae shows *Ponera* and *Isorchilus* to be sister to Laeliinae and *Meiracyllium* embedded within Laeliinae. *Microepidendrum subulatifolium* (A. Rich. & Gal.) W.E. Higgins is then sister to the remainder of the subtribe. A three-branch polytomy occurs in the outgroup taxa (FIGURE 1) and in *Encyclia* s.s. (FIGURE 2). *Cattleya* appears to be paraphyletic, since the two taxa sampled do not form a clade. The *Encyclia* s.s. clade is sister to the *Homalopetalum-Domingoa-Meiracyllium* clade. *Euchile* is sister to *Prosthechea*, and *Oestlundia* is sister to the *Alamania-Encyclia keinastii* (Rchb.f.) Dressler & G.E. Pollard clade. The clade of *Prosthechea pygmaea* (Hook.) W.E. Higgins and *P. pseudopygmaea* (Finet) W.E. Higgins (*Encyclia* sect. *Hormidium*) is embedded in *Prosthechea*.

### DISCUSSION

Four species appear twice in the topology; *Dinema polybulbon* (Sw.) Lindl., *Microepidendrum subulatifolium* (A. Rich. & Gal.) W.E. Higgins, *Euchile mariae* (Ames) Withner, and *Oestlundia luteorosea* (Rich. & Gal.) W.E. Higgins. These species were sampled twice for the following reasons: *Dinema* because it is a monotypic genus; *Microepidendrum subulatifolium* because of its unexpected position in the topology, *Euchile mariae* because sampling occurred before its sister taxon was obtained, and *Oest-*

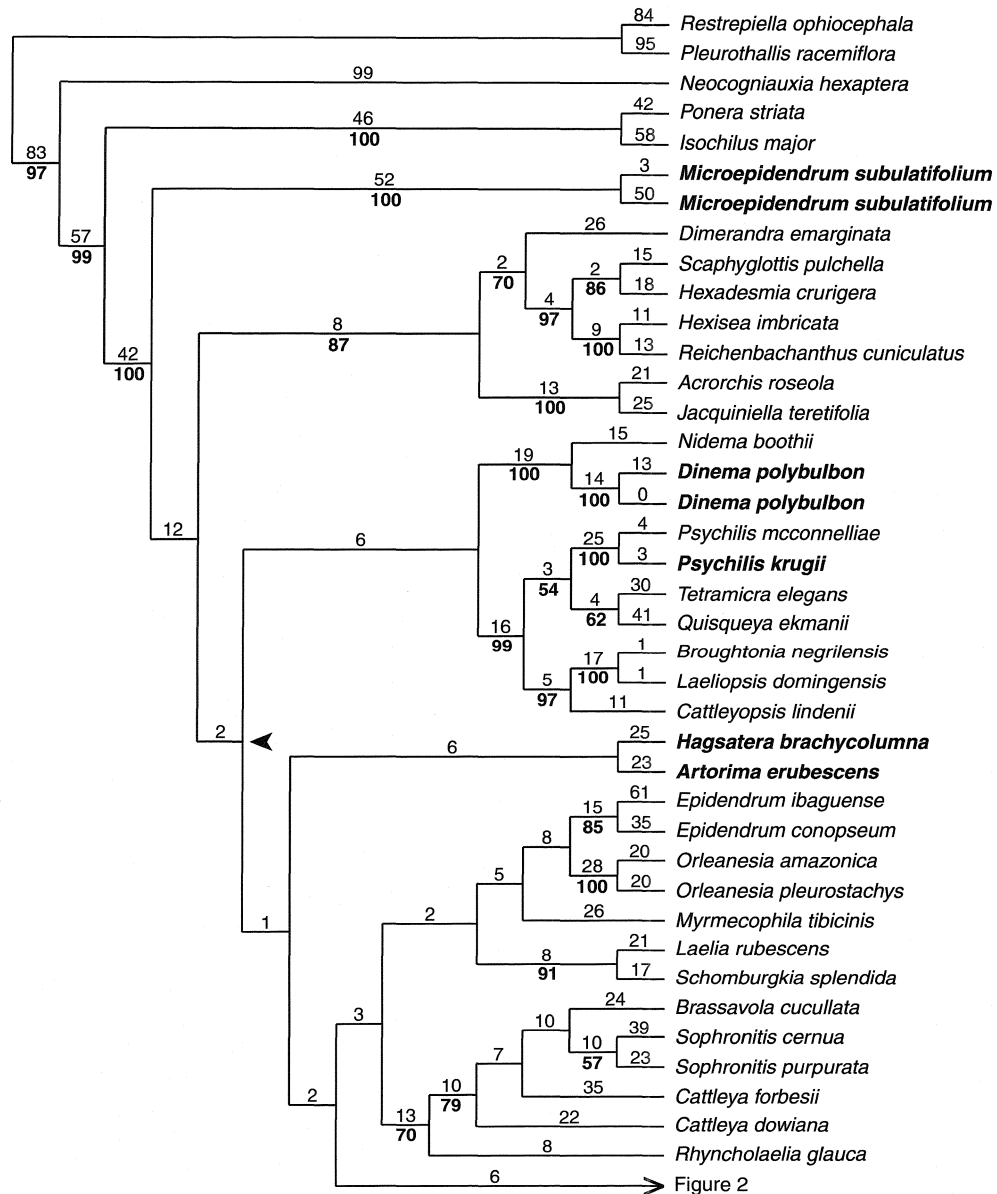


FIGURE 1. Selected tree (1 of 9) of Laeliinae topology based on the combined successively reweighted matrix (ITS, *trnL-F*, *matK*, and plastid indels). Taxa in bold are former members of *Encyclia* s.l. Fitch branch lengths (ACCTRAN) are above the branches, with equally weighted bootstrap indices below. The node that collapses in the strict consensus tree forming a trichotomy is marked with an arrow. This phylogeny continues in FIGURE 2.

*lundia luteorosea* because its specimens were unvouchered.

The topology of the strict consensus tree is in general agreement with a previously published ITS phylogeny of Laeliinae (van den Berg et al. 2000) using 295 taxa. *Neocogniauxia* is sister to Laeliinae plus Ponerinae sensu van den Berg and his coauthors (2000), and Meiracylliinae is

embedded within Laeliinae. Thus a monophyletic Laeliinae will include *Meiracyllium* but may exclude *Neocogniauxia*, *Ponera*, and *Isochilus*. The notable differences between the two studies are in the *Encyclia* alliance clade (FIGURE 2). In the three-gene study, *Nidema*, *Dinema*, *Dimerandra*, *Hagsatera*, and *Artorima* do not appear in the *Encyclia* alliance clade. Additionally,

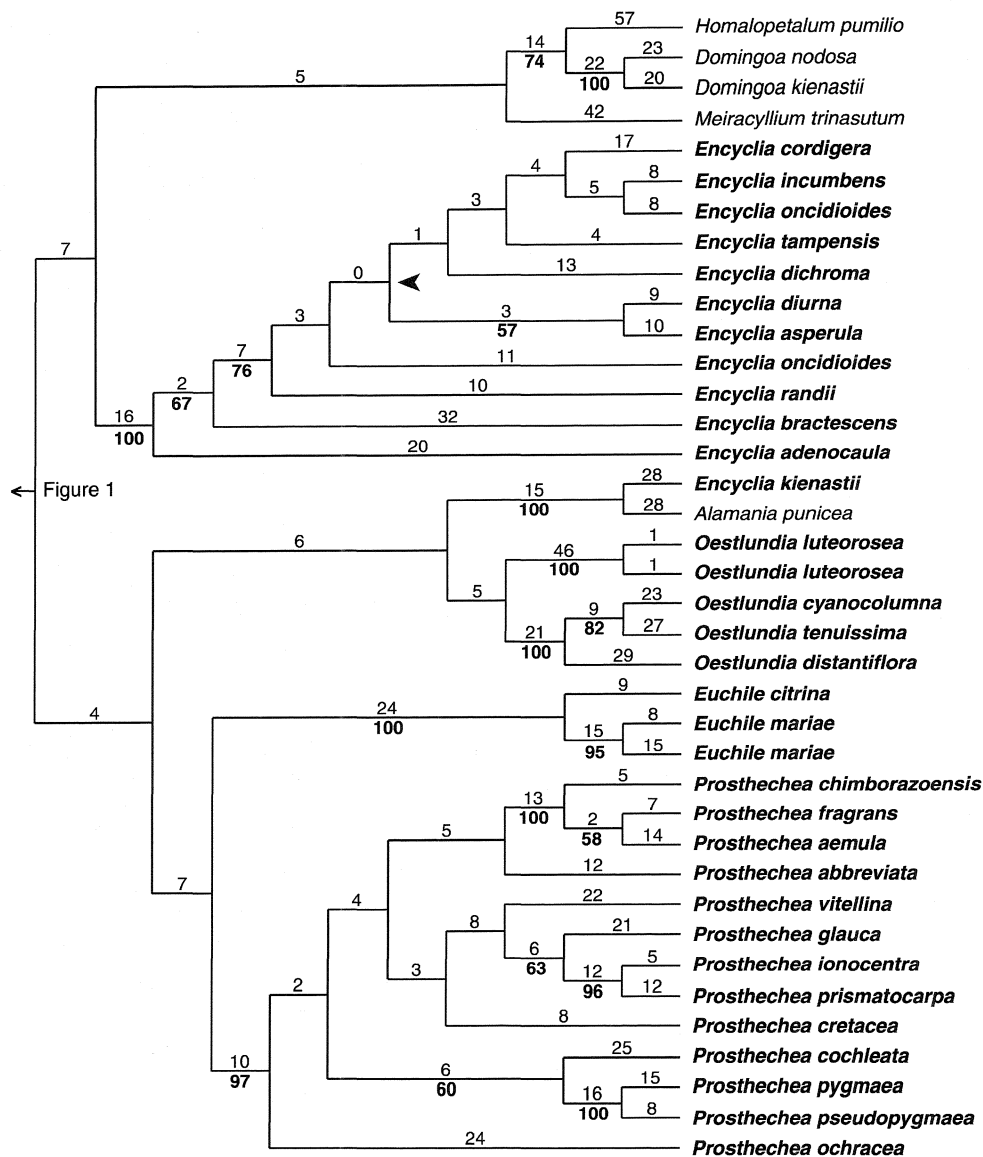


FIGURE 2. A selected tree of Laeliinae topology based on the combined successively reweighted matrix (ITS, *trnL-F*, *matK*, and plastid indels) highlights the *Encyclia* Alliance. Fitch branch lengths (ACCTRAN) are above the branches, with equally weighted bootstrap indices below. The node that collapses in the strict consensus tree forming a trichotomy is marked with an arrow. This tree connects to Laeliinae (FIGURE 1) at the base. Taxa in bold are former members of *Encyclia* s.l.

*Dimerandra*, *Hagsatera*, and *Artorima* do not appear in the *Encyclia* alliance clade. Additionally, *Hagsatera* is sister to *Artorima* in the strict consensus topology, although this position is not supported by bootstrap values. *Psychilis* is sister to *Tetramicra* and *Quisqueya*. These genera are included with *Broughtonia*, *Laeliopsis*, and *Catleyopsis* in a Caribbean clade that has 99% bootstrap support.

The subtribal relationships and most terminal clades are supported by bootstrap values; however, the topology at midlevels in Laeliinae does not have bootstrap support. Within Laeliinae, the following clades of interest have strong bootstrap support: *Encyclia* s.s., *Prosthechea*, *Euchile*, and *Dinema*. The *Oestlundia* clade does not have bootstrap support. Although *Homalopetalum*, *Domingoa*, *Meiracyllium*, *Encyclia*, *Ala-*

TABLE 4. Morphological characteristics distinguishing genera segregated from *Encyclia* sensu lato.

Characteristic	<i>Encyclia</i> sensu stricto	<i>Prosthechea</i>	<i>Euchile</i>	<i>Oestlundia</i>
Pseudobulbs	Usually ovoid or conic-ovoid	Fusiform, usually flattened	Conic-ovoid or fusiform-ovoid	Ovoid to conic-ovoid
Leaves	Thick	Usually thin	Thin	Thin
Glycoside crystals	Absent	Usually present	Absent	Absent
Inflorescence	Scape without spathe	Scape or sessile with prominent spathe	Scape without spathe	Scape without spathe
Flower	Resupinate	Usually non-resupinate	Resupinate	Resupinate
Labellum callus	2 fleshy ridges	Usually a thick pad	Laminar ridges	Papillae
Labellum adnate	Usually at base	Half of column	At base	At base
Column	Often winged	Never winged	Not winged	Not winged
Column mid-tooth	Short, usually broadly deltoid	Large, narrowly deltoid, ligulate, or subflabellate	Large, truncate, subequal to lateral teeth	Short, rounded, shorter than lateral teeth
Mid-tooth appendage	Absent	Fleshy, knob-like, obtuse, or truncate	Absent	Absent
Mid-tooth to anther	Appressed	Not appressed	Not appressed	Appressed
Column sinuses	Broad shallow	Deep usually acute	Deep	Shallow
Lateral teeth	Short	Stout	Stout	Wing-like
Rostellum	Vertical	Vertical	Vertical	Horizontal
Seed capsule	fusiform	3-winged or sharply 3-angled	Weakly triangular	Fusiform
Capsule suture	Strap absent	Covered by strap of tissue	Strap absent	Strap absent

mania, *Oestlundia*, *Euchile*, and *Prosthechea* form a clade (FIGURE 2) in the strict consensus, the relationships of the genera, and the adjacent outgroups in Laeliinae are not supported by bootstrap values. This Laeliinae phylogeny (FIGURE 1) also demonstrates that *Rhyncholaelia* Schltr. is not included in *Laelia* Lindl. and that *Myrmecophila* Rolfe is not included in *Schomburgkia* Lindl. In addition, the apparent paraphyly of *Cattleya* does not have bootstrap support and probably results from a low taxon number sampling error.

A different genus is required for *Microepidendrum subulatifolium*, since this species is clearly not an *Epidendrum* or *Encyclia* and does not form a clade with another genus. *Microepidendrum subulatifolium* is caespitose with very slender stems, new growth red; roots thick, verrucose, root tip red; leaves subcylindric, acute, strict; inflorescence racemose or paniculate, rachis flexuous, few-flowered; flower color sepals and petals yellow-brown, lip white, callus yellow, anther burgundy; sepals, elliptic-lanceolate, complicate-acute, recurved; petals oblanceolate-linear, acute or subacute; lip adnate to 3/5 of column, simple suborbicular, retuse, strongly plicate-undulate, margins erose, reflexed; callus ovate flattened disk, with 3 papillose keels; column small, clavate, three apical teeth subequal,

midtooth obtuse surpassed by anther; capsule ellipsoid. Transfer of *Encyclia kienastii* to *Ala-mania* remains questionable since we have not seen the voucher specimen, although the placement of *E. kienastii* does have bootstrap support.

Although the type species for *Encyclia* may be extinct and was not sequenced, the morphology of *E. viridiflora* clearly places it in the *Encyclia* s.s. group. *Encyclia viridiflora* has a separate 3-lobed lip that encircles the winged column, a lip callus of two parallel ridges, four polinia, a rounded pseudobulb, and a branched inflorescence. Additional research is needed to examine the polyphyly of *Cattleya* and the placement of *Encyclia kienastii*. A larger three-gene analysis of Laeliinae is in progress (C. van den Berg, W. Higgins, R. Dressler, W. Whitten, M. Arenas, A. Culham, & M. Chase unpubl. data) to examine differences between plastid and nuclear genomes. Additionally, relationships in *Encyclia* s.l. are being examined using the total evidence approach (holomorphology) of combining morphology and DNA (Higgins 2000).

A good classification should have maximum general utility, i.e., natural, easy to use, concise, predictive, aiding memory, and stable (Gilmour 1940, Backlund & Bremer 1998). Currently recognized genera in Laeliinae are built on the Linnaean principle of reproductive characters serv-

ing as the major source of generic-level characters (Judd et al. 1999), but convergence in pollination-related floral characters may result in polyphyletic genera. The previous classifications of *Encyclia* conflict with the molecular-based cladograms presented here. *Encyclia* in the broad sense includes many different taxa that are not closely related. For example, the taxa shown in bold face type in FIGURE 1 that have been included in the genus are spread across the subtribe. When FIGURE 2 is compared to former classifications of *Encyclia*, the relationships between the former sections are not supported: *Encyclia* section *Hormidium* (*E. pygmaea* & *E. pseudopygmaea*) is embedded in *Encyclia* section *Osmophytum* (*Prosthechea*); *Encyclia* section *Leptophyllum* (*Oestlundia*) is sister to *Alamania* and is not a member of *Encyclia* subgenus *Encyclia*; and *Encyclia* subgenus *Dinema* (*Dinema*) is not included in *Encyclia* s.l.

The recognition of *Encyclia* s.s., *Euchile*, *Prosthechea*, and *Oestlundia* at the generic level is more predictive, based on their morphological characteristics, than is *Encyclia* s.l. The following morphological characters distinguish the genera: *Encyclia* s.s. has a two fleshy-ridged callus on the lip; the column has a small mid-tooth appressing the anthercap and two short large deltoid lateral teeth; and the column is often winged. *Euchile* has glaucous leaves, a lip that encircles the column, a nectary at the base of the column, and three large truncate teeth on the column. Higgins (1997) placed *Euchile mariae* and *E. citrina* in *Prosthechea*; however, since this clade is sister to the remainder of *Prosthechea*, Withner's *Euchile* could be recognized as a distinct genus. The column structure of *Euchile* differs in that the midtooth is not ligulate. *Prosthechea* has fusiform pseudobulbs often flattened and a prominent spathe; the callus is a thick pad, lip adnate one-half of column, three large knob-like teeth on column, ligulate midtooth; and the seed capsule is 3-winged/angled with a strap covering the suture (Higgins 1999). *Oestlundia* has a small mid-tooth that appresses the anther and two large wing-like lateral teeth; the rostellum is in a horizontal position between the lateral teeth, and the small pseudobulbs have grass-like leaves. These morphological characters are summarized in TABLE 4. The diversity of morphological features among the major clades of *Encyclia* s.l. make this group cumbersome at the generic level. When *Encyclia* s.l. is divided in smaller segregate genera, these smaller genera form predictive groups that are easily defined using morphological as well as molecular characters. Thus, a narrow delimitation of *Encyclia* is preferred as a more user-friendly classification.

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## FOLIAR ANATOMY OF FIVE *VRIESEA* SECT. *XIPHION* (BROMELIACEAE) SPECIES

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**ABSTRACT.** The present work aims to extend the knowledge of the genus *Vriesea* Lindley, contributing data on the foliar anatomy of five species: *Vriesea bituminosa* Wawra, *V. longiscapa* Ule, *V. triligulata* Mez, *V. gigantea* Gaudich., and *V. fosteriana* L.B.Sm. Transverse and longitudinal cuts were made manually in fully expanded leaves. The usual histochemical tests were performed. Features typical to the Bromeliaceae were peltate trichomes, single layered epidermis, stomata located only on the abaxial surface of the leaves, a hypodermis with mechanical and water-storing functions, and collateral vascular bundles. Observed differences among the species were number of trichomes in both lamina and sheath, number of cells on the trichome shield, and number of layers of the aquiferous hypodermis. Many of the observed characteristics confirm the xerophytic nature of the leaves. The anatomical organization of the leaves in the studied Bromeliaceae could be related both to environmental pressures as well as to phylogenetic relationships.

**RESUMO.** O presente trabalho tem como objetivo ampliar o conhecimento acerca do gênero *Vriesea* Lindley fornecendo dados sobre a anatomia foliar de cinco espécies: *Vriesea bituminosa* Wawra, *V. longiscapa* Ule, *V. triligulata* Mez, *V. gigantea* Gaudich. e *V. fosteriana* L.B.Sm. O material botânico foi cortado a mão livre. Testes histoquímicos usuais foram realizados. Os resultados mostraram que as espécies exibem as características típicas da família: folhas hipostomáticas, epiderme simples dotada de tricomas peltados multicelulares, hipoderme com funções mecânica e de reserva de água, tecido clorofiliano diferenciado em paliádico e esponjoso do tipo brácteiforme e feixes vasculares colaterais. Foram notadas diferenças entre as espécies com relação à quantidade de tricomas ao longo da folha; número de células que compõem o escudo dos tricomas e número de camadas da hipoderme aquífera. As características observadas confirmam a natureza xerófitica das folhas. A organização anatômica das folhas das Bromeliaceae estudadas pode ser uma resposta a pressões ambientais assim como refletir relações filogenéticas.

**Key words:** Bromeliaceae, *Vriesea*, leaf anatomy, epiphytes

### INTRODUCTION

Comprising nearly 2700 species (Benzing 2000) in 56 genera (Grant & Zijlstra 1998), the Bromeliaceae family presently represents an extremely diversified group of vascular plants, which has colonized a variety of neotropical habitats (Dahlgren et al. 1985). Specialization related to water-storage, represented by sheath development as well as xeromorphic leaf structure in many species, is a remarkable feature of this family, not yet sufficiently investigated. Family representatives are found both in xeric and mesic environments, from sea level to mountaintops, and account for a considerable portion of the epiphytic vegetation in neotropical forests (Benzing 1990).

Although some anatomic studies have been conducted (Tomlinson 1969, Benzing 1970, 1976, Braga 1977, Souza & Neves 1996, Sajo et al. 1998), the existing data on particular groups are fragmented and little explored, as for example the genus *Vriesea* Lindl. Considering the present infra-generic division of *Vriesea*, the Section *Xiphion* comprises ca. 130 species (Luther & Sieff 1994, 1997a, 1997b, Luther 2001). These species are characterized predominantly by an inflorescence with dull colors; corolla with cream, yellow, or reddish petals; androecium and gynoecium included within the corolla; and stigma with the convolute-blade type morphology (Grant 1993, 1995a, 1995b).

The present work provides data on the foliar anatomy of five species of the *Xiphion* section of *Vriesea*, which are discussed in the light of

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