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Phylogenetic relationships among tribes in Xylocopinae (Apidae) and implications on nest structure evolution

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ABSTRACT

The subfamily Xylocopinae has been recognized as the most basal lineage within the family Apidae, comprising four tribes; Allodapini, Ceratinini, Xylocopini and Manueliini. Relationships among the tribes are not well resolved with morphological data. In particular, Manueliini and Xylocopini have each been placed as the most basal lineage in separate analyses of the subfamily. While relationships within each tribe, excepting Manueliini, have been investigated using molecular data, these data have not been applied to examine the relationships among tribes, which remain controversial. Here we present results of molecular phylogenetic analyses using sequences of CoI, Cyt*b* and EF-1αF1 from members of the four tribes of Xylocopinae. We used available data from other studies in combination with data generated for the three species of Manueliini. Competing phylogenetic hypotheses regarding the alternate positions proposed to Manueliini and Xylocopini were evaluated through statistical tests. The basal position of either Manueliini or Xylocopini has contrasting implications on the evolutionary history of nest architecture, which mediates the potential for contact between adult and immature individuals. Our results indicate that Manueliini is the most basal lineage of Xylocopinae, in agreement with an evolutionary transition from nests having completely sealed cells to nests lacking cells. A nest structure with closed cells prevents physical interactions between adult and immature stages, whereas an open structure provides the opportunity for interactions that may play an important role in the emergence of sociality.

1. Introduction

The subfamily Xylocopinae includes four tribes: Manueliini, Xylocopini, Ceratinini and Allodapini (systematics reviewed in Michener, 2007). This group has been considered a valuable model to study early stages and evolution of sociability (e.g. Michener, 1985; Schwarz et al., 1997, 1998, 2007; Tierney et al., 2002), since it includes species ranging from solitary to social levels of organization (Michener, 1969, 1974, 1985). Information on nesting attributes, which is necessary to understand the complexity of social systems in Xylocopinae (Michener, 1990), has been reported for several species; however, data on nesting biology (e.g. nest architecture) have not been interpreted within a phylogenetic framework including representative species from each tribe.

1.1. Nest structure in Xylocopinae

The nests of Manueliini (Daly et al., 1987; Flores-Prado et al., 2008a), Xylocopini (excepting *Proxylocopa* subgenus) and Ceratinini species consist of either branched or simple burrows, transversely divided by partitions made of bits of wood to form a series of cells. In those tribes, cell building by females includes the construction of a food mass, which is the substrate for oviposition, followed by cell closure. The nest is formed by sequential repetition of these steps (see reviews in Michener, 1969, 1985, 1990). Both modification of part of this nest building pattern and removal of cell partitions by the mother, which provides the opportunity for contact with her immature progeny or between them, have been proposed as a prerequisite for the evolution of sociality in bees (Michener, 1969, 1974; Sakagami and Maeta, 1977).

In Manueliini, omission or removal of cell partitions by the mother during the breeding period (i.e. in cells containing immature individuals) is not evident from previous studies (Claude-Joseph, 1926; Daly et al., 1987; Flores-Prado et al., 2008a). Although for Xylocopini it has been pointed out that cells are invariantly sealed by partitions during the breeding period (Michener, 1985, 1990), removal and omission of partitions has been reported for some species (Sakagami and Laroca, 1971; Silveira, 2002). However, this nest architecture consisting of opened cells between sealed cells is far more common in Ceratinini (reviewed in Sakagami and Laroca, 1971; Sakagami and Maeta, 1977; Maeta

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et al., 1997). The most extreme example of open nest architecture is exhibited in Allodapini where none of the nests examined so far exhibit any cell partitions (reviewed in Michener, 1971, 1974; Schwarz et al., 1997, 2007). Therefore, alternative evolutionary histories for the nesting behaviors in Xylocopinae can be inferred depending on the phylogenetic relationships among tribes under consideration.

1.2. Phylogenetic hypotheses in Xylocopinae

The subfamily Xylocopinae has been hypothesized as the most basal lineage of the Apidae (Michener, 2007). It was included within the old family Anthophoridae (paraphyletic taxon no longer recognized), but cladistic analysis of morphological data led to its placement within Apidae (Roig-Alsina and Michener, 1993). Manueliini has been proposed as a relict lineage of Xylocopinae (Daly et al., 1987; Michener, 1979, 2007). This tribe consists of the single genus *Manuelia* Vachal containing only three species that occur predominantly in Chile: *Manuelia gayi*, *Manuelia postica* and *Manuelia gayatina* (Sakagami and Michener, 1987; Michener, 2007). Analyses of the relationships among the Xylocopinae tribes by Sakagami and Michener (1987), Roig-Alsina and Michener (1993) and Engel (2001) identified two alternative placements of *Manuelia*.

Roig-Alsina and Michener (1993) used morphological data to obtain two minimum-length trees of Xylocopinae that differ in the positions of Manueliini and Xylocopini. One topology placed Manueliini as the most basal position within the Xylocopinae with Xylocopini as a secondary divergence, while the sequence of divergence events is reversed in the alternative topology. Engel (2001) examined the relationships among the tribes of Xylocopinae, also on the basis of morphological characters, but including a Baltic amber fossil. The results of the analysis were in agreement with one of the topologies presented by Roig-Alsina and Michener (1993), wherein Xylocopini is the most basal lineage in Xylocopinae: (Xylocopini (Manueliini (Ceratinini, Allodapini))).

Molecular phylogenetic studies have been undertaken for species of Apidae, including species belonging to different tribes of Xylocopinae (Cameron, 1993; Schwarz et al., 1998; Mardulyn and Cameron, 1999; Ascher et al., 2001; Cameron and Mardulyn, 2001; Leys et al., 2002; Bull et al., 2003; Rehan et al., 2010). However, Manueliini species have not yet been included as part of the ingroup in molecular phylogenetic analyses. This study generates DNA sequence data from the three species of Manueliini for a phylogenetic study of the relationships among tribes of Xylocopinae. Resolution of the competing hypotheses on the relative positions of tribes Manueliini and Xylocopini is critical to inferences concerning the evolutionary history of nesting biology in Xylocopinae. In particular, an early diversification of Manueliini is more compatible with a transition within the subfamily from nests containing cells invariantly sealed by partitions to non-partitioned nests. The aim of this study is to reveal the relationships among tribes of Xylocopinae using nucleotide data and to test the competing hypotheses on the basal lineages within the subfamily (Manueliini versus Xylocopini). The results of this work contribute new insights into the phylogeny of Xylocopine bees, while generating the phylogenetic framework necessary to examine the evolution of nesting biology at this basal lineage of Apidae.

2. Materials and methods

2.1. Samples and sequencing

Samples of *M. postica*, *M. gayi* and *M. gayatina* were collected in Central Chile and used to obtain nucleotide sequences from regions of the CoI (cytochrome oxidase I) and Cyt*b* (cytochrome *b*) genes in mtDNA and a region of the EF-1αF1 (elongation factor copy F1) nuclear gene. These genes have been used in previous studies of molecular phylogenetics within three tribes of Xylocopinae: Xylocopini (Leys et al., 2000), Allodapini (Bull et al., 2003; Schwarz et al., 2004, 2006), and Ceratinini (Bull et al., 2003; Cronin, 2004; Fuller et al., 2005; Schwarz et al., 2006).

Single bees were homogenized and DNA extracted using the DNeasy Tissue Kit (Qiagen). PCR amplification was performed by mixing 1.0 μl of DNA with 49 μl of PCR master mix containing 1× reaction buffer, 0.1 mM each dNTP, 0.2 μM each primer, and 2.5 U of Taq Polymerase (New England Biolabs). Primer sequences of Folmer et al. (1994) were used to amplify CoI. Alignments of reference sequences, from different species of Apidae, were used to design primers in conserved regions of Cyt*b* and EF-1aF1. Respectively, forward and reverse primers, amplicon size, and annealing temperature used for PCR were as follows: Cyt*b*: (cb1 = 5′TATGTACTACCATGAGGACAAATATC3′ and cb2 = 5′ATTACACCTCCTAATTTATTAGGAAT3′), 651 bp, 60 °C; EF-1aF1: (EF-1For2 = 5′AAG GAG GC[C/G] CAG GAG ATG GG3′ and EF-1Rev2 = 5′[T/C]TC [G/C]AC [T/C]TT CCA TCC GTA CC3′), 453 bp, 62 °C.

An MJ Research thermocycler was used to incubate the reactions for 2 min at 95 °C, and cycle 35 times at 95 °C for 0.5 min, at the annealing temperature for 0.5 min, and 72 °C for 1 min followed by 10 min at 72 °C. PCR products were purified with the MinElute PCR Purification Kit (Qiagen). Purified PCR products were sequenced with Big Dye Terminator Chemistry V3 (ABI). 5–10 ng of amplified target was added to 4.5 μl of Big Dye reaction mix with 0.4 μM of primer to a total volume of 10 μl. Reactions were ramped to 96 °C at 2.5 °C/s and cycled 30 times for 10 s at 96 °C, 5 s at 50 °C, and 2 min at 60 °C. Sequences were cleaned using Wizard (r) Magnesil Green Sequencing Reaction Clean-Up System (Promega) and analyzed with an ABI 3730 DNA sequencer. Sequences were deposited on GenBank under accessions HM461878–HM461886 (Table 1).

Sequences from species representing the tribes Xylocopini, Allodapini and Ceratinini, and also from *Apis mellifera* were obtained from Genbank (Table 1). *A. mellifera* was used as outgroup since it belongs to the Apinae subfamily which, together Nomadinae, is the sister clade of Xylocopinae. Alignments were obtained using ClustalX (Thompson et al., 1997) and visually inspected using Bioedit (Hall, 1999).

2.2. Analyses of noise in phylogenetic signal

Saturation of phylogenetic signal was measured by plotting transitional and transversional uncorrected distances for each codon position against maximum likelihood distances for the complete gene fragment estimated under the GTR+G model of nucleotide substitution. Pairwise matrices for corrected and uncorrected distances were obtained using PAUP4.0b10 (Swofford, 2002).

In order to test for congruence among data partitions, 10^4 replicates of the partition homogeneity test (IDL, Farris et al., 1994, 1995) were run in PAUP, comparing the phylogenetic signal between both mitochondrial genes and between mtDNA and EF-1αF1.

Since the IDL test showed incongruence of phylogenetic signal between mtDNA and EF-1αF1, suggesting distortion of phylogenetic signal in EF-1αF1 (see Section 3), homogeneity of base frequencies was tested on the three codon positions of EF-1αF1. First, base frequencies across taxa were analyzed using a χ^2 as implemented in PAUP. Second, a χ^2 test of the base frequencies observed in each species compared with the expected frequencies derived from the entire dataset was implemented in Tree-Puzzle v5 (Schmidt et al., 2002).

Table 1
Accession of nucleotide sequences used in the phylogenetic analysis of Xylocopinae.

Tribe	Species	Accessions (reference)		
		CoI	Cyt*b*	Ef-F1
–	*Apis mellifera*	AY114482.1 (Unpublished)	L06178.1 (Crozier and Crozier, 1993)	X52884.1 (Walldorf et al., 1990)
Allodapini	*Braunsapis unicolor*	DQ149658.1 (Schwarz et al., 2006)	AF072666.1 (Unpublished)	AJ416776.1 (Bull et al., 2003)
Allodapini	*Brevineura ploratula*	DQ149674.1 (Schwarz et al., 2006)	AJ416824.1 (Bull et al., 2003)	AJ416769.1 (Bull et al., 2003)
Allodapini	*Compsomelissa borneri*	DQ149675.1 (Schwarz et al., 2006)	AJ416840.1 (Bull et al., 2003)	AJ416784.1 (Bull et al., 2003)
Allodapini	*Exoneura robusta*	DQ149661.1 (Schwarz et al., 2006)	AJ416815.1 (Bull et al., 2003)	AJ416760.1 (Bull et al., 2003)
Allodapini	*Exoneurella tridentata*	DQ149665.1 (Schwarz et al., 2006)	AF072670.1 (Reyes et al.)*	AJ416766.1 (Bull et al., 2003)
Ceratinini	*Ceratina flavipes*	AY250190.1 (Cronin, 2004)	AY250200.1 (Cronin, 2004)	AY250210.1 (Cronin, 2004)
Ceratinini	*Ceratina iwatai*	AY250191.1 (Cronin, 2004)	AY250201.1 (Cronin, 2004)	AY250211.1 (Cronin, 2004)
Ceratinini	*Ceratina japonica*	AY250192.1 (Cronin, 2004)	AY250202.1 (Cronin, 2004)	AJ416849.1 (Bull et al., 2003)
Ceratinini	*Ceratina okinawana okinawana*	AY250194.1 (Cronin, 2004)	AY250204.1 (Cronin, 2004)	AY250214.1 (Cronin, 2004)
Manueliini	*Manuelia gayatina*	HM461879 (This study)	HM461882 (This study)	HM461885 (This study)
Manueliini	*Manuelia gayi*	HM461878 (This study)	HM461881 (This study)	HM461884 (This study)
Manueliini	*Manuelia postica*	HM461880 (This study)	HM461883 (This study)	HM461886 (This study)
Xylocopini	*Xylocopa bombylans*	AY005227.1 (Leys et al., 2000)	AY005254.1 (Leys et al., 2000)	AY005281.1 (Leys et al., 2002)
Xylocopini	*Xylocopa frontalis*	AY005248.1 (Leys et al., 2000)	AY005275.1 (Leys et al., 2000)	AY005302.1 (Leys et al., 2002)
Xylocopini	*Xylocopa pubescens*	AY005236.1 (Leys et al., 2000)	AY005263.1 (Leys et al., 2000)	AY005290.1 (Leys et al., 2002)
Xylocopini	*Xylocopa virginica virginica*	AY005231.1 (Leys et al., 2000)	AY005258.1 (Leys et al., 2000)	AY005285.1 (Leys et al., 2002)

2.3. Phylogeny of Xylocopinae

Phylogenetic analyses were executed in PAUP 4.0b10 for maximum parsimony (MP) and maximum likelihood (ML), and in MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001) for Bayesian inference (BA). Optimal models of nucleotide substitution, supported by the Akaike Information Criterion test (AIC; Akaike, 1974), were evaluated using ModelTest (Posada and Crandall, 1998, 2001) for ML and MrModeltest 2.0 (Nylander, 2004) for BA. Parameters for the priors of topology inference, used in BA, were also tested and selected using MrModeltest 2.0. The confidence values for each clade in MP and ML were assessed by bootstrap resampling (Felsenstein, 1985) with 10^3 pseudoreplicates, heuristic searching, and random-addition of sequences. In the BA analysis, two runs were conducted using 4 million generations in four independent chains. The number of generations needed to reach the stationary state were evaluated by plotting the likelihood values ($-\ln L$). Only generations above the stationary were included in the computation of the consensus tree, applying the 50% majority rule.

2.4. Hypothesis testing

The two alternate hypotheses on the basal lineage of Xylocopinae ("Xylocopini-basal" versus "Manueliini-basal") were compared using the approximately unbiased test (AU test; Shimodaira and Hasegawa, 2001; Shimodaira, 2002) as implemented in the package Consel, version 0.1i. First, maximum likelihood trees enforced either to "Xylocopini-basal" or the "Manueliini-basal" constrains were obtained in PAUP, using heuristic search under maximum likelihood optimization criterion. GTR+I+G was used as model for nucleotide substitutions, since it was the best-fit model according to the AIC test. The respective parameters were estimated for each tree during the heuristic search to ensure accurate estimations of likelihoods, especially for suboptimal trees. Site-wise likelihoods were estimated for each tree and used as input in the AU test. The scaled bootstrapping and estimation of probabilities and confidence intervals were performed using the default options in the respective programs included in Consel.

3. Results and discussion

This study represents the first analysis of the phylogenetic relationships among the four extant tribes of Xylocopinae using molecular data. The three species belonging to Manueliini were included in the analyses, but sampling within Xylocopini, Ceratinini and Allodapini was limited to a small number of species, representing a broadly distributed set of lineages. Therefore, this study does not contribute new insight on relationships within tribes. Instead, this work was especially conceived to contrast the two hypotheses concerning the basal lineage within this subfamily.

3.1. Substitution saturation on third codon positions

For pairwise comparisons of mtDNA sequences, uncorrected distances for nucleotide transversions increased with corrected GTR+G distances (Fig. 1). On the other hand, corrected GTR+G distances were not predictive of uncorrected transitions. These results indicate saturation for transitions at third codon positions for both mitochondrial genes affecting nearly all the dataset, thus the consequences of homoplasious phylogenetic signal from third codon positions were evaluated in subsequent analyses. We found that the uniformly low proportion of transitions at the third codon position of mtDNA genes should minimize the effects on the phylogentic analyses. Contrary to the commonly observed pattern of molecular divergence among species, uncorrected distances for EF-1αF1 third codon positions were higher than GTR+G distances being, on average, 4.49 times for transitions and 2.41 for transversions. This pattern suggests a lack of fit between model parameters and nucleotide substitution patterns within the dataset, which has the potential to distort the phylogenetic signal due to something other than substitution saturation (i.e. heterogeneity of nucleotide composition among OTUs).

3.2. Congruence between partitions

Maximum parsimony trees derived from mitochondrial and from EF-1αF1 genes (Fig. 2), including the third codon position, showed generalized incongruence between both topologies. The phylogeny derived from EF-1αF1 is strongly incompatible with previous knowledge on the phylogenetic relationships among the tribes of Xylocopinae, as indicated by studies using morphological data strongly supporting the derived clade Ceratinini + Allodapini and only differing on the basal placements of Manueliini and Xylocopini (Sakagami and Michener, 1987; Roig-Alsina and Michener, 1993; Engel, 2001).

Statistical tests of phylogenetic signal revealed concordance between the two mitochondrial genes, either including or excluding

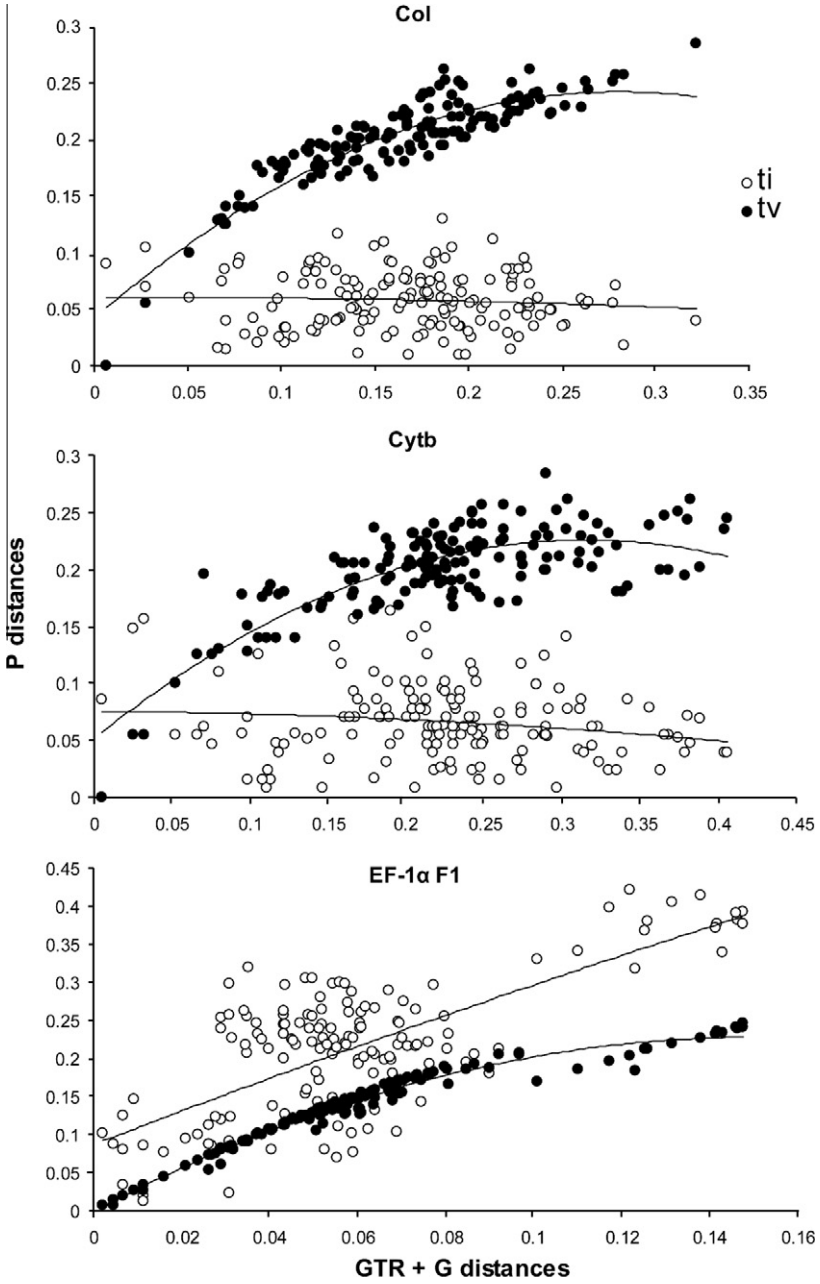

Fig. 1. Saturation on third codon positions. Comparison between GTR+G distance (*X* axis) and uncorrected distances for third codon positions (*Y* axis) estimated for CoI, Cyt*b*, and EF-F1 nucleotide sequences. Polynomial trend lines fitted either to transitions or transversions are shown.

third codon positions (respectively, $P = 0.602$ and 0.621, homogeneity test). However, conflict between phylogenetic signal derived from mitochondrial genes and EF-1αF1 was detected when third codon positions were included ($P = 0.032$, homogeneity test). This discordance vanished when third codon positions were excluded from the entire dataset ($P = 0.991$, homogeneity test) or from EF-1αF1 alone ($P = 0.997$, homogeneity test). Overall these results indicate that the discordant phylogenetic signal between mitochondrial genes and EF-1αF1 resides within the third codon positions of EF-1αF1. Analysis of nucleotide composition revealed a strong bias toward increased G + C at the third codon position of Xylocopini and Ceratinini (Table 2), which likely has a strong influence on the phylogenetic inference. Therefore, third codon positions in the EF-1αF1 data set were excluded in subsequent phylogenetic analyses.

3.3. Phylogeny of Xylocopinae

Phylogenetic analyses of concatenated sequences of the two mtDNA genes with the first and second codon positions of EF-1αF1 generated a topology which placed Manueliini as the basal tribe followed by Xylocopini and the sister tribes Ceratinini and Allodapini (Fig. 3), in agreement with the phylogenetic relationships proposed by Sakagami and Michener (1987). The same topology was recovered from analyses that included and excluded the third nucleotide position of mtDNA genes, however, statistical support for among tribes nodes were higher when third codon positions were excluded. This molecular phylogenetic analysis provided low support for the node connecting Allodapini and Ceratinini (Fig. 3), yet this clade is strongly supported by a set of synapomorphic morphological characters (Roig-Alsina and Michener,

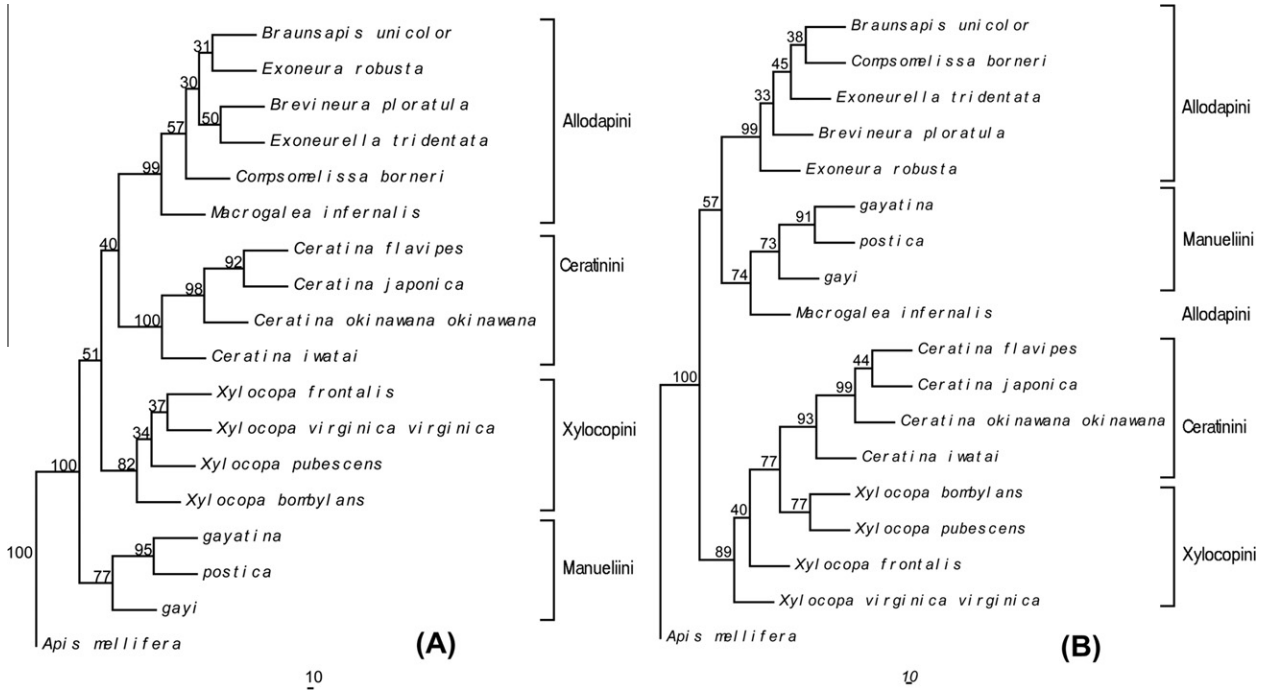

Fig. 2. Majority consensus trees obtained from maximum parsimony using (A) concatenated mitochondrial genes and (B) EF1-F1. Numbers at nodes indicate bootstrap values. Phylogenetic conflict between both partitions is clear and affects the relative positions of every tribe. The topology obtained from mitochondrial genes is strongly supported by morphological evidence.

Table 2
Base composition on the three codon positions of EF-1alfa in species of Xylocopinae. Data are mean frequencies and SD (between parentheses). χ^2 test of homogeneity of base frequencies across taxa (df = 51) showed homogeneity for first and second positions ($p = 1$), and heterogeneity for the third position ($p = 0$). χ^2 test of homogeneity of base frequencies for each of the 18 species (df = 3) showed p values above 0.95 for first and second positions. On the third codon position significant p values ($p < 0.01$), suggesting heterogeneity, were found on 13 species.

	First Codon position				Second Codon position				Third Codon position			
	A	C	G	T	A	C	G	T	A	C	G	T
Allodapini	0.31 (0)	0.15 (0.0052)	0.36 (0)	0.18 (0.0075)	0.32 (0)	0.25 (0)	0.15 (0)	0.29 (0)	0.15 (0.0273)	0.29 (0.0207)	0.37 (0.0293)	0.19 (0.0281)
Ceratinini	0.31 (0.0050)	0.17 (0.0050)	0.36 (0.0050)	0.17 (0.0058)	0.32 (0)	0.25 (0)	0.15 (0)	0.29 (0)	0.05 (0.0050)	0.44 (0.0141)	0.46 (0.0191)	0.05 (0.0050)
Xylocopini	0.3 (0.0050)	0.15 (0.0096)	0.36 (0)	0.18 (0.0115)	0.31 (0.0050)	0.26 (0)	0.14 (0)	0.29 (0.0050)	0.04 (0.0340)	0.49 (0.0436)	0.43 (0.0287)	0.05 (0.0316)
Manueliini	0.31 (0.0115)	0.14 (0.0115)	0.37 (0)	0.18 (0)	0.32 (0)	0.24 (0.0058)	0.15 (0)	0.29 (0)	0.13 (0.0058)	0.31 (0.0231)	0.35 (0.0058)	0.21 (0.0208)

1993). The low level of support provided by the molecular dataset is likely a consequence of the short branch connecting with the last common ancestor shared with the tribe Xylocopini.

Support for the basal placement of Manueliini was indicated by the relatively high bootstrap values obtained for the monophyly of Xylocopini + Ceratinini + Allodapini in addition to the moderate support for the monophyly of Manueliini (Fig. 3). The node connecting the three species of Manueliini predated the origin of any other tribe within Xylocopinae, being near to the origin of the subfamily. The basal placement of Manueliini and its relatively early origin, in addition to retention of morphological plesiomorphic characters, agree with Michener's viewpoint on this tribe as a "relict taxon" within Xylocopinae (Michener, 1979, 2007; Daly et al., 1987).

3.4. Hypothesis testing

The −log likelihoods for the alternate topologies enforcing the basal placement of Manueliini and Xylocopini were 7016.07 and 7024.61, respectively. The AU test resulted in a significant difference in the likelihoods between both constraints (Table 3). In addition, the posterior probability in the BA analyses for the basal placement of Xylocopini was zero (data not shown). In other words, the hypothesis "Xylocopini-basal" was never retained as a credible tree during the Markov chain – Monte Carlo coupled generations (MCMC) in the Bayesian phylogenetic analysis. In contrast, the basal placement of Manueliini was supported by the methods and strategies of phylogenetic reconstruction used in this study.

3.5. Implications of Manueliini basal position in the phylogeny of the Xylocopinae

As previously indicated, in Manueliini omission or removal of cell partitions by the mother during the breeding season (period in which nests contain immature developing individuals inside cells) has not been reported (Claude-Joseph, 1926; Daly et al., 1987; Flores-Prado et al., 2008a). Most nests in several Xylocopini species exhibit sealed cells during the breeding period (e.g. Dover, 1924; Rau, 1943; Hurd, 1955, 1958; Hurd and Moure, 1960; Balduf, 1962; Sakagami and Laroca, 1971; Gerling and Hermann, 1978; Camillo and Garófalo, 1982; Gerling et al., 1983; Camillo et al., 1986; Maeta et al., 1985, 1996; Thoenes and Buchmann, 1994; Steen and Schwarz, 2000; Ramalho et al., 2004; Gimenes et al.,

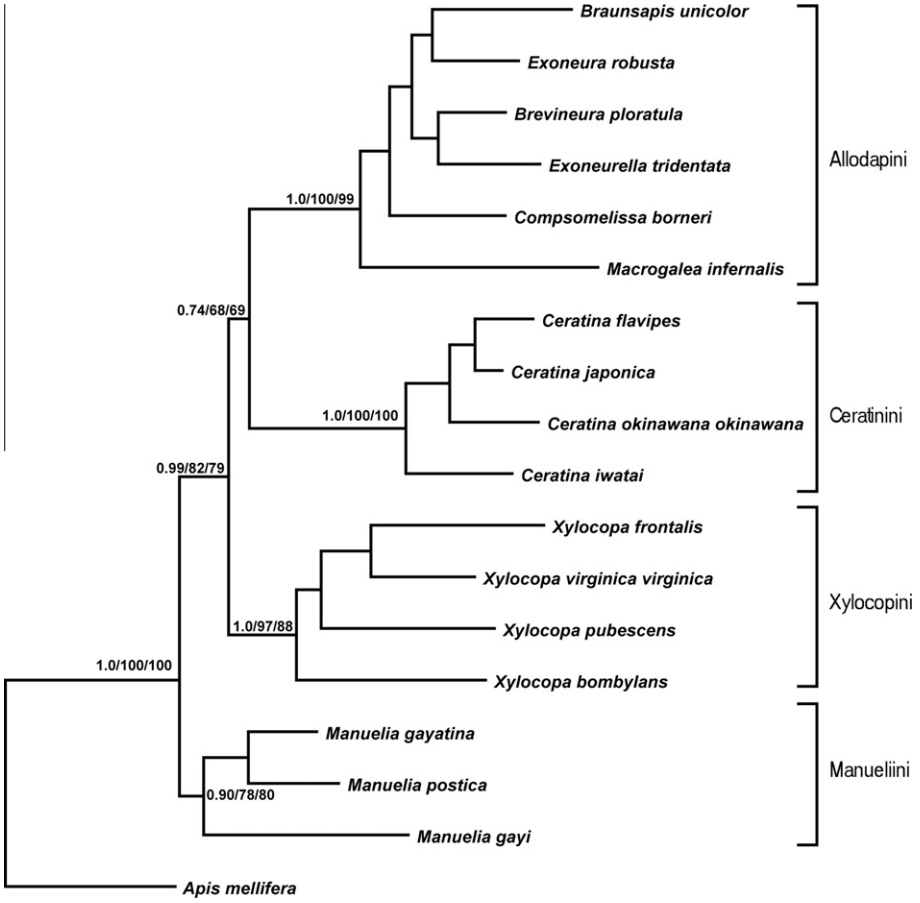

Fig. 3. Bayesian phylogeny among tribes of Xylocopinae inferred from CoI, Cyt*b* and EF-F1 nucleotide sequences. The numbers at the nodes indicate Bayesian posterior probability percentages (2×10^6 generations), and bootstrap values from maximum likelihood (10^3 replicates) and maximum parsimony (10^4 replicates), respectively. Supports within tribes are omitted. Dataset excluding mtDNA and EF1-F1 3rd codon positions.

Table 3
Contrast among topologies representing the two alternative hypotheses on the basal lineage of the subfamily Xylocopinae.

Topology	Partition			
	mtDNA		mtDNA + EF1	
	$-\ln L$	$P_{(AU\ test)}$	$-\ln L$	$P_{(AU\ test)}$
Manueliini basal	6268.55	0.821	7036.48	0.754
Xylocopini basal	6276.00	0.036	7045.40	0.042

2006). However, incomplete partitions (with a large orifice in the center) and occasional omission of partitions during breeding period have been reported in some species (Sakagami and Laroca, 1971; Silveira, 2002). By contrast, removal of cell partitions (Sakagami and Laroca, 1971; Sakagami and Maeta, 1977, 1987; Maeta and Katayama, 1978; Katayama and Maeta, 1979; Maeta et al., 1997), and omission of them during nest building (Shiokawa, 1966; Sakagami and Laroca, 1971; Sakagami and Maeta, 1977; Maeta et al., 1997; González et al., 2004) are behavioral traits common in several species of Ceratinini. Incomplete partitions have also been identified (González et al., 2004). Therefore, contact of mother and her immature progeny is quite common in Ceratinini, but likely less frequent than reported in Allodapini (Sakagami and Laroca, 1971). Allodapini species are unique in having nests without cells. Immature individuals are frequently in contact with adult bees, because larvae are progressively fed by females in most species (reviewed in Michener, 1971, 1974; Schwarz et al., 2007). The phylogenetic relationships resolved by this analysis, supports an evolutionary transition from nests with completely sealed cells that do not permit physical interaction between immature individuals (i.e. Manueliini nests) to nests lacking cells like those described in Allodapini.

In Xylocopinae, the occasional omission or removal of cell partitions has been regarded as an intermediate stage between nests with cells arranged linearly and nests without partitions (Shiokawa, 1966). This intermediate structure may represent a "preadaptation" to the total abandonment of the unit cell system (Michener, 1974), as occurs in all species of Allodapini, suggesting a transition from an isolated development of the immature individuals inside cells to a system in which mother bees and their immatures interact in the simple burrow without partitions (Maeta and Katayama, 1978). Nests consisting of tunnels made in wood, twigs or tree branches like those constructed by "Carpenter bees" (i.e. Manueliini, Ceratinini and Xylocopini) convergently arose in some species of the Lithurgini tribe (Megachilidae: Megachilinae) (O'Toole and Raw, 1991) (Fig. 4). As observed in species of Xylocopinae, occasional omission of some cell partitions inside nests has been observed in *Lithurgus atratiformis* (Houston, 1971), *Lithurgus chrysurus* (Roberts, 1978), and *Lithurgus collaris* (Hannan and Maeta, 2007), whereas total absence of cell partitions has been reported in *Lithurgus corumbae* (Garófalo et al., 1981). Comparison of nest architecture in Megachilinae has led to the hypothesis that occasional omission of cell partitions represents a transition toward progressive provisioning (as found in Allodapine bees) (Roberts, 1978). Garófalo et al. (1981) proposed an alternative hypothesis where omission of cell partitions is explained as an initial evolutionary step leading to the total absence of partitions and, consequently, individual cells.

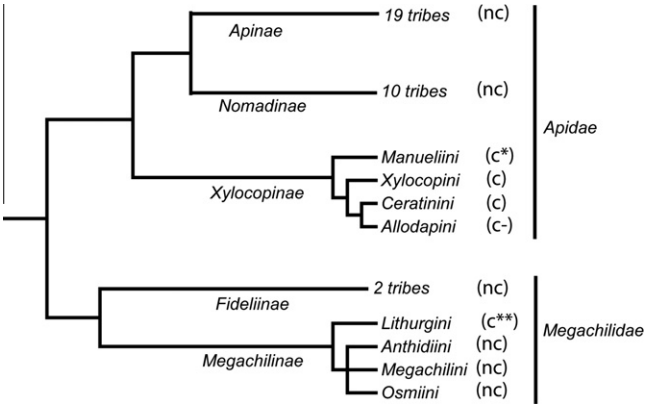

Fig. 4. Topology showing the phylogenetic relationship among the subfamilies of Apidae and Megachilidae (based on Michener, 2007). Manueliini exhibit nests constituted by cells, and omission of partitions is not observed. Xylocopini exhibits nests constituted by cells, and occasional omission of partitions. Ceratinini exhibits nests constituted by cells, and frequent omission of partitions. Allodapini exhibits nests without cells because omission of partitions is complete. Lithurgini, including species that exhibit nests always constituted by cells, nests composed by cells but occasional omission of partitions, and nests lacking cells. c = Canonical carpenter bees. c^{*} = We are considering this taxon as a carpenter bee, since it nests like canonical carpenter bees (Flores-Prado et al., 2008a). c- = Despite this taxon nests in wood, it is not a carpenter bee since does not divide nests. c^{**} = Considered as a carpenter bee by other authors (see O'Toole and Raw, 1991). nc = Non carpenter bees.

Considering the evidences summarized above, it is likely that omission or removal of partitions in Xylocopinae arose first in the last common ancestor of the Xylocopini + Ceratinini + Allodapini clade and, as shown in Fig. 4, the absence of cell partitions in Xylocopinae has converged with those observed in carpenter bees belonging to the tribe Lithurgini.

Finally, Manueliini can be used as a model to study evolution of sociality, since it is a relict taxon (Michener, 1979; Daly et al., 1987) exhibiting several morphological ancestral features (Michener, 2007) and containing species having unusual traits for solitary species, such as (i) guarding behavior by mother during the breeding period, (ii) existence of hibernating assemblages, (iii) mutual tolerance between nestmate females (Daly et al., 1987; Flores-Prado et al., 2008a) and (iv) nestmate recognition capacity (Flores-Prado et al., 2008b). The three first behavioral traits have been proposed as precursors of sociality in species of bees (Michener, 1969, 1974; Sakagami and Maeta, 1977), and the last is typical in eusocial species. To evaluate if these (and other) behavioral characters are precursors of sociality within Xylocopinae, a phylogenetic comparative approach is required including as many taxa as possible in addition to information on nesting biology from social and non-social species, in order to reconstruct ancestral states of social life in the subfamily Xylocopinae. That approach could contribute with new insights about the transitions from solitary to social modes of life.

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