Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 32 (2004) 309–323
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Phylogeny of the orchid bees (Hymenoptera: Apinae: Euglossini): DNA and morphology yield equivalent patterns

Alice Michel-Salzat,[a,1] Sydney A. Cameron,[a,*] and Marcio L. Oliveira[b]

[a] *Department of Entomology, 320 Morrill Hall, 505 S. Goodwin Ave. University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*
[b] *Coordenação de Pesquisas em Entomologia, Instituto Nacional de Pesquisas da Amazônia, Av. André Araújo 2936, Petrópolis, Caixa postal 478 69011-970, Manaus, AM, Brazil*

Received 24 August 2003; revised 9 December 2003
Available online 1 March 2004

Abstract

Orchid bees (Euglossini) are spectacular long-tongued Neotropical bees important in the pollination of Neotropical long-corolla flowers, particularly some orchids. Besides remarkably long tongues, males in particular exhibit other flower-related adaptations, including setal brushes on the foretarsi used for rasping the petals of orchids while collecting aromatic compounds. These compounds are stored in large swollen tibiae and are thought to play an important role in courtship behavior. Euglossini are also unusual in lacking sociality; they are the only tribe among the corbiculate bees that are not eusocial, and two of the genera are cleptoparasitic. Each genus exhibits distinct behavioral traits including nest architecture and host–parasite interactions, yet their evolution is unknown. Despite previous phylogenetic studies of on morphological characters, the relationships among the five euglossine genera remain under debate. We investigate euglossine generic relationships using DNA sequence data from four genes and new morphological characters. The morphological and molecular data yield congruent evolutionary patterns, and combining the data gives a fully resolved and well supported phylogeny of Euglossini.

Keywords: Corbiculate bees; Neotropics; Molecular phylogeny; Combined data; Nuclear genes; Opsin; EF-1α

1. Introduction

Orchid bees comprise the tribe Euglossini and have fascinated biologists for centuries (Cameron, 2004; Darwin, 1862; Merian, 1705, pl. 48). Found only in the New World tropics, they are spectacular in color, form and behavior (Roubik and Hanson, 2004). The males are primary pollinators of certain groups of Neotropical orchids (Dodson and Frymire, 1961; Dodson, 1967, 1975; Dressler, 1982; van der Pijl and Dodson, 1966), and although elusive in the field, they have come under scientific focus in studies of incipient sociality (Bennett, 1965; Eberhard, 1988; Santos and Garófalo, 1994; Zucchi et al., 1969), mimicry (Dressler, 1979, 1982), and sexual selection (Eltz et al., 1999, 2003; Peruquetti, 2000). Their ecological status as important tropical plant pollinators makes them particularly valuable targets for conservation, and they are used increasingly as models in field studies of tropical forest conservation (Becker et al., 1991; Oliveira, 2001; Oliveira and Campos, 1995). Their mostly solitary status amidst the highly eusocial bees places them in an important position to advance the understanding of social evolution in insects, especially true for groups exhibiting facultative social interactions within the nest (reviewed in Cameron, 2004).

Euglossini are one of four tribes comprising the corbiculate bees (Shuckard, 1866, cited in Engel, 2001) within the subfamily Apinae (Roig-Alsina and Michener, 1993), which also includes the highly eusocial stingless bees (Meliponini) and honey bees (Apini), and the "primitively" eusocial bumble bees (Bombini) (Kimsey, 1984a; Michener, 2000). Euglossines differ significantly from their social relatives in morphology

* Corresponding author. Fax: 1-217-244-3499.
E-mail address: scameron@life.uiuc.edu (S.A. Cameron).
[1] Present address: INSERM U445, département d'immunologie, ICGM, 27 rue du Faubourg Saint-Jacques, 75014 Paris, France.

1055-7903/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2003.12.009

and behavior, including their exceptionally long tongues and brilliant iridescent coloring. Most strikingly, male morphology is uniquely modified for collecting aromatic compounds from certain orchids and other plants. Modifications include the brush-like setal tufts on the foretarsi used to sop up aromatics from the orchid labellum, and the comb-like structures on the midtarsi that transfer the fragrant compounds from the foretarsi into large inflated hind tibiae, where they are absorbed through a transverse opening, or tibial slit, into a spongy storage chamber (Michener et al., 1978). The exact function of the stored aromatics is still debated, although accumulating evidence points to a role in mating behavior and sexual selection (Eltz et al., 1999, 2003; Peruquetti, 2000; reviewed in Cameron, 2004).

Although many euglossine species develop communal or semisocial nests (sensu Michener, 2000), two of the five genera are cleptoparasitic and none have evolved the obligate eusociality of their corbiculate relatives—there is no evidence from fossils or extant taxa to suggest that eusociality has been lost in this group (Engel, 2001). Why has eusociality never evolved among euglossines despite their closest relatives exhibiting some of the most complex social systems known in insects? Are the two cleptoparasitic genera independently derived or closely related to their hosts, as in other bees (Michener, 2000)? Besides new fossil discoveries and comparative behavioral data, answering these questions ultimately requires knowledge of the phylogeny of the corbiculate tribes and of the Euglossini.

With respect to corbiculate tribal phylogeny, the placement of Euglossini is ambiguous. Morphological investigation by Roig-Alsina and Michener (1993), with reanalysis by Schultz et al. (1999, 2001) and the addition of fossil data (Engel, 2001) places euglossines basally within the corbiculate clade, as sister group to the remaining corbiculate tribes. A recent behavioral phylogeny also supports this placement of Euglossini (Noll, 2002). Collective gene sequences from several molecular investigations resolve euglossines as sister group to Apini (Cameron and Mardulyn, 2001). However, strong support for the placement of Euglossini is lacking for both molecular and morphological data (Lockhart and Cameron, 2001), most likely because of the rapid radiation of the entire corbiculate clade (Lockhart and Cameron, 2001) as it evolved with the angiosperms during the Cretaceous (Engel, 2001).

Euglossini (approximately 190 described species, Roubik and Hanson, 2004) is divided into five monophyletic genera (Kimsey, 1987). The largest genus is *Euglossa* Latreille with 109 species organized into six subgenera (Dressler, 1978, 1982; Moure, 1989). Relatively small in body size, with vivid green to purple or coppery iridescence and sparse hair, they are predominantly solitary, initiating single-foundress nests, although communal and semisocial nests also occur (Eberhard, 1988; Garófalo, 1998). Nests are constructed entirely of plant resins. The phylogeny of *Euglossa* is still to be investigated.

Eufriesea Cockerell are relatively large, robust and hairy bees, often with muted iridescence and banded patterns of pubescence on the abdomen. *Eufriesea* has roughly 64 species (Roubik and Hanson, 2004), all highly seasonal and difficult to study. They are intermediate in size between *Euglossa* and the exceptionally large *Eulaema*, and some mimic the coloration of *Eulaema* (Dressler, 1979, 1982). Nests are composed of resins and wood chips, and are frequently found in communal aggregations, with each female working independently on her own nest (Myers and Loveless, 1976; Kimsey, 1982). *Eufriesea* was revised by Kimsey (1982) but as with *Euglossa*, no phylogenetic hypothesis is available.

Eulaema Lepeletier comprises 15 currently described species (Dressler and Opsina-Torres, 1997; Kimsey and Dressler, 1986; Ospina-Torres and Sandino-Franco, 1997) (although Oliveira (2000); proposes additional species) divided into two monophyletic subgenera *Apeulaema* Moure and *Eulaema s.s.* Lepeletier (Oliveira, 2004). They are bumble bee-like in appearance, often with black and yellow or orange banding. Some species, if not all, can make communal nests (Nates-Parra and González, 2000; Zucchi et al., 1969), although many are solitary (Cameron and Ramírez, 2001). Nests are constructed of mud and lined with resins. Oliveira (2004) recently revised *Eulaema* and examined their phylogeny.

The two remaining genera, *Exaerete* Hoffmannsegg and *Aglae* Lepeletier and Serville, are obligate cleptoparasites of other members of their tribe, *Eulaema* and *Eufriesea* (Bennett, 1972; Garófalo and Rozen, 2001). They are large, elongate, steely metallic blue to brilliant green bees. *Exaerete* has five described species (Kimsey, 1979) known to parasitize *Eufriesea* and *Eulaema*; *Aglae* is a monotypic genus, described from the single species *Aglae caerulea*, which parasitizes *Eulaema*. Both of these cleptoparasitic genera show the morphological loss of female characters associated with pollen collection for rearing brood, as seen in other clepto- and social parasites. The ecology and behavior of both genera are poorly known, and recent observations of variable host-attack strategies in *Exaerete* (Garófalo and Rozen, 2001) suggest interesting behavioral variation. There are no phylogenetic hypotheses of *Exaerete*.

Knowledge of the evolution of generic traits will ultimately rely upon well-supported inter- and infrageneric phylogenies. In this report, we focus on the higher level (intergeneric) relationships. A phylogeny of the genera can clarify the direction, timing and potential correlations of novel innovations such as divergent nest building strategies and social interactions. It can also help to trace the origins of new life history strategies, cleptoparasitism in particular. For example, "Emery's rule" (discussed in Wilson, 1971), which claims that

parasites most closely resemble their hosts, would suggest that *Exaerete* and *Aglae* evolved from *Eufriesea* and *Eulaema*, but it is not possible to test this idea because there is no single well-supported generic phylogeny of Euglossini. While Kimsey (1982, 1987), Michener (1990), Engel (1999), and Oliveira (2000, 2004) examined generic relationships based upon morphological characters, each analysis has led to a different hypothesis (Fig. 1). All but Oliveira's investigation (Fig. 1E) depended on a small character set from Kimsey (1982), slightly modified from study to study, with only one or two synapomorphies supporting any of the internal nodes. Oliveira added additional new characters to his analysis.

Essentially, these conflicting topologies differ over the position of *Aglae*, reported as a basal sister group to the rest of the tribe (Kimsey, 1982; Oliveira, 2000, 2004) or as a terminal sister group to *Eulaema* (Engel, 1999; Kimsey, 1987; Michener, 1990). The topology in Fig. 1C is a less resolved pattern of topology B; Engel's hypothesis (Fig. 1D) diverges further as *Eufriesea* moves out of the lineage containing *Eulaema* to attach to *Euglossa* + *Exaerete*. Oliveira's augmented character analysis yields a fifth hypothesis (Fig. 1E), more similar to that of Kimsey's original study (Fig. 1A). Kimsey (1982) and Oliveira (2000, 2004) both infer *Aglae* as sister group to the other genera, and *Eufriesea* + *Eulaema* as the terminal clade. They disagree in the splitting of *Exaerete* + *Euglossa*. The conflict or lack of resolution from these morphological analyses clearly suggests the need for additional new characters.

Our investigation of euglossine generic relationships is the first molecular analysis, and is based on DNA sequences from four genes: two widely used mitochondrial genes (16S rDNA and COI) and two protein-encoding nuclear genes (long-wavelength rhodopsin, LW *Rh* (also known as opsin), and the F2 copy of elongation factor-1α, EF-1α), shown to be useful in determining generic relationships in insects. The 16S and COI genes have been used extensively in phylogenetic analyses of Hymenoptera (repeatedly in bees), at both lower and higher taxonomic levels (see for 16S: Cameron, 1993; Cameron and Mardulyn, 2001; Cameron and Williams, 2003; Dowton and Austin, 1994; Whitfield and Cameron, 1998; see for COI: Crozier et al., 1989; Koulianos and Schmid-Hempel, 2000; Leys et al., 2000; Pedersen, 1996, 2002; Schwarz et al., 2003; Sipes and Wolf, 2001). LW *Rh* has been shown to be useful in several studies of higher and lower level relationships in insects (Briscoe, 2001; Cameron and Mardulyn, 2001; Carulli et al., 1994; Kawakita et al., 2003; Lockhart and Cameron, 2001; Mardulyn and Cameron, 1999; Rokas et al., 2002), although levels of the phylogenetic utility of the marker have been debated (Ascher et al., 2001; Cameron and Mardulyn, 2003). EF-1α, particularly the F2 copy, has proven useful in several recent phylogenetic studies of insects (see for example, Cameron, 2003; Cruickshank et al., 2001; Danforth et al., 2003; Johnson and Whiting, 2002; Jordal, 2002; Monteiro and Pierce, 2001; Rokas et al., 2002; Sipes and Wolf, 2001).

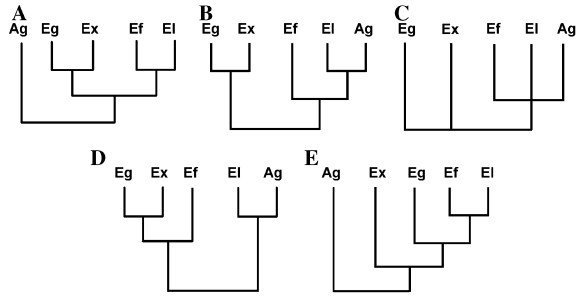

Fig. 1. Previously proposed phylogenies for Euglossini (A) Kimsey, 1982; (B) Kimsey, 1987; (C) Michener, 1990; (D) Engel, 1999; and (E) Oliveira, 2000. Ag refers to the genus *Aglae*, Ex to *Exaerete*, Eg to *Euglossa*, Ef to *Eufriesea*, and El to *Eulaema*.

In addition to analyses of DNA sequences, we examine the congruence between the DNA and the morphological character set reported by one of us (MLO) (Oliveira, 2004). We report that the phylogeny inferred from these morphology data (Fig. 1E) is congruent with the results based on the combined gene sequences. Additional taxon sampling is encouraged and would likely resolve any residual uncertainty of relationship in these combined analyses.

2. Material and methods

2.1. Taxa examined

We obtained DNA sequences from *Euglossa* (4 species), *Eufriesea* (3 species), *Eulaema* (9 species), *Exaerete* (3 species), and *Aglae caerulea* (Table 1). Species of the three other tribes of corbiculate bees (Apini, Meliponini, and Bombini) were used as outgroups. A list of the species, their taxonomic classification, and GenBank Accession nos. are given in Table 1. The use of exemplars to represent each euglossine genus is justified on the basis that the genera have been shown to be monophyletic in numerous independent studies (Cameron, 1993; Cameron and Mardulyn, 2001; Engel, 1999; Kimsey, 1987). We included a representative from each subgenus of *Eulaema* and *Eufriesea*, and from three of the six *Euglossa* subgenera. Detailed information on collecting localities can be provided upon request. Voucher specimens of the examined species are retained at the Illinois Natural History Survey, University of Illinois at Urbana-Champaign.

2.2. Obtaining DNA sequences

Total genomic DNA samples were obtained from thoracic tissue ground in an extraction buffer (0.05 M

Table 1
Systematic positions of the specimens used in this study and accession numbers in EMBL, GenBank, and DDBJ databases

Tribe	Genus	Species	Accession number			
			16S	COI	LW *Rh*	EF-1α
Apini	*Apis*	*dorsata*	AF153098*	AJ581104	AF091733*	AJ582381
		mellifera	AF250955*	AJ581105	AF091732*	AF015267*
Bombini	*Bombus*	*terrestris*	AF181582*	L26573*	AF091722*	AJ582380
Meliponini	*Melipona*	*bicolor*	AF466146*	AF370439*	—	—
		compressipes	AF181589*	—	—	—
		sp.	—	—	AF344607*	—
Euglossini	*Aglae*	*caerulea*	AJ581103	AJ582627	AJ581739	AJ582383
	Eufriesea	*caerulescens*	L22904*	AF091725*	AF091725*	—
		flaviventris	AJ581090	AJ581109	AJ581735	AJ582376
		xantha (synonymized as *vidua* by Moure, 1999)	AJ581091	AJ581110	AJ581736	AJ582382
	Euglossa	*bidentata*	AJ581088	—	AJ581742	—
		championi	AJ581089	—	AJ581740	AJ582375
		imperialis 1	AF181584*	—	AF091720*	AJ582374
		imperialis 2	AJ581085	AJ581106	—	AJ582373
		intersecta 1	AJ581086	AJ581107	AJ581741	AJ582377
		intersecta 2	AJ581087	AJ581108	—	—
	Eulaema	*bombiformis*	AJ581100	AJ582624	—	AJ582368
		cingulata	AJ581098	AJ581117	AJ581728	—
		meriana	AJ581095	AJ581114	AJ581731	AJ582370
		mocsaryi	AJ581099	—	AJ581729	—
		nigrita	AJ581097	AJ581116	AJ581732	AJ582369
		peruviana	AJ581092	AJ581111	AJ581734	—
		polychroma	AJ581094	AJ581113	AJ581730	—
		polyzona	AJ581093	AJ581112	AJ581733	AJ582371
		speciosa	AJ581096	AJ581115	AJ581727	AJ582372
	Exaerete	*frontalis*	—	—	AF091718*	—
		smaragdina	AJ581101	AJ582625	AJ581738	AJ582379
		sp.	AJ581102	AJ582626	AJ581737	AJ582378

* Indicates a sequence published in a previous study, —Indicates no sequence.

Tris–HCl, 0.01 M EDTA, 0.5% SDS, 50 mM NaCl) and incubated with proteinase K (final concentration: 0.15 mg/ml) 4 h at 40 °C. The incubation was followed by standard phenol–chloroform extraction and ethanol precipitation. DNA pellets were resuspended in 70 μl of ddH_2O and stored at −20 °C.

Double-stranded PCR products were amplified in an Eppendorf Mastercycler gradient (Eppendorf AG, Hamburg) following standard protocols (initial denaturation 5 min at 94 °C, followed by denaturation 1 min at 94 °C, annealing 1 min at 47–52 °C, extension 1 min at 67–72 °C for 35 cycles, and final extension: 5 min at 72 °C). 16S fragments of ~530 bp were amplified using 16SWb (Dowton and Austin, 1994) and 874-16S LR (Cameron et al., 1992). COI fragments were amplified using AP-L-2013 and AP-H-2931 primers (Pedersen, 1996). The ~940 bp fragments correspond to positions 1991–2931 of the COI sequence published for *Apis mellifera ligustica* (Crozier and Crozier, 1993). Fragments of the LW *Rh* gene, ~700 bp, including 502 bp of coding sequence, were amplified with the primers LW*Rh* F and LW*Rh* R given in Mardulyn and Cameron (1999). The amplified region comprises two introns and corresponds to the nucleotide positions 421–922 in the *Apis mellifera* long-wavelength rhodopsin sequence (introns excluded) (Chang et al., 1996). Fragments of ~1000 bp of the EF-1α gene were amplified using the F2-specific primers HaF2For1 and F2Rev (Danforth et al., 1999; Sipes and Wolf, 2001). For each specimen and gene, at least two independent PCR products were obtained and sequenced. The PCR products were purified using the Qiaquick kit (QIAGEN Genomics, Germantown, USA) according to the manufacturer's protocol, or were eluted from an agarose gel using the Perfectprep kit (Eppendorf). Sequencing was carried out using the PCR primers, with BigDye version 3.0 (Applied Biosystems, Foster City, USA), according to the manufacturer's protocol. The sequences were run on an ABI 377 capillary sequencer. Both strands were sequenced for all taxa.

The sequences were deposited in GenBank and Accession nos. are given in Table 1. Some additional sequences were retrieved from GenBank and added to our data set (those with asterisks in Table 1).

2.3. Sequence alignment and summary statistics

DNA sequences were edited using BioEdit version 5.0.9 (Hall, 1999) and aligned with CLUSTAL X (Thompson et al., 1997) using default parameters (gap opening, 10; gap extension, 0.2). The alignments of COI and the coding regions of LW *Rh* and EF-1α were unambiguous. For 16S, several variable AT-rich regions were identified. To minimize the impact of alignment on phylogenetic analysis in this study, we excluded regions of 16S-ambiguous alignment from all analyses. Whereas this had the consequence of eliminating some potentially useful regions for 16S sequences, we wanted to eliminate alignment decisions as a potential source of difference among trees estimated from different gene regions (Johnson and Whiting, 2002; Lutzoni, 1997). By the same token, we excluded from the analyses the variable noncoding regions of LW *Rh* (as in Mardulyn and Cameron, 1999) and EF-1α. All the alignments used for this study are available upon request.

Uncorrected pairwise sequence divergences (*p*-distances) and base frequencies were calculated for each gene fragment using the computer program MEGA v2.1 (Kumar et al., 2001); *p*-distances were used to estimate the divergence among sequences and to compare the mean divergence observed for each gene. Homogeneity of base frequencies among sequences was tested with the BaseFreqs option of PAUP* v 4.0 (Swofford, 2001) beta version b10.

2.4. Morphology

The morphological characters are described in detail by MLO (Oliveira, 2004). The character state matrix of 37 characters for our study is given in Table 2 and the character list from MLO (2004) is given in Appendix A.

2.5. Phylogenetic analyses

Maximum parsimony (MP) and maximum likelihood (ML) analyses were implemented in PAUP* 4.0 (Swofford, 2001), b10, with the heuristic search option and step-wise addition of 100 random taxon addition sequence replicates. The MP analyses were conducted using equal weights for all positions. Alternative weightings were tested for the COI and LW *Rh* data sets (down-weighted third position transitions: Ts/Tv = 1/2; 1/5; 0). The most appropriate substitution models for ML analyses were determined using Modeltest v. 3.04 (Posada and Crandall, 1998). Support values (BV) were estimated with bootstrap analyses (1000 replicates for MP, 100 replicates for ML).

The molecular data sets were tested for heterogeneity using the partition homogeneity test (Farris et al., 1994), implemented in PAUP*, to assess the appropriateness of combining the data partitions. We conducted a test between each pair of gene partitions (6 tests) and among all four partitions simultaneously (1 test) using 100 replicates for each test. Because none of the three exemplars of *Melipona* (an outgroup) yielded sequences from all four genes (Table 1), we combined the 16S and COI sequences from *Melipona bicolor* and the LW *Rh* sequences of *M. sp.* (obtained from GenBank) into a single combined *M. sp.* sequence in order to retain the maximum phylogenetic information. This is justified on the basis that *Melipona* is a monophyletic group (Michener, 2000).

In a separate MP analysis (1000 bootstrap replicates), morphological characters were weighted equally and treated as unordered. Molecular and morphological data sets were also combined into a single matrix for parsimony analysis. All characters were unweighted. The molecular and morphological partitions were first tested for heterogeneity in PAUP*, using the partition homogeneity test (100 replicates).

3. Results

3.1. Sequence data characteristics

The type and number of molecular characters, *p*-distance values (range and mean), and significance values of the base composition homogeneity tests for each gene fragment are given in Table 3. Based on *p*-distances, the mitochondrial genes (16S and COI) were the most variable (57.6 and 48.3% variable sites relative to all sites, respectively). Variable sites for the nuclear genes (EF-1α and LW *Rh*) were 28.1 and 37.8%, respectively (Table 3). COI had the highest absolute number of parsimony-informative characters (most of them at the third codon position) because it was the largest fragment, but 16S had proportionately more informative characters (Table 3). There was no heterogeneity of base composition across taxa; although nonsignificant, the *p*-value for COI was low (0.7) because the outgroup sequences had a higher A-content and lower T-content than average. When these sequences were removed from the data set, the test of homogeneity of base composition resulted in $p = 0.99$ ($\chi^2 = 26.14$, df = 45).

3.2. Phylogenetic inference

3.2.1. Morphology

MP analysis of the 37 morphological characters led to two equally parsimonious trees, the strict consensus of which is shown in Fig. 2. The trees differed only in the respective positions of *Exaerete* and *Euglossa*. The monophyly of Euglossini and of each of the four multispecies genera was supported by high bootstrap values (100, 81, 93, and 99, respectively). *Aglae* was the basal

Table 2
Morphological data matrix (missing data = ?)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Outgroups																																						
Apis dorsata	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	?	?	0	0	0	0	0	?	0	0	0	0	0	0	0
Apis mellifera	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	?	?	0	0	0	0	0	?	0	0	0	0	0	0	
Bombus terrestris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0
Melipona bicolor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	?	?	0	0	0	0	0	?	0	0	0	0	0	0	0
Melipona compressipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	?	?	0	0	0	0	0	?	0	0	0	0	0	0	0
In group																																					
Aglae caerulea	1	0	1	0	0	0	1	2	0	1	0	1	0	1	1	0	1	1	0	0	0	1	1	1	0	2	1	0	0	0	1	0	0	0	0	1	0
Eufriesea caerulescens	1	0	0	1	0	0	0	1	0	1	0	0	0	0	1	1	1	0	1	0	0	1	0	0	1	1	0	1	1	1	0	0	0	1	1	1	1
Eufriesea flaviventris	1	0	0	1	0	0	0	1	0	1	0	0	0	0	1	1	1	0	1	0	0	1	0	0	1	1	0	1	1	1	0	0	0	1	1	1	1
Eufriesea vidua	1	0	0	1	0	0	0	1	0	1	0	0	0	0	1	1	1	0	1	0	0	1	0	0	1	1	0	1	1	1	0	0	0	1	1	1	1
Euglossa bidentata	1	1	0	2	0	0	0	2	0	1	0	0	1	0	1	1	1	0	1	0	0	1	1	1	0	2	0	1	2	0	0	0	1	1	0	1	0
Euglossa championi	1	1	0	2	0	0	0	2	0	1	0	0	1	0	1	1	1	0	1	0	0	1	1	1	0	2	0	1	2	0	0	0	1	1	0	1	0
Euglossa imperialis	1	1	0	2	0	0	0	2	0	1	0	0	1	0	1	1	1	0	1	0	0	1	1	1	0	2	0	1	2	0	0	0	1	1	0	1	0
Euglossa intersecta	1	1	0	2	0	0	1	2	0	1	0	0	1	0	1	1	1	0	1	0	0	1	1	1	0	2	0	1	2	0	0	0	1	1	0	1	0
Eulaema bombiformis	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	0
Eulaema cingulata	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	0
Eulaema meriana	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	0
Eulaema mocsaryi	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	0
Eulaema nigrita	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	0
Eulaema peruviana	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	0
Eulaema polychroma	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	0
Eulaema polyzona	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	0
Exaerete frontalis	1	0	1	0	0	0	1	2	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	0	0	2	1	0	2	0	0	0	0	1	0	1	0
Exaerete smaragdina	1	0	1	0	0	0	1	2	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	0	0	2	1	0	2	0	0	0	0	1	0	1	0

Table 3
Type and number of molecular characters analyzed for each examined data set; uncorrected distances (*p*-distances), test of homogeneity of base composition, and evolutionary model used ML phylogenetic analyses

Data set	No. of taxa	Total no. characters analyzed	No. of variable characters	No. of parsimony-informative characters	*p*-distances ingroup (range/mean) (%)	Base composition homogeneity test	Model used for ML analyses shape of gamma distribution
16S	26	453	261 (57.6%)	142 (31.3%)	4.1–32.7 (11.5)	$\chi^2 = 29.85$, df = 75, $p = 1.00$	TVM + G 0.6740
COI	20	871	421 (48.3%)	219 (25.1%)	6.1–21.6 (10.9)	$\chi^2 = 51.72$, df = 57, $p = 0.7$	F81 + G 0.4014
EF-1α	24	693	195 (28.1%)	102 (14.7%)	1.5–6.0 (4.3)	$\chi^2 = 14.03$, df = 45, $p = 1.00$	HKY + G 0.3452
LW *Rh*	17	482	182 (37.8%)	136 (28.2%)	0.6–18.0 (6.5)	$\chi^2 = 23.34$, df = 69, $p = 1.00$	HKY + G 0.3700
Three-gene combined	17	2017	821 (40.7%)	394 (19.5%)	2.3–14.9 (9.4)	$\chi^2 = 21.85$, df = 42, $p = 0.99$	TVM + G 0.5347
Four-gene combined	13	2499	1008 (40.3%)	537 (21.5%)	2.1–12.6 (8.7)	$\chi^2 = 16.81$, df = 36, $p = 1.00$	TVM + G 0.5053

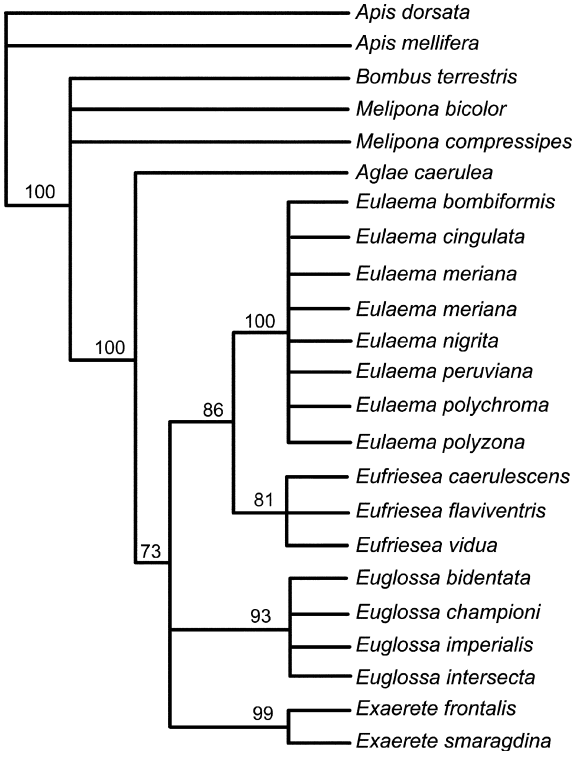

Fig. 2. Strict consensus of the two equally parsimonious trees based on morphology (length = 54; CI = 0.694; RI = 0.611). Bootstrap values >50 (1000 replicates) are indicated above the nodes.

sister genus to the remaining four euglossine genera (BV = 73), and *Eufriesea* and *Eulaema* were terminal sister groups (BV = 86). The relationships between *Euglossa* and *Exaerete* relative to the *Eufriesea* + *Eulaema* clade were ambiguous and resulted in the two equally parsimonius trees: *Aglae* ((*Exaerete* + *Euglossa*) + (*Eufriesea* + *Eulaema*)) and *Aglae* (*Exaerete* (*Euglossa* (*Eufriesea* + *Eulaema*))).

3.2.2. Genes

We compared the phylogenies from each gene fragment using MP and ML. The ML models were selected using Modeltest 3.04 and are given in Table 3.

MP and ML analyses of the 16S data essentially led to the same tree. MP analysis resulted in a single tree (Fig. 3), although many nodes were poorly supported—only 8 nodes were supported with bootstrap values >70% (Fig. 3A). Among the outgroup taxa, the two *Apis* species grouped together as sister group to the remaining bees, and the two *Melipona* species formed a sister group to *Bombus terrestris* (BV = 92). Each euglossine genus was monophyletic with high bootstrap values for *Exaerete* and *Euglossa* (BV = 96 and 87; Fig. 3).

In both MP and ML analyses, the COI data resulted in well-supported monophyletic genera (BV > 85) but the relationships among them were poorly supported. MP led to two equally parsimonious trees (Fig. 3). As COI has been reported to exhibit homoplasy at the third codon position (Koulianos and Schmid-Hempel, 2000; Schwarz et al., 2003; Sipes and Wolf, 2001), we applied several weighting schemes to give more weight to first and second positions. However, the results remained basically unchanged, with only small variations in bootstrap values (results not shown).

MP and ML analyses of the LW *Rh* data resulted in essentially compatible trees, the differences occurring only in poorly supported nodes considered as polytomies. MP analysis produced 10 equally parsimonious trees, shown as a majority-rule consensus tree in Fig. 3. The euglossine genera, except *Eufriesea*, were monophyletic with high bootstrap support (BV > 70), and *Aglae* fell outside as possible sister group to the other genera. However, neither MP nor ML methods resulted in well-supported generic relationships. Down-weighting third positions as described in Mardulyn and Cameron

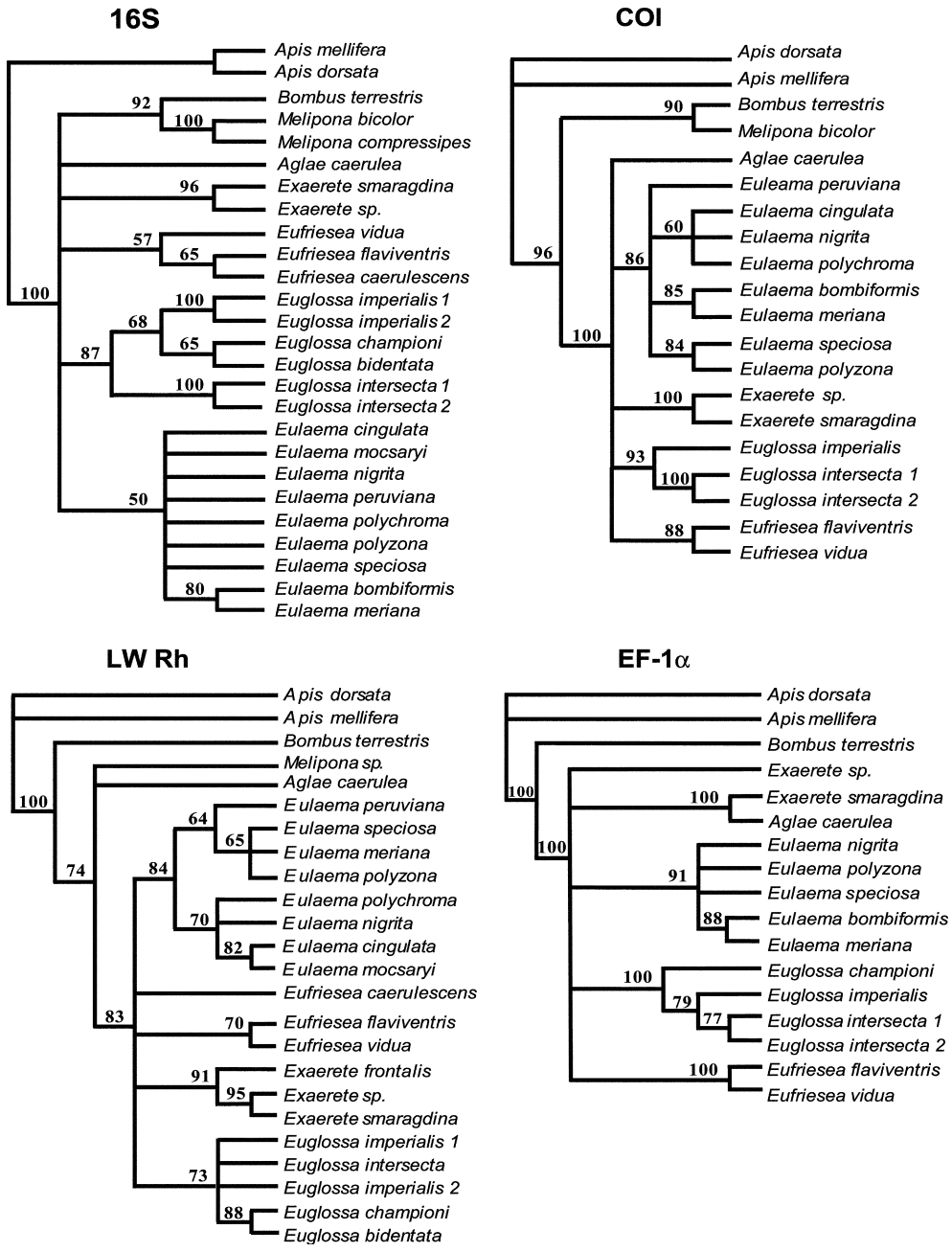

Fig. 3. Maximum parsimony trees resulting from independent analyses of four molecular data sets. Bootstrap values (1000 replicates) are indicated on the corresponding nodes. Nodes with low support (BV < 50) are represented as polytomies. 16S data set: 1 MP tree, length = 592, CI = 0.591, RI = 0.574; COI: majority-rule consensus of 2 MP trees, length = 911, CI = 0.591, RI = 0.554; LW *Rh*: majority-rule consensus of 10 MP trees, Length = 375, CI = 0.629, RI = 0.706; EF-1α: majority-rule consensus of 9 MP trees, length = 298, CI = 0.782, RI = 0.734.

(1999) did not increase resolution among genera in this analysis (results not shown).

MP and ML analyses of EF-1α resulted in compatible trees with strong support for the monophyly of *Eulaema, Euglossa* and *Eufriesea* (BV = 91; 100; and 100, respectively) but not *Exaerete*. However, there was no resolution of the generic relationships except that of *Aglae* + *Exaerete*, the latter represented by a single exemplar. MP analysis resulted in nine equally parsimonious trees (Fig. 3), with 7 ingroup nodes supported by high bootstrap values.

In summary, analyses of the individual data partitions usually resolved each of the euglossine genera but provided no unambiguous support of the relationships among the genera. The morphology partition showed strong support for a sister group relationship between *Eufriesea* and *Eulaema*, with some support for *Aglae* as sister group to the remaining genera.

Table 4
Results of the ILD tests on the different partition combinations (100 replicates)

	16S/COI	16S/LW *Rh*	16S/EF-1α	COI/LW *Rh*	COI/EF-1α	LW *Rh*/EF-1α	16S/COI/LW *Rh*/EF-1α	Molecular/ morphology
P value of ILD test	0.46	0.01*	0.31	0.01*	0.65	0.19	0.01*	1.00

* Indicates weak heterogenity.

3.3. Combining data

Partition homogeneity tests between sequence data partitions indicated that 16S, COI, and EF-1α were homogeneous ($p > 0.19$) (Table 4). Comparisons involving LW *Rh* revealed weak heterogeneity ($p = 0.01$—a value $p > 0.01$ is not significant (Cunningham, 1997)). Given these results, we compared the trees obtained by combining the three significantly homogeneous partitions (16S, COI, and EF-1α) with those obtained by combining all four gene partitions. We included *Melipona* and *Bombus* as outgroups (EF-1α sequences were missing for *Melipona*). Missing sequences were coded as missing data. However, *p*-distances and tests of homogeneity of base composition were calculated with *Melipona* excluded (Table 3).

MP and ML analyses (ML models given in Table 3) were applied to these combined data partitions. The three- and four-gene partitions gave similar proportions of variable and parsimony-informative characters, as well as similar *p*-distances (Table 3). Under parsimony, the three-gene partition led to a single tree (Fig. 4A), as did the four-gene partition (Fig. 5A). Considering only the well-supported nodes (BV > 70), MP and ML topologies were compatible for both the three- and four-gene partitions (compare Figs. 4A and B; Figs. 5A and B). Regarding the MP trees, the three-gene partition (Fig. 4A) resulted in a less resolved topology (10 of 14 nodes supported by BV ⩾ 70; nodes <50 are collapsed) than that of the four-gene partition (10 of 12 nodes supported by BV ⩾ 74, Fig. 5A). With the three-gene partition, there was no strong support for generic groupings. Euglossini was monophyletic (BV = 100), as were each of the genera ($99 < BV < 100$). With respect to the ML trees, support for any generic relationships was low ($55 < BV < 69$) (Fig. 4B).

Aglae emerged as sister group to the remaining four genera with strong support ($88 < BV < 99$) in the four-partition trees (Figs. 5A and B), matching the morphology result (Fig. 2). The three-gene ML tree (Fig. 4B) shows *Aglae* as sister group to *Exaerete* but with low support (BV = 69). *Eulaema* + *Eufriesea* formed an apical clade in reconstructions of the four-gene partition (Figs. 5A and B) (BV = 74 for MP and 62 for ML). The relationships of *Euglossa* and *Exaerete* were not resolved unambiguously: *Exaerete* was sister to *Aglae* (Fig. 4B) (with low support as mentioned above) or to the non-parasitic genera (Fig. 5B). In the only well supported relationship for *Euglossa*, it is shown as sister group to *Eufriesea* + *Eulaema*. (four-gene MP, BV = 76, Fig. 5A).

The four-gene partition was combined with the morphological data (2262 characters; 1115 variable characters [41.9%], 612 parsimony-informative characters [23.0%]). MP analysis resulted in a single tree (Fig. 6).

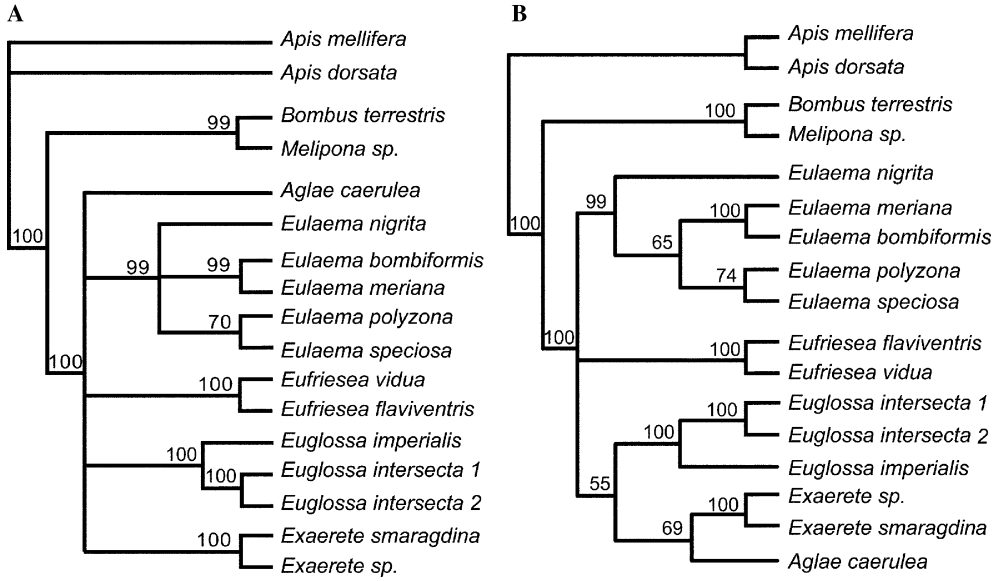

Fig. 4. Three-gene partition MP (A) and ML (B) trees. Bootstrap values (1000 replicates for MP, 100 replicates for ML) are indicated on the corresponding nodes. (A) MP tree length = 1847, CI = 0.633, RI = 0.555; (B) ML tree ln $L = -11474.93$.

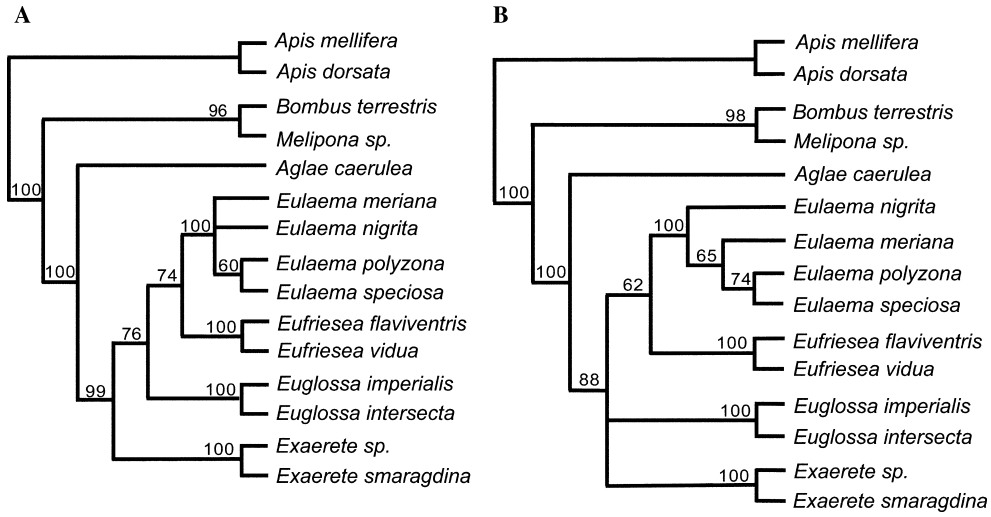

Fig. 5. Four-gene partition MP (A) and ML (B) trees. Bootstrap values (1000 replicates for MP, 100 replicates for ML) are indicated on the corresponding nodes. (A) MP tree length = 1838, CI = 0.681, RI = 0.572; (B) ML tree ln $L = -13109.07$.

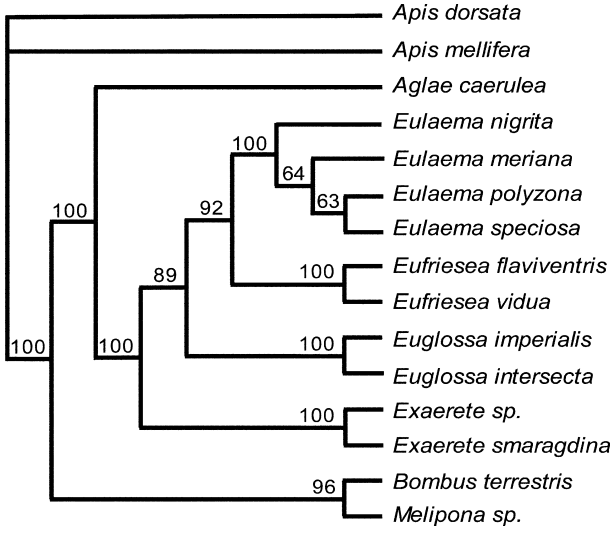

Fig. 6. Phylogeny of Euglossini derived from the combined analysis of 2625 molecular characters and 37 morphological characters (MP analysis; bootstrap 1000 replicates). Length = 2003, CI = 0.694, HI = 0.306.

Euglossini was monophyletic (BV = 100) as was each genus (BV = 100). *Aglae* was sister group to the remainder of the tribe (BV = 100) and *Eulaema* + *Eufriesea* formed the apical-most clade (BV = 92). *Euglossa* was sister group to *Eulaema* + *Eufriesea* (BV = 89), with *Exaerete* as sister group to (*Euglossa* (*Eulaema* + *Eufriesea*)) (BV = 100).

4. Discussion

4.1. Individual data partitions

The four gene fragments used in these analyses of euglossine relationships were selected to represent different structures and functions. Despite potential differences in patterns of variation, each data partition gives a compatible topology, except for the placement of *Aglae* in the EF-1α tree. This suggests that the models used to analyze the data were appropriate for recovering accurate phylogenetic signal for the taxa examined (Gaucher et al., 2001; Miyamoto and Fitch, 1995). While the individual gene fragments were useful for demonstrating monophyly of the genera, alone they were insufficient for resolving relationships among the genera. Both the equal and differential weighting schemes used in the parsimony analyses, down-weighting transitions at third codon positions of LW *Rh* and EF-1α (results not shown), were employed to decrease possible misleading effects of mutational saturation (Huang et al., 2000; Meyer, 1994). Nonetheless, all weighting schemes resulted in principally congruent topologies.

4.2. Combining data partitions

Whether to combine different data partitions that give significantly conflicting signal in phylogenetic analyses is still under debate (Bull et al., 1993; de Queiroz et al., 1995; Huelsenbeck et al., 1996; Miyamoto and Fitch, 1995; Scotland et al., 2003). The conditional combination approach (Bull et al., 1993; de Queiroz, 1993; de Queiroz et al., 1995) recommends that only homogeneous data sets be combined. Thus, combining the data probably maximizes the amount of information available overall (Vogler and Welsh, 1997; Chippindale et al., 1999). Partition homogeneity tests indicated that the LW *Rh* data were weakly heterogeneous relative to the other partitions. As alignment decisions can lead to rate heterogeneity (Lutzoni, 1997; Whiting et al., 1997; Sullivan, 1996), we excluded the highly variable intron

regions. Recent studies of the partition homogeneity test show that large differences in substitution rates among gene fragments may be interpreted as significant heterogeneity even if the underlying phylogeny is the same for both partitions (Barker and Lutzoni, 2002; Dolphin et al., 2000). Indeed, results of our analyses of the three- and four-gene data sets were mostly congruent, although the four-gene partition provided better resolution and stronger bootstrap values. Moreover, it is thought that increasing the size of a data set to more than 1000 characters increases the chances of recovering the correct phylogeny (DeBry and Olmstead, 2000; Hillis et al., 1994; Hillis, 1995), even with missing data (Wiens, 2003). Therefore, in our analyses, combining the data from each gene fragment is appropriate for assessing accurate relationships among Euglossini.

The molecular and morphological partitions were also homogeneous, and the branch support for the topology inferred from these combined partitions is higher than that of any of the other analyses. It is possible that branch support might be improved even further by employing a mixed-model Bayesian analysis (Ronquist and Huelsenbeck, 2003) to make full use of the patterns of evolution within each gene partition. It is unlikely, however, that the topology would change given the strong congruence between the molecular and morphological data and high bootstrap support of the overall combined phylogeny. Complete taxon sampling of each genus will provide a test of this hypothesis.

In summary, analysis of the combined partitions strongly supports monophyly of the Euglossini and monophyly of each euglossine genus. The relationships among the five genera are unambiguous, with strong support for cleptoparasitic *Aglae* as sister group to the other genera. The nonparasitic *Eulaema* + *Eufriesea* comprise an apical clade, with nonparasitic *Euglossa* as sister group. Cleptoparasitic *Exaerete* is sister group to the three nonparasitic genera.

4.3. Comparison with prior phylogenetic hypotheses

The five previous morphology-based euglossine phylogenies using a largely overlapping character set have resulted in four different topologies (Figs. 1A, B, D, E—C is a less resolved version of B). These incongruent results could be explained by the choice and polarization of the characters. Euglossines are indeed distinct morphologically, and many characters useful for showing generic relationships do not exist in potential outgroups (Kimsey, 1984b, 1987). The earliest studies (Kimsey, 1982, 1987) were prior to the common use of standardized algorithms for global outgroup analysis, and did not include outgroup characters in the data matrices. Polarity of the characters was determined mainly by "assuming that the dominant condition was the most primitive one, or (...) the more elaborate the more derived" (Kimsey, 1987, p. 64). Obviously, such assumptions may lead to bias in the reconstruction of phylogeny, particularly in the placement of the root. Many characters used by Kimsey (1982, 1987) were reinterpreted by Engel (1999). For example, the presence of a sternal groove was considered plesiomorphic by Kimsey (1987) but derived by Engel (1999); the linear volsella is a derived character grouping *Eufriesea*, *Eulaema*, and *Aglae* in Kimsey (1987), but was reinterpreted as a plesiomorphy for the tribe by Engel (1999). Where both Kimsey and Engel considered the shape of the male tibial slit to be broad and ovoid in *Eufriesea*, *Eulaema*, and *Aglae*, MLO (Appendix A) considers *Aglae* instead to have the narrow, curved condition described in *Exaerete* and *Euglossa*. Considering the small number of characters used in these morphological studies (25 in Kimsey, 1987; 15 in Engel, 1999; 37 in Oliveira, Appendix A), the obvious difficulties in describing and polarizing the characters could lead to contradictory phylogenetic conclusions.

Each euglossine genus is also distinct and specialized, thus many morphological characters observed in euglossines are autapomorphic for a particular genus. For example, out of 25 characters used in Kimsey's analysis (1987), 19 were autapomophies for genera and therefore not useful for phylogenetic purposes. Engel (1999) implies many homoplasious character transitions (11 homoplasious transitions for 12 unreversed changes), which could be considered difficult to polarize, or could reflect misinterpretation of the evolution of the characters. The monospecific *Aglae*, which is parasitic, is particularly unique among euglossine genera (Engel, 1999; Kimsey, 1987), being "so streamlined and simplified externally and [having] such highly derived male genitalia that it is very different from all other euglossines." (Kimsey, 1987, p. 68). This could explain why *Aglae* has been placed in two widely different positions in the different morphology trees (compare trees in Fig. 1). Most analyses have placed *Exaerete* as sister group to *Euglossa*, but MLO finds only a single synapomorphy (his character 29) for that relationship. MLOs reexamination of euglossine morphology has yielded a character state matrix that is entirely congruent with our overall molecular data set (Table 4), which gives strong support for the placement of *Exaerete* as sister group to the (*Euglossa* (*Eulaema* + *Eufriesea*)) clade.

Interestingly, *Aglae* diverged prior to the nonparasitic genera, one of which (*Eulaema*) is its current host. This pattern clearly violates "Emery's rule" (see Wilson, 1971), which states that parasites in Hymenoptera more closely resemble their hosts than any other group. The implication is that parasites generally evolve from the same lineage as that of their host. Of course, host shifts may have taken place in combination with extinction of past hosts. It is also possible that *Aglae* and *Exaerete* at one time included nonparasitic species that served as

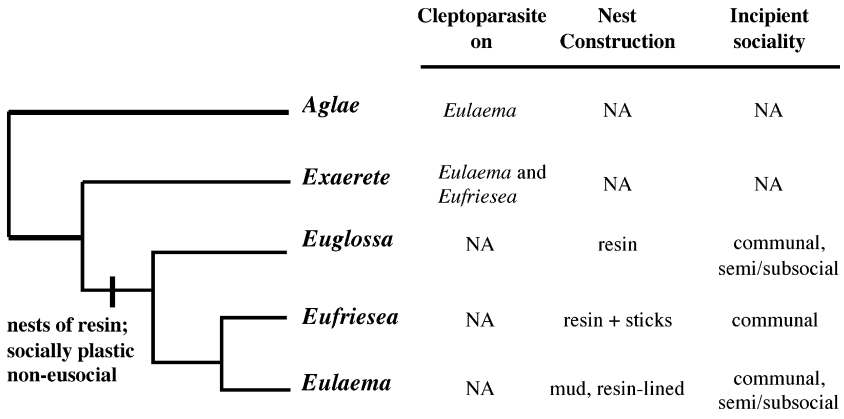

Fig. 7. Nest construction and levels of social interaction hypothesized for common ancestor of the nonparasitic Euglossini.

hosts but which are now extinct, or as yet undescribed. Most likely, the common ancestor of the euglossines was nonparasitic, with *Aglae* evolving from a nonparasitic relative that later became extinct. Without fossils, this remains speculative. Similarly, *Exaerete*, the other cleptoparasitic genus, appears not to have evolved from among its current hosts, *Eufriesea* and *Eulaema*. In fact, none of the extant nonparasitic genera had evolved by the time the extant cleptoparasitic genera were diverging.

Regarding the evolution of variable nesting behavior among the nonparasitic Euglossini, Fig. 7 would suggest a resin-collecting common ancestor, perhaps similar to present-day *Euglossa*, with later additions of sticks and wood chips (*Eufriesea*) or the use of mud (*Eulaema*) in nest building. It appears that most of the evolutionary diversification in the use of nest building material occurred in the early stages of divergence of the genera and that each genus is fixed for use of one type or the other. For instance, to our knowledge there are no mud-building *Euglossa*, nor any *Eulaema* that build nests of resin (though they may line brood cells with resin) or use wood chips. Because phenotypic variation is ultimately interpretable in the light of both environmental adaptation and phylogenetic history, our phylogeny provides a framework for future work on comparative nest architecture within and between genera.

Cooperation in cell provisioning, foraging, and guarding are indicators of division of labor and eusociality, especially if backed up with evidence that some females in the nest are unmated and have slender ovaries. Social behavior of the putative nonparasitic euglossine common ancestor was probably not eusocial, but facultatively communal (terms described in Michener, 1974, 2000) with occasional formation of semi- or subsocial nests (Fig. 7). It is not known, nor may it ever be fully understood, why the orchid bees never bridged the gap between simple social interactions and true sociality (Michener, 1974, 2000). Nonetheless, field studies of large communal, semi- and sub-social nests are essential to filling the gaps in our understanding of euglossine social behavior.

Note added in proof

While this paper was in review, a sixth species, intermediate between *Exaerete frontalis* and *E. smaragdina* was reported (Oliveira, M.L., Nemésio, A., 2003 *Exaerete lepeletiere* (Hymenoptera: Apidae: Apini: Euglossina): a new cleptoparasitic bee from Amazonia. Lundiana 4, 117–120), but was not available for analysis.

Acknowledgments

We especially thank those individuals who contributed specimens (Mike Sharkey [Colombia project] and Benjamin Bembé), assisted with identification of specimens (Dressler, 1978, 1982), and provided technical assistance (Heather Hines). We thank two anonymous reviewers for useful comments on the manuscript. This work was supported by NSF (GER-9450117) and USDA (NRICGP-2002-35302-11553) Grants to S.A.C.

Appendix A. Morphological characters and character states

1. Metallic shine on integument of head and thorax: absent (0); present (1).
2. Medial carina of clypeus: absent (0); present (1).
3. Postocellar carina: absent (0); present (1).
4. Length of the first antennal flagellum: shorter than or equal to second (0); longer than the second (1); equal to second plus third (2).
5. Genal projection (Character 2 in Kimsey, 1987): absent (0); present (1).
6. Clypeus laterally elevated above frons: slightly elevated (0); strongly elevated (1).
7. Labial palpus (Character 1 in Kimsey, 1987; 2 in Kimsey, 1982 and 2 in Engel, 1999): four-segmented (0); two-segmented (1).
8. Pilosity of thorax (Character 12 in Kimsey, 1982): very dense (0); dense (1); sparce (2).

9. Hypoepimeral knob (Character 11 in Engel, 1999): absent (0); present (1).
10. Mesoscutellar posterior margin: slightly convex (0); slightly linear (1).
11. Medial line of mesoscutum: grooved (0); carinate (1).
12. Scutellum in profile view (Character 3 in Kimsey, 1987 and 2 in Engel, 1999): convex (0); depressed (1).
13. Scutellar tuft (Character 4 in Kimsey, 1987 and 3 in Engel, 1999): absent (0); present (1).
14. Scutellar posterior margin: slightly convex (0); slightly concave (1).
15. Jugal comb (Character 15 in Engel, 1999): absent (0); present (1).
16. Sternal groove (Character 5 in Kimsey, 1987 and 4 in Engel, 1999): absent (0); present (1).
17. Midtibial carina (Character 5 in Engel, 1999): absent (0); present (1).
18. Shape of midtibial carina (Character 9 in Kimsey, 1987): incomplete (0); complete (1).
19. Midtibial apicolateral projection (Character 8 in Kimsey, 1987): absent (0); present (1).
20. Internal midtarsal tooth (Character 12 in Engel, 1999): absent (0); present (1).
21. Hindfemoral dentition (Character 13 in Engel, 1999): absent (0); present (1).
22. Hindtibial slit (Characters 13-15 in Kimsey, 1987 and 14 in Engel, 1999): absent (0); present (1).
23. Shape of hindtibial slit (Characters 13-15 in Kimsey, 1987 and 6 in Engel, 1999): broad, ovoid (0); narrow, curved (1).
24. Length of hindtibial slit (Character 5 in Kimsey, 1987 and 7 in Engel, 1999): reaching tibial apex (0); not reaching tibial apex (1).
25. Apex of hindtibia: (Character 8 in Engel, 1999): rounded (0); pointed (1).
26. Pilosity on outer surface of hindtibia: dense (0); slightly dense (1); sparse (2).
27. Shape of corbicula (Character 4 in Kimsey, 1982): normal (0); reduced (1).
28. Length of hindtibia (Character 6 in Kimsey, 1982): more than two times the width (0); less than two times the width (1).
29. Length of fifth hindtarsus: equal to fifth midtarsus (0); shorter than fifth midtarsus (1); longer than fifth midtarsus (2).
30. Length of external hindtibial spur: shorter than the internal spur (0); equal to the internal spur (1).
31. Antero-superior margin of first abdominal tergum: slightly concave or linear (0); with a frontal projection (1).
32. Length of first abdominal tergum (exposed portion): half of the second (0); 1/3 of the second (1).
33. Tufts on second sternum (Character 17 in Kimsey, 1987): absent (0); present (1).
34. Apical lobe of seventh sternum in profile view: depressed (0); expanded (1).
35. Ventrolateral projection of gonocoxite (Character 19 in Kimsey, 1987 and 9 in Engel, 1999): absent (0); present (1).
36. Hindtibial auricle: absent (0); present (1).
37. Gonostylus (Character 10 in Kimsey, 1982): simple (0); bilobed (1).

References

Ascher, J.S., Danforth, B.N., Ji, S., 2001. Phylogenetic utility of the major opsin in bees (Hymenoptera: Apoidea): a reassessment. Mol. Phylogenet. Evol. 19, 76–93.

Barker, F.K., Lutzoni, F.M., 2002. Spurious rejection of phylogenetic congruence by the ILD test: a simulation study. Syst. Biol. 51, 625–637.

Becker, P., Moure, J.S., Peralta, F.J.A., 1991. More about euglossine bees in Amazonian forest fragments. Biotropica 23, 586–591.

Bennett, F.D., 1965. Notes on a nest of *Eulaema terminata* Smith (Hymenoptera, Apoidea) with a suggestion of the occurrence of a primitive social system. Insectes Sociaux, Paris 12, 81–92.

Bennett, F.D., 1972. Observations on *Exaerete* spp. and their hosts *Eulaema terminata* and *Euplusia surinamensis* (Hymen., Apidae, Euglossinae) in Trinidad. J. N. Y. Entomol. Soc. 80, 137–145.

Briscoe, A.D., 2001. Functional diversification of lepidopteran opsins following gene duplication. Mol. Biol. Evol. 18, 2270–2279.

Bull, J.J., Huelsenbeck, J.P., Cunningham, C.W., Swofford, D.L., Waddell, P.J., 1993. Partitioning and combining data in phylogenetic analysis. Syst. Biol. 42, 384–397.

Cameron, S.A., 1993. Multiple origins of advanced eusociality in bees inferred from mitochondrial DNA sequences. Proc. Natl. Acad. Sci. USA 90, 8687–8691.

Cameron, S.A., 2003. Data from the elongation factor-1α gene corroborates the phylogenetic pattern from other genes revealing common ancestry of bumble bees and stingless bees (Hymenoptera: Apinae). III Scminario Mesoamericano sobre Abejas sin Aguijón, Tapachula, Chiapas, Mexico, pp. 132–135.

Cameron, S.A., 2004. Phylogeny and biology of neotropical orchid bees (Euglossini). Ann. Rev. Entomol. 49, 377–404.

Cameron, S.A., Mardulyn, P., 2001. Multiple molecular data sets suggest independent origins of highly eusocial behavior in bees (Hymenoptera : Apinae). Syst. Biol. 50, 194–214.

Cameron, S.A., Mardulyn, P., 2003. The major opsin gene is useful for inferring higher level phylogenetic relationships of the corbiculate bees. Mol. Phylogenet. Evol. 28, 610–613.

Cameron, S.A., Ramírez, S., 2001. Nest architecture and nesting ecology of the orchid bee *Eulaema meriana* (Hymenoptera : Apinae : Euglossini). J. Kansas Entomol. Soc. 74, 142–165.

Cameron, S.A., Williams, P.H., 2003. Phylogeny of bumble bees in the new world subgenus *Fervidobombus* (Hymenoptera, Apidae): congruence of molecular and morphological data. Mol. Phylogenet. Evol. 28, 552–563.

Cameron, S.A., Derr, J.N., Austin, A.D., Woolley, J.B., Wharton, R.A., 1992. The application of nucleotide sequence data to phylogeny of the Hymenoptera: a review. J. Hymenop. Res. 1, 63–79.

Carulli, J.P., Cen, D.-M., Stark, W.S., Hartl, D.L., 1994. Phylogeny and physiology of *Drosophila* opsin. J. Mol. Evol. 38, 250–262.

Chang, B.S.W., Ayers, D., Smith, W.C., Pierce, N.E., 1996. Cloning of the gene encoding honeybee long-wavelength rhodopsin: a new class of insect visual pigments. Gene 173, 215–219.

Chippindale, P.T., Davéé, V.K., Whitmore, D.H., Robinson, J.V., 1999. Phylogenetic relationships of North American Damselflies of the genus *Ischnura* (Odonata: Zygoptera: Coenagrionidae) based on sequences of three mitichondrial genes. Mol. Phylogenet. Evol. 11, 110–121.

Crozier, R.H., Crozier, Y.C., 1993. The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. Genetics 133, 97–117.

Crozier, R.H., Crozier, Y.C., Mackinlay, A.G., 1989. The COI and COII region of honeybee mitochondrial DNA: evidence for variation in insect mitochondrial evolutionary rates. Mol. Biol. Evol. 6, 399–411.

Cruickshank, R.H., Johnson, K.P., Smith, V.S., Adams, R.J., Clayton, D.H., Page, R.D.M., 2001. Phylogenetic analysis of partial sequences of elongation factor 1α identifies major groups of lice (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 19, 202–215.

Cunningham, C.W., 1997. Can three incongruence tests predict when data should be combined? Mol. Biol. Evol. 14, 733–740.

Danforth, B.N., Conway, L., Ji, S., 2003. Phylogeny of eusocial *Lasioglossum* reveals multiple losses of eusociality within a primitively eusocial clade of bees (Hymenoptera: Halictidae). Syst. Biol. 52 (1), 23–36.

Danforth, B., Sauquet, H., Packer, L., 1999. Phylogeny of the bee genus *Halictus* (Hymenoptera: Halictidae) based on parsimony and likelihood analyses of nuclear EF-1α sequence data. Mol. Phylogenet. Evol. 13, 605–618.

Darwin, C., 1862. On the Various Contrivances by which British and Foreign Orchids are Fertilised by Insects. John Murray, London.

DeBry, R.W., Olmstead, R.G., 2000. A simulation study of reduced tree-search effort in bootstrap resampling analysis. Syst. Biol. 49, 171–179.

de Queiroz, A., 1993. For consensus (sometimes). Syst. Biol. 42, 368–372.

de Queiroz, A., Donogue, M.J., Kim, J., 1995. Separate versus combined analysis of phylogenetic evidence. Annu. Rev. Ecol. Syst. 26, 657–681.

Dodson, C.H., 1967. Relationships between pollinators and orchid flowers. Atas Simp. Biota Amaz. 5, 1–72.

Dodson, C.H., 1975. Coevolution of orchids and euglossine bees. In: Gilbert, L., Raven, P.H. (Eds.), Coevolution of Animals and Plants. University of Texas Press, Austin, pp. 91–99.

Dodson, C.H., Frymire, G.P., 1961. Natural pollination of orchids. Missouri Bot. Gard. Bull. 69, 133–152.

Dolphin, K., Belshaw, R., Orme, C.D.L., Quicke, D.L.J., 2000. Noise and incongruence: interpreting results of the incongruence length difference test. Mol. Phylogenet. Evol. 17, 401–406.

Dowton, M., Austin, A.D., 1994. Molecular phylogeny of the insect order Hymenoptera: apocritan relationships. Proc. Natl. Acad. Sci. USA 91, 9911–9915.

Dressler, R.L., 1978. An infrageneric classification of *Euglossa*, with notes on some features of special taxonomic importance (Hymenoptera; Apidae). Rev. Biol. Trop. 26, 187–198.

Dressler, R.L., 1979. *Eulaema bombiformis, Eulaema meriana* and Muellerian mimicry in related species (Hymenoptera, Apidae). Biotropica 11, 144–151.

Dressler, R.L., 1982. Biology of the orchid bees (Euglossini). Annu. Rev. Ecol. Syst. 13, 373–394.

Dressler, R.L., Opsina-Torres, R., 1997. Una nueva especie *Eulaema* (Hymenoptera: Apidae) del Chocó, Colombia. Caldasia 19 (1-2), 95–100.

Eberhard, W.G., 1988. Group nesting in two species of *Euglossa* bees (Hymenoptera: Apidae). J. Kansas Entomol. Soc. 61, 406–411.

Eltz, T., Roubik, D.W., Whitten, M.W., 2003. Fragrances, male display and mating behavior of *Euglossa hemichlora*: a flight cage experiment. Physiol. Entomol. 28, 251–260.

Eltz, T., Whitten, W.M., Roubik, D.W., Linsenmair, K.E., 1999. Fragrance collection, storage, and accumulation by individual male orchid bees. J. Chem. Ecol. 25, 157–176.

Engel, M.S., 1999. The first fossil *Euglossa* and phylogeny of the orchid bees (Hymenoptera: Apidae; Euglossini). Am. Museum Novitates 3272, 1–14.

Engel, M.S., 2001. Monophyly and extensive extinction of advanced eusocial bees: insights from an unexpected eocene diversity. Proc. Natl. Acad. Sci. USA 98, 1661–1664.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.

Garófalo, C.A., Camillo, E., Augusto, S.C., de Jesus, B.M.V., Serrano, J.C., 1998. Nest structure and communal nesting in *Euglossa* (*Glossura*) *annectans* Dressler (Hymenoptera, Apidae, Euglossini). Rev. Bras. Zool. 15, 589–596.

Garófalo, C.A., Rozen Jr., J.G., 2001. Parasitic behavior of *Exaerete smaragdina* with descriptions of its mature oocyte and larval instars (Hymenoptera: Apidae: Euglossini). Am. Museum Novitates, 1–26.

Gaucher, E.A., Miyamoto, M.M., Benner, S., 2001. Function–structure analysis of proteins using covarion-based evolutionary approaches: elongation factors. Proc. Natl. Acad. Sci. USA 98, 548–552.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hillis, D.M., 1995. Approaches for assessing phylogenetic accuracy. Syst. Biol. 44, 3–16.

Hillis, D.M., Huelsenbeck, J.P., Cunningham, C.W., 1994. Application and accuracy of molecular phylogenies. Science 264, 671–677.

Huang, Y., Orti, G., Sutherlin, M., Duhachek, A., Zera, A., 2000. Phylogenetic relationships of North American field crickets inferred from mitochondrial DNA data. Mol. Phylogenet. Evol. 17, 48–57.

Huelsenbeck, J.P., Bull, J.J., Cunningham, C.W., 1996. Combining data in phylogenetic analysis. Trends Ecol. Evol. 11, 152–158.

Johnson, K.P, Whiting, M.F., 2002. Multiple genes and the monophyly of Ischnocera (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 22, 101–110.

Jordal, B.H., 2002. Elongation factor 1α resolves the monophyly of the haplodiploid ambrosia beetles Xyleborini (Coleoptera: Curculionidae). Ins. Mol. Biol. 11, 453–463.

Kawakita, A., Sota, T., Ascher, J.S., Ito, M., Tanaka, H., Kato, M., 2003. Evolution and phylogenetic utility of alignment gaps within intron sequences of three nuclear genes in bumble bees (*Bombus*). Mol. Biol. Evol. 20, 87–92.

Kimsey, L.S., 1979. An illustrated key to the genus *Exaerete* with descriptions of male genitalia and biology (Hymenoptera: Euglossini, Apidae). J. Kansas Entomol. Soc. 52, 735–746.

Kimsey, L.S., 1982. Systematics of bees of the genus *Eufriesea* (Hymenoptera, Apidae). University of California Publications in Entomology 95, 1–125.

Kimsey, L.S., 1984a. A re-evaluation of the phylogenetic relationships of the Apidae (Hymenoptera). Systematic Entomology. 9, 431–435.

Kimsey, L.S., 1984b. The behavioural and structural aspects of grooming and related activities in euglossine bees (Hymenoptera: Apidae). J. Zool. (London) 204, 541–550.

Kimsey, L.S., 1987. Generic relationships within the Euglossini (Hymenoptera: Apidae). Syst. Entomol. 12, 63–72.

Kimsey, L.S., Dressler, R.L., 1986. Synonymic species list of Euglossini. Pan Pac. Entomol. 62, 229–236.

Koulianos, S., Schmid-Hempel, H.P., 2000. Phylogenetic relationships among bumble bees (*Bombus*, Latreille) inferred from mitochondrial cytochrome *b* and cytochrome oxidase I sequences. Mol. Phylogenet. Evol. 14, 335–341.

Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. Analysis software MEGA2: Molecular Evolutionary Genetics. Arizona State University, Tempe, USA.

Leys, R., Cooper, S.J.B., Schwarz, M.P., 2000. Molecular phylogeny of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae), based on mitochondrial DNA sequences. Mol. Phylogenet. Evol. 17, 407–418.

Lockhart, P.J., Cameron, S.A., 2001. Trees for bees. Trends Ecol. Evol. 16, 84–88.

Lutzoni, F.M., 1997. Phylogeny of lichen- and non-lichen-forming Omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. Syst. Biol. 46, 373–406.

Mardulyn, P., Cameron, S.A., 1999. The major opsin in bees (Insecta : Hymenoptera): a promising nuclear gene for higher level phylogenetics. Mol. Phylogenet. Evol. 12, 168–176.

Merian, M.S., 1705. Metamorphosis Insectorum Surinamensis (Amsterdam).

Meyer, A., 1994. Shortcomings of the cytochrome *b* gene as a molecular marker. Trends Ecol. Evol. 9, 278–280.

Michener, C.D., 1974. The Social Behavior of the Bees. Harward University Press, Cambridge, MA.

Michener, C.D., 1990. Classification of the Apidae (Hymenoptera). Univ. Kans. Sci. Bull. 54, 75–164.

Michener, C.D., 2000. The Bees of the World. Johns Hopkins University Press, Baltimore and London.

Michener, C.D., Winston, M.L., Jander, R., 1978. Pollen manipulation and related activities and structures in bees of the family Apidae. Univ. Kansas Sci. Bull. 51, 575–601.

Miyamoto, M.M., Fitch, W.M., 1995. Testing species phylogenies and phylogenetic methods with congruence. Syst. Biol. 44, 64–76.

Monteiro, A., Pierce, N.E., 2001. Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from COI, COII and EF-1α gene sequence. Mol. Phylogenet. Evol. 18, 264–281.

Moure, J.S., 1989. *Glossuropoda*, novo subgênero de *Euglossa*, e duas espécies novas da Amazônia, do mesmo subgênero. Memorias do Instituto Oswaldo Cruz 84, 387–389.

Moure, J.S, 1999. Novas espécies e notas sobre Euglossinae do Brasil e Venezuela (Hymenoptera, Apidae). Revista Brasileira de Zoología 16 (Supl.), 91–104.

Myers, J., Loveless, M.D., 1976. Nesting aggregations of the euglossine bee *Euplusia surinamensis* (Hymenoptera: Apidae): individual interactions and the advantage of living together. Can. Entomol. 108, 1–6.

Nates-Parra, G., González, V.H., 2000. Notes on the nest of *Eulaema polychroma* (Hymenoptera: Apidae: Euglossini). Actualidades Biólogicas 22, 83–90.

Noll, F.B., 2002. Behavorial phylogeny of corbiculate Apidae (Hymenoptera; Apinae), with special reference to social behavior. Cladistics 18, 137–153.

Oliveira, M.L., 2000. O genero *Eulaema* Lepeletier, 1841 (Hymenoptera, Apidae, Euglossini): Filogenia, biogeografia e relaçòes com as Orchidaceae. PhD thesis, Universidade de Sao Paulo, Ribeirào Preto SP, pp. 160.

Oliveira, M.L., 2001. Stingless bees (Meliponini) and orchid bees (Euglossini) in "terra firme" tropical forests and forest fragments. In: Bierregaard Jr., R.O., Gascon, C., Lovejoy, T.E., Mesquita, R. (Eds.), Lessons from Amazonia: The Ecology and Conservation of a Fragmented Forest. Yale University Press, New Haven, pp. 208–218 (Chapter 17).

Oliveira, M.L., 2004. Novas hipóteses de relacionamentos filogenéticos para os gêneros de Euglossini e para as espécies de *Eulaema* Lepeletier, 1841 (Hymenoptera: Apidae: Euglossini). Acta Amazonica (in press).

Oliveira, M.L., Campos, L.A.O., 1995. Abundância, riqueza e diversidade de abelhas Euglossinae (Hymenoptera: Apidae) em florestas contínuas de terra firme na Amazônia Central. Revta bras. Zool. 12, 547–556.

Ospina-Torres, R., Sandino-Franco, J.C., 1997. *Eulaema chocoana*, nueva especie de abeja euglosina de la costa pacifica colombiana. Caldasia 19, 165–174.

Pedersen, B.V., 1996. A phylogenetic analysis of cuckoo bumblebees (*Psithyrus*, Lepeletier) and bumblebees (*Bombus*, Latreille) inferred from sequences of the mitochondrial gene cytochrome oxidase. Mol. Phylogenet. Evol. 5, 289–297.

Pedersen, B.V., 2002. European bumblebees (Hymenoptera: Bombini)—phylogenetic relationships inferred from DNA sequences. Insect Syst. Evol. 33, 361–386.

Peruquetti, R.C., 2000. Function of fragrances collected by Euglossini males (Hymenoptera: Apidae). Entomologia Generalis 25, 33–37.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Roig-Alsina, A., Michener, C.D., 1993. Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). Univ. Kansas Sci. Bull. 55, 123–173.

Rokas, A., Nylander, J.A., Ronquist, F., Stone, G.N., 2002. A maximum-likelihood analysis of eight phylogenetic markers in gallwasps (Hymenoptera: Cynipidae): implications for phylogenetic studies. Mol. Phylogenet. Evol. 22, 206–219.

Ronquist, F., Huelsenbeck, J.P., 2003. Mr Bayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Roubik, D.W., Hanson, P.E., 2004. Orchid bees: biology and field guide. San Jose, CR: INBIO (in press).

Santos, M.L., Garófalo, C.A., 1994. Nesting biology and nest re-use of *Eulaema nigrita* (Hymenoptera: Apidae, Euglossini). Insectes Sociaux 41, 99–110.

Schultz, T.R., Engel, M.S., Prentice, M., 1999. Resolving conflict between morphological and molecular evidence for the origin of eusociality in the "corbiculate" bees (Hymenoptera: Apidae): a hypothesis-testing approach. Univ. Kansas Nat. Hist. Mus. Spec. Publ. 24, 125–138.

Schultz, T.R., Engel, M.S., Ascher, J.S., 2001. Evidence for the origin of eusociality in the corbiculate bees (Hymenoptera: Apidae). J. Kans. Entomol. Soc. 74, 10–16.

Schwarz, M.P., Bull, N.J., Cooper, S.J.B., 2003. Molecular phylogenetics of allodapine bees, with implications for the evolution of sociality and progressive rearing. Syst. Biol. 52, 1–14.

Scotland, R.W., Olmstead, R.G., Bennett, J.R., 2003. Phylogenetic reconstruction: the role of morphology. Syst. Biol. 52, 539–548.

Sipes, S.P., Wolf, P.G., 2001. Phylogenetic relationships within *Diadasia*. A group of specialist bees. Mol. Phylogenet. Evol. 19, 144–156.

Sullivan, J., 1996. Combining data with different distributions of among-site rate variation. Syst. Biol. 45, 375–380.

Swofford, D.L., 2001. PAUP*, Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, MA.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

van der Pijl, L., Dodson, C.H., 1966. Orchid Flowers, Their Pollination and Evolution. University of Miami Press, Coral Gables, FL.

Vogler, A.P., Welsh, A., 1997. Phylogeny of North American *Cicindela* tiger beetles inferred from multiple mitochondrial DNA sequences. Mol. Phylogenet. Evol. 8, 225–235.

Whitfield, J.B., Cameron, S.A., 1998. Hierarchical analysis of variation in the mitochondrial 16S rRNA gene among Hymenoptera. Mol. Biol. Evol. 15, 1728–1743.

Whiting, M.F., Carpenter, J.C., Wheeler, Q.D., Wheeler, W.C., 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. 46, 1–68.

Wiens, J.J., 2003. Missing data, incomplete data, and phylogenetic accuracy. Syst. Biol. 52, 528–538.

Wilson, E.O., 1971. Insect Societies. Harvard University Press, Cambridge, MA.

Zucchi, R., Sakagami, S.F., de Camargo, J.M.F., 1969. Biological observations on a neotropical parasocial bee, *Eulaema nigrita*, with a review on the biology of Euglossinae (Hymenoptera, Apidae). A comparative study. J. Fac. Sci. Hokkaido Univ. Ser. VI 17, 271–380.