



## REVIEW

### Investigating Eusociality in Bees while Trusting the Uncertainty

EAB ALMEIDA, DS PORTO

Universidade de São Paulo, Ribeirão Preto, SP, Brazil

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##### Corresponding author

Eduardo A. B. Almeida  
 Laboratório de Biologia Comparada e  
 Abelhas (LBCA), Depto. de Biologia  
 Faculdade de Filosofia, Ciências e Letras  
 de Ribeirão Preto (FFCLRP)  
 Universidade de São Paulo  
 Avenida Bandeirantes, 3900. 14040-901  
 Ribeirão Preto, SP, Brazil  
 E-Mail: eduardo@ffclrp.usp.br

#### Abstract

Phylogenetic hypotheses and estimates of divergence times have already been used to investigate the evolution of social behavior in all lineages of bees. The interpretation of the number of origins of eusocial behavior and the timing of these events depends on reliable phylogenetic hypotheses for the clades in which these lineages are nested. Three to six independent origins of eusocial behavior are interpreted to have occurred in bee taxa that differentiated in the Late Cretaceous, or much later in the Paleogene. Only two groups of bees exhibit the behaviors that qualify their members to be considered obligate (i.e. 'fixed-caste') eusocial, the honey bees (Apini) and the stingless bees (Meliponini). The evolutionary history of corbiculate bees remains uncertain in many respects, but phylogenetic research has been paving the path for comprehensive comparative approaches likely to shed light on the origin of diversity of forms and behaviors of these bees. In total, corbiculate bees encompass about 1,000 species, roughly 5% of the described species diversity of bees. These bees are rather heterogeneous in terms of social organization, particularly stingless bees and orchid bees, which display a fascinating range of behavioral variation. Using phylogenetic tools, it has been possible to infer that caste polymorphism, division of labor and other traits of corbiculate bees probably started evolving over 80 million years ago. Phylogenetic hypotheses must be interpreted as more or less uncertain scenarios for studying the biological diversity, but when trusted they can provide powerful tools to investigate the evolution of social behaviors.

#### Introduction

During the last century, research on bee behavior and morphology has largely become increasingly comparative, incorporating the diversity of traits found in the variety of species of these insects (e.g., Michener, 1944, 1974, 2007; Engel, 2011; Danforth et al. 2013). Remarkably detailed investigations had been previously made on bees (e.g., Schmid & Kleine, 1861; Girdwoyn, 1876; Macloskie, 1881; Briant, 1884; Carlet, 1884; Bordas, 1894; Abonyi, 1903; Hommel, 1904, 1905; Arnhart, 1906; Snodgrass, 1910, 1935, 1942, 1956), particularly with *Apis mellifera* Linnaeus, 1758 - the honeybee. This has long been a species with close proximity of mankind (the species of *Apis* are distributed all over the Old World), with economic interest because of honey production (pollination became a hotly debated topic more recently, e.g., Oldroyd, 2007; Cameron et al., 2010; Potts et al.,

2010; Albrecht et al., 2012; Bartolomeus et al., 2013; Garibaldi et al., 2013), and certainly because of its fascinating social organization. Along with ants, bumble bees, termites, social wasps, and unusual species of mammals (mole-rats: Jarvis [1981]), honey bees have had a prime importance for the understanding of the cohesion observed among organisms of some species that allow them to live close together, interact, share tasks related to survival and reproduction. Arthropods comprise about half of the described biodiversity, and the total number of known origins of "eusociality" is approximately 12 in this clade (Wilson, 1971; Wilson & Hölldobler, 2005). Sociality, in this way, is a very rare condition in nature, indicating that it is not a frequently acquired trait, particularly because it involves reproductive division of labor (Wilson, 1971) and all potential conflicts related to this (e.g., Trivers & Hare, 1976; Reeve & Keller, 1999; Tóth et al., 2004).



If the evolution of sociality was elementary, one might ask why there are relatively few eusocial species. Although the number of social species is reduced in comparison to the known biodiversity, sociality can be viewed as ubiquitous because social organisms, particularly insects, are everywhere and tend to be very common. Some estimates indicate that social organisms, particularly termites and ants, might comprise almost 30% of the biomass of a tropical rainforest (Fittkau & Klinge, 1973); and that the number of workers in a single colony of ants may reach more than 20 million (Hölldobler & Wilson, 1990; Wilson & Hölldobler, 2005). Among termites, this number can be as high as 13 million individual workers (Lee, 2002), honey bees are documented to have colonies comprising between 10 to 100 thousand workers (Bourke, 1999) and nests of eusocial stingless bees of the genus *Scaptotrigona* (Meliponini) may encompass 50 thousand workers (Lindauer & Kerr, 1960). These huge colonies account for the ubiquity and the heavy biomass of social organisms.

The study of the honey bee and research of all the traits related to its social behavior have been a challenge posed by researchers of several fields over the centuries. In addition to external and internal morphology (e.g., Snodgrass, 1910, 1942, 1956), there were several landmarks in ethological investigation made with *A. mellifera* (the most famous must be Karl von Frisch's 1946 study that deciphered the "waggle dance" of honey bees — see also the excellent summaries by Winston, 1987; Seeley, 1995, 2010; Page, 2013); genetics (*A. mellifera* mitochondrial genome sequencing [Crozier & Crozier, 1993]; discovery of important insulin/TOR signaling pathways and receptors related to queen caste determination [Patel et al., 2007]; discovery of the royalactin by Kamakura, 2011); physiology (food contents versus caste determination in *A. mellifera*; hormones and pathways related to social behaviors, queen dominance, etc. [e.g. Weaver, 1955; Laidlaw et al., 1956; Hartfelder & Engels, 1998; Hartfelder, 2000; Sullivan et al., 2000]); and more recently genomics (Honeybee Genome Sequencing Consortium, 2006).

A natural progress in the research of social behavior is to make it comparative, to unravel similarities and differences among the diversity of organisms that display some level of sociality. There exist intricate physiological (hormonal/neurobiological) interactions of different signaling pathways that accounts for the complexly self-organized colony of *A. mellifera*, perhaps just a fortuitous result of the evolutionary odds, perhaps a set of specific mechanistic components interacting with each other, responsible for ordering colony-level functions. One of the exciting challenges resulting from the enormous body of knowledge about *A. mellifera* is the understanding of how social interactions would be expressed in other bee species that also exhibit complex behaviors related to the self-organization of their societies (e.g., Page, 2013), and how comparative research could help elucidate the evolution of social behaviors.

## Eusociality

Among the various levels of intraspecific interactions that can be interpreted as social interactions, this work will focus on the eusocial behavior of bees, more specifically on obligate eusociality *sensu* Crespi and Yanega (1994; commonly referred to as "advanced eusocial" or "highly eusocial" behavior). Michener (1974) presented a comprehensive classification of kinds of social behaviors documented among bees in his chapter 5 "Kinds of Societies among Bees". Eusocial organisms may be characterized by life in "colonies, which are family groups", "consisting of individuals of two generations, mothers and daughters"; and by "division of labor, with some individuals functioning as egg layers or queens and others as workers, that is with more or less recognizable castes" (Michener, 1974: p.46). "Eusociality" has been defined in slightly different ways over the years, but the presence of castes seems to be a main trait for the recognition of this phenomenon (Crespi & Yanega, 1994). Other behaviors that are often cited as necessarily co-occurring in eusocial organisms are the superposition of generations and the cooperation in the tasks necessary for survival and reproduction. One important distinction between eusocial bees refers to the morphological polymorphism between females of reproductive and non-reproductive castes, and the capacity of gynes (i.e., potential or actual reproductive individual: queen) of surviving alone (Michener, 1974). When the morphological distinction is more clearly marked and a gyne is not able to start a nest by herself (Michener 1974, 2007), hence neither caste can be said to be totipotent (Crespi & Yanega, 1994), there is a phenomenon defined as dependent colony foundation, which was neatly reviewed by Cronin et al. (2013). Lack of gyne totipotency thus is key for the recognition of "advanced eusocial" or "highly eusocial", or "obligate eusocial" behavior. In this paper we choose to favor the term "fixed-caste eusociality", similar to that proposed by Crespi and Yanega (1994), because it transmits a less teleological notion than the former two. Michener (1974: p.47) remarked that it "*is tempting to look at the sequence of increasingly complex societies (...) as an evolutionary sequence*", but in fact multiple evolutionary changes toward simpler as well as more complex behaviors have occurred throughout bee history. Terms with ability to introduce misconceptions, such as "primitive" and "advanced" should, hence, be avoided and replaced by "totipotent-caste" and "fixed-caste" when possible. There are at least five independent origins of eusociality among bees, as discussed below, but obligate eusociality is rare and can only be found in the honey bees (Apini: *Apis*) and the stingless bees (Meliponini).

## Phylogenetic research and the understanding of social evolution

Recent insights given by phylogenetic work have allowed estimation of divergence ages between distinct lineages of social organisms (e.g., Cardinal & Danforth, 2011). The two most distantly related eusocial taxa, mammals and insects, diverged between the Precambrian and the Early Cambrian, at the early

split of the common ancestor of bilaterian animals that gave rise to Protostomia and Deuterostomia (Blair, 2009; Edgecombe et al., 2011). Focusing on the eusocial insects, termites diverged from the lineage that gave rise to Hymenoptera in the beginning of the Mesozoic or earlier (Ware et al., 2010; Cardinal & Danforth, 2011), and began their differentiation and diversification about 235 Mya, during the Late Triassic. Within Hymenoptera the origin of eusocial behavior in ants probably occurred in the early Cretaceous, which occurred independently from vespid wasps (some time in the Cretaceous: Grimaldi & Engel [2005], Brady et al. [2006a], Moreau et al. [2006], Hines et al. [2007]); and bees, which comprise three to six independent origins of eusocial behavior in taxa that differentiated in the Late Cretaceous, or much later in the Paleogene (Brady et al., 2006b; Chenoweth et al., 2007; Cardinal & Danforth, 2011; Gibbs et al., 2012; Danforth et al., 2013).

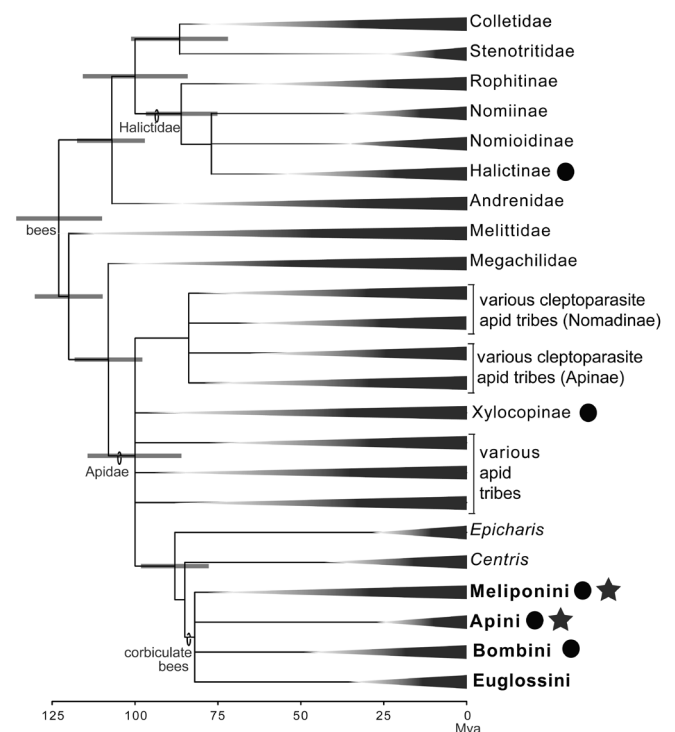
These discrepancies in divergence ages bring one certainty and several possible questions. We can say we are confident that eusociality is not a homologous condition that can be properly used in a comparative (i.e., historical) context. Therefore, caution must be exercised at all times when comparisons are made between taxa showing social behaviors because their traits may not be directly comparable (i.e., homologous; Nixon & Carpenter, 2012). A more radical interpretation of sociality, as a non-homologous trait, is its interpretation as an emerging property or epiphenomenon resulting from the co-occurrence of complex suite of traits related to the coexistence and certain interactions among individuals of a species. Given that eusociality surely is not a homologous characteristic, there are various interesting queries that can be posed about the evolution of social behaviors, such as the following: (a) When can we assume that at least some of the traits comprised in the definition of “eusociality” to be homologous?; (b) Do the defining traits of “eusociality” appear together or they evolve in a stepwise manner?; (c) Is it possible to relate multiple evolution of “eusocial” behaviors with general evolutionary factors or they are mostly intrinsic properties of the organisms that possess them?

It is very possible that many of those questions will be confidently answered in the years to come partly because of the empirical knowledge that is fundamental for depiction of life’s diversity, as well as the growing phylogenetic basis necessary for historical comparative research. Below we are going to discuss the relative confidence and uncertainty regarding the phylogenetic history of bees (and its relevance for comparative research); the most diverse taxon of obligate eusocial bees: the Meliponini; and the importance of the growing body of knowledge about orchid bees to the understanding of behaviors in corbiculate bees.

#### *Relative Uncertainty of Phylogenetic Hypotheses Available for Bees*

Our understanding of bee phylogeny has been improving (Michener, 1944, 2007; Roig-Alsina & Michener, 1993; Alexander & Michener, 1995; Danforth et al., 2013) and, with it, a growing comparative framework for understanding the evolution of social behavior is being constructed. The higher-level (i.e., family-

level) classification of bees has become very remarkably stable over the last decades particularly because of intense phylogenetic research within Apoidea (e.g., Roig-Alsina & Michener, 1993; Alexander & Michener, 1995; Melo, 1999; Danforth et al., 2006a, b, 2013; Michener, 2007; Engel 2011; Debevec et al., 2012; Cardinal & Danforth 2013; Hedke et al., 2013). There are still portions of the tree of bees that can be represented by incongruent competing hypotheses (e.g., Alexander & Michener, 1995; Engel, 2011; Danforth et al., 2013), but the accumulated scientific progress is nevertheless clear. Phylogenetic hypotheses and estimates of divergence times have been used to investigate the evolution of social behavior in all lineages of bees (reviewed by Schwarz et al., 2007; Cardinal & Danforth, 2011; Danforth et al., 2013). The interpretation of the number of origins of eusocial behavior and the timing these events depends on reliable phylogenetic hypotheses for the clades in which these lineages are nested. A summary of bee phylogeny is presented in Figure 1, depicting in particular detail clades where eusocial behavior is present



**Fig 1.** Phylogenetic relationships within bees (Hymenoptera: Apoidea: Anthophila). The overall relationships depicted are those summarized by Danforth et al. (2013). Bee lineages are represented at family-level resolution for taxa not comprising eusocial representatives, or in more detail in cases where eusocial species are present (indicated by solid circles, stars indicate the bee taxa considered obligate eusocial) — see Michener (2007) and Schwarz et al. (2007) for details. Unresolved nodes (i.e., polytomies) and gray shaded branches indicate regions of the tree of bees upon which uncertainty and conflicts have been most noticeable in studies published during the last decade. Branch lengths are proportional to the evolutionary time, and a ruler scaled in million years before the present is given beneath the tree (divergence ages and the estimates for clade ages were taken primarily from Cardinal & Danforth [2013], and complemented by estimates by Brady et al. [2006b], Cardinal et al. [2010], Almeida et al. [2012], Gibbs et al. [2012], and Martins et al. [2014]).

in all of its component taxa or part of them. It is worth noticing that hypotheses about the factors and processes favoring the evolution of social interactions (e.g., Michener, 1974, 1985; Wcislo & Tierney, 2009) can be dissociated from a number and timing of evolutionary events, although there are obvious advantages in jointly investigating these two fields.

Whereas the hypotheses of phylogenetic relationships among the eusocial representative taxa of allopapine bees (Apidae: Xylocopinae) and Halictinae (Halictidae) have become more stable over the years (Brady et al., 2006b; Chenoweth et al., 2007; Schwarz et al., 2007; Gibbs et al., 2012), relationships among the corbiculate bees have remained largely uncertain based on phylogenetic investigations and discussions published since the 1970's. Nevertheless, two points must be made about the systematics of corbiculate bees: (a) there is almost no doubt about the naturalness (i.e., monophyly) of the corbiculate-bee clade (e.g., Roig-Alsina & Michener, 1993; Cardinal & Danforth, 2011); and (b) there is little or no controversy over the natural boundaries of the four most distinctive corbiculate lineages, which are classified as the tribes Apini, Bombini, Euglossini, and Meliponini. The most recent common ancestor of corbiculate bees is estimated to have begun differentiating from the closest apid lineages at about 95-72 million years ago, during the Late Cretaceous (Cardinal et al., 2010; Cardinal & Danforth, 2011, 2013; Martins et al., 2014). The group comprises the well-known honey bees (*Apis*, 11 species — Apini), bumble bees (*Bombus*, approximately 260 species — Bombini), orchid bees (5 genera, approximately 240 species — Euglossini), and stingless bees (ca. 60 genera, over 500 species — Meliponini) (Michener, 2007; Rasmussen & Cameron, 2010; Camargo & Pedro, 2012; Moure et al., 2012; Ascher & Pickering, 2014). In total, corbiculate bees encompass about 5% of the described species diversity of bees known to this day, and the group is far from homogeneous in terms of social organization (particularly Meliponini and Euglossini). In contrast, the closest relatives to the corbiculate clade are the bee genera *Centris* and *Epicharis* (Cardinal & Danforth, 2011; Martins et al., 2014), which do not display any kind of social behavior (Michener, 2007; Cardinal & Danforth, 2011; Martins et al., 2014).

It should be a rather straightforward problem the search for the phylogenetic relationships among Apini, Bombini, Euglossini, and Meliponini, because there are only three possible unrooted tree topologies, based on the mathematical properties of trees (Felsenstein, 1978). For each of these unrooted networks, there are five possible rooted tree topologies, as shown in Figure 2. After comparing the empirical support each of the 15 possible cladograms received over the history, it is amazing to realize that nine of them have had at least one paper in their favor (Fig 2), although some of them are supported by many results (hypotheses H1-c, H1-d, H2-c, H2-e shown in Fig 2). The conflicting empirical support received by the various phylogenetic hypotheses of corbiculate bees has been hard to explain. The gap between hypotheses listed as H1 and those

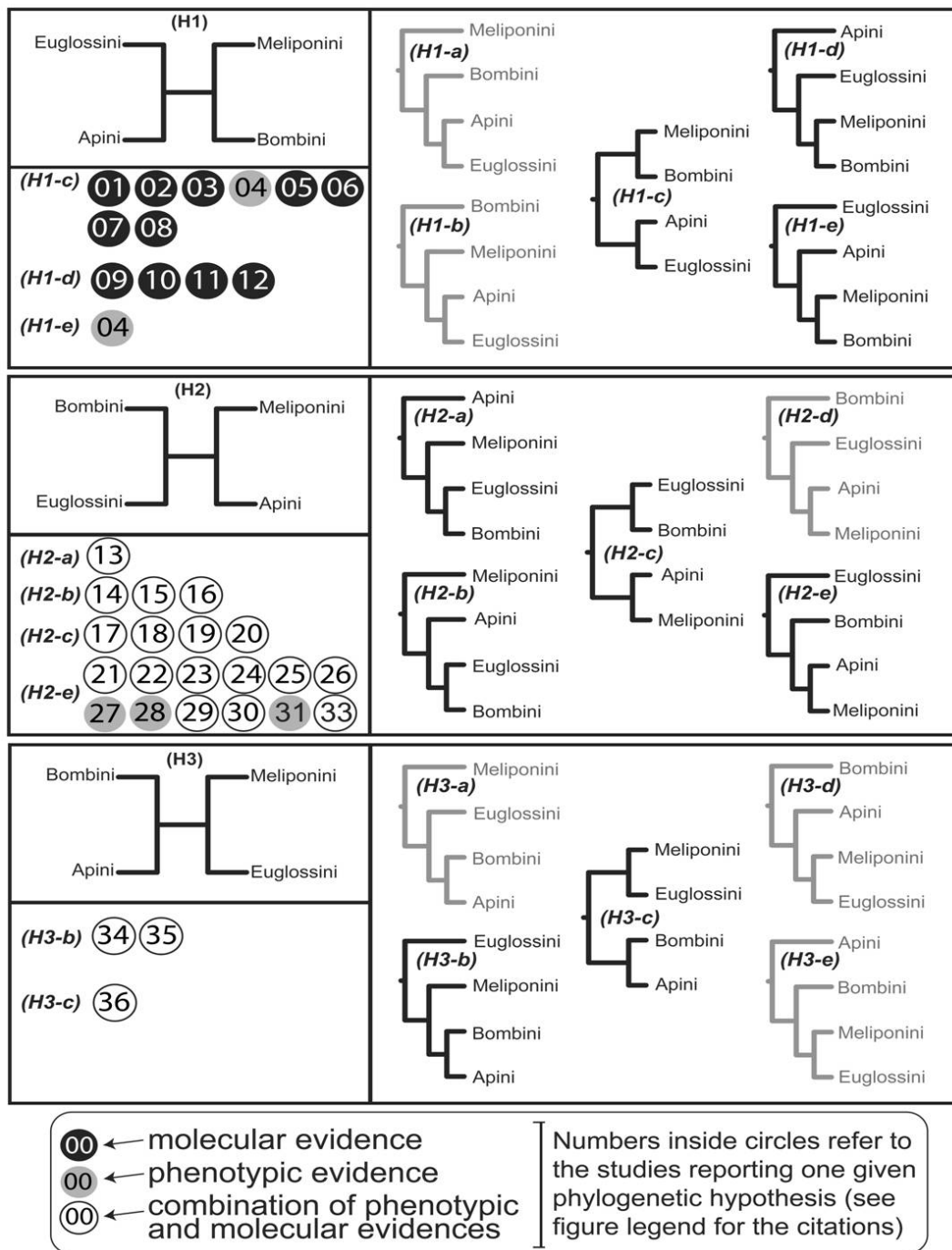
portrayed as H2 in Figure 2 is clearly related to the source of the data employed for the investigation, as molecular datasets largely support the former, and phenotypic characters (behavior and morphology) favor the latter in most cases.

Potential convergence in parts of the phenotype and artifacts related to molecular evolution have been pointed as the most likely causes for the contention (Winston & Michener, 1977; Michener, 1990; Ascher et al., 2001; Cameron & Mardulyn, 2001; Lockhart & Cameron, 2001; Kawakita et al., 2008), but this has not resolved the controversy about how Apini, Bombini, Euglossini, and Meliponini are related to each other. The early diversification of corbiculate bees probably happened in ways that make the ancient phylogenetic signal hardly detectable. In part, this might be explained by the extinction of various lineages of corbiculate bees since the Cretaceous (e.g., Engel, 2001a, b; Engel et al., 2009) and possibly by rapid divergence among some lineages. Choice of outgroups is a sensitive issue when working with corbiculate bee relationships (e.g., Canevazzi & Noll, 2014), which is illustrated by the unstable placement of different lineages of Apidae near or within the corbiculate clade (Table 1 of Cardinal & Packer, 2007). Finally, it is worth speculating the rooting is also an issue when selecting one of the alternative rooted tree topologies (right column of Fig 2) from one of the unrooted trees shown on the left of the same figure. Character data will support one of the three topologies on the left, whereas rooting (more specifically, the placement of the root-node) will be decisive for the proposal of one rooted tree-topology as the most likely scenario for the evolutionary connections among the four corbiculate-bee lineages. The consequences of the instability of placement of the root-node are far from trivial, as for example, in H2 the relationships can vary from Apini as sister-group of all remaining corbiculates (Fig 2, hypothesis H2-a), Meliponini as sister to the other three apine tribes (Fig 2, hypothesis H2-b), or Meliponini as sister to Apini (Fig 2, hypotheses H2-c and H2-e). These are the possibilities only within the realm of the hypothesis termed 'H2' in Figure 2, but there are also three possible rootings accepted for H1 and two possibilities within H3, as can be seen in the same figure.

#### *Phylogenetic Uncertainty and Behavioral Evolution within the corbiculate clade.*

How do the incongruences and the uncertainty about the phylogeny of this group of bees affect our understanding of the evolution of obligate sociality in the corbiculate clade?

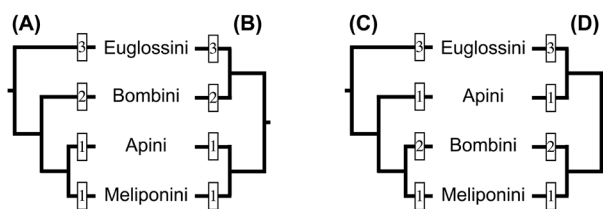
If the phylogenetic hypotheses sustained by the majority of morphological and behavioral data obtained so far is correct, thus we should assume that the direct ancestor of Apini+Meliponini also presented some (or all) the traits necessary to be considered obligate eusocial too (Fig 3A, B). Alternatively, it is possible that the immediate ancestor of Apini+Meliponini+Bombini (Fig 3C) or of all four taxa (Fig 3D) already presented some or all the traits observed in obligate eusocial bees. Although the debate about



**Fig 2.** The corbiculate bee clade comprises four monophyletic groups: Apini, Bombini, Euglossini, and Meliponini. There are only three possible unrooted tree topologies that enable the representation of alternative scenarios for these four taxa, which are illustrated by the diagrams on the left (H1-H3). On the right column, the five possible rooted topologies are given to the corresponding three diagrams. Published hypotheses supporting each of the rooted tree topologies are given inside circles and can be tracked by the following referencing system: <01> Cameron (1991); <02> Koulianos et al. (1999); <03> Schultz et al. (1999); <04> Cameron & Mardulyn (2001); <05> Kawakita et al. (2008); <06> Cardinal et al. (2010); <07> Cardinal & Danforth (2011); <08> Cardinal & Danforth (2013); <09> Sheppard & McPheron (1977); <10> Cameron (1993); <11> Koulianos et al. (1999); <12> Mardulyn & Cameron (1999); <13> Kerr (1987); <14> Winston & Michener (1977); <15> Kimsey (1984); <16> Sakagami & Maeta (1984); <17> Michener (1974); <18> Michener (1990); <19> Schultz et al. (1999); <20> Serrão (2001); <21> Michener (1944); <22> Maa (1953); <23> Michener (1990); <24> Prentice (1991); <25> Roig-Alsina & Michener (1993); <26> Chavarría & Carpenter (1994); <27> Schultz et al. (1999); <28> Ascher et al. (2001); <29> Engel (2001a, b); <30> Noll (2002); <31> Cardinal & Packer (2007); <32> Payne (2013); <33> Canevazzi & Noll (2014); <34> Plant & Paulus (1987); <35> Peixoto & Serrão (2001); <36> Pereira-Martins & Kerr (1991). Placement of outgroup taxa (i.e., non-corbiculate bees) was ignored when accounting for the support of published hypotheses in relation to the 15 possible rooted cladograms. All unrooted tree topologies were favored by at least one published study as noticeable by references listed in the figure; and nine out of the fifteen possible rooted tree topologies were favored by at least one study (black trees on the right of the figure). Three points are worth highlighting when evaluating the support given by the various types of data and/or authors about these alternative phylogenetic scenarios: (1) there is a clear prevalence of molecular data supporting hypotheses shown in H1; (2) there is a clear prevalence of phenotypic genotypic data supporting hypotheses shown in H2; (3) scenarios shown in H3 have received lower support by empirical data than H1-2.

single versus multiple origins of obligate eusociality has generated enthusiastic discussions over the years, it must be pondered that this is an over-simplification of a more complex case.

It is far less informative because the relation of homology being sought and investigated is not about “obligate eusociality” itself, but the suite of traits that evolve and are the requisites for this condition to emerge. If research programs are targeted on obligate eusociality, growth of knowledge about the evolution of this epiphenomenon will be likely hindered. An innovative examination of key traits most directly related to the appearance of eusociality in bees was that by Cardinal and Danforth (2011), who reconstructed states of phenotypic characters that co-occur in eusocial bees. This resulted in the reconstruction of evolutionary paths taken by the following five characters in the corbiculate clade: (a) castes/division of labor, (b) adults of two generations, (c) morphologically distinct gynes, (d) progressive feeding, and (e) swarming. A simplified version in which levels of sociality are treated as character-states was also considered, but it is way less informative than the former (Cardinal & Danforth, 2011: Fig 1C, their “traditional” model). In all scenarios evaluated, totipotent-caste eusociality was the most likely condition estimated for the most recent common ancestor of all corbiculate bee species (Cardinal & Danforth, 2011). Worth noting too is the extensive behavioral comparison by Noll (2002), who expanded the term “social” into no fewer than 42 characters! The continued detailed investigations of morphological and behavioral changes, as well as the genetic basis for eusociality to appear (e.g., Toth et al., 2007; Woodard et al., 2011) will be key for the advancement in this field.



**Fig 3.** Schematic representation of four commonly proposed hypotheses of phylogenetic relationships among the four tribes of corbiculate bees (Apidae). Hypotheses A and B are favored by the several datasets comprising phenotypical characters (Fig 2: hypothesis H2-e and H2-c), whereas Hypotheses C and D are recurrent results when these relationships are inferred using molecular datasets (Fig 2: hypothesis H1-e and H1-c). Numbers at each of the terminal branches represent conditions of sociality: 1: fixed-caste eusociality; 2: totipotent-caste eusociality; 3: social behaviors present, but not eusociality. Tree topologies A-C correspond to hypotheses H2-e, H2-c, H1-e, H1-c (Fig. 2), respectively.

*Fascinating Behavioral Variation and the Need of Comparative Research: Stingless Bees and Orchid Bees (Apidae: Meliponini and Euglossini)*

When comparing the four major lineages of corbiculate bees, the Meliponini occupy a very special position in all measures of their diversity. There are about 500 described species of stingless bees, which are distributed in various tropical regions of the planet (the distribution was wider in the past as documented by the fossil

record: e.g., Engel [2011]), there is a great morphological diversity, which is reflected by the approximately 60 genera currently accepted, and great behavioral variation. The diversity of biologies of stingless bees has been documented by several researchers, many of which made important contributions to document it in comparative manners (e.g., Ihering, 1903, 1930; Kerr, 1948, 1969, 1987; Sakagami, 1982; Engels & Imperatriz-Fonseca, 1990; Imperatriz-Fonseca & Zucchi, 1995; Faustino et al., 2002; Tapy & Gilley, 2004; Tóth et al., 2004; Santos-Filho et al., 2006). Observations, experiments and predictions exist for attributes such as queen-policing, mode of queen production, worker egg-laying and cost of worker reproduction, relatedness between sisters in a colony (e.g., Engels & Imperatriz-Fonseca, 1990; Imperatriz-Fonseca & Zucchi, 1995; Tóth et al., 2004), making stingless bees a biologically diverse and with an incredibly open field for comparative research. Many comparisons made in the past lacked an explicit phylogenetic framework, which have the potential of illuminating which evolutionary scenarios are more likely to explain the observed phenotypic diversity. One notable exception was the evaluation of the diversity in composition of the cuticular chemistry within the Meliponini using an explicit phylogenetic framework (Leonhardt et al., 2013).

Different authors contributed to the understanding of phylogenetic relationships within Meliponini (e.g., Schwarz, 1948; Moure, 1961; Wille, 1979; Michener, 1990; Camargo & Pedro, 1992a, b; Costa et al., 2003; Rasmussen & Cameron, 2010). A summary of the three most comprehensive phylogenetic hypotheses (in terms of taxon sampling) is presented in Fig 4. The great diversity of forms, questionable proposals for generic delimitation, and wide geographical distribution certainly made the task of studying all stingless bees very challenging. The comparison of three hypotheses proposed independently along the last 24 years (Fig 4A-C) makes it clear that certain portions of the tree are very stable for example, *Trigonisca*, *Leurotrigona* and related lineages have been placed relatively distant from the remaining Neotropical genera, whereas placement of certain taxa have varied widely with the analysis. The genus *Melipona*, for example, is placed as sister to the majority of Neotropical meliponine genera in one hypothesis (Fig 4A; Rasmussen & Cameron, 2010), sister of a smaller Neotropical clade (Fig 4B; Camargo & Pedro, 1992a), or sister of all other stingless bee genera (Fig 4C; Michener, 1990).

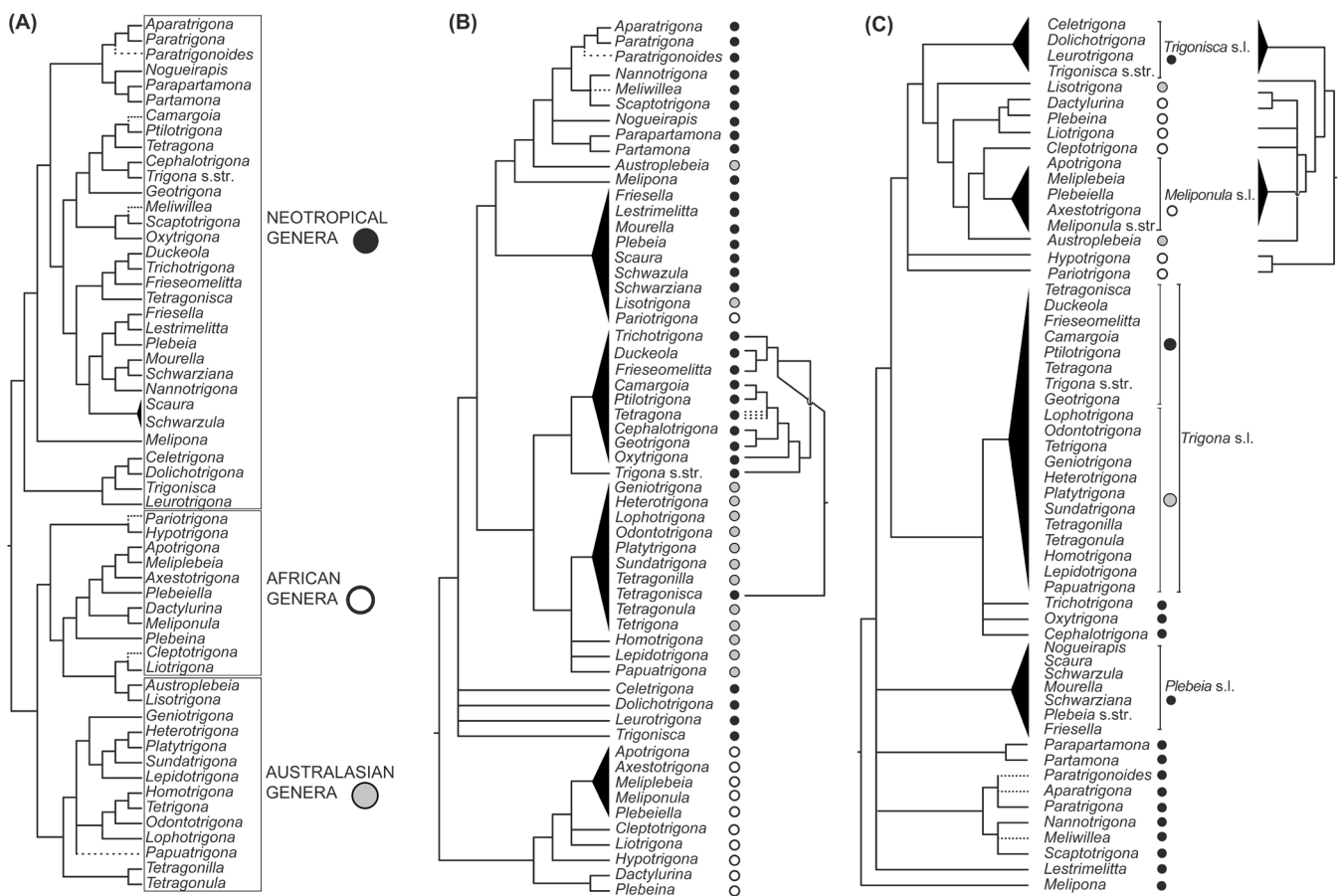
The Neotropical genus *Melipona* has had an important place in the discussion of behavioral evolution of stingless bees, because of its rather unique morphological and biological characteristics. Some authors have argued for a classification of stingless bees in which *Melipona* would be clustered in its own subgroup (i.e., Meliponini s.str.), apart from the *Trigona*-group (Trigonini), comprising all remaining genera of Meliponini (e.g., Moure, 1961). *Melipona* is the sole stingless bee genus where polygynic colonies (multiple queens) are known (Bego, 1989; Alves et al., 2011); these bees are also unique for the breeding queens, males and workers in identical cells, instead of having distinctly larger royal cells as

in the other Meliponini (Michener, 1974; Engels & Imperatriz-Fonseca, 1990); and the occurrence of worker parasitism related to male production (Alves et al., 2009) and alien queens infiltrate colonies whose own queen had recently died (Wenseleers et al., 2011). A robust phylogenetic framework for the Meliponini can make possible the investigation of the unique traits of *Melipona*, which might be autapomorphic for these bees, or be better interpreted as retention of plesiomorphic states in some cases. Behaviors related to the evolution of obligate eusociality will then be reinterpreted in historical terms.

Orchid bees (Euglossini) are another key-group for discussing the evolution of social interactions in the corbiculate clade (e.g., Soucy et al., 2003). Whereas research focused on the other corbiculate tribes (Apini, Bombini, Meliponini) mainly clarify the maintenance of elaborate social behaviors, investigation on Euglossini species are especially important because they would be able to potentially shed light on issues

concerning the appearance of these social behaviors. Two genera are strictly cleptoparasitic on other euglossine bees, *Aglae* and *Exaerete*, whereas species of *Euglossa*, *Eulaema*, and *Eufriesea* are solitary, communal or “primitively social” (Zucchi et al., 1969; Dressler, 1982; Garófalo, 1985; Cameron, 2004; Augusto & Garófalo, 2009). Although orchid bees are closely related to the eusocial honey bees, bumble bees, and stingless bees, no species of Euglossini has been reported to display eusocial behaviors comparable to what is known for the remaining corbiculate tribes. Some species in *Euglossa*, however, present clusters of behavioral traits that are somewhat similar to those observed in Bombini. The decision to classify them as totipotent-caste eusocial in this case would be, perhaps, arbitrary.

Unfortunately, little is known about the nesting biology and social interactions of most of the species of Euglossini. If we consider all the described species, only a small amount of them



**Fig 4.** Summary of the phylogenetic relationships among the genera of stingless bees (Apidae: Meliponini) as proposed by (A) Rasmussen and Cameron (2010) – molecular dataset; (B) Camargo and Pedro (1992a), accompanied by a more detailed account of relationships among Neotropical genera later studied by Camargo and Pedro (2003) on the left – both morphological datasets; and (C) Michener (1990) – morphological dataset – showing two alternative scenarios for the inner relationships of one of the clades. The genus-level classification was standardized to match Rasmussen and Cameron’s classification and thus make the three hypotheses readily comparable. When one of the three analyses did not include a given genus, a putative position was defined for it and dashed lines represented the placement of this genus. For example, the following five genera were not originally included in the analysis by Rasmussen and Cameron (2010): *Camargoia*, *Cleptotrigona*, *Meliwillea*, *Papuatrigona*, and *Paratrigonoides*; putative placement of these in this summary tree was based on hypotheses and comments by various authors (Michener, 1990; Roubik et al., 1997; Camargo & Pedro, 2004; Camargo & Roubik, 2005), as previously indicated by Rasmussen and Cameron (2010).

(< 20%) has their nests described in some detail (Dressler, 1982; Kimsey, 1982; Garófalo, 1985, 1992; Garófalo et al., 1998; Cameron, 2004). The genus *Euglossa* is the best-studied group and probably the most interesting taxon for sociobiological investigation in Euglossini, with the nesting behavior described for at least six species (Garófalo, 1985, 1992; Augusto & Garófalo, 2009; Andrade-Silva & Nascimento, 2012). The interest on this genus is largely explained by the usual occurrence of multi-female nests (MFN) with some overlap of generations and task allocation with reproductive dominance (Augusto & Garófalo, 2009, 2011). MFN's are commonly attained by nest reactivation by the daughters in the presence of the mother (matrifilial associations), although it can be potentially accomplished also by two or more sisters of a same generation (i.e., sororal associations). When a nest is reactivated, the mother or older sister could act as the dominant (Ramírez-Arriaga et al., 1996; Pech et al., 2008; Augusto & Garófalo, 2009, 2011). In addition to *Euglossa*, there are reports of task allocation and reproductive dominance (division of labor) in the genus *Eulaema* (Bennett, 1965; Dodson, 1966), which is not verified in communal nests of *Eufriesea* (Dressler, 1982; Kimsey, 1982).

In this context, a phylogenetic framework is indispensable to fully understand the evolution of social-related traits in Euglossini (e.g., Ramírez et al., 2010), and corbiculate bees as a whole, without making erroneous assumptions on the homology of behavioral complexes. Noll (2002) remarked that many components of what are called “primitive” and “advanced” social behavior should be viewed as sets of independent characters. In this way, analyses of complex traits related to sociality should be done very carefully as in the following comparisons: (a) single-female nests (solitary) vs. multi-female nests (MFN), and matrifilial or sororal associations; (b) communal nests vs. existence of task allocation (e.g., guarding, provisioning) and evidence of reproductive dominance (i.e., oophagy, threatening/aggressive behavior). The knowledge about the co-occurrence of these traits and the instances that this occurs in the euglossine phylogeny is central for grasping the fine aspects of social evolution. Nonetheless, phylogenetic relationships among the five genera of Euglossini remain highly uncertain (see Cameron, 2004; Cardinal & Danforth, 2011; Ramírez et al., 2011 for a sample of conflicting results). Better understanding of these relationships together with a much needed accumulation of behavioral data for orchid bees (particularly *Euglossa*) will provide the confidence needed for the resolution of enigmas regarding social evolution within Euglossini and the corbiculate clade as whole.

According to the resulting ancestral state reconstructions by Cardinal and Danforth (2011), the most likely ancestral state for corbiculate bees was facultative eusociality. Perhaps more relevant than the accuracy of the reconstruction itself is the indication of a strong signal of social interactions as pervasive in the early stages of evolution of corbiculate bees. Complex forms of communication, caste polymorphism and division of labor, and construction of complex nests could have begun its

unique path of evolution in corbiculate bees in the Cretaceous, over 80 million years ago (Cardinal & Danforth, 2011).

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